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(54) Title: BISPECIFIC CAR T-CELLS FOR SOLID TUMOR TARGETING

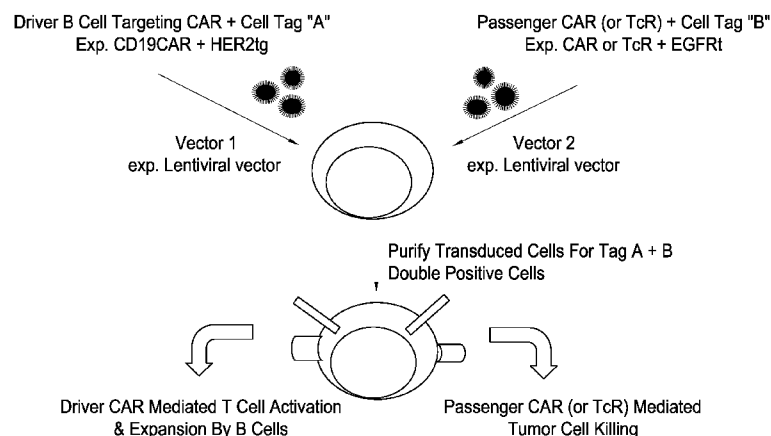


FIG. 1

(57) Abstract: Disclosed herein are methods of engineering a bi-specific T-cell expressing chimeric antigen receptors for promoting the in vivo expansion and activation of an effector cell and a second chimeric antigen receptor or TcR specific for a ligand on a tumor. Methods of administering to subjects in need, bi-specific chimeric antigen receptor bearing cells are also provided.

BISPECIFIC CAR T-CELLS FOR SOLID TUMOR TARGETING

INCORPORATION BY REFERENCE TO ANY PRIORITY APPLICATIONS

[0001] The present application claims the benefit of priority to U.S. Provisional Patent Application No. 62/202,698, filed August 7, 2015, the entire disclosure of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING, TABLE, OR COMPUTER PROGRAM LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled SCRI.95WO.TXT, created July 26, 2016, which is 45 kb in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] Aspects of the present invention described herein, include methods, cells and compositions for augmenting the therapeutic potency of adoptively transferred chimeric antigen receptor (CAR) bearing T-cells against solid tumors. In particular, methods, cells and compositions for CAR T-cell products that co-express two CARs in individual T-cells, such as a B cell targeting "driver" CAR for promoting in vivo expansion and activation of an effector cell, and a CAR or T-cell receptor (TcR) of a desired specificity for targeting a solid tumor (passenger CAR/TcR), are provided herein.

BACKGROUND OF THE INVENTION

[0004] A variety of cellular therapies have been integrated into the standard methods used in the treatment of cancer. The cellular therapy for patients suffering from cancer or disease is the injection of cellular material, such as living cells into a patient in need. This can include the infusion of polyclonal or antigen specific T-cells, lymphokine activated killer cells, natural killer cells, dendritic cells as well as macrophages. Advancements have been made in the development of chimeric antigen receptor (CAR)

bearing T-cells for adoptive T-cell therapies for cancer therapy, which are a promising therapeutic route for cancer immunotherapy and viral therapy.

[0005] CAR T-cell therapy is an immunotherapy in which the patient's own T-cells are isolated in a laboratory, genetically manipulated to express a synthetic receptor to recognize a particular antigen or protein, and reinfused into the patient. A CAR can be comprised of several domains. Without being limiting, the CAR can have 1) an antigen-binding region, typically derived from an antibody, (2) a transmembrane domain to anchor the CAR into the T-cells, and/or (3) 1 or more intracellular T-cell signaling domains. First-generation CARs commonly incorporated a single chain variable fragment (scFv) that is derived from a monoclonal antibody (mAb) plus a signaling motif from a TCR ζ chain. The second- and third-generation CARs are an improvement over the first-generation CARs with co-stimulatory activating motifs, which can lead to the enhanced proliferation, cytotoxicity and persistence of the CAR bearing cells *in vivo*. Clinical trials have shown some evidence of anti-tumor activity, with insufficient activation, persistence and homing to cancer tissue. Some anti-tumor responses have been reported in patients with B cell lymphoma, for example, and some neuroblastoma patients have reported partial response, stable disease and remission. Second- and third- generation CAR-modified T-cells have been shown to be able to provide enhanced activation signals, proliferation, production of cytokines and effector function of CAR-modified T-cells in pre-clinical trials. Initial clinical trials have been shown to exhibit some promising results.

[0006] Unfortunately, the field of immunotherapy is unable to adequately address the problems associated with CAR bearing T-cells. Problems can include the suboptimal expansion and activation cells as well as toxic effects that occur upon infusion of cells. There are several types of toxicities that can occur with the administration of CAR T-cells. Adverse reactions can include B cell aplasia, cytokine release syndrome (CRS) and tumor lysis syndrome upon infusion of CAR bearing T-cells. B cell aplasia can occur due to effective targeting of antigens on a B-cell. However, B cells are not a life sustaining type of tissue and can be an initial target for CAR T-cells.

[0007] Expanding the CAR T-Cells in the body can be associated with CRS. The symptoms of CRS can include fever, hypotension, hypoxia, and neurologic changes. Neurologic changes can include seizures, aphasia, and mental status changes. Additional

clinical and biochemical changes may occur, such as disseminated intravascular coagulation and/or transaminitis associated with marked elevations in ferritin and C-reactive protein, which are found to be similar to macrophage activation syndrome or hemophagocytic lymphohistiocytosis (HLH).

[0008] Adverse effects can also include failure to exhibit engraftment, which may be due to the limited migration of infused T-cells to the sites of tumor metastasis, and the limited immunostimulatory activity of solid tumors and their immunosuppressive environment. As the development for CARs is still in its infancy, advancements in the field of CAR T-cell therapy are much needed to circumvent the adverse effects that arise during therapy.

SUMMARY

[0009] In a first aspect, a nucleic acid encoding a chimeric antigen receptor is provided. The nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip

sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or

binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

[0010] In a second aspect, a nucleic acid encoding a chimeric antigen receptor is provided. The nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain

and/or CD3-zeta domain, and wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid; and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached to a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In

some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

[0011] In a third aspect, a nucleic acid encoding a chimeric antigen receptor is provided. The nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv

is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid sequence is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME,

SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment

thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

[0012] In a fourth aspect, a nucleic acid encoding a chimeric antigen receptor is provided. The nucleic acid comprises a first nucleic acid comprising a sequence encoding a

leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid sequence is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is

tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or

scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In

some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

[0013] In a fifth aspect, a nucleic acid encoding a bi-specific chimeric antigen receptor is provided. The nucleic acid encoding the bi-specific chimeric antigen receptor comprises a first nucleic acid sequence comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some

alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a

nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the

antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

[0014] In a sixth aspect, a nucleic acid encoding a bi-specific chimeric antigen receptor is provided. The nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end

of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, an eighth nucleic acid comprising a sequence encoding a linker, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, and a ninth nucleic acid comprising a sequence encoding a marker domain, wherein the ninth nucleic acid is covalently attached at a 3' end of the eighth nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21,

CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, IDO1, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence

set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or

binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

[0015] In a seventh aspect, a vector for expression of a chimeric antigen receptor specific for promoting in vivo expansion and activation of B cells is provided. The vector can comprise the nucleic acid of any one of the alternatives described herein. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a

sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid; and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached to a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In

some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFRSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B

cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. The leader sequence can comprise a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. The vector can be a viral vector in some alternatives. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

[0016] In an eighth aspect, a vector for expression of a chimeric antigen receptor or TcR specific for targeting a solid tumor is provided. The vector can comprise the nucleic acid of any one of the alternatives described herein. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid sequence is covalently

attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid sequence is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the

T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is

encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific

molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. The vector can be a viral vector in some alternatives. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

[0017] In a ninth aspect, a vector for expression of a bi-specific chimeric antigen receptor is provided. The bi-specific chimeric antigen receptor is specific for a B cell specific cell surface molecule and is specific for a cell surface tumor specific molecule. The vector can comprise the nucleic acid of any one of the alternatives described herein. In some alternatives, the nucleic acid encoding the bi-specific chimeric antigen receptor comprises a first nucleic acid sequence comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a

fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the seventh nucleic acid is covalently

attached at a 3' end of the sixth nucleic acid, an eighth nucleic acid comprising a sequence encoding a linker, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, and a ninth nucleic acid comprising a sequence encoding a marker domain, wherein the ninth nucleic acid is covalently attached at a 3' end of the eighth nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3,

MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface

tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence

comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. In some alternatives, the vector is a viral vector. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

[0018] In a tenth aspect, a chimeric antigen receptor or TcR specific for a B-cell specific cell surface molecule encoded by a nucleic acid or vector of any one of the alternatives described herein is provided. The vector can comprise the nucleic acid of any one of the alternatives described herein. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a

sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid; and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached to a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7,

TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. The leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence in some alternatives. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the

leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. In some alternatives, the vector is a viral vector. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

[0019] In an eleventh aspect, a chimeric antigen receptor or TcR specific for targeting a solid tumor, encoded by a nucleic acid or vector of any one of the alternatives described herein, is provided. The vector can comprise the nucleic acid of any one of the alternatives described herein. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid sequence is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid

comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid sequence is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some

alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface

tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives,

the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. The vector is a viral vector in some alternatives. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

[0020] In a twelfth aspect, a bi-specific chimeric antigen receptor specific for a B cell specific cell surface molecule and specific for a cell surface tumor specific molecule, encoded by a nucleic acid or vector of any one of the alternatives described herein is provided. The vector can comprise the nucleic acid of any one of the alternatives described herein. In some alternatives, the nucleic acid encoding the bi-specific chimeric antigen receptor comprises a first nucleic acid sequence comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is

covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, an eighth nucleic acid comprising a sequence encoding a linker, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, and a ninth nucleic acid comprising a sequence encoding a marker domain, wherein the ninth nucleic acid is covalently attached at a 3' end of the eighth nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In

some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alphaCD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is

encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the

antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. In some alternatives, the vector is a viral vector. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

[0021] In a thirteenth aspect, a cell comprising a first and second chimeric antigen receptor or TcR is provided. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor or TcR is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFRSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alphafetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR are inducibly expressed in said cell. In some alternatives, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR is under the control of a regulatory element. In some alternatives, the first chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the second chimeric antigen receptor or TcR comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some

alternatives, a first marker protein is co-expressed with the first chimeric antigen receptor and a second marker protein is co-expressed with the second chimeric antigen receptor or TcR. In some alternatives, the first marker protein co-expressed with the first chimeric antigen receptor is EGFRt and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is Her2tg or first marker protein co-expressed with the first chimeric antigen receptor is Her2tg and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is EGFRt. In some alternatives, the cell further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the cell expresses a soluble protein for therapy. In some alternatives, the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for L1CAM, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for ROR1, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain.

[0022] In a fourteenth aspect, a cell comprising a bi-specific chimeric antigen receptor is provided, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain, wherein the second binding domain is specific for a ligand on a tumor. In some alternatives,

the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the cell further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the cell expresses a soluble protein for therapy. In some alternatives, the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central

memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for L1CAM, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for ROR1, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for L1CAM. In some alternatives, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for ROR1.

[0023] In a fifteenth aspect, a method of making a cell having a chimeric antigen receptor is provided. The method can comprise the following steps: introducing into a cell a first nucleic acid or a first vector comprising a polynucleotide sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, introducing into the cell a second nucleic acid or a second vector comprising a polynucleotide sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor, expanding the cell and isolating the cell. In some alternatives, the first nucleic acid and the second nucleic acid reside on separate viral vectors. In some alternatives, the viral vectors are retroviral vectors, gammaretroviral vectors, foamy viral vector and/or lentiviral vectors. In some alternatives, the viral vectors are co-introduced into the cell as a composition comprising the viral vectors. In some alternatives, the vectors are a transposon, integrase vector system, and/or an mRNA vector. In some alternatives, expression of the first chimeric antigen receptor is linked to co-expression of EGFRt and expression of the second chimeric antigen receptor is linked to co-expression of Her2tg, or wherein expression of the first chimeric antigen receptor is linked to co-expression of Her2tg, and expression of the second chimeric antigen receptor is linked to co-expression of EGFRt. In some alternatives, the method further comprises introducing a vector comprising a

sequence encoding a soluble protein into said cell. In some alternatives, the soluble protein is a homeostatic cytokine. In some alternatives, the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the viral vectors further comprise a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system.

[0024] In a sixteenth aspect, a method of making a cell having a chimeric antigen receptor is provided, wherein the method comprises co-delivering into a cell two vectors, wherein the first vector comprises a first nucleic acid sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second vector wherein the second vector comprises a second polynucleotide sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor, expanding the cell and isolating the cell. In some alternatives, the vectors are plasmids and/or minicircle transposons. In some alternatives, the first nucleic acid and the second nucleic acid reside between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence. In some alternatives, the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons. In some alternatives, the method further comprises introducing a vector encoding the Sleeping Beauty transposase or PiggyBac transposase into the cell.

[0025] In a seventeenth aspect, a method of making a cell having a bi-specific chimeric antigen receptor is provided, wherein the method comprises introducing into a cell a nucleic acid comprising a polynucleotide sequence encoding a bi-specific chimeric antigen receptor that comprises a first binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second binding domain specific for a ligand on a solid tumor, expanding the cells and isolating the cells. In some alternatives, the polynucleotide resides on a viral vector. In some alternatives, the viral vector is a lentiviral, retroviral vector, foamy viral vector or a gammaretroviral vector. In

some alternatives, the bi-specific chimeric antigen receptor is co-expressed with a marker protein. In some alternatives, the marker protein is EGFRt or Her2tg. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a soluble protein into said cell. In some alternatives, the soluble protein is a homeostatic cytokine. In some alternatives, the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the viral vector further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system.

[0026] In an eighteenth aspect, a method of making a cell having a bi-specific chimeric antigen receptor is provided, wherein the method comprises introducing into a cell a vector, wherein the vector comprises a first nucleic acid encoding a bi-specific chimeric antigen receptor that comprises a first binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second binding domain wherein the second binding domain comprises a binding domain specific for a ligand on a solid tumor, expanding the cell and isolating the cell. In some alternatives, the vector is a plasmid or minicircle transposon. In some alternatives, the first nucleic acid resides between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence. In some alternatives, the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons. In some alternatives, the method further comprises introducing a vector encoding a Sleeping Beauty transposase or PiggyBac transposase into the cell.

[0027] In a nineteenth aspect, a composition is provided, wherein the composition comprises any one or more of the cells of any of the alternatives described herein. In some alternatives, the cell comprises a first and second chimeric antigen receptor or TcR. In some alternatives, the cell comprises a bi-specific chimeric antigen receptor, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and

activation of the B cell and a second binding domain, wherein the second binding domain is specific for a ligand on a tumor. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor or TcR is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alphafetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR are inducibly expressed in said cell. In some alternatives, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR is under the control of a regulatory element. In some alternatives, the first chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the second chimeric antigen receptor or TcR comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some

alternatives, a first marker protein is co-expressed with the first chimeric antigen receptor and a second marker protein is co-expressed with the second chimeric antigen receptor or TcR. In some alternatives, the first marker protein co-expressed with the first chimeric antigen receptor is EGFRt and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is Her2tg or first marker protein co-expressed with the first chimeric antigen receptor is Her2tg and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is EGFRt. In some alternatives, the cell further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the cell expresses a soluble protein for therapy. In some alternatives, the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for L1CAM, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for ROR1, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7,

TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the cell further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the cell expresses a soluble protein for therapy. In some alternatives, the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for L1CAM, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first chimeric

antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for ROR1, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for L1CAM. In some alternatives, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for ROR1.

[0028] In a twentieth aspect, a method of treating, ameliorating, or inhibiting a non-B cell related disease in a subject is provided. The method can comprise identifying a subject that does not have a B-cell related disease for therapy, introducing, providing, or administering any one or more of the cells of any of the alternatives provided herein or the cells made by any one or more of any of the alternatives provided herein or a composition of any of the alternatives described herein into a subject for therapy. In some alternatives, the method comprises introducing into a cell a vector, wherein the vector comprises a first nucleic acid encoding a bi-specific chimeric antigen receptor that comprises a first binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second binding domain wherein the second binding domain comprises a binding domain specific for a ligand on a solid tumor, expanding the cell and isolating the cell. In some alternatives, the vector is a plasmid or minicircle transposon. In some alternatives, the first nucleic acid resides between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence. In some alternatives, the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons. In some alternatives, the method further comprises introducing a vector encoding a Sleeping Beauty transposase or PiggyBac transposase into the cell. In some alternatives, the composition comprises any one or more of the cells of any of the alternatives described herein. In some alternatives, the cell comprises a first and second chimeric antigen receptor or TcR. In some alternatives, the cell comprises a bi-specific chimeric antigen receptor, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain, wherein the second binding domain is specific for a ligand on a tumor. In some alternatives,

the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor or TcR is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, IDO1, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR are inducibly expressed in said cell. In some alternatives, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR is under the control of a regulatory element. In some alternatives, the first chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the second chimeric antigen receptor or TcR comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, a first marker protein is co-expressed with the first chimeric antigen receptor and a second marker protein is co-

expressed with the second chimeric antigen receptor or TcR. In some alternatives, the first marker protein co-expressed with the first chimeric antigen receptor is EGFRt and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is Her2tg or first marker protein co-expressed with the first chimeric antigen receptor is Her2tg and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is EGFRt. In some alternatives, the cell further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the cell expresses a soluble protein for therapy. In some alternatives, the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T-cells, CD8+ memory T-cells, central memory CD8+ T-cells, regulatory CD8+ T-cells, IPS derived CD8+ T-cells, effector memory CD8+ T-cells and bulk CD8+ T-cells. In some alternatives, the cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T-cells, CD4+ memory T-cells, central memory CD4+ T-cells, regulatory CD4+ T-cells, IPS derived CD4+ T-cells, effector memory CD4+ T-cells and bulk CD4+ T-cells. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for L1CAM, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for ROR1, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alphaCD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the ligand on the tumor is a

cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the cell further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the cell expresses a soluble protein for therapy. In some alternatives, the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for L1CAM, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor

is specific for ROR1, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for L1CAM. In some alternatives, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for ROR1. In some alternatives, the composition comprises any one or more of the cells of any of the alternatives described herein or the cells made by any one or more of the alternative methods described herein. In some alternatives, the composition comprises CD8⁺ T cytotoxic lymphocyte cells and/or CD4⁺ T helper lymphocyte cells, wherein the CD8⁺ T cytotoxic lymphocyte cells are selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells and, wherein the CD4⁺ T helper lymphocyte cells are selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the composition has a ratio of CD4⁺ T helper lymphocyte cells to CD8⁺ T lymphocytes of 1:10 to 10:1. In some alternatives, the ratio of CD4⁺ T helper lymphocyte cells to CD8⁺ T lymphocytes is 1:1. In some alternatives, the subject does not have a B-cell related disease. In some alternatives, the subject does not have B-cell lymphoma, Hodgkin's lymphomas, non-Hodgkins lymphomas, Diffuse large B cell lymphoma, Follicular lymphoma, marginal zone lymphoma, Mucosa-Associated Lymphatic Tissue lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B cell lymphoma, Lymphoplasmacytic lymphoma, Waldenstrom macroglobulinemia, Nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B cell lymphoma, Intravascular large B-cell lymphoma, Primary effusion lymphoma, Lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, Primary central nervous system lymphoma, Primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, Diffuse large B-cell lymphoma associated with inflammation, Intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, ALK-positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-

cell lymphoma arising in HHV8-associated multicentric Castleman's disease, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, or nodular lymphocyte predominant Hodgkin's lymphoma. In some alternatives, the disease is a cancer. In some alternatives, the disease is an infection, wherein the infection is a bacterial or viral infection. In some alternatives, the cancer is a solid tumor. In some alternatives, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer. In some alternatives, the subject has refractory and relapsed neuroblastoma. In some alternatives, the subject is identified or selected to receive a non-B cell related disease therapy, anti-cancer therapy, anti-infection therapy, antibacterial therapy, anti-viral therapy, or anti-tumoral therapy. In some alternatives, the method further comprises measuring or evaluating an inhibition of said non-B cell related disease, cancer, infection, bacterial infection, viral infection, or tumor. In some alternatives, the method further comprises introducing, providing, or administering to said subject an additional therapeutic agent, such as a chemotherapeutic agent, an antiviral agent, or an antibacterial agent or an adjunct therapy such as radiation therapy and/or surgery before, during, or after introducing, providing, or administering any one or more of the cells of the alternatives described herein or the cells made by any one or more of the alternative methods described herein or the composition of any one of the alternatives described herein into the subject for therapy. In some alternatives, the composition comprises any one or more of the cells of any of the alternatives described herein or the cells made by any one or more of the methods of the alternatives described herein. In some alternatives, the composition comprises CD8⁺ T cytotoxic lymphocyte cells and/or CD4⁺ T helper lymphocyte cells, wherein the CD8⁺ T cytotoxic lymphocyte cells are selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells and, wherein the CD4⁺ T helper lymphocyte cells are selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived

CD4+ T-cells, effector memory CD4+ T-cells and bulk CD4+ T-cells. In some alternatives, the composition has a ratio of CD4+ T helper lymphocyte cells to CD8+ T lymphocytes of 1:10 to 10:1. In some alternatives, the ratio of CD4+ T helper lymphocyte cells to CD8+ T lymphocytes is 1:1. In some alternatives, the cells or compositions are introduced, provided, or administered to said subject by adoptive cell transfer. In some alternatives, the method further comprises introducing, providing, or administering a drug that induces expression of a chimeric antigen receptor or TcR. In some alternatives, the drug is a steroid. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the subject is a mammalian species. In some alternatives, the subject is a cow, sheep, pig, horse, dog, cat, primate or a human. In some alternatives, the subject is human. In some alternatives, the subject is of pediatric age. In some alternatives, the method further comprises evaluating the subject for symptoms of cytokine storm or B-cell aplasia. In some alternatives, the method further comprises administering to the subject a prodrug. In some alternatives, the prodrug is Erbitux, Herceptin, Ganciclovir, FK506 or a chemical inducer of dimerization. In some alternatives, the subject is suffering from refractory and relapsed neuroblastoma and wherein the method comprises administering the cell of any one of the alternatives described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] In addition to the features described above, additional features and variations will be readily apparent from the following descriptions of the drawings and exemplary alternatives. It is to be understood that these drawings depict typical alternatives, and are not intended to be limiting in scope.

[0030] **Figure 1** shows a T-cell expressing a “driver” B cell targeting chimeric antigen receptor (CAR) that is co-expressed with a “passenger” CAR that is directed to a tumor cell.

[0031] **Figure 2** shows a schematic of the primary sequence of a CAR described in the alternatives herein. As shown, the sequence encoding the CAR comprises a leader sequence (such as a sequence for targeting the protein to the cell surface), an antigen binding domain (variable light chain and variable heavy chain sequence of an immunoglobulin and a

linker, a spacer domain (IgG4 hinge CH3 region, a signaling domain (CD28 transmembrane region, 4-1BB domain, CD3 zeta), a T2A ribosome skip sequence, and a transduction marker (EGFRt or Her2tg marker).

[0032] **Figure 3** shows the marker Her2t and its variants that demonstrate variable binding affinity to Herceptin based on their respective linker. The CARs used were dual CD19CAR-T2A-Her2tG/ CD20CAR-T2A-EGFRt in the T-lymphocytes. **3A)** H9 T cells were transduced with 3ul of lentivirus containing the Her2t variant Her2t(CD28hinge), Her2t(IgG4hinge) or Her2tG (gly-ser linker). The transduced H9 cells were then cultured for 5 days and stained with biotinylated Herceptin (Herceptin-bio) and a streptavidin conjugated secondary fluorophore (SA-PE). Results demonstrate that the Her2t variant Her2tG displays the greatest ability to bind Herceptin, Her2t(IgG4hinge) with modest Herceptin binding and Her2t(CD28hinge) with the weakest Herceptin binding. **3B)** A timeline for the isolation (D0), growth (D0-21), selection (D14 and D21) and expansion (REP – D21) of CD4+ and CD8+ primary T cells isolated from PBMCs as per **Figure 4**. **3C)** CD8+ T cells were transduced with two separate lentiviruses containing CD19CAR-T2A-Her2tG or CD20CAR-T2A-EGFRt at an MOI = 1 for each lentivirus. Pre-selection CD8+ T cells were stained with Erbitux-APC, biotinylated-Herceptin and a streptavidin conjugated secondary fluorophore (SA-PE) seven days post transduction (D10 of culture), while Post-selection cells were stained on S1Sp1D12 (**See Figure 4**) **3D)** A western blot that indicates the proteins in the cells. Cell lysis for western blot analysis was carried out in RIPA buffer containing protease inhibitor cocktail. Cell lysates were analyzed by BCA assay (Pierce), equally loaded onto gels and western blots were probed with the primary antibody anti CD247 (CD3ζ) and the secondary IRDye 800CW conjugated goat anti-mouse antibody (LI-COR). Blots were imaged on the Odyssey Infrared Imaging System (LI-COR).

[0033] **Figure 4** shows a Western Blot of the cells of interest S1Sp1D12. The cells were purified for their respective marker/s. Dual CAR bearing CD8+ T cells carry CD19CAR-T2A-Her2tG and CD20CAR-T2A-EGFRt. This western blot is specific to the zeta portion of the CAR. The western demonstrates CAR expression in the CD8+ T cells. Most importantly, expression of both CARs (CD19CAR and CD20CAR) is demonstrated in the dual transduced CD8+ T cells.

[0034] **Figure 5: 5A)** 4-hour chromium release assay showing CD19- and CD20-CAR T cell specificity against K562 target panel cells. CD8 Tcm were co-cultured with K562 target cells at a 30:1, 10:1, 3.3:1 or 1.1:1 ratio. Only the dual transduced T cells were able to target all antigen expressing K562 cells. The CD19CAR-T2A-Her2t and CD19CAR-T2A-EGFRt CD8 Tcm demonstrate similar lytic capacity. As shown representations are Mock (X), CD19CAR-Her2tG (square), CD 19CAR-EGFRt (triangle), CD20CAR-EGFRt (diamond) and CD19CAR-Her2tG/CD20CAR-EGFRt (upside-down triangle) **5B)** 24-hour cytokine release assay. CD8 Tcm were co-cultured with K562 target cells at a 2:1 T cell-to-target ratio for 24 hours and then the supernatant was analyzed for the presence of effector cytokines. CD19CAR-T2A-Her2t transduced CD8 Tcm produced a more diverse repertoire and higher levels of effector cytokines relative to CD19CAR-T2A-EGFRt transduced CD8 Tcm. The panels are the same as **5A** and **5B** (Left to right in the bar graphs: K562 CD19, K562 CD20 and K562 CD19/CD20). **5C)** Similar results were seen for CD4 Tcm. As shown representations are Mock (X), CD19CAR-Her2tG (square), CD 19CAR-EGFRt (triangle), CD20CAR-EGFRt (diamond) and CD19CAR-Her2tG/CD20CAR-EGFRt (upside-down triangle).

[0035] **Figure 6** shows experimental results of Raji cells electroporated with plasmids containing CD19-targeted CRISPR guide sequences. **6A)** Seven days post electroporation the Raji cells were subjected to negative selection using CD19 microbeads. Post-depletion of CD19+ cells the CD19- cells were clonally selected and expanded for downstream experiments. **Figure 6B-C):** 4 hour chromium release assays and bioplex assay using the same cells from **Figure 5**. As shown representations are Mock (X), CD19CAR-Her2tG (square), CD 19CAR-EGFRt (triangle), CD20CAR-EGFRt (diamond) and CD19CAR-Her2tG/CD20CAR-EGFRt (upside-down triangle). Against Raji parental and three Raji CRISPR clones. Shown in 6C clockwise, the top left graph are the Raji parental, Raji CRISPR (3), Raji CRISPR (15) and Raji CRISPR (27). The results on the bottom graph are results from left to right, mock, CD19CAR-Her2tG, CD19CAR-EGFRt, CD20CAR-EGFRt and CD19CAR-Her2tG/CD20CAR-EGFRt (For IL-2, IFN- γ and TNF- α). **6D)** CD4+ chromium release assay.

[0036] **Figure 7** shows experimental results of NSG mice injected with 5×10^6 NSO-IL15 cells and then 10×10^6 Mock or CAR expressing T cells i.v. As shown in the top row, on

Day 14 post T cell injection, mouse bone marrow was harvested and subjected to flow analysis. The top row gates live, singlets. The middle row gates CD8+ x CD45+ cells. The bottom row looks at the CD45+ cell population and stains for the markers Her2tG and EGFRt. Results demonstrate that EGFRt and Her2tG can be used to efficiently track T cells in vivo. The panels in the middle row show cells gated for viable (93.6% lymphocytes), single (98.8%), and alive cells (99.9%). As shown are CD8 and CD45 staining from left to right: Mock, CD19CAR-T2A-Her2t, CD19CAR-T2A-EGFRt Tcm and CD19CAR-Her2tg/CD20CAR-EGFRt). At least 1×10^7 cells were recorded inside of the viable, single cell and alive gates. So although the CD45+ cells represent around 1% of the population, it is equivalent to 1×10^5 cells. The remaining cells are mouse bone marrow cells. Third row: Multisort purification of Her2t and EGFRt positive T cells. H9 cells (5×10^6 parental, Her2t⁺, EGFRt⁺, or Her2t⁺/EGFRt⁺) were mixed together and then subjected to purification. The cells were initially purified based on biotinylated Herceptin and anti-biotin multisort beads. The multisort beads were then removed and the positive fraction subsequently subjected to purification based on Erbitux-APC and anti-APC microbeads. The final positive fraction was dual positive for Her2t and EGFRt. D) Chromium release assays

[0037] **Figure 8** shows the experimental results of NSG mice injected with 0.5×10^6 Raji eGFP:ffluc cells i.v. and then 10×10^6 Mock or CAR expressing T cells at a 1:1 ratio of CD4:CD8. Serial tumor imaging was performed and total flux graphed to demonstrate tumor growth or the inhibition of tumor growth. Results demonstrate that the single CAR or dual CAR expressing T cells were able to inhibit tumor growth relative to Mock T cells. As shown representations are Mock (X), CD19CAR-Her2tG (square), CD 19CAR-EGFRt (triangle), CD20CAR-EGFRt (diamond) and CD19CAR-Her2tG/CD20CAR-EGFRt (upside-down triangle).

[0038] **Figure 9A** shows that CD4 and CD8 T-cells can be transduced with two separate CAR-encoding lentiviral vectors. CD4 and CD8 purified T-cells were stimulated with CD3/CD28 beads and then co-transduced with clinical grade virus encoding the 2nd generation 41BB- ζ short spacer FMC63CD19CAR or the 2nd generation 41BB- ζ short spacer CE7CAR. Each CAR was followed by an in-frame T2A-EGFRt. Transduced T-cells were purified by EGFRt and frozen on S1D14 (CD4) or S1D15 (CD8). The process development project was PD0170. Flow analysis demonstrated that transduced cells were purified to

homogeneity based on EGFRt expression. Results do not demonstrate what percentage of the CD19- or CE7CAR is represented in the EGFRt⁺ population or whether the T-cells are indeed dual transduced with virus.

[0039] **Figure 9B** shows that CD19 and CE7CAR dual transduced T-cells demonstrate specific lysis against CD19 or L1CAM positive target cell lines. Mock (CAR⁻) T-cells and dual transduced T-cells were co-cultured with target cell lines at different effector to target ratios for 4-hours. K562 are negative for both targets CD19 and L1CAM. TM-LCL, TM-LCL-OKT3, K562 + CD19, and Raji are CD19-positive alone. SK-N-DZ is L1CAM-positive alone. SK-N-DZ + CD19 are positive for both targets. Results demonstrate that the transduced CD8 or mixed CD4:CD8 T-cells elicit high levels of CD19CAR activity. There was a lower level of CE7CAR activity as demonstrated against the SK-N-DZ cell line. These results indicate that T-cells can be successfully transduced with two separate CAR-encoding viruses and the resultant T-cell population is able to recognize multiple antigens.

[0040] **Figure 9C** shows that CD19- and CE7CAR dual transduced T-cell populations produce cytokines against CD19 or L1CAM positive target cell lines. Dual transduced CD4 and CD8 T-cell populations were co-cultured for 24 hours with target cell lines at a 2:1 effector-to-target ratio. Following the 24 hour incubation period, supernatant from the co-cultures was analyzed for the presence of IL-2, IFN-g, or TNF-a by Bioplex assay. The dual transduced T-cells produced cytokine in response to all suspension cell (LCL and K562) targets expressing CD19. There was little to no cytokine produced in response to SK-N-DZ which is not uncommon with the 2nd Gen S-spacer CE7CAR even when expressed in 100% of the T-cell population. Since it was not known what percentage of the T-cell populations contain the CE7CAR this was not surprising when combined with the chromium data in **Fig 9B**.

[0041] **Fig 9D** shows that dual-transduced T-cells elicit antitumor activity in an intracranial xenograft tumor model. Cohorts of mice were inoculated with 0.2e6 SK-N-DZ that express GFP:ffluc, CD19t, and IL-2 (Day 0) and 2e6 dual transduced CD4:CD8 T-cells (1:1 ratio) (Day 7) intracranially (i.c.). Serial bioluminescence imaging of tumor in cohorts of mice treated with Mock (PBS only – left) or dual transduced CD4:CD8 CE7CAR T-cells (middle). Kaplan-Meier analysis (right) of survival in treatment and control groups. The

dual-transduced cells were able to regress and control tumor growth as evidenced by a prolonged decrease in bioluminescence imaging.

[0042] **Figure 10A** shows that CD4 and CD8 T-cells can be transduced with two separate CAR-encoding lentiviral vectors and dual positive populations can be identified by associated markers. CD4 and CD8 purified T-cells were stimulated with CD3/CD28 beads and then individually or co-transduced with lentivirus encoding the 2nd generation 41BB- ζ short spacer FMC63CD19CAR or/and the 2nd generation 41BB- ζ long mutant (L235D, N297Q) spacer CE7CAR. The CD19CAR was followed by an in-frame T2A-Her2tG and the CE7CAR by a T2A-EGFRt. Transduced T-cells were purified by EGFRt (CE7CAR) and frozen. Flow analysis was performed on day 9 and demonstrates that transduced cells were purified to homogeneity based on EGFRt expression. For the CD4 T-cells there was ~56% Her2tG (CD19CAR) positivity and ~44% dual-positivity for the CD8 T-cells.

[0043] **Figure 10B** shows that CD19 and CE7CAR dual transduced CD8⁺ T-cells demonstrate specific lysis against CD19 or L1CAM positive target cell lines. Mock (CAR⁻), single transduced (CD19CAR or CE7CAR) and dual transduced CD8⁺ T-cells were co-cultured with target cell lines at different effector to target ratios for 4-hours. K562 are negative for both targets CD19 and L1CAM. K562-OKT3 was used as a positive control. Be2 are L1CAM-positive alone. Be2+CD19t are positive for both targets. Results demonstrate that the single transduced CD8⁺ T-cells elicit specific lysis against their cognate antigen. However, the dual transduced T-cells efficiently recognized and lysed cells expressing CD19, L1CAM or both antigens. All CD8⁺ T-cells were able to elicit similar levels of cell lysis against the K562-OKT3. These results indicate that T-cells can be successfully transduced with two separate CAR-encoding viruses, purified by a selectable marker, and the resultant T-cell population is able to recognize multiple antigens.

[0044] **Figure 10C** shows that CD19- and CE7CAR dual transduced T-cell populations produce cytokines against CD19 or L1CAM positive target cell lines. Mock (CAR⁻), single transduced (CD19CAR or CE7CAR) and dual transduced CD4⁺ and CD8⁺ T-cells were co-cultured for 24 hours with target cell lines at a 2:1 effector-to-target ratio. Following the 24 hour incubation period, supernatant from the co-cultures was analyzed for the presence of IL-2, IFN-g, or TNF-a by Bioplex assay. Dual-transduced cells were able to release cytokine against all target expressing cell lines. While there was no difference in

cytokine production between individual CE7CAR-expressing and dual CAR expressing T-cells against Be2-CD19t, there was a difference in cytokine production between CD19CAR-expressing and dual transduced T-cells against K562-CD19t. This data coincides with the CD19CAR positivity of the dual transduced T-cell populations.

[0045] **Fig 10D** shows that dual-transduced T-cells elicit antitumor activity in an intracranial xenograft tumor model. Cohorts of mice were inoculated with 0.2e6 SK-N-DZ that express GFP:ffluc and IL-2 (Day 0) and 2e6 dual transduced CD4:CD8 T-cells (1:1 ratio) (Day 7) intracranially (i.c.). Serial bioluminescence imaging of tumor in cohorts of mice treated with Mock (untransduced - left), CE7CAR-expressing (top row, middle) or dual-transduced CD4:CD8 CE7CAR T-cells (top row, right). Kaplan-Meier analysis (bottom) of survival in treatment and control groups. Both the single CE7CAR-expressing and dual-transduced cells were able to regress and control tumor growth as evidenced by a prolonged decrease in bioluminescence imaging. A subset of mice treated with single CE7CAR-expressing T-cells was euthanized prior to the arbitrary end point of the study due to outgrowth of tumor. These results show that dual-transduced T-cells are able to eradicate tumor *in vivo* at levels similar to single CE7CAR-expressing T-cells. There was therefore no inhibition in CE7CAR activity for the dual-transduced T-cell population.

[0046] **Figure 11A** shows that CD4 and CD8 T-cells can be transduced with two separate CAR-encoding lentiviral vectors and dual positive populations can be identified by associated markers. CD4 and CD8 purified T-cells were stimulated with CD3/CD28 beads and then individually or co-transduced with lentivirus encoding the 2nd generation 41BB- ζ short spacer FMC63CD19CAR or/and the 2nd generation 41BB- ζ short spacer ROR1CAR. The CD19CAR was followed by an in-frame T2A-Her2tG and the ROR1CAR by a T2A-EGFRt. Transduced T-cells were purified by EGFRt (ROR1CAR) and frozen. Flow analysis was performed on day 9 and demonstrates that transduced cells were purified to homogeneity based on EGFRt expression. For the CD4 T-cells there was ~86.7% Her2tG (CD19CAR) positivity and ~88.1% dual-positivity for the CD8 T-cells.

[0047] **Figure 11B** shows that CD19 and ROR1CAR dual transduced CD8⁺ T-cells demonstrate specific lysis against CD19 or ROR1 positive target cell lines. Mock (CAR), single transduced (CD19CAR or ROR1CAR) and dual transduced CD8⁺ T-cells were co-cultured with target cell lines at different effector to target ratios for 4-hours. Single

ROR1CAR T-cells contain either the 2nd generation 41BB- ζ or CD28- ζ short spacer. K562 are negative for both targets CD19 and ROR1. K562-OKT3 was used as a positive control. Raji (CD19⁺) were transduced to express ROR1. Be2, SK-N-AS and SK-N-DZ are ROR1 positive. Results demonstrate that the single transduced CD8⁺ T-cells elicit specific lysis against their cognate antigen. However, the dual transduced T-cells efficiently recognized and lysed cells expressing CD19, ROR1 or both antigens (Raji ROR1). All CD8⁺ T-cells were able to elicit similar levels of cell lysis against the K562-OKT3. These results indicate that T-cells can be successfully transduced with two separate CAR-encoding viruses, purified by a selection marker, and the resultant T-cell population is able to recognize multiple antigens.

[0048] **Figure 11C** shows that CD19- and ROR1CAR dual transduced T-cell populations produce cytokines against CD19 or ROR1 positive target cell lines. Mock (CAR), single transduced (CD19CAR or ROR1CAR) and dual transduced CD4⁺ T-cells were co-cultured for 24 hours with target cell lines at a 2:1 effector-to-target ratio. Following the 24 hour incubation period, supernatant from the co-cultures was analyzed for the presence of IL-2, IFN-g, or TNF-a by Bioplex assay. Dual-transduced cells were able to release cytokine against all target expressing cell lines at similar levels to single CAR-expressing T-cells.

[0049] **Figure 11D** shows that CD19- and ROR1CAR dual transduced T-cell populations produce cytokines against CD19 or ROR1 positive target cell lines. Mock (CAR), single transduced (CD19CAR or ROR1CAR) and dual transduced CD8⁺ T-cells were co-cultured for 24 hours with target cell lines at a 2:1 effector-to-target ratio. Following the 24 hour incubation period, supernatant from the co-cultures was analyzed for the presence of IL-2, IFN-g, or TNF-a by Bioplex assay. Dual-transduced cells were able to release cytokine against all target expressing cell lines at similar levels to single CAR-expressing T-cells.

[0050] **Fig 11E** shows that dual-transduced T-cells elicit antitumor activity in an intracranial xenograft tumor model. Cohorts of mice were inoculated with 0.2e6 Be2 that express GFP:ffluc (Day 0) and 2e6 dual transduced CD4:CD8 T-cells (1:1 ratio) (Day 7) intracranially (i.c.). Serial bioluminescence imaging of tumor in cohorts of mice treated with Mock (untransduced - left), ROR1CAR-expressing (middle-left) or dual-transduced CD4:CD8 CE7CAR T-cells (middle right). Kaplan-Meier analysis (right) of survival in treatment and control groups. Both the single ROR1CAR-expressing and dual-transduced

cells were able to transiently regress tumor growth as evidenced by a short decrease in bioluminescence imaging. Both the single and dual transduced T-cells were able to significantly prolong survival. These results show that dual-transduced T-cells are able to treat tumor *in vivo* at levels similar to single ROR1CAR-expressing T-cells. There was therefore no inhibition in ROR1CAR activity for the dual-transduced T-cell population.

Definitions

[0051] The following definitions are provided to facilitate understanding of the alternatives or alternatives of the invention.

[0052] As used herein, “a” or “an” can mean one or more than one.

[0053] As used herein, the term “about” indicates that a value includes the inherent variation of error for the method being employed to determine a value, or the variation that exists among experiments.

[0054] “Chimeric antigen receptors” (CARs), as described herein, refers to genetically engineered protein receptors, which can confer specificity onto an immune effector cell, such as for example, a T-cell. Without being limiting, the use of CAR bearing T-cells can promote *in vivo* expansion and activation. The CARs can also be designed to redirect T-cells to target cells that express specific cell-surface antigens, where they can activate lymphocytes, such as T-cells, upon target recognition. The CARs graft the specificity of a monoclonal antibody or binding fragment thereof or scFv onto a T-cell, with the transfer of their coding sequence facilitated by vectors. In order to use CARs as a therapy for a subject in need, a technique called adoptive cell transfer is used in which T-cells are removed from a subject and modified so that they can express the CARs that are specific for an antigen. The T-cells, which can then recognize and target an antigen, are reintroduced into the patient. In some alternatives, CAR expressing lymphocytes are described, wherein the CAR expressing lymphocyte can be delivered to a subject to target specific cells. In some alternatives, the lymphocyte can express two CARs for bi-specificity. In some alternatives, the lymphocyte can express a CAR and a specific T-cell receptor (TcR) for bi-specificity. A TcR is a molecule on the surface of T lymphocytes or T-cells that can recognize antigens. In some alternatives, the lymphocyte can express a bi-specific CAR for bi-specificity, wherein the bi-specific CAR comprises a first domain for binding a B-cell specific ligand, and a

second domain for binding a tumor specific ligand on a tumor that is a non-B cell specific cancer. In some alternatives, the cancer is not a B-cell malignancy. As described herein, the B-cell specific CAR promotes in vivo expansion and activation of effector cells.

[0055] The structure of the CAR can comprise fusions of single-chain variable fragments (scFv) that are derived from monoclonal antibodies that are attached to transmembrane and cytoplasmic signaling domains. Most CARs can include an extracellular scFv that is linked to an intracellular CD3 ζ domain (first generation CAR). Additionally, the scFv can be linked to a co-stimulatory domain, which can increase their efficacy in the therapy of a subject in need (second generation CAR). When T-cells express this molecule they can recognize and kill target cells that express a specific antigen targeted by the CAR.

[0056] In some alternatives, a CAR directed against CD19 comprises an amino acid sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12

[0057] In some alternatives, a CAR directed against CD20 comprises an amino acid sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14.

[0058] In some alternatives, a CAR directed against CE7 comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16.

[0059] In some alternatives, a CAR directed against ROR1 comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18.

[0060] In some alternatives, a CAR directed against EGFR 806 comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20.

[0061] In some alternatives, a CAR directed against Her2 comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22.

[0062] In some alternatives, a CAR directed against GD2 comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24.

[0063] In some alternatives, a CAR directed against EphA2 comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26.

[0064] In some alternatives, a CAR directed against EphA2 comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28.

[0065] In some alternatives, the polynucleotide encoding the chimeric antigen receptor comprises a sequence encoding a GMCSF signal sequence. In some alternatives, the GMCSF signal sequence signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30.

[0066] In some alternatives, the polynucleotide encoding the chimeric antigen receptor comprises a sequence encoding a signal sequence. In some alternatives, the signal sequence signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the polynucleotide encodes a scFv specific for CD20.

[0067] "EGFR 806," as described herein, is a conformational epitope of wild type EGFR.

The chimeric antigen receptor can comprise a binding portion that is specific for a ligand. Without being limiting, the binding portion can comprise an antibody or binding fragment thereof or scFv, a receptor ligand or mutants thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives of the first chimeric antigen receptor, the first chimeric antigen receptor comprises a binding portion, wherein the binding portion comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutants thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the binding portion is specific for a ligand on a B-cell. In some alternatives of the second chimeric antigen receptor, the second chimeric antigen receptor comprises a binding portion, wherein the binding portion comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutants thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the binding portion is specific for a ligand on a tumor cell. In some alternatives, the tumor is not a tumor of a B-cell related cancer. As shown in **Figure 1**, vectors for the B-cell targeting CAR (Driver) and the tumor targeting

CAR (Passenger) are delivered by two separate lentiviral vectors. Cells that contain the specific markers co-expressed with the two CARs are then purified and used for therapy.

[0068] As shown in **Figure 2** is the schematic of the primary sequence of a CAR described in the alternatives herein. The CAR comprises a leader sequence, an antigen binding domain (variable light chain and variable heavy chain sequence of an immunoglobulin and a linker, a spacer domain (IgG4 hinge CH3 region, a signaling domain (CD28 transmembrane region, 4-1BB domain, CD3 zeta), a T2A ribosome skip sequence, and a transduction marker (EGFRt or Her2tg marker). CAR bearing T-cells described herein, in several alternatives are CD19CAR-T2A-Her2tG, CD19CAR-T2A-EGFRt, CD20CAR-T2A-EGFRt, or CD19CAR-T2A-Her2tG/CD20CAR-T2A-EGFRt.

[0069] “Co-stimulation” as described herein, refers to the activation of lymphocytes. In some alternatives of the chimeric antigen receptor, the chimeric antigen receptor comprises an endodomain, wherein the endodomain comprises co-stimulatory domains for co-stimulatory signaling. In some alternatives, the co-stimulatory domains comprise a transmembrane CD28 domain, 4-1BB domain and/or a CD3zeta domain. In some alternatives, the transmembrane CD28 domain comprises the amino acid sequence set forth in SEQ ID NO: 5; M F W V L V V V G G V L A C Y S L L V T V A F I I F W V). In some alternatives, the transmembrane CD28 domain is encoded by the acid sequence set forth in SEQ ID NO: 6 (SEQ ID NO: 6; ATGTTCTGGGTGCTGGTGGTGGTCGGAGGCGTGCTGGCCTGCTACAGCCTGCTGGTCACCGTGGCCTTCATCATCTTTTGGGTG). In some alternatives, the 4-1BB domain comprises the amino acid sequence set forth in SEQ ID NO: 7 (SEQ ID NO: 7; K R G R K K L L Y I F K Q P F M R P V Q T T Q E E D G C S C R F P E E E E G G C E L R V K). In some alternatives, the 4-1BB domain is encoded by the nucleic acid sequence set forth in SEQ ID NO: 8 (SEQ ID NO: 8; AAACGGGGCAGAAAGAACTCCTGTATATATTCAAACAACCATTTATGAGACCA GTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAA GAAGGAGGATGTGAACTGCGGGTGAAG). In some alternatives, the CD3-zeta domain comprises the amino acid sequence set forth in SEQ ID NO: 9 (SEQ ID NO: 9; F S R S A D A P A Y Q Q G Q N Q L Y N E L N L G R R E E Y D V L D K R R G R D P E M G G K P R R K N P Q E G L Y N E L Q K D K M A E A Y S E I G M K G E R R R G K G H D G L

Y Q G L S T A T K D T Y D A L H M Q A L P P R). In some alternatives, the CD3-zeta domain is encoded by the nucleic acid sequence set forth in SEQ ID NO: 10 (SEQ ID NO: 10;

TTCAGCAGAAGCGCCGACGCCCCTGCCTACCAGCAGGGCCAGAATCAGCTGTAC
AACGAGCTGAACCTGGGCAGAAGGGAAGAGTACGACGTCCTGGATAAGCGGAG
AGGCCGGGACCCTGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAG
GCCTGTATAACGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCG
GCATG
AAGGGCGAGCGGAGGCGGGGCAAGGGCCACGACGGCCTGTATCAGGGCCTG
TCCACCGCCACCAAGGATACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCA
AGG).

[0070] “Ligand” as described herein, refers to a substance that can form a complex with a biomolecule. By way of example and not of limitation, ligands can include substrates, proteins, small molecules, inhibitors, activators, nucleic acids and neurotransmitters. Binding can occur through intermolecular forces, for example ionic bonds, hydrogen bonds, and van der Waals interactions. Ligand binding to a receptor protein can alter the three dimensional structure and determine its functional state. The strength of binding of a ligand is referred to as the binding affinity and can be determined by direct interactions and solvent effects. A ligand can be bound by a “ligand binding domain.” A ligand binding domain, for example, can refer to a conserved sequence in a structure that can bind a specific ligand or a specific epitope on a protein. The ligand binding domain or ligand binding portion can comprise an antibody or binding fragment thereof or scFv, a receptor ligand or mutants thereof, peptide, and/or polypeptide affinity molecule or binding partner. Without being limiting, a ligand binding domain can be a specific protein domain or an epitope on a protein that is specific for a ligand or ligands.

[0071] A “B-cell ligand” or a “B-cell antigen” as described herein, refers to a target antigen that is expressed in a B-cell or B-cell surface of a subject, in which the subject or patient does not have a B-cell related disease. In some alternatives, the subject does not have a B cell related disease. In some alternatives, the subject does not have a B-cell related disease and/or is being treated for a solid tumor. Without being limiting, examples of B-cell related diseases can include B-cell lymphoma, Hodgkin’s lymphomas, non-Hodgkins

lymphomas, Diffuse large B cell lymphoma, Follicular lymphoma, marginal zone lymphoma, Mucosa-Associated Lymphatic Tissue lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B cell lymphoma, Lymphoplasmacytic lymphoma, Waldenstrom macroglobulinemia, Nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B cell lymphoma, Intravascular large B-cell lymphoma, Primary effusion lymphoma, Lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, Primary central nervous system lymphoma, Primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, Diffuse large B-cell lymphoma associated with inflammation, Intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, ALK-positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-cell lymphoma arising in HHV8-associated multicentric Castleman's disease, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, and nodular lymphocyte predominant Hodgkin's lymphoma. In some alternatives, the patient does not have a B-cell related disease. In some alternatives, the patient does not have B-cell lymphoma, Hodgkin's lymphomas, non-Hodgkins lymphomas, Diffuse large B cell lymphoma, Follicular lymphoma, marginal zone lymphoma, Mucosa-Associated Lymphatic Tissue lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B cell lymphoma, Lymphoplasmacytic lymphoma, Waldenstrom macroglobulinemia, Nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B cell lymphoma, Intravascular large B-cell lymphoma, Primary effusion lymphoma, Lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, Primary central nervous system lymphoma, Primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, Diffuse large B-cell lymphoma associated with inflammation, Intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, ALK-positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-cell lymphoma arising in HHV8-associated multicentric Castleman's disease, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma

and Burkitt lymphoma, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, or nodular lymphocyte predominant Hodgkin's lymphoma.

[0072] Although rare, lymphomas can also transform into a malignant tumor or mass and metastasize or spread to other organs that are not in the lymphatic system. In this case, a patient has B-cell lymphoma and a solid tumor. Furthermore, patients with B-cell diseases, such as systemic lupus erythematosus and rheumatoid arthritis, are also susceptible to cancers such as solid tumors, for example. In some alternatives, the subject does not have a B-cell disease and/or is being treated for a cancer. In some alternatives, the cancer is a solid tumor.

[0073] In some alternatives, the ligand binding domain is an antibody, or a portion thereof. In some alternatives, the ligand binding domain is an antibody or binding fragment thereof or scFv, a receptor ligand or mutants thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the ligand binding domain is an scFv. In some alternatives described herein, a ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148 or EMMPRIN/CD147. In some alternatives described herein, a ligand on a B-cell is B-cell specific.

[0074] “B-cells” as described herein refers to a type of lymphocyte in the humoral immunity of the adaptive immune system. B cells can be distinguished from other lymphocytes, such as T-cells and natural killer cells (NK cells), by the presence of a protein on the B cell's outer surface known as a B cell receptor (BCR). This specialized receptor protein allows a B cell to bind to a specific antigen. In mammals, immature B cells are formed in the bone marrow. The principal functions of B cells are to make antibodies in response to antigens, to perform the role of antigen-presenting cells (APCs), and to develop into memory B cells after activation by antigen interaction. B cells also release cytokines used for signaling immune regulatory functions. B cells have a short-life span and undergo

apoptosis when the inciting agent that induced immune response is eliminated. This occurs because of cessation of continuous exposure to various colony-stimulating factors, which is required for survival. In some alternatives described herein, a chimeric antigen receptor is provided, wherein the chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell. In some alternatives, binding of the B-cell ligand leads to the release of cytokines. In some alternatives described herein, a bi-specific chimeric antigen receptor is provided, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain is specific for a ligand on a tumor. In some alternatives, the chimeric antigen receptor is specific for a B-cell ligand, wherein the chimeric antigen receptor is specific for CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alphaCD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148 or EMMPRIN/CD147. In some alternatives, the first binding domain on the bi-specific chimeric antigen receptor is specific for CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alphaCD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, EMMPRIN/CD147 and/or tumor targets that are non-B cell tumors. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant version thereof, peptide, and/or polypeptide affinity molecule or binding partner.

[0075] In some alternatives, binding of a chimeric antigen receptor to a B-cell ligand elicits a cytokine response and supports T-cell expansion.

[0076] In some alternatives, a chimeric antigen receptor is provided, wherein the ligand or target molecule is a cell surface molecule that is found on tumor cells and is not substantially found on normal tissues, or restricted in its expression to non-vital normal tissues. In some alternatives, the tumor does not originate from a B-cell related cancer. In some alternatives, the ligand or target molecule is found on a tumor cell as well as on normal tissues. In some alternatives the cells expressing a CAR that is specific for a ligand on tumor cells and normal tissue further comprises a suicide gene to limit the time of therapy and increase their safety profile. Conditional suicide genes may also be applied to the donor T-cells to limit the attack on normal tissue that may express a tumor associated antigen or ligand.

[0077] “Effector cells” as described herein, refers to a lymphocyte that has been induced to differentiate into another cell type that can be capable of mounting a specific immune response, such as a terminally differentiated leukocyte that performs one or more specific functions. The main effector cells of the immune system, for example, are activated lymphocytes and phagocytes that are involved in destroying pathogens and removing them from the body. The effector cells can include large granular lymphocytes, such as, for example, natural killer cells and cytotoxic T lymphocytes. In some alternatives of the cells provided herein, the cell comprises a first and second chimeric antigen receptor, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor is specific for a ligand on a tumor. In some alternatives, the cells that undergo expansion and activation are lymphocytes, phagocytes, large granular lymphocytes, natural killer cells and/or cytotoxic T lymphocytes.

[0078] “Cancer antigen,” “tumor antigen” or “tumor marker” refers to an antigenic substance that is produced in a tumor cell, which can therefore trigger an immune response in the host. These cancer antigens can be useful as markers for identifying a tumor cell, which will be a potential candidate during treatment or therapy. There are several types of cancer or tumor antigens. There are tumor specific antigens (TSA) which are present only on tumor cells and not on healthy cells, as well as tumor associated antigens (TAA) which are present in tumor cells and also on some normal cells. In some alternatives of the methods and chimeric antigens provided herein, the chimeric antigen receptors are specific for tumor

specific antigens. In some alternatives, the chimeric antigen receptors are specific for tumor associated antigens. In some alternatives described herein, the tumor does not originate from a B-cell related cancer. In some alternatives, cells expressing a CAR that is specific for a TAA is further modified by the introduction of a suicide gene to limit the time of the CAR T-cell therapy and to reduce the attack of normal tissues expressing the TAA.

[0079] In some alternatives of the methods provided herein, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cell surface tumor specific molecule is ROR1. In some alternatives, the cancer antigen is expressed by a tumor, wherein the tumor is not a B-cell related cancer.

[0080] “Specific” or “Specificity” can refer to the characteristic of a ligand for the binding partner or alternatively, the binding partner for the ligand, and can include complementary shape, charge and hydrophobic specificity for binding. Specificity for binding can include stereospecificity, regioselectivity and chemoselectivity. In some alternatives, a chimeric antigen receptor is provided, wherein the chimeric antigen receptor is specific for a B-cell ligand. In some alternatives, a chimeric antigen receptor is provided, wherein the chimeric antigen receptor is specific for a tumor cell ligand.

[0081] “CE7” as described herein is an epitope on L1CAM.

[0082] In some alternatives, the lymphocyte can express a CAR and a specific T-cell receptor (TcR) for bi-specificity. In some alternatives, the specific T-cell receptor is specific for EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of

ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, ROR1 or NY-ESO-1.

[0083] “Inducibly expressed” or “inducible expression” refers to the initiation or increase in the production of a protein at the level of genetic transcription. Induction of a protein can occur if a gene encoding a protein of interest is under the control of a promoter. As used herein, a promoter can be constitutively active, repressible or inducible. If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known. In some alternatives described herein, the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR are inducibly expressed in said cell. In some alternatives, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR is under the control of a regulatory element. In some alternatives the repressible systems are controlled by a Tet-On/Off system using doxycycline. In some alternatives, the repressible systems are controlled by tamoxifen. In some alternatives, the expressible systems are controlled by riboswitch/small molecule mRNA regulation. In some alternatives, the expressible system is controlled by a riboswitch, shRNA or microRNA.

[0084] A “regulatory element” as described herein, can refer to a regulatory sequence, which is any DNA sequence that is responsible for the regulation of gene expression, such as promoters and operators. The regulatory element can be a segment of a nucleic acid molecule which is capable of increasing or decreasing the expression of specific genes within an organism. In some alternatives described herein, a cell is provided, wherein the cell comprises a first and second chimeric antigen receptor or TcR, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor or TcR is specific for a ligand on a tumor. In some alternatives, the first chimeric

antigen receptor and/or the second chimeric antigen receptor or TcR are inducibly expressed in said cell. In some alternatives, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR is under the control of a regulatory element.

[0085] “Transmembrane domain” as described herein is an integral protein that can span a cellular membrane.

[0086] A “promoter” is a nucleotide sequence that directs the transcription of a structural gene. In some alternatives, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. Without being limiting, these promoter elements can include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee et al., *Mol. Endocrinol.* 7:551 (1993); incorporated by reference in its entirety), cyclic AMP response elements (CREs), serum response elements (SREs; Treisman, *Seminars in Cancer Biol.* 1:47 (1990); incorporated by reference in its entirety), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly et al., *J. Biol. Chem.* 267:19938 (1992); incorporated by reference in its entirety), AP2 (Ye et al., *J. Biol. Chem.* 269:25728 (1994); incorporated by reference in its entirety), SP1, cAMP response element binding protein (CREB; Loeken, *Gene Expr.* 3:253 (1993); incorporated by reference in its entirety) and octamer factors (see, in general, Watson et al., eds., *Molecular Biology of the Gene*, 4th ed. (The Benjamin/Cummings Publishing Company, Inc. 1987; incorporated by reference in its entirety)), and Lemaigre and Rousseau, *Biochem. J.* 303:1 (1994); incorporated by reference in its entirety). As used herein, a promoter can be constitutively active, repressible or inducible. If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known. In some alternatives of the nucleic acid is provided, the nucleic acid comprises a promoter sequence. In some alternatives of the chimeric antigen, the chimeric antigen is inducibly expressed in response to an inducing agent. In some alternatives, the TcR is inducibly expressed in response to an inducing agent.

[0087] In some alternatives, promoters used herein can be inducible or constitutive promoters. Without being limiting, inducible promoters can include, for example, a tamoxifen inducible promoter, tetracycline inducible promoter, and doxocycline inducible promoter (e.g. tre) promoter. Constitutive promoters can include, for example, SV40, CMV, UBC, EF1alpha, PGK, and CAGG. In some alternatives, the regulatory is a promoter. In some alternatives, the promoter is a tamoxifen inducible promoter, a tetracycline inducible promoter, or a doxocycline inducible promoter (e.g. tre) promoter. In some alternatives provided herein, expression of a chimeric antigen receptor or a TcR on a cell is induced by tamoxifen and/or its metabolites. Metabolites for tamoxifen are active metabolites such as 4-hydroxytamoxifen (afimoxifene) and N-desmethyl-4-hydroxytamoxifen (endoxifen), which can have 30-100 times more affinity with an estrogen receptor than tamoxifen itself. In some alternatives, the tamoxifen metabolites are 4-hydroxytamoxifen (afimoxifene) and/or N-desmethyl-4-hydroxytamoxifen (endoxifen). In some alternatives, vectors are provided wherein the vector has a first promoter for the CAR/TcR and a second promoter for the marker protein.

[0088] An “antibody” as described herein, refers to a large Y-shape protein produced by plasma cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. The antibody protein can comprise four polypeptide chains; two identical heavy chains and two identical light chains connected by disulfide bonds. Each chain is composed of structural domains called immunoglobulin domains. These domains can contain about 70, 80, 90, 100, 110, 120, 130, 140, 150 amino acids or any number of amino acids in between in a range defined by any two of these values, and are classified into different categories according to their size and function. In some alternatives, the ligand binding domain comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutants thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the ligand binding domain is an antibody fragment, desirably, a binding portion thereof. In some alternatives, the antibody fragment or binding portion thereof present on a CAR is specific for a ligand on a B-cell. In some alternatives, the antibody fragment or binding portion thereof present on a CAR or TcR is specific for a ligand on a tumor cell. In some alternatives, the tumor is not derived from a B-cell related cancer. In some alternatives, the antibody fragment or binding portion thereof present on a

CAR is specific for a ligand present on a tumor cell. In some alternatives, the ligand binding domain is an antibody fragment or a binding portion thereof, such as a single chain variable fragment (scFv). In some alternatives, the ligand comprises a tumor specific mutation. In some alternatives, the antibody fragment or binding portion thereof present on a CAR comprises one or more domains from a humanized antibody, or binding portion thereof.

[0089] “ScFv” as described herein, is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. In some alternatives, a CAR is provided, wherein the CAR comprises a ScFv specific for a cell surface tumor molecule. In some alternatives, the chimeric antigen receptor comprises a scFV specific for CD19. In some alternatives, the scFV specific for CD19 comprises an amino acid sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the chimeric antigen receptor comprises a scFV specific for CD20. In some alternatives, the scFV specific for CD20 comprises an amino acid sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the chimeric antigen receptor comprises a scFV specific for CE7. In some alternatives, the scFV specific for CE7 comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the chimeric antigen receptor comprises a scFV specific for ROR1. In some alternatives, the scFV specific for ROR1 comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the chimeric antigen receptor comprises a scFV specific for EGFR 806. In some alternatives, the scFV specific for EGFR 806 comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the chimeric antigen receptor comprises a scFV specific for Her2. In some alternatives, the scFV specific for Her2 comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the chimeric antigen receptor comprises a scFV specific for GD2. In some alternatives, the scFV specific for GD2 comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the chimeric antigen receptor comprises a scFV specific for EphA2 (2H4). In some alternatives,

the scFV specific for EphA2 (2H4) comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the chimeric antigen receptor comprises a scFV specific for EphA2 (4H5). In some alternatives, the scFV specific for EphA2 (4H5) comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28.

[0090] “Marker domains” as described herein, refers to a protein that serves as a label for a cell. In some alternatives of the cells described herein, the cells co-express a marker protein for a specific chimeric antigen protein that is expressed. In some alternatives of the cells provided herein, the chimeric antigen receptor is co-expressed with a specific marker protein. In some alternatives of the cells provided herein, the cells comprise a nucleic acid encoding a chimeric antigen receptor. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B-cell specific cell surface molecule, and wherein the first nucleic acid is covalently attached to a 5’ end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid is covalently attached to a 3’ end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached to a 3’ end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the fifth nucleic acid is covalently attached to a 3’ end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached to a 3’ end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached to a 3’ end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence.

[0091] A “ribosome skip sequence” as described herein refers to a sequence that during translation, forces the ribosome to “skip” the ribosome skip sequence and translate the

region after the ribosome skip sequence without formation of a peptide bond. Several viruses, for example, have ribosome skip sequences that allow sequential translation of several proteins on a single nucleic acid without having the proteins linked via a peptide bond. As described herein, this is the “linker” sequence. In some alternatives of the nucleic acids provided herein, the nucleic acids comprise a ribosome skip sequence between the sequence for the chimeric antigen receptor and the sequence of the marker protein, such that the proteins are co-expressed and not linked by a peptide bond. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is a T2A sequence. In some alternatives, the T2A sequence comprises the amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives the marker protein is a EGFRt protein. In some alternatives, the marker protein is a Her2tg protein. By placing the marker gene after the ribosome skip motif, the expressed marker protein will not be bound to the CAR but can be used in determining or purifying cells that express the CAR of interest. In some alternatives, the T-cell is identified for being a bi-specific T-cell by the determination of two marker proteins wherein the first marker protein is indicative of the presence of the B-cell specific CAR, and the second marker is indicative of the presence of the tumor specific CAR. Determination of the marker proteins can be performed by immunoselection, antibody binding to the marker protein and other methods of selection known to those skilled in the art.

[0092] In some alternatives the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34.

[0093] “Internal ribosome entry site (IRES),” as described herein, is a nucleotide sequence that allows for translation initiation in the middle of a messenger RNA (mRNA) sequence as part of the greater process of protein synthesis.

[0094] “Bi-specific chimeric antigen receptor” refers to a CAR that comprises two domains, wherein the first domain is specific for a first ligand, and wherein the second domain is specific for a second ligand. In some alternatives, the first ligand is a B-cell specific protein. In some alternatives, the second ligand is a tumor-specific ligand.

[0095] As used herein, “nucleic acid” or “nucleic acid molecule” refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogous of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term “nucleic acid molecule” also includes so-called “peptide nucleic acids,” which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded. In some alternatives, a nucleic acid encoding a chimeric antigen receptor is provided. In some alternatives, a method of making a nucleic acid encoding a chimeric antigen receptor is provided. In some alternatives, a nucleic acid encoding a chimeric antigen receptor specific for a ligand on a B cell is provided. In some alternatives, a nucleic acid encoding a chimeric antigen receptor specific for a ligand on a tumor cell is provided. In some alternatives the nucleic acid is a DNA encoding a chimeric antigen receptor. In some alternatives, the nucleic acid is an mRNA encoding a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor is bi-specific.

[0096] “Vector” as described herein, is a nucleic acid vehicle that carries a generic material encoding a protein or mRNA of interest into another cell, such that it is replicated and/or expressed in the cell. There are several types of vectors. Without being

limiting, a vector can be a plasmid, viral vector, cosmid, artificial chromosome, or an mRNA. The vector can be linear or circular. In some alternatives provided herein, a viral vector is used to carry the nucleic acid encoding a chimeric antigen receptor. In some alternatives, the viral vector is a lentiviral vector. In some alternatives, the viral vector is a retroviral vector. In some embodiments, the viral vector is a gammaretroviral vector. In some alternatives, the vector is a foamy viral vector. In some alternatives, the vector is a plasmid. In some alternatives, the vector is an mRNA. In some alternatives, the vector is linear and comprises telomeres.

[0097] “Plasmid” as described herein, is a genetic structure in a cell that can replicate independently of the chromosomes. Without being limiting, the plasmid can be a small circular DNA strand in the cytoplasm of a bacterium or protozoan, or a linear nucleic acid.

[0098] “Minicircles,” as described herein, are small circular plasmid derivatives that have been freed from all prokaryotic vector parts. Minicircles can serve as an expression vector, where they have been applied as transgene carriers for the genetic modification of mammalian cells, with the advantage that, since they contain no bacterial DNA sequences, they are less likely to be perceived as foreign and destroyed. As such, typical transgene delivery methods involve plasmids, which contain foreign DNA. The smaller size of minicircles also extends their cloning capacity and facilitates their delivery into cells. Without being limiting, the preparation of minicircles can follow a two-step procedure, which can involve production of a parental plasmid (bacterial plasmid with eukaryotic inserts) in *E. coli* and induction of a site-specific recombinase at the end of this process but still in bacteria. These steps can be followed by the excision of prokaryotic vector parts via two recombinase-target sequences at both ends of the insert and recovery of the resulting minicircle (vehicle for the highly efficient modification of the recipient cell) and the miniplasmid by capillary gel electrophoresis (CGE).

[0099] An “inverted terminal repeat,” as described herein, is a sequence of nucleotides followed downstream by its reverse complement and occur at opposite ends of a transposon.

[0100] As described herein, “transposable element” (TE), transposon or retrotransposon, can be referred to as a DNA sequence that can change its position within the

genome. The transposon can create or reverse mutations and alter the cell's genome size. Transposition often results in duplication of the TE. TEs can make up a large fraction of the C-value of eukaryotic cells. "C-values," as described herein, refers to amount, in picograms, of DNA contained within a haploid nucleus of one half the amount in a diploid somatic cells of a eukaryotic organism. In some alternatives, the nucleic acid encoding a chimeric antigen receptor resides within a vector, wherein the vector is a minicircle transposon. In some alternatives, the vector comprises a transposon.

[0101] The "Sleeping Beauty transposon system" as described herein, is composed of a Sleeping Beauty (SB) transposase and a transposon that was designed in 1997 to insert specific sequences of DNA into genomes of vertebrate animals. DNA transposons can translocate from one DNA site to another in a simple, cut-and-paste manner. Transposition is a precise process in which a defined DNA segment is excised from one DNA molecule and moved to another site in the same or different DNA molecule or genome. In some alternatives of the vectors provided herein, the vector comprises a Sleeping Beauty transposon.

[0102] An SB transposase can insert a transposon into a TA dinucleotide base pair in a recipient DNA sequence. The insertion site can be elsewhere in the same DNA molecule, or in another DNA molecule (or chromosome). In mammalian genomes, including humans, there are approximately 200 million TA sites. The TA insertion site is duplicated in the process of transposon integration. This duplication of the TA sequence is a hallmark of transposition and used to ascertain the mechanism in some experiments. The transposase can be encoded either within the transposon or the transposase can be supplied by another source, in which case the transposon becomes a non-autonomous element.

[0103] "PiggyBac (PB) transposon," as described herein refers to a mobile genetic element that efficiently transposes between vectors and chromosomes via a "cut and paste" mechanism. During transposition, the PB transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector and efficiently moves the contents from the original sites and efficiently integrates them into TTAA chromosomal sites. The powerful activity of the PiggyBac transposon system enables genes of interest between the two ITRs in the PB vector to be easily mobilized into target genomes.

[0104] In some alternatives, the vector is a PiggyBac transposon. The PiggyBac (PB) transposon is a mobile genetic element that efficiently transposes between vectors and chromosomes via a "cut and paste" mechanism. During transposition, the PB transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector and efficiently moves the contents from the original sites and efficiently integrates them into TTAA chromosomal sites. The powerful activity of the PiggyBac transposon system enables genes of interest between the two ITRs in the PB vector to be easily mobilized into target genomes.

[0105] In some alternatives, a PB contains a promoter linked to a polynucleotide coding for a chimeric antigen receptor operably linked to a genetic tag. One or more PB transposons can be employed. In some alternatives, a PB comprises a promoter linked to a polynucleotide coding for a chimeric antigen receptor and a first genetic tag, another PB comprises a promoter linked to a polynucleotide coding for a chimeric antigen receptor, and a second and different genetic tag. Each element of the constructs is separated by a nucleic acid, such as that coding for a self-cleaving T2A sequence. In some alternatives, each PB differs from one another in the chimeric antigen receptor including but not limited to the spacer length and sequence, the intracellular signaling domain, and/or the genetic tag sequence.

[0106] "Adoptive cell transfer" as described herein, refers to the transfer of cells into a patient. In some alternatives, a method of treating, ameliorating, or inhibiting a non-B cell related disease in a subject is provided, wherein the method comprises introducing, providing, or administering any one or more of the cells or compositions of any of the alternatives described herein into a subject for therapy. In some alternatives, the cells are administered by adoptive cell transfer.

[0107] A "leader sequence" as described herein is also known as a signal sequence that can direct a protein to the cell surface. The leader sequence under the context of a CAR, refers to the first sequence of amino acids in a CAR that directs surface expression. This leader sequence, or signal sequence can be required for surface expression of a protein. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic

acid sequence set forth in SEQ ID NO: 30. In some alternatives, the signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32.

[0108] A “de-immunized spacer” as described herein, refers to a spacer that induces little to no immune response from a patient. In some alternatives, the chimeric antigen receptor comprises a spacer, wherein the spacer does not induce an immune response in a subject in need.

[0109] The de-immunized spacer can comprise a polypeptide chain that can range in length from a length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239 or 240 amino acids or a length within a range defined by any two of the aforementioned lengths. A de-immunized spacer can comprise any of the 20 amino acids in any order to create a desirable length of polypeptide chain in a chimeric antigen receptor, which includes the amino acids arginine, histidine, lysine, aspartic acid, glutamic acid, serine, threonine, asparagine, glutamine, cysteine, glycine, proline, alanine, valine, isoleucine, methionine, phenylalanine, tyrosine and/or tryptophan. A de-immunized spacer sequence can be a linker between the scFv and the transmembrane domain of the chimeric antigen receptor. In some alternatives, a method of making a nucleic acid encoding a chimeric antigen receptor is provided. In some alternatives, a nucleic acid encoding a chimeric antigen receptor is provided. In some alternatives, the nucleic acid comprises a sequence for a de-immunized spacer. In some alternatives, the de-immunized spacer comprises a sequence with a length of 10, 11, 12, 13,

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

[0110] In some alternatives, a spacer region has at least 10 to 229 amino acids, 10 to 200 amino acids, 10 to 175 amino acids, 10 to 150 amino acids, 10 to 125 amino acids, 10 to 100 amino acids, 10 to 75 amino acids, 10 to 50 amino acids, 10 to 40 amino acids, 10 to 30 amino acids, 10 to 20 amino acids, or 10 to 15 amino acids, or a length that is within a range defined by any two of the aforementioned amino acid lengths. In some alternatives, a spacer region has 12 amino acids or less but greater than 1 amino acid, 119 amino acids or less but greater than 1 amino acid, or 229 amino acids or less but greater than 1 amino acid.

[0111] In some alternatives, the spacer comprises an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 (SEQ ID NO: 1; E S K Y G P P C P P C P). In some alternatives, the spacer is encoded by a sequence set forth in SEQ ID NO: 2 (SEQ ID NO: 2; GAGAGCAAGTACGGACCGCCCTGCCCCCCTTGCCCT).

[0112] In some alternatives, the spacer comprises an IgG4-CH3 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 (SEQ ID NO: 3; E S K Y G P P C P P C P G Q P R E P Q V Y T L P P S Q E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S R L T V D K S R W Q E G N V F S C S V M H E A L H N H Y T Q K S L S L S L).

G K). In some alternatives, the spacer is encoded by a sequence set forth in SEQ ID NO: 4 (SEQ ID NO: 4; GAGAGCAAGTACGGACCGCCCTGCCCCCTTGCCCTGGCCAGCCTCGCGAGCCCCAGGTGTACACCCTGCCTCCCTCCCAGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACACTACAAGACCACCCTCCCGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGCCGGTGGCAGGAAGGCAACGTCTTTAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCTGGGCAAG).

[0113] In some alternatives, the de-immunized spacer comprises a IgG4-CH2 spacer (L235D, N297Q mutant). In some alternatives, the IgG4-CH2 spacer (L235D, N297Q mutant) comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40.

[0114] In some alternatives, the de-immunized spacer comprises a IgG4 hinge. In some alternatives, the de-immunized spacer comprises a CD8 α hinge. In some alternatives, the de-immunized spacer comprises a CD28hinge and their fusion to the CH2 and/or CH3 domains of IgG molecules.

[0115] “Signaling domain” as described herein is a domain on a chimeric antigen receptor that can promote cytokine release, *in vivo* T cell survival and tumor elimination. In some alternatives herein, a signaling domain comprises CD28, 4-1BB and/or CD3-zeta cytoplasmic domains.

[0116] “Steroid” as described herein refers to a small cyclic organic compound with a common characteristic comprising an arrangement of seventeen carbon atoms within a four ring structure. In some alternatives provided herein, the expression of a CAR is induced by a steroid, such as estrogen, testosterone, or a glucocorticoid or retinoic acid. In some alternatives described here, the steroid tamoxifen is used to induce expression of a CAR or TcR. In some alternatives, estrogen or glucocorticoid are used to induce expression of a CAR or TcR.

[0117] “Non-B cell related disease” refers to any cancer or disease that is not related to B-cell cancers or diseases. Therefore expression of specific proteins on the cell surface that are markers for a cancer are markers for diseases that are not caused by any B-cell related abnormalities in the alternatives described herein. Without being limiting, non-B

cell related diseases can include B-cell lymphoma, Hodgkin's lymphomas, non-Hodgkins lymphomas, Diffuse large B cell lymphoma, Follicular lymphoma, marginal zone lymphoma, Mucosa-Associated Lymphatic Tissue lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B cell lymphoma, Lymphoplasmacytic lymphoma, Waldenstrom macroglobulinemia, Nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B cell lymphoma, Intravascular large B-cell lymphoma, Primary effusion lymphoma, Lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, Primary central nervous system lymphoma, Primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, Diffuse large B-cell lymphoma associated with inflammation, Intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, ALK-positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-cell lymphoma arising in HHV8-associated multicentric Castleman's disease, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, or nodular lymphocyte predominant Hodgkin's lymphoma.

[0118] “Solid Tumors” as described herein, refers to a malignant cancerous mass of tissue. In some alternatives of the methods of treating, ameliorating, or inhibiting a non-B cell related disease in a subject provided herein, the method comprises introducing, providing, or administering any one or more of the cells or compositions of any of the alternatives herein or the cells made by any one or more of the methods of the alternatives herein into a subject for therapy. In some alternatives, the subject has a cancer. In some alternatives, the cancer is a solid tumor. In some alternatives, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer.

[0119] “Neuroblastoma” as described herein, refers to an extracranial solid cancer in childhood and infancy that is a neuroendocrine tumor that can arise from a neural crest element of the sympathetic nervous system. Without being limiting, a tumor can originate, for example, in the adrenal glands, and nerve tissues of the neck, chest, abdomen, and/or

pelvis. In some alternatives of the methods described herein, T-cells comprising a bi-specific chimeric antigen receptor or two chimeric antigen receptors for bi-specificity are administered to a subject in need by adoptive cell transfer. In some alternatives, the subject in need suffers from neuroblastoma. In some alternatives, the subject in need is a person of pediatric age. Pediatric age as described herein refers to a person of age 24 or under. A person of pediatric age is at age 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 years old or any other age within a range in between any two of the aforementioned values described.

[0120] “Refractory and relapsed neuroblastoma” as described herein, refers to the children who are considered “high risk” that do not respond completely to treatment and are labeled refractory. The children that are considered high-risk are removed from frontline therapy and are considered to be eligible for clinical trials that can use new therapies. Children who have had a good response to frontline therapy and achieved a disease reoccurrence (relapse) are considered high-risk and are also eligible for new therapies tested in clinical trials. In some alternatives of the methods described herein, the patients suffer from refractory and/or relapsed neuroblastoma. In some alternatives, the subject in need is a person of pediatric age. Pediatric age as described herein refers to a person of age 24 or under. A person of pediatric age is at age 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 years old or any other age within a range in between any two of the aforementioned values described. In some alternatives, the patient is receiving cancer therapy. In some alternatives, the cancer therapy comprises administration of ¹³¹I-MIBG to the patient.

[0121] “Suicide gene therapy,” “suicide genes” and “suicide gene systems” as described herein, can refer to methods to destroy a cell through apoptosis, which requires a suicide gene that will cause a cell to kill itself by apoptosis. Due to safety concerns for the patients in need of CAR therapy, strategies are being developed in order to prevent or abate adverse events. Adverse effects of CAR T-cell therapy can include “cytokine storms” which is a cytokine release syndrome in which the infused T-cells release cytokines into the bloodstream, which can lead to dangerously high fevers as well as a precipitous drop in blood pressure. To date, cytokine-release syndrome is a common problem in patients treated with CAR T-cells and patients with the most extensive disease, prior to receiving the CAR T-

cells were shown to be more likely to experience severe cases of cytokine-release syndrome. Off target adverse effects can also occur, if a tumor specific antigen is also expressed in healthy tissue.

[0122] Suicide gene therapy can be used to increase the safety of chimeric antigen receptor redirected T- cells and manage the adverse events that can occur following infusion of CAR bearing T-cells. Pharmacologic therapies, suicide genes or novel strategies are needed to limit the cytotoxic effect only to malignant cells. There are several methods for suicide gene therapy. Without being limiting, methods can include gene-directed enzyme producing therapy or virus directed enzyme prodrug therapy. For gene-directed enzyme producing therapy (GDEPT), a gene is taken from the cancer cell and then modified with other genes to form enzymes that are harmless to healthy cells. This foreign enzyme is inserted into the tumor cells where it releases a prodrug, which is a small molecule harmless to healthy cells, but destructive to cancerous cells. The modified suicide gene converts the non-toxic prodrug into a cytotoxic substance. For virus directed enzyme prodrug therapy, a virus, such as herpes simplex or cold virus, as the carrier, or vector, is used to deliver the modified genes to the cancer cells. Suicide gene therapy is not necessarily expected to completely eliminate the need for chemotherapy and radiation treatment for all cancerous tumors. The damage inflicted upon the tumor cells, however, makes them more susceptible to the chemo or radiation. This approach has already proven effective against prostate and bladder cancers. The application of suicide gene therapy is being expanded to several other forms of cancer as well. Cancer patients often experience depressed immune systems, so they can suffer some side effects of the use of a virus as a delivery agent. Management of adverse effects of CAR T-cell therapy can be performed by expressing the CARs under the control of a promoter. As previously described in several reviews, T-lymphocytes that express a CAR can further be genetically modified *ex vivo* with a suicide gene. Without being limiting, the suicide gene can be a gene encoding for a factor that is able to convert at a cellular level a non-toxic prodrug into a toxic compound. During adverse effect that may follow infusion of CAR T-cells by adoptive cell transfer, the prodrug can be administrated to the subject suffering from adverse effects, and the prodrug can selectively eliminate suicide gene modified T-cells without interfering with the process of immune reconstitution operated by the non-modified T-cells. Suicide systems using the herpes simplex thymidine kinase (Hsv-

tk)/ganciclovir (GCV) suicide system have been described. (Casucci *et al.* 2011, Journal of Cancer 2011, 2; incorporated in its entirety herein). In some alternatives, a cell comprising a first and second chimeric antigen receptor is provided, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor is specific for a ligand on a tumor. In some alternative, a cell comprising a bi-specific chimeric antigen receptor is provided, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain is specific for a ligand on a tumor. In some alternatives, the cell further comprises a suicide gene system.

[0123] In some alternatives, the suicide gene system is an EGFRt suicide gene system, wherein the patient is administered Erbitux as the prodrug. In some alternatives, the subject is administered 0.04mg/cm² Erbitux a day. In some alternatives, wherein Erbitux is the prodrug, 0.04mg/cm² can be considered the initial dose and in some alternatives includes a weekly 0.025mg/ cm² dose of Erbitux after the initial dose. In some alternatives, wherein a rash develops on the patient, the weekly dose can go down to 0.03 mg/cm², 0.02 mg/cm² or 0.01mg/ cm², or any other dosage between any two of the aforementioned values described.. In some alternatives, the suicide gene system is a Her2tG suicide gene system, wherein the patient is administered Herceptin as the prodrug. In some alternatives, the subject is administered 2 mg/kg, 3 mg/kg or 4mg/kg Herceptin or any dosage between any two of the aforementioned values described. In some alternatives of the methods of making a cell comprising a first and second chimeric antigen receptor or TcR, the method further comprises introducing into a cell a nucleic acid encoding a suicide gene. In some alternatives, the nucleic acid encoding a suicide gene is resides on a vector. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentiviral vector or a retroviral vector. In some alternatives, the Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36.

[0124] “Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system,” as described herein, is a suicide gene system using the thymidine kinase gene from the herpes simplex virus in combination with the prodrug ganciclovir.

[0125] In some alternatives of the methods of making a cell comprising a first and second chimeric antigen receptor or TcR, the method further comprises introducing into a cell a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system. In some alternatives, the nucleic acid encoding the suicide gene is resides on a vector. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentiviral vector or a retroviral vector. In some alternatives, the suicide gene resides on the vector carrying the nucleic acid encoding the chimeric antigen receptor, wherein the suicide gene system is located upstream or downstream from the nucleic acid encoding the chimeric antigen receptor, and wherein the suicide gene system is separated from the nucleic acid encoding the chimeric antigen receptor by a gene encoding a cleavable linker. In some alternatives, 1000 mg of Ganciclovir is administered to a subject in need three times a day with food.

[0126] In some alternatives, wherein Ganciclovir is the prodrug, the patient can be administered Ganciclovir as induction therapy at a 5 mg/kg intravenously (IV) at a constant rate for 1, 2, or 3 hours or any amount of time in between any two of the aforementioned values, every 12 hours for 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or 21 days or any number of days within a range in between any two aforementioned values. In some alternatives, wherein Ganciclovir is the prodrug, the amount of Ganciclovir in the patient is maintained, wherein the patient is administered Ganciclovir intravenously (IV) at 5 mg/kg at a constant rate over 1, 2 or 3 hours or any amount of time within a range in between any two of the aforementioned values. In some alternatives, the Ganciclovir is administered once a day, for seven days a week. In some alternatives, wherein Ganciclovir is the prodrug, the amount of Ganciclovir in the patient is maintained, wherein the patient is administered Ganciclovir intravenously (IV) at 6 mg/kg at a constant rate over 1, 2 or 3 hours or any amount of time in between any two of the aforementioned values, once a day, for five days a week.

[0127] In some alternatives of the methods of making a cell comprising a first and second chimeric antigen receptor or TcR, the method further comprises introducing into a cell a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Caspase based suicide gene system. Without being limiting, examples of inducible caspase suicide gene systems can include the inducible caspase-9 suicide gene system and the caspace-8 suicide gene system. The use of inducible caspase 9 (iCasp9) mediated suicide is based on the conditional dimerization of pro-apoptotic molecules that are constructed from human proteins and are less likely to be immunogenic. In some alternatives, wherein the iCasp9 suicide system is used, the iCasp9 protein comprises a human FK506 binding protein.

[0128] In some alternatives of the suicide gene system is a Caspase system, such as the Caspase 9 suicide gene system. In some alternatives, the prodrug is FK506 or another chemical inducer of dimerization. In some alternatives of the methods of making a cell comprising a first and second chimeric antigen receptor or TcR, the cell comprises introducing into a cell a nucleic acid encoding a suicide gene. In some alternatives, the nucleic acid encoding a suicide gene is resides on a vector. In some alternatives, the vector is a viral vector. In some alternatives, the viral vector is a lentiviral vector or a retroviral vector. In some alternatives, the subject is administered a prodrug to induce apoptosis. In some alternatives, the prodrug is FK506. In some alternatives, the subject is administered a dosage of 0.075 mg/kg/day, 0.1 mg/kg/day, 0.125 mg/kg/day, 0.150 mg/kg/day, 0.175 mg/kg/day or 0.2 mg/kg/day or any dosage within a range in between any two of the aforementioned values described.

[0129] In some alternatives, the suicide gene system comprises a suicide vector for tamoxifen inducible apoptosis. In particular, an inducible apoptosis system with a Fas-estrogen receptor fusion protein can be used in order to rapidly eliminate transduced cells. In some alternatives, the system can induce apoptosis independently from endogenously expressed estrogen.

[0130] “Prodrug” as described herein, is a medication that is administered in a pharmacologically inactive form which is then converted to an active form through a normal metabolic process. In some alternatives, a method of treating, ameliorating, or inhibiting a non-B cell related disease in a subject is provided wherein the method comprise introducing,

providing, or administering any one or more of the cells or compositions of any of the alternatives described herein or the cells made by any one or more of the methods of any of the alternatives described herein into a subject for therapy. In some alternatives, the method further comprises evaluating the subject for symptoms of cytokine storm or B-cell aplasia. In some alternatives, the subject is suffering from high fevers, increase or decrease of blood pressure, hypotension, hypoxia, seizures, or aphasia. In some alternatives, the subject has an elevation in ferritin and/or C-reactive protein. In some alternatives of the methods provided herein, wherein the cells comprise a suicide gene system, the methods further comprise administering to the subject a prodrug.

[0131] In some alternatives, wherein the transduced T-cell comprises a suicide gene system, wherein the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or inducible Caspase 9 suicide gene system, the prodrug is FK506. In some alternatives, the subject is administered 0.075-0.2mg/kg/day of FK506. In some alternatives, the subject is administered a dosage of 0.075 mg/kg/day, 0.1 mg/kg/day, 0.125 mg/kg/day, 0.150 mg/kg/day, 0.175 mg/kg/day or 0.2 mg/kg/day or any dosage within a range in between any two of the aforementioned values described.

[0132] In some alternatives, wherein the transduced T-cell comprises a suicide gene system, wherein the suicide gene system is the suicide gene system comprises a suicide vector for tamoxifen inducible apoptosis, the prodrug is tamoxifen. In some alternatives, the subject is administered 20mg/day tamoxifen.

[0133] In some alternatives, wherein the transduced T-cell comprises a suicide gene system, wherein the suicide gene system is the Herpes Simplex Virus Thymidine Kinase (HSVTK) suicide gene system comprises a suicide vector for tamoxifen inducible apoptosis, the prodrug is GCV. In some alternatives, the subject is administered 1000mg orally 3x/day GCV with food.

[0134] “Engraftment” as described herein, refers to the incorporation of grafted tissue into the body of the host. Several characteristics of effective CAR T-cells include showing signs of adequate engraftment, which is required for responses. For example, detection of the CAR transgene by polymerase chain reaction is not informative about the surface expression of the CAR, which is the only form that matters for efficacy. Thus, the

availability of reagents to specifically detect CARs at the cell surface by flow cytometry or other methods known to those skilled in the art is crucial to understand the activity and engraftment of CAR T-cells. In the alternatives described herein, the therapeutic potency of the adoptively transferred CARs are improved by allowing a B-cell targeting CAR to drive the activation, proliferation and dispersion of infused CAR T-cells that have a second CAR that provides for redirected killing of the solid tumor. In some alternatives described herein, the methods and cells comprising a CAR with B-cell specificity led to the surprising effect of having an improved level of engraftment compared to T-cells that only comprised CARs specific for a tumor ligand. As described in the alternatives herein, the obstacle of failure to exhibit engraftment is overcome by allowing a B cell targeting CAR to drive the activation, proliferation and dispersion of infused CAR T-cells that have a CAR that provides for redirected killing of the solid tumor.

[0135] "Subject" or "patient," as described herein, refers to any organism upon which the alternatives described herein may be used or administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Subjects or patients include, for example, animals. In some alternatives, the subject is mice, rats, rabbits, non-human primates, and humans. In some alternatives, the subject is a cow, sheep, pig, horse, dog, cat, primate or a human.

DETAILED DESCRIPTION

[0136] Although the invention is described in various exemplary alternatives and implementations as provided herein, it should be understood that the various features, aspects, and functionality described in one or more of the individual alternatives are not limited in their applicability to the particular alternative with which they are described. Instead, they can be applied alone or in various combinations to one or more of the other alternatives of the invention, whether the alternatives are described or whether the features are presented as being a part of the described alternative. The breadth and scope of the present invention should not be limited by any exemplary alternatives described or shown herein.

[0137] Aspects of the present invention relate to methods, cells and compositions for augmenting the therapeutic potency of adoptively transferred chimeric antigen receptor

(CAR) bearing T-cells against solid tumors. In particular, methods, cells and compositions for CAR T-cell products that co-express two CARs in individual T-cells, a B cell targeting "driver" CAR for promoting in vivo expansion and activation of an effector cell, and a CAR or T-cell receptor (TcR) of a desired specificity for targeting a solid tumor (passenger CAR/TcR), are provided herein.

Cells expressing a first and second chimeric antigen receptor

[0138] In accordance with some preferred alternatives, there are cells provided wherein the cells comprise a first and second chimeric antigen receptor or TcR, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor or TcR is specific for a ligand on a tumor. In some alternatives of the cells, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFRSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, EMMPRIN/CD147 and/or tumor targets on tumors that are non-B cell tumors. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives,

the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR are inducibly expressed in said cell. In some alternatives, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR is under the control of a regulatory element. In some alternatives, the first chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the second chimeric antigen receptor or TcR comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, a first marker protein is co-expressed with the first chimeric antigen receptor and a second marker protein is co-expressed with the second chimeric antigen receptor or TcR. In some alternatives, the first marker protein co-expressed with the first chimeric antigen receptor is EGFRt and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is Her2tg or first marker protein co-expressed with the first chimeric antigen receptor is Her2tg and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is EGFRt.

[0139] In some alternatives, the lymphocyte can express a CAR and a specific T-cell receptor (TcR) for bi-specificity. In some alternatives, the specific T-cell receptor is specific for EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1.

[0140] In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen,

abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cell surface tumor specific molecule is ROR1. In some alternatives, the first chimeric antigen receptor and/or the second chimeric antigen receptor are inducibly expressed in said cell. In some alternatives, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor is under the control of a regulatory element. In some alternatives, the first chimeric receptor comprises a first ligand binding domain and the second chimeric receptor comprises a second ligand binding domain. In some alternatives, the ligand binding domain comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutants thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the first chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand, peptide, and/or polypeptide affinity molecule. In some alternatives, the second chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand, peptide, and/or polypeptide affinity molecule. In some alternatives, a first marker protein is co-expressed with the first chimeric antigen receptor and a second marker protein is co-expressed with the second chimeric antigen receptor. In some alternatives, the first marker protein co-expressed with the first chimeric antigen receptor is EGFRt and the second marker protein co-expressed with the second chimeric antigen receptor is Her2tg or first marker protein co-expressed with the first chimeric antigen receptor is Her2tg and the second marker protein co-expressed with the second chimeric antigen receptor is EGFRt. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS

derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

[0141] Some alternatives described herein, incorporate a novel strategy to augment the therapeutic potency of adoptively transferred CAR redirected T cells against solid tumors. The alternatives also include CAR T cell products that co-express two CARs in individual T-cells (CD4⁺ and CD8⁺ T-cells subsets) incorporating a B cell targeting "driver" CAR (specific for CD19, CD22, CD20, CD10, CD79c, ROR1 or another cell surface molecule expressed by B cells) for promoting in vivo expansion and activation and a CAR or TcR of a desired specificity for targeting a solid tumor (passenger CAR/TcR). Dual CAR (or CAR/TcR) T- cells can be identified and selected by using two cell surface tags developed in the alternatives described herein, that consist of modified human EGFR and HER2 polypeptides, EGFRt and HER2tg, respectively. Additionally, single CARs that house a B cell targeting domain and solid tumor targeting domain are also envisioned to achieve bi-specificity for B cells and solid tumors. A variety of gene transfer methods are envisioned to generate bispecific CAR T-cells inclusive of viral vectors, non-viral transposon vectors, and mRNA. Additionally, a genetic system is envisioned in which the B cell targeting CAR is housed in a drug regulated expression format such as TamR. A preferred alternative of this system is the co-expression of a CD19 specific CAR, ideally housing a human scFv binding domain, deimmunized extracellular spacer and 4-1 BB:zeta second generation cytoplasmic tail, in conjunction with either EGFRt or HER2tg (or another appropriate cell surface tag) coexpressed via a genetic element that links the cDNA's, such as a ribosome skip sequence or IRES, along with a CAR or TcR developed for solid tumor therapy co-expressed with the second cell surface tag. One iteration of this system allows for separate lentiviral vectors to house each CAR/tag (or TcR/tag) and the use of a mixture of the two lentiviruses to co-transduce human T cells. These alternatives anticipates cell selections (immunomagnetic, cell sorting, other) that allows for purification of transduced T cells that express both cell surface tags and their corresponding CARs.

[0142] The dual CAR (or CAR/TcR) T-cells can be identified and selected by using two cell surface tags developed and described herein, and can consist of marker proteins (e.g., modified human EGFR and HER2 polypeptides, EGFRt and HER2tg,

respectively). Additionally, single CARs that house a B cell targeting domain and solid tumor targeting domain are also envisioned to achieve bi-specificity for B cells and solid tumors, and are provided herein. A variety of gene transfer methods are envisioned to generate bispecific CAR T-cells inclusive of viral vectors, non-viral transposon vectors, and mRNA. Additionally, a genetic system is envisioned in which the B cell targeting CAR is housed in a drug regulated expression format such as TamR. A preferred alternative of this system is the co-expression of a CD19 specific CAR, which can also house a human scFv binding domain, deimmunized extracellular spacer and 4-1 BB:zeta second generation cytoplasmic tail, in conjunction with an cell surface tag (e.g. EGFRt or HER2tg) or the CAR can be co-expressed via a genetic element that links the cDNA's, such as a ribosome skip sequence or IRES, along with a CAR or TcR developed for solid tumor therapy co-expressed with the second cell surface tag. One alternative of this system allows for separate lentiviral vectors to house each CAR/tag (or TcR/tag) and the use of a mixture of the two lentiviruses to co-transduce human T-cells. The alternatives described herein, anticipates cell selections (immunomagnetic, cell sorting, other) that allows for purification of transduced T-cells that express both cell surface tags and their corresponding CARs.

[0143] The novel feature of bispecific CAR T-cells housing a driver CAR that targets normal B cells and a second solid tumor targeting passenger CAR, arises from the observations made from the several alternatives described herein, that CD19 CAR T-cells infused into leukemia patients undergo multi-log expansion and are activated to mediate the regression of systemic leukemia inclusive of the central nervous system (CNS). This phenomenon has been observed in patients in remission who have CD19 CAR T-cell expansion in response to activation by non-malignant B cells. The expansion mediated by B cells reflects their unique immune-stimulatory properties for CD19 CAR T-cells and their distribution in the blood, lymph nodes, and bone marrow where infused CAR T-cells migrate following intravenous infusion.

[0144] In contrast, CAR T-cells targeting solid tumors such as CE7 CAR T-cells used in clinical trials involving patients with metastatic neuroblastoma fail to exhibit engraftment, presumably due to limited migration of infused T-cells to sites of tumor metastasis, and the limited immune-stimulatory activity of solid tumors and their immunosuppressive environment. The cells described herein overcomes this obstacle by

allowing a B cell targeting CAR to drive the activation, proliferation and dispersion of infused CAR T-cells that have a second passenger CAR that provides for redirected killing of the solid tumor.

[0145] In several of the alternatives described herein, data has been generated that can demonstrate the following: 1) Human T-cells can be co-transduced with two lentiviruses that house a CD19CAR-T2AEGFRt and a second CAR (antiCD20)-T2A-HER2t; 2) Co-transduced human T-cells have a portion of cells that co-express both EGFRt and HER2t. 3) Double positive T-cells can be purified to homogeneity using immunomagnetic selection reagents specific for EGFRt (Erbix) and HER2 (Herceptin); 4) Dual CAR T-cells retain CD19 redirected specificity and an acquired second specificity (example CD20); 5) The TamR regulated transgene expression genetic system allows for tamoxifen dependent expression of the CD19CAR; 6) Ongoing experiments are testing CD19 driver x L1CAM passenger dual CAR expressing T-cells to be activated by B cells and subsequently exhibit enhanced anti-solid tumor activity.

[0146] The anticipated use of the alternatives described herein, is to augment the curative potential of CAR T-cell therapy for human cancer, specifically solid tumors. This driver/passenger CAR product could be used in the treatment any patient harboring a solid tumor for which there is a passenger CAR and overcome suboptimal activation and expansion following administration to patient by the driver CAR that recognizes a B cell lineage cell surface antigen, such as CD19. In one alternative, the driver CAR is placed into a drug regulated expression system, such as TamR-tf, allowing for cycles of driver CAR expansion followed by periods of "rest" and B cell reconstitution. Further, boosting of this effect to achieve augmented tumor targeting is anticipated by the infusion of patient derived B cells, T-APCs that express the cell surface B cell antigen. This strategy could be employed in the setting by which the tumor specificity is achieved by isolating tumor reactive T-cells through their endogenous TcR or an introduced TcR and the driver is the B cell antigen specific CAR.

[0147] In some alternatives, it is demonstrated that the dual CAR constructs can be introduced using co-transduction with lentivirus. It is contemplated to condense the vectorization into a single vector, e.g., using a nonviral transposon systems such as Sleeping Beauty Transposon Transposase.

[0148] CAR bearing T-cells exhibit the potential for prolonged B cell aplasia in treated patients. Accordingly, the TamR regulated expression system is contemplated for use to turn ON and OFF CD19 driver CAR reactivity, allowing for episodic B cell reconstitution. Alternately, CAR T-cells are equipped with a suicide gene to allow for periods of B cell recovery, and or the re-infusion of patient B cells after CAR T-cell ablation. In some alternatives, the B-cell targeting CAR is under the control of a drug.

[0149] However, other adverse effects of the CAR bearing T-cells of the present invention can also include cytokine storms. Therefore management of the adverse effects of CAR T-cell therapy can be performed by expressing the CARs under the control of a promoter or by the incorporation of a suicide gene system by ex vivo methods known to those skilled in the art. In some alternatives of the cells provided herein, the cells further comprise a suicide gene system.

Cells expressing a bi-specific chimeric antigen receptor

[0150] As described herein, cells comprising a bi-specific CAR are also provided. In some alternatives, a cell is provided wherein the cell comprises a bi-specific chimeric antigen receptor, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, EMMPRIN/CD147 and/or tumor targets that are non-B cell tumors. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME,

SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cell surface tumor specific molecule is ROR1. In some alternatives, the epitope for binding L1CAM is a CE7 epitope. In some alternatives, the target for binding L1CAM is the CE7 epitope. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant version thereof, peptide, and/or polypeptide affinity molecule or binding partner.

[0151] T-cells expressing a bi-specific CAR, wherein the bi-specific CAR comprises two binding domains is provided, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain is specific for a ligand on a tumor, are also anticipated to augment the therapeutic potency of adoptively transferred CAR redirected T cells against solid tumors, as the B-cell binding domain promotes in vivo expansion and activation of effector cells. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant version thereof, peptide, and/or polypeptide affinity molecule or binding partner.

[0152] Bi-specific CAR bearing T-cells have the potential for prolonged B cell aplasia in treated patients. This is mitigated by use of the TamR regulated expression system to turn ON and OFF the bi-specific CAR reactivity, allowing for episodic B cell reconstitution. Alternately, bi-specific CAR T-cells could be equipped with a suicide gene to allow for periods of B cell recovery, and or the re-infusion of patient B cells after bi-specific CAR T-cell ablation. In some alternatives, the bi-specific CAR is under the control of a drug.

[0153] In some instances, bi-specific CAR bearing T-cells can cause cytokine storms. Management of these adverse effects of bi-specific CAR T-cell therapy can be performed by expressing the bi-specific CARs under the control of a promoter or by the incorporation of a suicide gene system by ex vivo methods known to those skilled in the art.

In some alternatives of the cells provided herein, the cells further comprise a suicide gene system.

Compositions

[0154] In some alternatives of the methods and compositions, the methods and composition are used to treat, ameliorate or eliminate cancer in a human suffering from cancer. In some alternatives of the methods and compositions provided herein, the patients in need are people of pediatric age with relapsed refractory neuroblastoma. In some alternatives, the cancer is a solid tumor. In some alternatives, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer.

[0155] The cell product will be a defined composition CD4+ and CD8+ subset product. The product will be co-transduced with the SCR lentivirus that directs the expression of the CD19CAR(4- 1BB:zeta) and EGFRt along with the CE7R CAR(4-1 BB:zeta) and EGFRt. In some alternatives described herein trials have used a CAR/lentivirus pair that used EGFRt and HER2tg.

[0156] As described herein, are developed CD19 specific CARs and solid tumor targeting CARs. The alternatives described herein are dual specific CAR products through expressing multiple CARs in T-cells or engineering of bispecific CARs. The concept of using a driver CAR specific for B cells co-expressed with a passenger CAR or TcR having solid tumor specificity as a strategy for driving high levels of engraftment for augmented tumor responses is original.

[0157] In some alternatives, a composition is provided, wherein the composition comprises any one or more of the cells described herein. In some alternatives, the cell comprises a bi-specific chimeric antigen receptor, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45,

CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, EMMPRIN/CD147 and/or tumor targets that are non-B cell tumors. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cell surface tumor specific molecule is ROR1. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant version thereof, peptide, and/or polypeptide affinity molecule or binding partner.

[0158] In some alternatives, the cell comprises a bi-specific chimeric antigen receptor. In some alternatives, the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148,

EMMPRIN/CD147 and/or tumor targets that are non-B cell tumors. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cell surface tumor specific molecule is ROR1. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant version thereof, peptide, and/or polypeptide affinity molecule or binding partner.

[0159] Pharmaceutical excipient,” or pharmaceutical vehicle as described herein can refer to a carrier or inert medium used as a solvent in which the medicinally active agent or T-cells for treatment is formulated and or administered. Vehicles can include polymeric micelles, liposomes, lipoprotein-based carriers, nano-particle carriers, dendrimers, and/or other vehicles for T-cells that are known to one skilled in the art. An ideal vehicle or excipient can be non-toxic, biocompatible, non-immunogenic, biodegradable, and can avoid recognition by the host's defense mechanisms.

[0160] In some alternatives, a composition or product combination for human therapy is provided, wherein the composition or product combination comprises a pharmaceutical excipient and at least one population of genetically modified T-cells of any of the alternatives described herein. In some alternatives, the excipients are pharmaceutical vehicles. In some alternatives, the pharmaceutical vehicles include pharmaceutical compositions.

[0161] The composition can further comprise a vehicle, or pharmaceutical vehicle. Vehicles as described herein can refer to a substance of no therapeutic value that is

used to convey an active medicine or cells for administration. Pharmaceutical vehicle as described herein can refer to a carrier or inert medium used as a solvent in which the medicinally active agent is formulated and or administered. An ideal vehicle can be non-toxic, biocompatible, non-immunogenic, biodegradable, and can avoid recognition by the host's defense mechanisms. In several alternatives described herein, compositions are described which comprise vehicles or excipients that help maintain the integrity of the T-cells. In some alternatives, the vehicles are pharmaceutical vehicles. In some alternatives, the pharmaceutical vehicles include pharmaceutical compositions.

[0162] In some alternatives, the composition comprises CD8⁺ T cytotoxic lymphocyte cells and/or CD4⁺ T helper lymphocyte cells, wherein the CD8⁺ T cytotoxic lymphocyte cells are selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells, and wherein the CD4⁺ T helper lymphocyte cells are selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the composition has a ratio of CD4⁺ T helper lymphocyte cells to CD8⁺ T lymphocytes of 1:10 to 10:1. In some alternatives, the ratio of CD4⁺ T helper lymphocyte cells to CD8⁺ T lymphocytes is 1:1.

Methods of making a cell comprising two chimeric antigen receptors for bi-specificity

[0163] In some alternatives, a method of making a cell comprising a chimeric antigen receptor is provided. In some alternatives, the method comprises introducing into a cell a first nucleic acid comprising a polynucleotide sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, introducing into the cell a second nucleic acid comprising a polynucleotide sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor, expanding the cell and isolating the cell. In some alternatives, the first nucleic acid and the second nucleic acid reside on two separate vectors. In some alternatives, the first nucleic acid and the second nucleic acid reside on the same vector. In some alternatives, the first nucleic

acid and the second nucleic acid reside on two separate viral vectors. In some alternatives, the first nucleic acid and the second nucleic acid reside on the same vectors. In some alternatives, the viral vectors are retroviral vectors, gammaretroviral vectors, foamy viral vectors and/or lentiviral vectors. In some alternatives of the method, the vectors are introduced to the cell in a composition comprising a mixture of the two vectors. In some alternatives, the first and/or the second nucleic acid are introduced by a plasmid or a minicircle transposon. In some alternatives, the introducing the first and second nucleic acid is performed concurrently, wherein the first and second nucleic acid are prepared as a composition for delivery to the cell. In some alternatives, expression of the first chimeric antigen receptor is linked to co-expression of EGFRt and expression of the second chimeric antigen receptor is linked to co-expression of Her2tg, or wherein expression of the first chimeric antigen receptor is linked to co-expression of Her2tg, and expression of the second chimeric antigen receptor is linked to co-expression of EGFRt. In some alternatives, the method further comprises further comprising introducing a vector comprising a sequence encoding a soluble protein into said cell. In some alternatives, the soluble proteins are dominant negative versions of inhibitory proteins or constitutively active versions of pro-proliferative, T cell signal regulating or tumor microenvironment responsive proteins. In some alternatives, the soluble protein is a bi-specific T-cell engager (BiTE). BiTEs, as described herein are artificial bispecific monoclonal antibodies that can be used as anti-cancer drugs. In some alternatives, the BiTE is a Blinatomomab or Solitomab. BiTEs are fusion proteins comprising single chain variable fragments of different antibodies or amino acid sequences from four different genes. In some alternatives, the soluble proteins are homeostatic cytokines, fusion proteins or peptides. In some alternatives, the homeostatic cytokines are IL2, IL7, IL12 and/or IL15. In some alternatives of the method, the method further comprises stimulating the cells. In some alternatives, cells are stimulated (S1) with 50U/ml interleukin-2 (IL-2) for CD8 cells. In some alternatives, cells are stimulated with interleukin-7 (IL-7) for CD4 cells. In some alternatives cells are stimulated with anti-CD3/CD28 beads.

[0164] In some aspects, utilization of Her2tg or EGFRt tag as a genetic tag/marker allows for the *ex vivo* selection and purification of homogenous populations of cellular therapeutics that express the transgene of interest. In addition, Her2tg can be used to

track cellular therapeutics *in vivo*; for instance, Her2tg can be used as a target for Herceptin staining of blood, bone marrow and cerebrospinal fluid aspirates to check for the persistence of transgene-expressing cellular therapeutics to follow cancer remission to therapeutic persistence in a patient. Her2tg extends the therapeutic reach of CAR therapy by allowing for the concerted purification of cells expressing multiple transgenes when used with another genetic tag such as EGFRt. In some alternatives, the method further comprises selecting the cells by immunomagnetic selection or cell sorting. In some alternatives, the method further comprises selecting the cells by immunobinding the marker protein on the cell surface. In some alternatives, the marker protein is Her2tg and/or EGFRt.

Method of making a cell comprising two chimeric antigen receptors with a transposase system.

[0165] In some alternatives, a method of making a cell comprising a chimeric antigen receptor is provided. In some alternatives, the method comprises co-delivering into a cell two vectors, wherein a first vector comprises a first nucleic acid encoding a first chimeric antigen receptor, wherein the chimeric antigen receptor comprises a binding domain specific for a ligand on a B cell, which promotes the *in vivo* expansion and activation of the B cell, and a second vector comprising a second nucleic acid sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor, expanding the cell and isolating the cell. In some alternatives, the first nucleic acid and the second nucleic acid reside between a two inverted terminal repeat gene sequences. In some alternatives, the inverted terminal repeat gene sequences are Sleeping Beauty inverted terminal repeat gene sequences or PiggyBaC inverted terminal repeat gene sequences. In some alternatives, the vector is a plasmid or a minicircle transposon. In some alternatives, the method further comprises introducing into the cell a polynucleotide, wherein the polynucleotide sequence encodes an mRNA, wherein the mRNA encodes a transposase. In some alternatives, the transposase is a Sleeping Beauty transposase or a Piggyback transposase. In some alternatives, expression of the first chimeric antigen receptor is linked to co-expression of EGFRt and expression of the second chimeric antigen receptor is linked to co-expression of Her2tg, or wherein expression of the first chimeric antigen receptor is linked to co-expression of Her2tg, and expression of the second chimeric antigen receptor is linked to co-expression of EGFRt. In some alternatives, the method further comprises further

comprising introducing a vector comprising a sequence encoding a soluble protein into said cell.

[0166] In some alternatives, the vector contains a promoter linked to a polynucleotide coding for a chimeric antigen receptor operably linked to a genetic tag. One or more PiggyBac transposons can be employed. In some alternatives, a PB comprises a promoter linked to a polynucleotide coding for a chimeric antigen receptor and a first genetic tag, another PiggyBac comprises a promoter linked to a polynucleotide coding for a chimeric antigen receptor, and a second and different genetic tag. Each element of the constructs is separated by a nucleic acid, such as that coding for a self-cleaving T2A sequence. In some alternatives, each PiggyBac differs from one another in the chimeric antigen receptor including but not limited to the spacer length and sequence, the intracellular signaling domain, and/or the genetic tag sequence.

[0167] In some aspects, utilization of Her2tg or EGFRt tag as a genetic tag/marker allows for the *ex vivo* selection and purification of homogenous populations of cellular therapeutics that express the transgene of interest. In addition, Her2tg can be used to track cellular therapeutics *in vivo*; for instance, Her2tg can be used as a target for Herceptin staining of blood, bone marrow and cerebrospinal fluid aspirates to check for the persistence of transgene-expressing cellular therapeutics to follow cancer remission to therapeutic persistence in a patient. Her2tg extends the therapeutic reach of CAR therapy by allowing for the concerted purification of cells expressing multiple transgenes when used with another genetic tag such as EGFRt. In some alternatives, the method further comprises selecting the cells by immunomagnetic selection or cell sorting. In some alternatives, the method further comprises selecting the cells by immunobinding the marker protein on the cell surface. In some alternatives, the marker protein is Her2tg and/or EGFRt.

Methods of making a cell comprising a bi-specific chimeric antigen receptor for bi-specificity

[0168] In some alternatives, a method of making a cell having a bi-specific chimeric antigen receptor is provided, wherein the method comprises introducing into a cell a nucleic acid comprising a polynucleotide sequence encoding a bi-specific chimeric antigen receptor that comprises a first binding domain specific for a ligand on a B cell, which promotes the *in vivo* expansion and activation of the B cell, and a second binding domain

specific for a ligand on a solid tumor, expanding the cells and isolating the cells. In some alternatives, the polynucleotide resides on a viral vector. In some alternatives, the viral vector is a lentiviral vector or a retroviral vector. In some embodiments, the viral vector is a gammaretroviral vector. In some alternatives, the bi-specific chimeric antigen receptor is co-expressed with a marker protein. In some alternatives, the marker protein is EGFRt or Her2tg. In some alternatives, the method further comprises introducing a vector encoding a soluble protein into said cell.

[0169] In some alternatives, the method further comprises selecting the cells by immunomagnetic selection or cell sorting. In some alternatives, the method further comprises selecting the cells by immunobinding the marker protein on the cell surface.

[0170] In some aspects, utilization of Her2tg or EGFRt tag as a genetic tag/marker allows for the *ex vivo* selection and purification of homogenous populations of cellular therapeutics that express the transgene of interest. In addition, Her2t can be used to track cellular therapeutics *in vivo*; for instance, Her2t can be used as a target for Herceptin staining of blood, bone marrow and cerebrospinal fluid aspirates to check for the persistence of transgene-expressing cellular therapeutics to follow cancer remission to therapeutic persistence in a patient. Her2t extends the therapeutic reach of CAR therapy by allowing for the concerted purification of cells expressing multiple transgenes when used with another genetic tag such as EGFRt. In some alternatives, the method further comprises selecting the cells that express the bi-specific CAR by immunomagnetic selection or cell sorting. In some alternatives, the method further comprises selecting the cells expressing the bi-specific marker by immunobinding the marker protein on the cell surface. In some alternatives, the marker protein is Her2tg and/or EGFRt.

Methods of making a cell comprising a bi-specific chimeric antigen receptor for bi-specificity using a transposase system

[0171] In some alternatives, a method of making a cell comprising a bi-specific chimeric antigen receptor is provided. In some alternatives, the method comprises introducing into a cell a vector, wherein the vector comprises a first nucleic acid encoding a bi-specific chimeric antigen receptor comprising a first binding domain and a second binding domain, wherein the first binding domain is specific for a ligand on a B cell, which promotes the *in vivo* expansion and activation of the B cell, and the second binding domain is specific

for a ligand on a solid tumor, expanding the cell and isolating the cell. In some alternatives, the first nucleic acid and the second nucleic acid reside between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence. In some alternatives, the first inverted terminal repeat gene sequence and the second inverted terminal repeat gene sequence are Sleeping Beauty inverted terminal repeat gene sequences or PiggyBaC inverted terminal repeat gene sequences. In some alternatives, the vector is a plasmid or a minicircle transposon. In some alternatives, the method further comprises introducing into the cell a polynucleotide, wherein the polynucleotide sequence encodes an mRNA, wherein the mRNA encodes a transposase. In some alternatives, the transposase is a Sleeping Beauty transposase or a Piggyback transposase. In some alternatives, expression of the bi-specific chimeric antigen receptor is linked to co-expression of EGFRt or Her2tg. In some alternatives, the method further comprises further comprising introducing a vector comprising a sequence encoding a soluble protein into said cell.

[0172] In some alternatives, the vector contains a promoter linked to a polynucleotide coding for a chimeric antigen receptor operably linked to a genetic tag. One or more PiggyBac transposons can be employed. In some alternatives, a PB comprises a promoter linked to a polynucleotide coding for a chimeric antigen receptor and a first genetic tag, another PiggyBac comprises a promoter linked to a polynucleotide coding for a chimeric antigen receptor, and a second and different genetic tag. Each element of the constructs is separated by a nucleic acid, such as that coding for a self-cleaving T2A sequence. In some alternatives, each PiggyBac differs from one another in the chimeric antigen receptor including but not limited to the spacer length and sequence, the intracellular signaling domain, and/or the genetic tag sequence.

[0173] In some aspects, utilization of Her2tg or EGFRt tag as a genetic tag/marker allows for the *ex vivo* selection and purification of homogenous populations of cellular therapeutics that express the transgene of interest. In addition, Her2tg can be used to track cellular therapeutics *in vivo*; for instance, Her2tg can be used as a target for Herceptin staining of blood, bone marrow and cerebrospinal fluid aspirates to check for the persistence of transgene-expressing cellular therapeutics to follow cancer remission to therapeutic persistence in a patient. Her2tg extends the therapeutic reach of CAR therapy by allowing for the concerted purification of cells expressing multiple transgenes when used with another

genetic tag such as EGFRt. In some alternatives, the method further comprises selecting the cells by immunomagnetic selection or cell sorting. In some alternatives, the method further comprises selecting the cells by immunobinding the marker protein on the cell surface. In some alternatives, the marker protein is Her2tg and/or EGFRt.

Methods of treating, ameliorating, or inhibiting a non-B cell related disease in a subject

[0174] In some alternatives, a method of treating, ameliorating, or inhibiting a non-B cell related disease in a subject is provided, wherein the method comprises introducing, providing, or administering any one or more of the cells or compositions of any of the alternatives described herein or the cells made by any one or more of the methods of any of the alternatives described herein into a subject for therapy. In some alternatives, the cells that are administered are provided by an allogeneic transfer. In some alternatives, the cells that are administered are provided by an autologous transfer. In some alternatives the cells comprise a first and second chimeric antigen receptor, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor is specific for a ligand on a tumor. In some alternatives of the cells, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFRSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, EMMPRIN/CD147 and/or tumor targets on tumors that are non-B cell tumors.

[0175] In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01,

IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cell surface tumor specific molecule is ROR1. In some alternatives, the first chimeric antigen receptor and/or the second chimeric antigen receptor are inducibly expressed in said cell. In some alternatives, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor is under the control of a regulatory element. In some alternatives, the first chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand, peptide, and/or polypeptide affinity molecule. In some alternatives, the second chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand, peptide, and/or polypeptide affinity molecule. In some alternatives, a first marker protein is co-expressed with the first chimeric antigen receptor and a second marker protein is co-expressed with the second chimeric antigen receptor. In some alternatives, the first marker protein co-expressed with the first chimeric antigen receptor is EGFRt and the second marker protein co-expressed with the second chimeric antigen receptor is Her2tg or first marker protein co-expressed with the first chimeric antigen receptor is Her2tg and the second marker protein co-expressed with the second chimeric antigen receptor is EGFRt. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells.

[0176] In some alternatives, the cell comprises a bi-specific chimeric antigen receptor, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34,

CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, EMMPRIN/CD147 and/or tumor targets that are non-B cell tumors. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cell surface tumor specific molecule is ROR1. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner.

[0177] In some alternatives, the subject is monitored for B-cell aplasia. In some alternatives, the subject is administered a drug to induce the B-Cell specific CAR expression.

[0178] In some alternatives, expression of a CAR is under the control of a first promoter inducible by a drug. The drug is selected based on safety record, favorable pharmacokinetic profile, tissue distribution, a low partition coefficient between the extracellular space and cytosol, low immunogenicity, low toxicities, and/or high expression in lymphocytes. In a specific alternative, a drug is selected that is FDA approved, provides for transgene expression in lymphocytes, does not activate other undesirable gene expression, and induces a promoter that does not contain any xenogeneic components. In some alternatives, the inducible promoter is activated by a transcriptional activator that

interacts with a drug. The transcriptional activator is activated or able to bind to and activate the inducible promoter in the presence of the drug.

[0179] A specific alternative of a drug is a drug that binds to an estrogen receptor ligand binding domain of a transcriptional activator. In some alternatives, the drug includes tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, a specific alternative of a drug is a drug that binds to an estrogen receptor ligand binding domain of a transcriptional activator. In some alternatives, the drug is estrogen or glucocorticoid.

[0180] Tamoxifen, CAS RN: 10540-29-1, is also known as 2-(4-((1Z)-1,2-diphenyl-1-butenyl)phenoxy)-N,N-dimethyl- ethanamine, or (Z)-2-(para-(1,2-Diphenyl-1-butenyl)phenoxy)-N,N-dimethylamine (IUPAC), and has a molecular formula of $C_{26}H_{29}NO$, M.W. 371.52. Tamoxifen is a Selective Estrogen Receptor Modulator with tissue-specific activities. Tamoxifen acts as an anti-estrogen (inhibiting agent) agent in the mammary tissue, but as an estrogen (stimulating agent) in cholesterol metabolism, bone density, and cell proliferation in the endometrium. Tamoxifen is frequently administered orally as a pharmaceutically acceptable salt. For example, Tamoxifen citrate (RN 54965-24-1, M.W. 563.643) is indicated for treatment of metastatic breast cancer, and as an adjuvant for the treatment of breast cancer in women following mastectomy axillary dissection, and breast irradiation. Tamoxifen citrate is also indicated to reduce incidence of breast cancer in women at high risk for breast cancer.

[0181] Metabolites of tamoxifen in rat, mouse and human breast cancer patients, including major metabolites N-desmethyltamoxifen (RN 31750-48-8, M.W. 357.494) and 4-hydroxytamoxifen (4-OHT) (RN 68392-35-8, M.W. 387.52, Afimoxifene), are disclosed in Robinson et al., Metabolites, pharmacodynamics, and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. Drug Metab Dispos January 1991 19:36-43, which is incorporated by reference herein in its entirety. Additional cytochrome P-450 metabolites are disclosed in Crewe et al., 2002, including cis-4-hydroxytamoxifen (RN 174592, M.W. 387.52; Afimoxifene, E-isomer), and 4'-hydroxytamoxifen ((Z)-4-(1-(4-(2-(dimethylamino)ethoxy)phenyl)-1-phenylbut-1-en-2-yl)phenol). See Crewe et al., 2002, Metabolism of Tamoxifen by recombinant human cytochrome P-450 enzymes: Formation of the 4-hydroxy, 4'-hydroxy and N-desmethyl metabolites and isomerization of trans-4-

hydroxytamoxifen, Drug Metab Dispos, 30(8): 869-874, FIG. 1, which is incorporated herein in its entirety by reference.

[0182] Compounds with structural similarity to tamoxifen include, but are not limited to, cis-tamoxifen (RN 13002-65-8, M.W. 371.521), 4-methyltamoxifen (RN 73717-95-5, M.W. 385.548), N-desmethyltamoxifen (RN 31750-48-8, M.W. 357.494), (Z)-desethyl methyl tamoxifen (RN 15917-50-7, M.W. 357.494), (E)-desethyl methyl tamoxifen (RN 31750-45-5, M.W. 357.494), trans-4-hydroxytamoxifen (RN 68047-06-3, M.W. 387.52), Afimoxifene (RN 68392-35-8, M.W. 387.52, 4-hydroxytamoxifen), Afimoxifene, E-isomer (RN 174592-47-3, M.W. 387.52), 4-chlorotamoxifen (RN 77588-46-6, M.W. 405.966), 4-fluorotamoxifen (RN 73617-96-6, M.W. 389.511), Toremifene (RN 89778-26-7, M.W. 405.966), desethyl tamoxifen (RN 19957-51-8, M.W. 343.47), (E)-desethyl tamoxifen (RN 97151-10-5, M.W. 343.47), (Z)-desethyl tamoxifen (RN 97151-11-6, M.W. 343.47), Miproxifene (RN 129612-87-9, M.W. 429.6), 2-(p-(beta-ethyl-alpha-phenylstyryl)phenoxy)triethylamine (RN 749-86-0, M.W. 399.575), Droloxifene (RN 82413-20-5, M.W. 387.52), 4-iodo-tamoxifen (RN 116057-68-2, M.W. 497.413), dihydrotamoxifen (RN 109640-20-2, M.W. 373.537), (E)-N,N-dimethyl-2-(4-(1-(2-methylphenyl)-2-phenyl-1-butenyl)phenoxy)ethanamine (RN 97150-96-4, M.W. 385.548), or 4-hydroxytoremifene (RN 110503-62-3, M.W. 421.965); and/or pharmaceutically acceptable salts and/or hydrates or solvates thereof.

[0183] For example, citrate salts of tamoxifen, or citrate salts of compounds with structural similarity to tamoxifen, include, but are not limited to tamoxifen citrate (RN 54965-24-1, M.W. 563.64), 2-(p-(1,2-diphenyl-1-butenyl)phenoxy)-N,N-dimethylethylamine citrate (RN 7244-97-5, 563.64), (E)-tamoxifen citrate (RN 76487-65-5, M.W. 563.64), Toremifene citrate (RN 89778-27-8, M.W. 598.088), Droloxifene citrate (RN 97752-20-0, M.W. 579.64), 2-(p-(1,2-bis(p-methoxyphenyl)-1-butenyl)phenoxy)triethylamine citrate (RN 42920-39-8, M.W. 651.748), 2-(4-(1,2-diphenylethenyl)phenoxy)-N,N-diethyl-ethanamine 2-hydroxy-1,2,3-propanetricarboxylate (RN 40297-42-5, M.W. 563.643), 2-(p-(alpha-phenylstyryl)phenoxy)triethylamine citrate (RN 102433-95-4, M.W. 563.64), 2-(p-(2-(p-methoxyphenyl)-1-phenyl-1-butenyl)phenoxy)triethylamine citrate (1:1) (RN 42824-34-0, M.W. 637.72), 2-(p-(1-(p-methoxyphenyl)-2-phenylpropenyl)phenoxy)triethylamine citrate (RN 13554-24-0, M.W. 607.696), 2-(p-(alpha-(p-

methoxyphenyl)styryl)phenoxy)triethylamine citrate monohydrate (RN 13542-71-7, M.W. 593.669), 2-(p-(p-methoxy-alpha-phenylphenethyl) phenoxy)triethylamine citrate (RN 16421-72-0, M.W. 595.685), alpha-(p-(2-(diethylamino)ethoxy)phenyl)-beta-ethyl-p-methoxy-alpha-phenylphenethyl alcohol citrate (1:1) (RN 35263-93-5, M.W. 639.737), 1-(p-(2-(diethylamino)ethoxy)phenyl)-2-(p-methoxyphenyl)-1-phenylethanol citrate (M.W. 611.68), alpha-p-(2-(diethylamino)ethoxy)phenyl)-beta-ethyl-alpha-(p-hydroxyphenyl)-p-methoxyphenethyl alcohol citrate (RN 35263-96-8, M.W. 655.737), and/or 2-(p-(p-methoxy-alpha-methylphenethyl)phenoxy)-triethylamine citrate (RN 15624-34-7, M.W. 533.614).

[0184] In some alternatives, an affective amount of the drug for inducing expression of a chimeric antigen receptor is an amount that provides for an increase in transgene expression over uninduced and/or basal level of expression. In some alternatives, this amount can be readily determined using known dosages and pharmacokinetic profile of the drug.

[0185] In some alternatives, the inducible promoter has a low level of basal activity. When a lentiviral vector is used, the level of basal activity in uninduced cells is 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% or less, as compared to when cells are induced to express the gene. The level of basal activity can be determined by measuring the amount of the expression of the transgene (e.g. marker gene) in the absence of the inducer (e.g. drug) using flow cytometry.

[0186] In some alternatives, the inducible promoter provides for a high level of induced activity, as compared to uninduced or basal activity. In some alternatives, the level of activity in the induced state is 2, 4, 6, 8, or 10 fold or greater than the activity level in the uninduced state. In some alternatives, transgene expression under control of the inducible promoter is turned off in the absence of a transactivator in less than 10, 8, 6, 4, 2, or 1 days excluding 0 days.

[0187] In some alternatives, an inducible promoter can be designed and/or modified to provide for a low level of basal activity, a high level of inducibility, and/or a short time for reversibility. In some alternatives, the inducible promoter is the 7xHBD/mE1b promoter.

[0188] In some alternatives, the subject is administered a drug to induce a CAR that is specific for tumor targeting. In some alternatives, the subject is administered

tamoxifen, or its active metabolite, wherein the tamoxifen, or its active metabolite induce expression of the B-Cell specific CAR. In some alternatives, wherein the subject is diagnosed with B-cell aplasia, the administration of tamoxifen or its metabolite is stopped. In some alternatives, the method further comprises cycles of administering tamoxifen or its metabolite and periods of rest. In some alternatives, the method further comprises administering to the subject in need, subject derived B-cells. In some alternatives, the method further comprises administering T-APCs that express the cell surface B cell ligand or antigen. In some alternatives, the subject does not have a B-cell related disease. In some alternatives, the disease is a cancer. In some alternatives, the disease is an infection. In some alternatives, the infection is a bacterial or viral infection. In some alternatives, the cancer is a solid tumor. In some alternatives, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer. In some alternatives, the subject is identified or selected to receive a non-B cell related disease therapy, anti-cancer therapy, anti-infection therapy, antibacterial therapy, anti-viral therapy, or anti-tumoral therapy. In some alternatives, the method further comprises further comprising measuring or evaluating an inhibition of said non-B cell related disease, cancer, infection, bacterial infection, viral infection, or tumor. In some alternatives, the method further comprises measuring or evaluating an inhibition of said non-B cell related disease, cancer, infection, bacterial infection, viral infection, or tumor. In some alternatives, the method further comprises introducing, providing, or administering to said subject an additional therapeutic agent, such as a chemotherapeutic agent, an antiviral agent, or an antibacterial agent or an adjunct therapy such as radiation therapy and/or surgery before, during, or after introducing, providing, or administering any one or more of the cells or compositions provided herein or the cells made by any one or more of the methods provided herein into the subject. In some alternatives, the cells or compositions are introduced, provided, or administered to said subject by adoptive cell transfer. In some alternatives, the method further comprises introducing, providing, or administering a drug that induces expression of a chimeric antigen receptor. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the drug is a steroid. In some alternatives, the subject is a mammalian species. In

some alternatives, the subject is a cow, sheep, pig, horse, dog, cat, primate or a human. In some alternatives, the subject is human.

[0189] In some alternatives, the method further comprises evaluating the subject for symptoms of cytokine storm or B-cell aplasia. In some alternatives, the subject is suffering from high fevers, increase or decrease of blood pressure, hypotension, hypoxia, seizures, or aphasia. In some alternatives, the subject has an elevation in ferritin and/or C-reactive protein. In some alternatives of the methods provided herein, wherein the cells comprise a suicide gene system, the methods further comprise administering to the subject a prodrug.

[0190] In some alternatives, the subject does not have B-cell lymphoma, Hodgkin's lymphomas, non-Hodgkins lymphomas, Diffuse large B cell lymphoma, Follicular lymphoma, marginal zone lymphoma, Mucosa-Associated Lymphatic Tissue lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B cell lymphoma, Lymphoplasmacytic lymphoma, Waldenstrom macroglobulinemia, Nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B cell lymphoma, Intravascular large B-cell lymphoma, Primary effusion lymphoma, Lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, Primary central nervous system lymphoma, Primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, Diffuse large B-cell lymphoma associated with inflammation, Intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, ALK-positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-cell lymphoma arising in HHV8-associated multicentric Castleman's disease, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, or nodular lymphocyte predominant Hodgkin's lymphoma. In some alternatives, the subject has breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas or ovarian cancer. In some alternatives, the subject has refractory and relapsed neuroblastoma.

Nucleic acids encoding a chimeric antigen receptor or a bi-specific chimeric antigen receptor and method of making the nucleic acids encoding a chimeric antigen receptor or a bi-specific chimeric antigen receptor

[0191] In some alternatives, a nucleic acid encoding a chimeric antigen receptor is provided wherein the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B-cell specific cell surface molecule, and wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker sequence is a ribosome skip sequence or an IRES sequence. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a steroid e.g., a compound capable of binding to the estrogen receptor, or an estrogen receptor ligand. In some alternatives, the steroid is flustravand.

[0192] In some alternatives, a nucleic acid encoding a chimeric antigen receptor is provided wherein the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or

scFv is specific for a cell surface molecule expressed by B cells, and wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached to a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker sequence is a ribosome skip sequence or an IRES sequence. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by flustravant. In some alternatives, the first promoter is inducible by a steroid (e.g. estrogen, glucocorticoid).

[0193] In some alternatives, a nucleic acid encoding a bi-specific chimeric antigen receptor is provided, wherein the nucleic acid comprises a first nucleic acid sequence comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently

attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the linker sequence is a ribosome skip sequence or an IRES sequence. In some alternatives, the fifth nucleic acid sequence further comprises an IRES sequence at the 3' end of the fifth nucleic acid sequence. In some alternatives, the second nucleic acid encodes an antibody or binding fragment thereof or scFv, and wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule and the third nucleic acid comprises a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule or wherein the second nucleic acid encodes an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule and the third nucleic acid comprises a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific B cell specific cell surface molecule.

[0194] In some alternatives, a nucleic acid encoding a suicide gene system is provided.

Vectors

[0195] In some alternatives, a vector for expression of a chimeric antigen receptor specific for promoting in vivo expansion and activation of B cells is provided, wherein the vector comprises the nucleic acid of any of the alternatives described herein. In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or

binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B-cell specific cell surface molecule, and wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker sequence is a ribosome skip sequence or an IRES sequence. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a steroid.

[0196] In some alternatives, the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface molecule expressed by B cells, and wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid, a seventh nucleic acid

comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached to a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker sequence is a ribosome skip sequence or an IRES sequence. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by flustravant. In some alternatives, the first promoter is inducible by a steroid.

[0197] In some alternatives, the nucleic acid comprises a first nucleic acid sequence comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the linker sequence is a ribosome skip sequence or an

IRES sequence. In some alternatives, the fifth nucleic acid sequence further comprises an IRES sequence at the 3' end of the fifth nucleic acid sequence. In some alternatives, the second nucleic acid encodes an antibody or binding fragment thereof or scFv, and wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule and the third nucleic acid comprises a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule or wherein the second nucleic acid encodes an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule and the third nucleic acid comprises a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific B cell specific cell surface molecule.

[0198] In some alternatives, the vector expresses a chimeric antigen receptor specific for promoting in vivo expansion and activation of B cells. In some alternatives, the vector expresses a chimeric antigen receptor specific for targeting a solid tumor. In some alternatives, the vector comprises a first sequence encoding a first promoter sequence, wherein the first promoter sequence promotes expression of the chimeric antigen receptor, and wherein the vector comprises a second sequence encoding a second promoter sequence, wherein the second promoter sequence promotes expression of a marker protein.

[0199] In some alternatives, the vector further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the vector comprises two inverted repeats, wherein the nucleic acid resides between the two inverted repeats. In some alternatives, the vector is a minicircle. In some alternatives, the inverted repeats are Sleeping Beauty or PiggyBac inverted repeats.

Additional Alternatives

Significant expression of the markers EGFRt and HertG along with their appended CARs.

[0200] As shown in **Figure 3**, the marker Her2t and its variants demonstrate variable binding affinity to Herceptin based on their respective linker. The CARs used were dual CD19CAR-T2A-Her2tG/ CD20CAR-T2A-EGFRt in the T-lymphocytes. As shown in

Figure 3A, H9 T cells were transduced with 3ul of lentivirus containing the Her2t variant Her2t(CD28hinge), Her2t(IgG4hinge) or Her2tG (gly-ser linker). The transduced H9 cells were then cultured for 5 days and stained with biotinylated Herceptin (Herceptin-bio) and a streptavidin conjugated secondary fluorophore (SA-PE). Results demonstrate that the Her2t variant Her2tG displays the greatest ability to bind Herceptin, Her2t(IgG4hinge) with modest Herceptin binding and Her2t(CD28hinge) with the weakest Herceptin binding. These cells were never stimulated with CD3/CD28 beads (**Figure 3C**). Shown in **Figure 3B**, is a timeline for the isolation (D0), growth (D0-21), selection (D14 and D21) and expansion (REP – D21) of CD4⁺ and CD8⁺ primary T cells isolated from PBMCs as per Figure 4 below. These are CD8⁺ T cells transduced with two separate lentiviruses containing CD19CAR-T2A-Her2tG or CD20CAR-T2A-EGFRt at an MOI = 1 for each lentivirus. Pre-selection CD8⁺ T cells were stained with Erbitux-APC, biotinylated-Herceptin and a streptavidin conjugated secondary fluorophore (SA-PE) seven days post transduction (D10 of culture), while Post-selection cells were stained on S1Sp1D12 (*See Figure 4*). As shown in **Figure 3D**, cell lysis for western blot analysis was carried out in RIPA buffer containing protease inhibitor cocktail. Cell lysates were analyzed by BCA assay (Pierce), equally loaded onto gels and western blots were probed with the primary antibody anti CD247 (CD3ζ) and the secondary IRDye 800CW conjugated goat anti-mouse antibody (LI-COR). Blots were imaged on the Odyssey Infrared Imaging System (LI-COR).

Selection of CD4 and CD8 T-cells

[0201] CD4 and CD8 bulk T cells were isolated from human peripheral blood mononuclear cells (PBMCs) derived from blood discard kits of healthy donors (Puget Sound Blood Center). PBMCs from each donor were split into two groups (CD4 or CD8 bulk isolation) and Automacs depleted using CD4 or CD8 isolation kits (Miltenyi Biotec) as per the manufacturer's protocol. Isolated cells were then stimulated (S1) with 50U/ml interleukin-2 (IL-2) for CD8, 5ng/ml interleukin-7 (IL-7) for CD4, 1ng/ml interleukin-15 (IL-15) and anti-CD3/CD28 beads (Life Technologies). CD4 and CD8 T cells were transduced on day 3 after activation using protamine sulfate (1:100 dilution) and a virus MOI of 1 followed by centrifugation at 800xg for 30 minutes at 32°C. The Her2t⁺ or EGFRt⁺ subset of each cell line was enriched by immunomagnetic selection with biotin-conjugated

Herceptin or Erbitux and anti-biotin microbeads (Miltenyi). Selected CD19CAR⁺ and/or CD20CAR⁺ T cells were expanded 12-18 days post transduction by stimulation with irradiated (8000 rad) TM-LCL (Sp1) at a T cell:TM-LCL ratio of 1:7 in the presence of 50U/ml IL-2 (CD8), 5ng/ml IL-7 (CD4) and 1ng/ml IL-15. Cells were harvested on Day 12 (D12) post stimulation and subjected to flow analysis.

[0202] The cells of interest in the western blot are S1Sp1D12 (*see* **Figure 3**) and have already been purified for their respective marker/s. The dual transduced cells are the CD19CAR-T2A-Her2tG and CD20CAR-T2A-EGFRt containing CD8⁺ T cells. This western blot is specific to the zeta portion of the CAR. It does not speak to the expression of the two marker proteins Her2tG or EGFRt (*see* **Figure 3C** and **Figure 4**). The western blot demonstrates CAR expression in the CD8⁺ T cells. Most importantly, expression of both CARs (CD19CAR and CD20CAR) in the dual transduced CD8⁺ T cells is seen.

Specificity of CD19 and CD20 CAR T-cells against K562 target panel cells

[0203] CD8 Tcm were co-cultured with K562 target cells at a 30:1, 10:1, 3.3:1 or 1.1:1 ratio. Only the dual transduced T cells were able to target all antigen expressing K562 cells. As shown in **Figure 5A**, a 4-hour chromium release assay was performed showing CD19- and CD20-CAR T cell specificity against K562 target panel cells. CD8 Tcm were co-cultured with K562 target cells at a 30:1, 10:1, 3.3:1 or 1.1:1 ratio. Only the dual transduced T cells were able to target all antigen expressing K562 cells. The CD19CAR-T2A-Her2t and CD19CAR-T2A-EGFRt CD8 Tcm demonstrate similar lytic capacity. A 24-hour cytokine release assay was also performed using CD19, CD20 and CD19/CD20 bearing cells against K562 targets. CD8 Tcm were co-cultured with K562 target cells at a 2:1 T cell-to-target ratio for 24 hours and then supernatant was analyzed for the presence of effector cytokines. CD19CAR-T2A-Her2t transduced CD8 Tcm produced a more diverse repertoire and higher levels of effector cytokines relative to CD19CAR-T2A-EGFRt transduced CD8 Tcm. The panels are the same as in **Figures 5A and 5B** (Left to right: K562 CD19, K562 CD20 and K562 CD19/CD20). Similar results were seen for CD4 Tcm. The CD19CAR-T2A-Her2t and CD19CAR-T2A-EGFRt CD8 Tcm demonstrate similar lytic capacity, showing the efficacy of the CAR bearing T-cells. As shown in **Figure 5C**, similar results were seen for CD4 Tcm.

Raji Cells electroporated with plasmids containing CD19 targeted CRISPR guide sequences

[0204] Shown in **Figure 6A-D** are the experimental results of Raji cells electroporated with plasmids containing CD19-targeted CRISPR guide sequences. Raji cells were subjected to negative selection using CD19 microbeads. Post-depletion of CD19⁺ cells the CD19⁻ cells were clonally selected and expanded for downstream experiments. Seven days post electroporation the Raji cells were subjected to negative selection using CD19 microbeads. (**Figure 6A**). 4 hour chromium release assays and bioplex assays were then performed using the same cells from **Figure 5B and C**. A chromium release assay was also performed using CD4⁺ cells.

NSG mice injected with CAR expressing T-cells.

[0205] Shown in **Figure 7** are the experimental results of NSG mice injected with 5e⁶ NSO-IL15 cells and then 10e⁶ Mock or CAR expressing T cells i.v. On Day 14 post T cell injection, mouse bone marrow was harvested and subjected to flow analysis. Shown in the top row are gates live and singlets. The middle row gates show CD8⁺ x CD45⁺ cells. The bottom row looks at the CD45⁺ cell population and stains for the markers Her2tG and EGFRt. Results demonstrate that EGFRt and Her2tG can be used to efficiently track T cells in vivo. (Figure 7, top row). Shown in the middle row of Figure 7, are cells gated for viable (93.6% lymphocytes), single (98.8%), and alive cells (99.9%). CD8 and CD45 staining of the cells are shown from left to right as Mock, CD19CAR-T2A-Her2t, CD19CAR-T2A-EGFRt Tcm, and CD19CAR-Her2tg/CD20CAR-EGFRt. At least 1x10⁷ cells were recorded inside of the viable, single cell and alive gates. So although the CD45⁺ cells represent around 1% of the population, it is equivalent to 1x10⁵ cells. The remaining cells are mouse bone marrow cells. Shown in the last row of **Figure 7**, are the Multisort purification of Her2t and EGFRt positive T cells. For the experiment, H9 cells (5x10⁶ parental, Her2t⁺, EGFRt⁺, or Her2t⁺/EGFRt⁺) were mixed together and then subjected to purification. The cells were initially purified based on biotinylated Herceptin and anti-biotin multisort beads. The multisort beads were then removed and the positive fraction subsequently subjected to purification based on Erbitux-APC and anti-APC microbeads. The final positive fraction was dual positive for Her2t and EGFRt.

Mice expressing CAR T-Cells to inhibit tumor growth

[0206] eGFP:ffluc expressing Raji target cells (10^6) were i.v. injected into 6-10 week old mice. Seven days later a total of 10^7 CAR-Her2t⁺, CAR-EGFRt⁺ or dual CAR expressing T cells (1:1 ratio CD4:CD8) were injected intravenously (IV). Bioluminescent imaging was performed weekly by intraperitoneal (i.p.) injection of 4.29mg/mouse D-luciferin (Xenogen), anesthetization by isoflurane and imaging 10 minutes post D-luciferin injection using the IVIS Spectrum Imaging System (Perkin Elmer). Luciferase activity was analyzed using Living Image Software Version 4.3 (Perkin Elmer) and photon flux was analyzed within regions of interest. Shown in **Figure 8**, the single CAR or dual CAR expressing T cells were able to inhibit tumor growth relative to Mock T cells.

CD4 and CD8 T-cells can be transduced with two separate CAR-encoding lentiviral vectors

[0207] CD4 and CD8 purified T-cells were stimulated with CD3/CD28 beads and then co-transduced with clinical grade virus encoding the 2nd generation 41BB- ζ short spacer FMC63CD19CAR or the 2nd generation 41BB- ζ short spacer CE7CAR. Each CAR was followed by an in-frame T2A-EGFRt. Transduced T-cells were purified by EGFRt and frozen on S1D14 (CD4) or S1D15 (CD8). The process development project was PD0170. Shown in **Figure 9A**, the flow analysis demonstrates that transduced cells were purified to homogeneity based on EGFRt expression. Results do not demonstrate what percentage of the CD19- or CE7CAR is represented in the EGFRt⁺ population or whether the T-cells are indeed dual transduced with virus. FMC63 CD19CAR comprises the amino acid sequence set forth in SEQ ID NO: 11 and is encoded by the sequence set forth in SEQ ID NO: 12. In some alternatives, FMC63 CD19CAR comprises the amino acid sequence set forth in SEQ ID NO: 11 and is encoded by the sequence set forth in SEQ ID NO: 12.

CD19 and CE7CAR dual transduced T-cells demonstrate specific lysis against CD19 or L1CAM positive target cell lines.

[0208] Mock (CAR⁻) T-cells and dual transduced T-cells were co-cultured with target cell lines at different effector to target ratios for 4-hours. K562 are negative for both targets CD19 and L1CAM. TM-LCL, TM-LCL-OKT3, K562 + CD19, and Raji are CD19-positive alone. SK-N-DZ are L1CAM-positive alone. SK-N-DZ + CD19 are positive for both targets. Shown in **Figure 9B**, the transduced CD8 or mixed CD4:CD8 T-cells elicit high levels of CD19CAR activity. There was a lower level of CE7CAR activity as demonstrated

against the SK-N-DZ cell line. These results indicate that T-cells can be successfully transduced with two separate CAR-encoding viruses and the resultant T-cell population is able to recognize multiple antigens.

CD19- and CE7CAR dual transduced T-cell populations produce cytokines against CD19 or L1CAM positive target cell lines.

[0209] Dual transduced CD4 and CD8 T-cell populations were co-cultured for 24 hours with target cell lines at a 2:1 effector-to-target ratio. Following the 24 hour incubation period, supernatant from the co-cultures was analyzed for the presence of IL-2, IFN- γ , or TNF- α by Bioplex assay as shown in **Figure 9C**. The dual transduced T-cells produced cytokine in response to all suspension cell (LCL and K562) targets expressing CD19. There was little to no cytokine produced in response to SK-N-DZ which is not uncommon with the 2nd Gen S-spacer CE7CAR even when expressed in 100% of the T-cell population. Since it is not known what percentage of the T-cell populations contain the CE7CAR this is not surprising when combined with the chromium data in **Figure 9B**.

Dual-transduced T-cells elicit antitumor activity in an intracranial xenograft tumor model.

[0210] Cohorts of mice were inoculated with 0.2e6 SK-N-DZ that express GFP:ffluc, CD19t, and IL-2 (Day 0) and 2e6 dual transduced CD4:CD8 T-cells (1:1 ratio) (Day 7) intracranially (i.c.). Shown in **Figure 9D** are the results from serial bioluminescence imaging of tumors in cohorts of mice treated with Mock (PBS only – left) or dual transduced CD4:CD8 CE7CAR T-cells (middle). Kaplan-Meier analysis (right) of survival in treatment and control groups. The dual-transduced cells were able to regress and control tumor growth as evidenced by a prolonged decrease in bioluminescence imaging. However, the tumor did grow out over time and the mice slowly succumbed to tumor growth and were euthanized.

CD4 and CD8 T-cells can be transduced with two separate CAR-encoding lentiviral vectors and dual positive populations can be identified by associated markers.

[0211] CD4 and CD8 purified T-cells were stimulated with CD3/CD28 beads and then individually or co-transduced with lentivirus encoding the 2nd generation 41BB- ζ short spacer FMC63CD19CAR or/and the 2nd generation 41BB- ζ long mutant (L235D, N297Q) spacer CE7CAR. The CD19CAR was followed by an in-frame T2A-Her2tG and the

CE7CAR by a T2A-EGFRt. Transduced T-cells were purified by EGFRt (CE7CAR) and frozen. Flow analysis was performed on day 9 and demonstrates that transduced cells were purified to homogeneity based on EGFRt expression (**See Figure 10A**). For the CD4 T-cells there was ~56% Her2tG (CD19CAR) positivity and ~44% dual-positivity for the CD8 T-cells.

CD19 and CE7CAR dual transduced CD8⁺ T-cells demonstrate specific lysis against CD19 or L1CAM positive target cell lines.

[0212] Mock (CAR⁻), single transduced (CD19CAR or CE7CAR) and dual transduced CD8⁺ T-cells were co-cultured with target cell lines at different effector to target ratios for 4-hours. K562 are negative for both targets CD19 and L1CAM. K562-OKT3 was used as a positive control. Be2 are L1CAM-positive alone. Be2+CD19t are positive for both targets. Results demonstrate that the single transduced CD8⁺ T-cells elicit specific lysis against their cognate antigen. However, the dual transduced T-cells efficiently recognized and lysed cells expressing CD19, L1CAM or both antigens. All CD8⁺ T-cells were able to elicit similar levels of cell lysis against the K562-OKT3. These results indicate that T-cells can be successfully transduced with two separate CAR-encoding viruses, purified by a selection marker, and the resultant T-cell population is able to recognize multiple antigens (See Figure 10B).

CD19- and CE7CAR dual transduced T-cell populations produce cytokines against CD19 or L1CAM positive target cell lines.

[0213] Mock (CAR⁻), single transduced (CD19CAR or CE7CAR) and dual transduced CD4⁺ and CD8⁺ T-cells were co-cultured for 24 hours with target cell lines at a 2:1 effector-to-target ratio. Following the 24 hour incubation period, supernatant from the co-cultures was analyzed for the presence of IL-2, IFN- γ , or TNF- α by Bioplex assay, as shown in Figure 10C. Dual-transduced cells were able to release cytokine against all target expressing cell lines. While there was no difference in cytokine production between individual CE7CAR-expressing and dual CAR expressing T-cells against Be2-CD19t, there was a difference in cytokine production between CD19CAR-expressing and dual transduced T-cells against K562-CD19t. This coincides with the CD19CAR positivity of the dual transduced T-cell populations.

Dual-transduced T-cells elicit antitumor activity in an intracranial xenograft tumor model.

[0214] Cohorts of mice were inoculated with 0.2e6 SK-N-DZ that express GFP:ffluc and IL-2 (Day 0) and 2e6 dual transduced CD4:CD8 T-cells (1:1 ratio) (Day 7) intracranially (i.c.). Serial bioluminescence imaging of tumor in cohorts of mice treated with Mock (untransduced - left), CE7CAR-expressing (middle) or dual-transduced CD4:CD8

CE7CAR T-cells (right). Kaplan-Meier analysis (bottom) of survival in treatment and control groups. Both the single CE7CAR-expressing and dual-transduced cells were able to regress and control tumor growth as evidenced by a prolonged decrease in bioluminescence imaging. A subset of mice treated with single CE7CAR-expressing T-cells was euthanized prior to the arbitrary end point of the study due to outgrowth of tumor. These results show that dual-transduced T-cells are able to eradicate tumor *in vivo* at levels similar to single CE7CAR-expressing T-cells. There was therefore no inhibition in CE7CAR activity for the dual-transduced T-cell population (See Figure 10D).

CD4 and CD8 T-cells can be transduced with two separate CAR-encoding lentiviral vectors and dual positive populations can be identified by associated markers.

[0215] CD4 and CD8 purified T-cells were stimulated with CD3/CD28 beads and then individually or co-transduced with lentivirus encoding the 2nd generation 41BB- ζ short spacer FMC63CD19CAR or/and the 2nd generation 41BB- ζ short spacer ROR1CAR. The CD19CAR was followed by an in-frame T2A-Her2tG and the ROR1CAR by a T2A-EGFRt. Transduced T-cells were purified by EGFRt (ROR1CAR) and frozen. Flow analysis was performed on day 9 and demonstrates that transduced cells were purified to homogeneity based on EGFRt expression (See Figure 11A). For the CD4 T-cells there was ~86.7% Her2tG (CD19CAR) positivity and ~88.1% dual-positivity for the CD8 T-cells.

CD19 and ROR1CAR dual transduced CD8⁺ T-cells demonstrate specific lysis against CD19 or ROR1 positive target cell lines.

[0216] Mock (CAR⁻), single transduced (CD19CAR or ROR1CAR) and dual transduced CD8⁺ T-cells were co-cultured with target cell lines at different effector to target ratios for 4-hours. Single ROR1CAR T-cells contain either the 2nd generation 41BB- ζ or CD28- ζ short spacer. K562 are negative for both targets CD19 and ROR1. K562-OKT3 was used as a positive control. Raji (CD19⁺) were transduced to express ROR1. Be2, SK-N-AS and SK-N-DZ are ROR1 positive. Results demonstrate that the single transduced CD8⁺ T-cells elicit specific lysis against their cognate antigen. However, the dual transduced T-cells efficiently recognized and lysed cells expressing CD19, ROR1 or both antigens (Raji ROR1). All CD8⁺ T-cells were able to elicit similar levels of cell lysis against the K562-OKT3. These results indicate that T-cells can be successfully transduced with two separate CAR-

encoding viruses, purified by a selection marker, and the resultant T-cell population is able to recognize multiple antigens (See **Figure 11B**).

CD19- and ROR1CAR dual transduced T-cell populations produce cytokines against CD19 or ROR1 positive target cell lines.

[0217] Mock (CAR), single transduced (CD19CAR or ROR1CAR) and dual transduced CD4⁺ T-cells were co-cultured for 24 hours with target cell lines at a 2:1 effector-to-target ratio. Following the 24 hour incubation period, supernatant from the co-cultures was analyzed for the presence of IL-2, IFN-gamma, or TNF- α by Bioplex assay. Dual-transduced cells were able to release cytokine against all target expressing cell lines at similar levels to single CAR-expressing T-cells (See **Figure 11C**).

CD19- and ROR1CAR dual transduced T-cell populations produce cytokines against CD19 or ROR1 positive target cell lines.

[0218] Mock (CAR), single transduced (CD19CAR or ROR1CAR) and dual transduced CD4⁺ T-cells were co-cultured for 24 hours with target cell lines at a 2:1 effector-to-target ratio. Following the 24 hour incubation period, supernatant from the co-cultures was analyzed for the presence of IL-2, IFN-gamma, or TNF- α by Bioplex assay. Dual-transduced cells were able to release cytokine against all target expressing cell lines at similar levels to single CAR-expressing T-cells (See **Figure 11D**).

Dual-transduced T-cells elicit antitumor activity in an intracranial xenograft tumor model.

[0219] Cohorts of mice were inoculated with 0.2e6 Be2 that express GFP:ffluc (Day 0) and 2e6 dual transduced CD4:CD8 T-cells (1:1 ratio) (Day 7) intracranially (i.c.). Serial bioluminescence imaging of tumor in cohorts of mice treated with Mock (untransduced - left), ROR1CAR-expressing (middle-left) or dual-transduced CD4:CD8 CE7CAR T-cells (middle right). Kaplan-Meier analysis (right) of survival in treatment and control groups. Both the single ROR1CAR-expressing and dual-transduced cells were able to transiently regress tumor growth as evidenced by a short decrease in bioluminescence imaging. However, mice succumbed to Be2 tumor outgrowth quickly thereafter. Both the single and dual transduced T-cells were able to significantly prolong survival. These results show that dual-transduced T-cells are able to treat tumor *in vivo* at levels similar to single

ROR1CAR-expressing T-cells (See **Figure 11D**). There was therefore no inhibition in ROR1CAR activity for the dual-transduced T-cell population.

More alternatives

A nucleic acid encoding a chimeric antigen receptor specific for a B cell specific cell surface molecule

[0220] In some alternatives, a nucleic acid encoding a chimeric antigen receptor is provided, wherein the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid; and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface

molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFRSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and

is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

A nucleic acid encoding an inducible chimeric antigen receptor specific for a B cell specific cell surface molecule

[0221] In some alternatives, a nucleic acid encoding a chimeric antigen receptor is provided wherein the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the

eighth nucleic acid is covalently attached to a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-

1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

A nucleic acid encoding a chimeric antigen receptor specific for a cell surface tumor specific molecule

[0222] In some alternatives, a nucleic acid encoding a chimeric antigen receptor is provided wherein the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid sequence is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene

system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in

SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In

some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

A nucleic acid encoding an inducible chimeric antigen receptor specific for a cell surface tumor specific molecule

[0223] In some alternatives, a nucleic acid encoding a chimeric antigen receptor is provided, wherein the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid sequence is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded

by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1v the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain

comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or

scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

A nucleic acid encoding a bi-specific chimeric antigen receptor

[0224] In some alternatives, a nucleic acid encoding a bi-specific chimeric antigen receptor is provided, wherein the nucleic acid comprises a first nucleic acid sequence comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-

stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alphaCD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFRSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3,

MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface

tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence

comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

A nucleic acid encoding an inducible bi-specific chimeric antigen receptor

[0225] In some alternatives, a nucleic acid encoding a bi-specific chimeric antigen receptor is provided, wherein the nucleic acid comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, an eighth nucleic acid comprising a sequence encoding a linker, wherein the eighth nucleic acid is covalently attached at a 3' end of the

seventh nucleic acid, and a ninth nucleic acid comprising a sequence encoding a marker domain, wherein the ninth nucleic acid is covalently attached at a 3' end of the eighth nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFRSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250,

HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in

SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain

comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

A vector for expression of a chimeric antigen receptor specific for promoting in vivo expansion and activation of B cells

[0226] In some alternatives, a vector for expression of a chimeric antigen receptor specific for promoting in vivo expansion and activation of B cells is provided, wherein the vector comprises the nucleic acid of any one of the alternatives provided herein. In some alternatives, the nucleic acid encoding a chimeric antigen receptor comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid, and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the nucleic acid encoding a chimeric antigen receptor comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a

sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached to a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFRSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide

encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain

comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. In some alternatives, the vector is a viral vector. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

A vector for the expression of a chimeric antigen receptor or TcR specific for targeting a solid tumor

[0227] In some alternatives, a vector for expression of a chimeric antigen receptor or TcR specific for targeting a solid tumor is provided, wherein the vector comprises the nucleic acid of any one of alternatives provided herein. In some alternatives, the nucleic acid encoding a chimeric antigen receptor comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid sequence is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the nucleic acid encoding a chimeric antigen receptor

comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid sequence is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a

steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set

forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth

in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. In some alternatives, the vector is a viral vector. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

A vector for the expression of a bi-specific chimeric antigen receptor

[0228] In some alternatives, a vector for expression of a bi-specific chimeric antigen receptor is provided, wherein the bi-specific chimeric antigen receptor is specific for a B cell specific cell surface molecule and is specific for a cell surface tumor specific molecule, the vector comprising the nucleic acid of any one of the alternatives provided herein. In some alternatives, the nucleic acid encoding a bi-specific chimeric antigen receptor comprises a first nucleic acid sequence comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid

comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the nucleic acid encoding a bi-specific chimeric antigen receptor comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, an eighth nucleic acid comprising a sequence encoding a linker, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, and a ninth nucleic acid comprising a sequence encoding a marker domain, wherein the ninth nucleic acid is covalently attached at a 3' end of the eighth nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip

sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer

antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the

antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. In some alternatives, the vector is a viral vector.

In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

A chimeric antigen receptor or TcR specific for a B-cell specific cell surface molecule

[0229] In some alternatives, a chimeric antigen receptor or TcR specific for a B-cell specific cell surface molecule encoded by a nucleic acid or vector of any one of the alternatives is provided. In some alternatives, the vector is for expression of a chimeric antigen receptor specific for promoting in vivo expansion and activation of B cells, wherein the vector comprises the nucleic acid of any one of the alternatives provided herein. In some alternatives, the nucleic acid encoding a chimeric antigen receptor comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid, and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the nucleic acid encoding a chimeric antigen receptor comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or

binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached to a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFRSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes

Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth

in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. In some alternatives, the vector is a viral vector. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

A chimeric antigen receptor or TcR specific for a cell surface tumor specific molecule

[0230] In some alternatives, a chimeric antigen receptor or TcR specific for a cell surface tumor specific molecule encoded by a nucleic acid or vector of any one of the alternatives is provided. In some alternatives, the vector is for expression of a chimeric antigen receptor or TcR specific for targeting a solid tumor, wherein the vector comprises the nucleic acid of any one of alternatives provided herein. In some alternatives, the nucleic acid encoding a chimeric antigen receptor comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid sequence is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid and a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the nucleic acid encoding a chimeric antigen receptor

comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid sequence is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a

steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set

forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth

in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. In some alternatives, the vector is a viral vector. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

A bi-specific chimeric antigen receptor specific for a B cell specific cell surface molecule and specific for a cell surface tumor specific molecule

[0231] In some alternatives, a bi-specific chimeric antigen receptor specific for a B cell specific cell surface molecule and specific for a cell surface tumor specific molecule encoded by a nucleic acid or vector of any one of the alternatives is provided. In some alternatives, the bi-specific chimeric antigen receptor is specific for a B cell specific cell surface molecule and is specific for a cell surface tumor specific molecule, the vector comprising the nucleic acid of any one of the alternatives provided herein. In some alternatives, the nucleic acid encoding a bi-specific chimeric antigen receptor comprises a first nucleic acid sequence comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling

domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, and an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the nucleic acid encoding a bi-specific chimeric antigen receptor comprises a first nucleic acid comprising a sequence encoding a leader sequence, a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid, a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid, a fourth nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid, a fifth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid, a sixth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid, a seventh nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, an eighth nucleic acid comprising a sequence encoding a linker, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, and a ninth nucleic acid comprising a sequence encoding a marker domain, wherein the ninth nucleic acid is covalently attached at a 3' end of the eighth nucleic acid, thereby having said

nucleic acid encoding a bi-specific chimeric antigen receptor. In some alternatives, the linker is a ribosome skip sequence or an IRES sequence. In some alternatives, the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence. In some alternatives, the ribosome skip sequence is T2A. In some alternatives, the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34. In some alternatives, the linker further comprises an IRES sequence at the 5' end of the linker. In some alternatives, the first promoter is inducible by tamoxifen and/or its metabolites. In some alternatives, the first promoter is inducible by a drug. In some alternatives, the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain. In some alternatives, the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the nucleic acid further comprises a polynucleotide encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the drug is a steroid, such as a ligand for the estrogen receptor. In some alternatives, the steroid is tamoxifen and/or its metabolites. In some alternatives, the cell surface tumor specific molecule is a cancer antigen. In some alternatives, the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC,

p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the spacer is an IgG4 hinge spacer. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4. In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40. In some alternatives, the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6. In some alternatives, the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8. In some alternatives, the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20. In some alternatives, the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific

molecule is specific for ROR1. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2. In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26. In some alternatives, the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5). In some alternatives, the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28. In some alternatives, the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence. In some alternatives, the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30. In some alternatives, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32. In some alternatives, the marker domain comprises Her2tG. In some alternatives, Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36. In

some alternatives, the marker domain comprises EGFRt. In some alternatives, EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38. In some alternatives, the vector is a viral vector. In some alternatives, the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector. In some alternatives, the vector is a transposon, integrase vector system, or an mRNA vector.

A cell comprising a first and second chimeric antigen receptor or TcR

[0232] In some alternatives, a cell comprising a first and second chimeric antigen receptor or TcR is provided, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor or TcR is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR are inducibly expressed in said cell. In some alternatives, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR is under the control of a regulatory element. In some alternatives, the first chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the second chimeric antigen receptor or TcR comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant

thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, a first marker protein is co-expressed with the first chimeric antigen receptor and a second marker protein is co-expressed with the second chimeric antigen receptor or TcR. In some alternatives, the first marker protein co-expressed with the first chimeric antigen receptor is EGFRt and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is Her2tg or first marker protein co-expressed with the first chimeric antigen receptor is Her2tg and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is EGFRt. In some alternatives, the cell further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the cell expresses a soluble protein for therapy. In some alternatives, the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for L1CAM, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for ROR1, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain.

A cell comprising a bi-specific chimeric antigen receptor

[0233] In some alternatives, a cell comprising a bi-specific chimeric antigen receptor is provided, wherein the bi-specific chimeric antigen receptor comprises two

binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain, wherein the second binding domain is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the cell further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the cell expresses a soluble protein for therapy. In some alternatives, the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T-cells, CD8+ memory T-cells, central memory CD8+ T-cells,

regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for L1CAM. In some alternatives, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for ROR1.

A method of making a cell having a chimeric antigen receptor

[0234] In some alternatives, a method of making a cell having a chimeric antigen receptor is provided, wherein the method comprises introducing into a cell a first nucleic acid or a first vector comprising a polynucleotide sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, introducing into the cell a second nucleic acid or a second vector comprising a polynucleotide sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor, expanding the cell, and isolating the cell. In some alternatives, the first nucleic acid and the second nucleic acid reside on separate viral vectors. In some alternatives, the viral vectors are retroviral vectors, gammaretroviral vectors, foamy viral vector and/or lentiviral vectors. In some alternatives, the viral vectors are co-introduced into the cell as a composition comprising the viral vectors. In some alternatives, the vectors are a transposon, integrase vector system, and/or an mRNA vector. In some alternatives, expression of the first chimeric antigen receptor is linked to co-expression of EGFRt and expression of the second chimeric antigen receptor is linked to co-expression of Her2tg, or wherein expression of the first chimeric antigen receptor is linked to co-expression of Her2tg, and expression of the second chimeric antigen receptor is linked to co-expression of EGFRt. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a soluble protein into said cell. In some alternatives, the soluble protein is a homeostatic cytokine. In some alternatives, the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives,

the viral vectors further comprise a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives of the method, the method further comprises stimulating the cells. In some alternatives, cells are stimulated with interleukin-2 (IL-2) for CD8 cells. In some alternatives, cells are stimulated with interleukin-7 (IL-7) for CD4 cells. In some alternatives cells are stimulated with anti-CD3/CD28 beads.

A method of making a cell having a chimeric antigen receptor

[0235] In some alternatives, a method of making a cell having a chimeric antigen receptor is provided, wherein the method comprises co-delivering into a cell two vectors, wherein the first vector comprises a first nucleic acid sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second vector wherein the second vector comprises a second polynucleotide sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor, expanding the cell and isolating the cell. In some alternatives, the vectors are plasmids and/or minicircle transposons. In some alternatives, the first nucleic acid and the second nucleic acid reside between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence. In some alternatives, the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons. In some alternatives, the method further comprises introducing a vector encoding the Sleeping Beauty transposase or PiggyBac transposase into the cell. In some alternatives of the method, the method further comprises stimulating the cells. In some alternatives, cells are stimulated with interleukin-2 (IL-2) for CD8 cells. In some alternatives, cells are stimulated with interleukin-7 (IL-7) for CD4 cells. In some alternatives cells are stimulated with anti-CD3/CD28 beads.

A method of making a cell having a bi-specific chimeric antigen receptor

[0236] In some alternatives, a method of making a cell having a chimeric antigen receptor is provided, wherein the method comprises co-delivering into a cell two vectors, wherein the first vector comprises a first nucleic acid sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second vector wherein the second vector comprises a second polynucleotide sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor, expanding the cell, and isolating the cell. In some alternatives, the vectors are plasmids and/or minicircle transposons. In some alternatives, the first nucleic acid and the second nucleic acid reside between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence. In some alternatives, the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons. In some alternatives, the method further comprises introducing a vector encoding the Sleeping Beauty transposase or PiggyBac transposase into the cell. In some alternatives of the method, the method further comprises stimulating the cells. In some alternatives, cells are stimulated with interleukin-2 (IL-2) for CD8 cells. In some alternatives, cells are stimulated with interleukin-7 (IL-7) for CD4 cells. In some alternatives cells are stimulated with anti-CD3/CD28 beads.

A method of making a cell having a bi-specific chimeric antigen receptor

[0237] In some alternatives, a method of making a cell having a bi-specific chimeric antigen receptor is provided, wherein the method comprises introducing into a cell a nucleic acid comprising a polynucleotide sequence encoding a bi-specific chimeric antigen receptor that comprises a first binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second binding domain specific for a ligand on a solid tumor, expanding the cells and isolating the cells. In some alternatives, the polynucleotide resides on a viral vector. In some alternatives, the viral vector is a lentiviral, retroviral vector, foamy viral vector or a gammaretroviral vector. In some alternatives, the polynucleotide resides on a transposon, integrase vector system, and/or an mRNA vector. In some alternatives, the bi-specific chimeric antigen receptor is co-

expressed with a marker protein. In some alternatives, the marker protein is EGFRt or Her2tg. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a soluble protein into said cell. In some alternatives, the soluble protein is a homeostatic cytokine. In some alternatives, the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the viral vector further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives of the method, the method further comprises stimulating the cells. In some alternatives, cells are stimulated with interleukin-2 (IL-2) for CD8 cells. In some alternatives, cells are stimulated with interleukin-7 (IL-7) for CD4 cells. In some alternatives cells are stimulated with anti-CD3/CD28 beads.

A method of making a cell having a bi-specific chimeric antigen receptor

[0238] In some alternatives, a method of making a cell having a bi-specific chimeric antigen receptor is provided, wherein the method comprises introducing into a cell a vector, wherein the vector comprises a first nucleic acid encoding a bi-specific chimeric antigen receptor that comprises a first binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second binding domain wherein the second binding domain comprises a binding domain specific for a ligand on a solid tumor; expanding the cell and isolating the cell. In some alternatives, the vector is a plasmid or minicircle transposon. In some alternatives, the first nucleic acid resides between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence. In some alternatives, the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons. In some alternatives, the method further comprises introducing a vector encoding a Sleeping Beauty transposase or PiggyBac transposase into the cell. In some alternatives of the method, the method further comprises stimulating the cells. In some alternatives, cells are stimulated with interleukin-2

(IL-2) for CD8 cells. In some alternatives, cells are stimulated with interleukin-7 (IL-7) for CD4 cells. In some alternatives cells are stimulated with anti-CD3/CD28 beads.

Compositions

[0239] In some alternatives, a composition comprising any one or more of the cells of any one or more of the alternatives, is provided. In some alternatives, the cell comprises a first and second chimeric antigen receptor or TcR, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor or TcR is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR are inducibly expressed in said cell. In some alternatives, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor

or TcR is under the control of a regulatory element. In some alternatives, the first chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the second chimeric antigen receptor or TcR comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, a first marker protein is co-expressed with the first chimeric antigen receptor and a second marker protein is co-expressed with the second chimeric antigen receptor or TcR. In some alternatives, the first marker protein co-expressed with the first chimeric antigen receptor is EGFRt and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is Her2tg or first marker protein co-expressed with the first chimeric antigen receptor is Her2tg and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is EGFRt. In some alternatives, the cell comprises a bi-specific chimeric antigen receptor, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain, wherein the second binding domain is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, IDO1, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A,

Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the cell further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the cell expresses a soluble protein for therapy. In some alternatives, the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells. In some alternatives, the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for L1CAM, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for ROR1, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for L1CAM. In some alternatives, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for ROR1.

A method of treating, ameliorating, or inhibiting a non-B cell related disease in a subject

[0240] In some alternatives, a method of treating, ameliorating, or inhibiting a non-B cell related disease in a subject is provided, wherein the method comprises identifying a subject that does not have a B-cell related disease for therapy, introducing, providing, or administering any one or more of the cells of any one or more of the alternatives herein or the cells made by any one or more of the methods of any one or more of the alternatives herein or the composition of any one or more of the alternatives herein into a subject for therapy. In some alternatives, the composition comprises any one or more of the cells of any one or more of the alternatives herein or the cells made by any one or more of the methods of any one of the alternatives described herein. In some alternatives, the composition comprises CD8+ T cytotoxic lymphocyte cells and/or CD4+ T helper lymphocyte cells, wherein the CD8+ T cytotoxic lymphocyte cells are selected from the group consisting of naïve CD8+ T-cells, CD8+ memory T-cells, central memory CD8+ T-cells, regulatory CD8+ T-cells, IPS derived CD8+ T-cells, effector memory CD8+ T-cells and bulk CD8+ T-cells and, wherein the CD4+ T helper lymphocyte cells are selected from the group consisting of naïve CD4+ T-cells, CD4+ memory T-cells, central memory CD4+ T-cells, regulatory CD4+ T-cells, IPS derived CD4+ T-cells, effector memory CD4+ T-cells and bulk CD4+ T-cells. In some alternatives, the composition has a ratio of CD4+ T helper lymphocyte cells to CD8+ T lymphocytes of 1:10 to 10:1. In some alternatives, the ratio of CD4+ T helper lymphocyte cells to CD8+ T lymphocytes is 1:1. In some alternatives, the subject does not have a B-cell related disease. In some alternatives, the subject does not have B-cell lymphoma, Hodgkin's lymphomas, non-Hodgkins lymphomas, Diffuse large B cell lymphoma, Follicular lymphoma, marginal zone lymphoma, Mucosa-Associated Lymphatic Tissue lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B cell lymphoma, Lymphoplasmacytic lymphoma, Waldenstrom macroglobulinemia, Nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B cell lymphoma, Intravascular large B-cell lymphoma, Primary effusion lymphoma, Lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, Primary central nervous system lymphoma, Primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the

elderly, Diffuse large B-cell lymphoma associated with inflammation, Intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, ALK-positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-cell lymphoma arising in HHV8-associated multicentric Castleman's disease, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, or nodular lymphocyte predominant Hodgkin's lymphoma. In some alternatives, the disease is a cancer. In some alternatives, the disease is an infection, wherein the infection is a bacterial or viral infection. In some alternatives, the cancer is a solid tumor. In some alternatives, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer. In some alternatives, the subject has refractory and relapsed neuroblastoma. In some alternatives, the subject is identified or selected to receive a non-B cell related disease therapy, anti-cancer therapy, anti-infection therapy, antibacterial therapy, anti-viral therapy, or anti-tumoral therapy. In some alternatives, the method further comprises measuring or evaluating an inhibition of said non-B cell related disease, cancer, infection, bacterial infection, viral infection, or tumor. In some alternatives, the method further comprises introducing, providing, or administering to said subject an additional therapeutic agent, such as a chemotherapeutic agent, an antiviral agent, or an antibacterial agent or an adjunct therapy such as radiation therapy and/or surgery before, during, or after introducing, providing, or administering any one or more of the cells of any one of the alternatives described herein or the cells made by any one or more of the methods of any one of the alternatives described herein or the composition of any one of the alternatives described herein into the subject for therapy. In some alternatives, the composition comprises any one or more of the cells of any one of the alternatives described herein or the cells made by any one or more of the methods of any one of the alternatives described herein. In some alternatives, the composition comprises CD8⁺ T cytotoxic lymphocyte cells and/or CD4⁺ T helper lymphocyte cells, wherein the CD8⁺ T cytotoxic lymphocyte cells are selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺

T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells and, wherein the CD4⁺ T helper lymphocyte cells are selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells. In some alternatives, the composition has a ratio of CD4⁺ T helper lymphocyte cells to CD8⁺ T lymphocytes of 1:10 to 10:1. In some alternatives, the ratio of CD4⁺ T helper lymphocyte cells to CD8⁺ T lymphocytes is 1:1. In some alternatives, the composition comprises any one or more of the cells of any one or more of the alternatives. In some alternatives, the cell comprises a first and second chimeric antigen receptor or TcR, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor or TcR is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives of the cell, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives of the cell, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives of the cell, the first chimeric antigen receptor and/or the second chimeric antigen

receptor or TcR are inducibly expressed in said cell. In some alternatives of the cell, expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR is under the control of a regulatory element. In some alternatives of the cell, the first chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives of the cell, the second chimeric antigen receptor or TcR comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives of the cell, a first marker protein is co-expressed with the first chimeric antigen receptor and a second marker protein is co-expressed with the second chimeric antigen receptor or TcR. In some alternatives, the first marker protein co-expressed with the first chimeric antigen receptor is EGFRt and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is Her2tg or first marker protein co-expressed with the first chimeric antigen receptor is Her2tg and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is EGFRt. In some alternatives of the cell, the cell comprises a bi-specific chimeric antigen receptor, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain, wherein the second binding domain is specific for a ligand on a tumor. In some alternatives, the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alphaCD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147. In some alternatives of the cell, the ligand on the tumor is a cancer antigen. In some alternatives, the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1,

BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1. In some alternatives of the cell, the cancer antigen is L1CAM. In some alternatives, the cancer antigen is ROR1. In some alternatives, the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner. In some alternatives, the cell further comprises a nucleic acid encoding a suicide gene system. In some alternatives of the cell, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the cell expresses a soluble protein for therapy. In some alternatives of the cell, the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T-cells, CD8+ memory T-cells, central memory CD8+ T-cells, regulatory CD8+ T-cells, IPS derived CD8+ T-cells, effector memory CD8+ T-cells and bulk CD8+ T-cells. In some alternatives, the cell is a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T-cells, CD4+ memory T-cells, central memory CD4+ T-cells, regulatory CD4+ T-cells, IPS derived CD4+ T-cells, effector memory CD4+ T-cells and bulk CD4+ T-cells. In some alternatives, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for L1CAM, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives of the cell, the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for ROR1, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain. In some alternatives of the cell, the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for L1CAM. In some alternatives of the cell, the first binding domain is specific for a ligand

on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for ROR1. In some alternatives, the method of making a cell having a chimeric antigen receptor comprises introducing into a cell a first nucleic acid or a first vector comprising a polynucleotide sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, introducing into the cell a second nucleic acid or a second vector comprising a polynucleotide sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor, expanding the cell, and isolating the cell. In some alternatives, the first nucleic acid and the second nucleic acid reside on separate viral vectors. In some alternatives, the viral vectors are retroviral vectors, gammaretroviral vectors, foamy viral vector and/or lentiviral vectors. In some alternatives, the viral vectors are co-introduced into the cell as a composition comprising the viral vectors. In some alternatives, the vectors are a transposon, integrase vector system, and/or an mRNA vector. In some alternatives, expression of the first chimeric antigen receptor is linked to co-expression of EGFRt and expression of the second chimeric antigen receptor is linked to co-expression of Her2tg, or wherein expression of the first chimeric antigen receptor is linked to co-expression of Her2tg, and expression of the second chimeric antigen receptor is linked to co-expression of EGFRt. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a soluble protein into said cell. In some alternatives, the soluble protein is a homeostatic cytokine. In some alternatives, the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the viral vectors further comprise a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives the method of making a cell having a chimeric antigen receptor comprises co-delivering into a cell two vectors, wherein the first vector comprises a first nucleic acid sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which

promotes the in vivo expansion and activation of the B cell, and a second vector wherein the second vector comprises a second polynucleotide sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor, expanding the cell and isolating the cell. In some alternatives, the vectors are plasmids and/or minicircle transposons. In some alternatives, the first nucleic acid and the second nucleic acid reside between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence. In some alternatives, the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons. In some alternatives, the method further comprises introducing a vector encoding the Sleeping Beauty transposase or PiggyBac transposase into the cell. In some alternatives, the method of making a cell having a chimeric antigen receptor comprises co-delivering into a cell two vectors, wherein the first vector comprises a first nucleic acid sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second vector wherein the second vector comprises a second polynucleotide sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor, expanding the cell, and isolating the cell. In some alternatives, the vectors are plasmids and/or minicircle transposons. In some alternatives, the first nucleic acid and the second nucleic acid reside between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence. In some alternatives, the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons. In some alternatives, the method further comprises introducing a vector encoding the Sleeping Beauty transposase or PiggyBac transposase into the cell. In some alternatives, the method of making a cell having a bi-specific chimeric antigen receptor comprises introducing into a cell a nucleic acid comprising a polynucleotide sequence encoding a bi-specific chimeric antigen receptor that comprises a first binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second binding domain specific for a ligand on a solid tumor, expanding the cells and isolating the cells. In some alternatives, the polynucleotide resides on a viral vector. In some alternatives, the viral vector is a lentiviral, retroviral vector, foamy viral vector or a gammaretroviral vector. In some alternatives, the polynucleotide resides on a transposon,

integrase vector system, and/or an mRNA vector. In some alternatives, the bi-specific chimeric antigen receptor is co-expressed with a marker protein. In some alternatives, the marker protein is EGFRt or Her2tg. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a soluble protein into said cell. In some alternatives, the soluble protein is a homeostatic cytokine. In some alternatives, the homeostatic cytokine is IL2, IL7, IL12 or IL15. In some alternatives, the viral vector further comprises a nucleic acid encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the method further comprises introducing a vector comprising a sequence encoding a suicide gene system. In some alternatives, the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system. In some alternatives, the method of making a cell having a bi-specific chimeric antigen receptor comprises introducing into a cell a vector, wherein the vector comprises a first nucleic acid encoding a bi-specific chimeric antigen receptor that comprises a first binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second binding domain wherein the second binding domain comprises a binding domain specific for a ligand on a solid tumor; expanding the cell and isolating the cell. In some alternatives, the vector is a plasmid or minicircle transposon. In some alternatives, the first nucleic acid resides between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence. In some alternatives, the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons. In some alternatives, the method further comprises introducing a vector encoding a Sleeping Beauty transposase or PiggyBac transposase into the cell.

WHAT IS CLAIMED IS:

1. A nucleic acid encoding a chimeric antigen receptor comprising:
 - a first nucleic acid comprising a sequence encoding a leader sequence;
 - a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid;
 - a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid;
 - a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid;
 - a fifth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid;
 - a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid; and
 - a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor.
2. A nucleic acid encoding a chimeric antigen receptor comprising:
 - a first nucleic acid comprising a sequence encoding a leader sequence;
 - a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid;
 - a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule, and wherein the third nucleic acid is covalently attached to a 3' end of the second nucleic acid;

a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached to a 3' end of the third nucleic acid;

a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached to a 3' end of the fourth nucleic acid;

a sixth nucleic acid comprising a sequence encoding a signaling domain, wherein the signaling domain comprises a 4-1BB domain and/or CD3-zeta domain, and wherein the sixth nucleic acid is covalently attached to a 3' end of the fifth nucleic acid;

a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached to a 3' end of the sixth nucleic acid; and

an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached to a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor.

3. A nucleic acid encoding a chimeric antigen receptor comprising:

a first nucleic acid comprising a sequence encoding a leader sequence;

a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid;

a third nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the third nucleic acid sequence is covalently attached at a 3' end of the second nucleic acid;

a fourth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid;

a fifth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid;

a sixth nucleic acid comprising a sequence encoding a linker, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid; and

a seventh nucleic acid comprising a sequence encoding a marker domain, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor.

4. A nucleic acid encoding a chimeric antigen receptor comprising:

a first nucleic acid comprising a sequence encoding a leader sequence;

a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid;

a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid;

a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid sequence is covalently attached at a 3' end of the third nucleic acid;

a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid;

a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain, and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid;

a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid; and

an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a chimeric antigen receptor.

5. A nucleic acid encoding a bi-specific chimeric antigen receptor comprising:

a first nucleic acid sequence comprising a sequence encoding a leader sequence;

a second nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific

molecule, and wherein the first nucleic acid is covalently attached at a 5' end of the second nucleic acid:

a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid;

a fourth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid;

a fifth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid;

a sixth nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid;

a seventh nucleic acid comprising a sequence encoding a linker, wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid; and

an eighth nucleic acid comprising a sequence encoding a marker domain, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor.

6. A nucleic acid encoding a bi-specific chimeric antigen receptor comprising:

a first nucleic acid comprising a sequence encoding a leader sequence;

a second nucleic acid comprising a sequence encoding a first promoter inducible by a drug, wherein the first nucleic acid is covalently attached to a 5' end of the second nucleic acid;

a third nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the third nucleic acid is covalently attached at a 3' end of the second nucleic acid,

a fourth nucleic acid comprising a sequence encoding an antibody or binding fragment thereof or scFv, wherein the antibody or binding fragment thereof or scFv is specific for a B cell specific cell surface molecule or is specific for a cell surface tumor specific molecule, and wherein the fourth nucleic acid is covalently attached at a 3' end of the third nucleic acid;

a fifth nucleic acid comprising a sequence encoding a de-immunized extracellular spacer, wherein the fifth nucleic acid is covalently attached at a 3' end of the fourth nucleic acid;

a sixth nucleic acid comprising a sequence encoding a transmembrane domain, wherein the sixth nucleic acid is covalently attached at a 3' end of the fifth nucleic acid;

a seventh nucleic acid comprising a sequence encoding a signaling domain sequence, wherein the signaling domain comprises a co-stimulatory domain, wherein the co-stimulatory domain comprises a 4-1BB domain, CD3-zeta domain and/or CD28-zeta domain and wherein the seventh nucleic acid is covalently attached at a 3' end of the sixth nucleic acid;

an eighth nucleic acid comprising a sequence encoding a linker, wherein the eighth nucleic acid is covalently attached at a 3' end of the seventh nucleic acid; and

a ninth nucleic acid comprising a sequence encoding a marker domain, wherein the ninth nucleic acid is covalently attached at a 3' end of the eighth nucleic acid, thereby having said nucleic acid encoding a bi-specific chimeric antigen receptor.

7. The nucleic acid of any one of Claims 1-6, wherein the linker is a ribosome skip sequence or an IRES sequence.
8. The nucleic acid of Claim 7, wherein the ribosome skip sequence is a P2A, T2A, E2A or F2A sequence.
9. The nucleic acid of Claim 8, wherein the ribosome skip sequence is T2A.
10. The nucleic acid of Claim 9, wherein the T2A sequence comprises an amino acid sequence set forth in SEQ ID NO: 33 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 34.
11. The nucleic acid of any one of Claims 1-10, wherein the linker further comprises an IRES sequence at the 5' end of the linker.
12. The nucleic acid of any one of Claims 2, 4 or 6-11, wherein the first promoter is inducible by tamoxifen and/or its metabolites.

13. The nucleic acid of any one of Claims 2, 4 or 6-12, wherein the first promoter is inducible by a drug.
14. The nucleic acid of any one of Claims 1-13, wherein the sequence encoding the transmembrane domain further comprises an IRES sequence at the 3' end of the sequence encoding the transmembrane domain.
15. The nucleic acid of any one of Claims 1, 2 or 5-14, wherein the B-cell specific cell surface molecule is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147.
16. The nucleic acid of any one of Claims 1-15, wherein the nucleic acid further comprises a polynucleotide encoding a suicide gene system.
17. The nucleic acid of Claim 16, wherein the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system.
18. The nucleic acid of any one of Claims 13-17, wherein the drug is a steroid, such as a ligand for the estrogen receptor.
19. The nucleic acid of Claim 18, wherein the steroid is tamoxifen and/or its metabolites.
20. The nucleic acid of any one of Claims 3-19, wherein the cell surface tumor specific molecule is a cancer antigen.
21. The nucleic acid of claim 20, wherein the cell surface tumor specific molecule is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-foetoprotein, kallikrein4, KIF20A, Lengsin, M-CSF, MCSP, mdm-2,

Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1.

22. The nucleic acid of Claim 20 or 21, wherein the cancer antigen is L1CAM.

23. The nucleic acid of Claims 20 or 21, wherein the cancer antigen is ROR1.

24. The nucleic acid of any one of Claims 1-23, wherein the spacer is an IgG4 hinge spacer.

25. The nucleic acid of Claim 24, wherein the spacer comprises an amino acid sequence set forth in SEQ ID NO: 1 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 2.

26. The nucleic acid of any one of Claims 1-23, wherein the spacer comprises an amino acid sequence set forth in SEQ ID NO: 3 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 4.

27. The nucleic acid of any one of Claims 1-23, wherein the spacer comprises an amino acid sequence set forth in SEQ ID NO: 39 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 40.

28. The nucleic acid of any one of Claims 1-27, wherein the CD28-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 5 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 6.

29. The nucleic acid of any one of Claims 1-28, wherein the 4-1BB domain comprises an amino acid sequence set forth in SEQ ID NO: 7 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 8.

30. The nucleic acid of any one of Claims 1-29, wherein the CD3-zeta domain comprises an amino acid sequence set forth in SEQ ID NO: 9 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 10.

31. The nucleic acid of any one of Claims 1, 2, 5-30, wherein the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD19.

32. The nucleic acid of Claim 31, wherein the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set

forth in SEQ ID NO: 11 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12.

33. The nucleic acid of any one of Claims 1, 2, 5-30, wherein the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule is specific for CD20.

34. The nucleic acid of Claim 33, wherein the antibody or binding fragment thereof or scFv specific for the B cell specific cell surface molecule comprises an amino sequence set forth in SEQ ID NO: 13 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14.

35. The nucleic acid of any one of Claims 3-34, wherein the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for L1CAM.

36. The nucleic acid of Claim 35, wherein the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for a CE7 epitope on L1CAM.

37. The nucleic acid of Claim 36, wherein the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 15 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16.

38. The nucleic acid of any one of Claims 3-34, wherein the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for ROR1.

39. The nucleic acid of Claim 38, wherein the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 17 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18.

40. The nucleic acid of any one of Claims 3-34, wherein the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EGFR 806.

41. The nucleic acid of Claim 40, wherein the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 19 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20.

42. The nucleic acid of any one of Claims 3-34, wherein the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for Her2.

43. The nucleic acid of Claim 42, wherein the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 21 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22.

44. The nucleic acid of any one of Claims 3-34, wherein the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for GD2.

45. The nucleic acid of Claim 44, wherein the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 23 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 24.

46. The nucleic acid of any one of Claims 3-34, wherein the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (2H4).

47. The nucleic acid of Claim 46, wherein the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 25 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 26.

48. The nucleic acid of any one of Claims 3-34, wherein the antibody or binding fragment thereof or scFv specific for a cell surface tumor specific molecule is specific for EphA2 (4H5).

49. The nucleic acid of Claim 48, wherein the antibody or binding fragment thereof or scFv comprises an amino acid sequence set forth in SEQ ID NO: 27 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 28.

50. The nucleic acid of any one of Claims 1-49, wherein the leader sequence comprises a Granulocyte-macrophage colony-stimulating factor signal sequence.

51. The nucleic acid of Claim 50, wherein the Granulocyte-macrophage colony-stimulating factor signal sequence comprises an amino acid sequence set forth in SEQ ID NO: 29 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 30.

52. The nucleic acid of any one of Claims 1-49, wherein the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 31 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 32.

53. The nucleic acid of any one of Claims 1-52, wherein the marker domain comprises Her2tG.

54. The nucleic acid of Claim 53, wherein Her2tG comprises an amino acid sequence set forth in SEQ ID NO: 35 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 36.

55. The nucleic acid of any one of Claims 1-52, wherein the marker domain comprises EGFRt.

56. The nucleic acid of Claim 55, wherein EGFRt comprises an amino acid sequence set forth in SEQ ID NO: 37 and is encoded by a nucleic acid sequence set forth in SEQ ID NO: 38.

57. A vector for expression of a chimeric antigen receptor specific for promoting in vivo expansion and activation of B cells, wherein the vector comprises the nucleic acid of any one of Claims 1-2, 7-19, 24-34 or 50-56.

58. A vector for expression of a chimeric antigen receptor or TcR specific for targeting a solid tumor, wherein the vector comprises the nucleic acid of any one of Claims 3-4 or 7-14, 16-30 or 35-56.

59. A vector for expression of a bi-specific chimeric antigen receptor, wherein the bi-specific chimeric antigen receptor is specific for a B cell specific cell surface molecule and is specific for a cell surface tumor specific molecule, the vector comprising the nucleic acid of any one of Claims 5-56.

60. The vector of any one of Claims 57-59, wherein the vector is a viral vector.

61. The vector of Claim 60, wherein the vector is a lentiviral vector, retroviral vector, gammaretroviral vectors or a foamy viral vector.

62. The vector of any one of Claims 57-59, wherein the vector is a transposon, integrase vector system, or an mRNA vector.

63. A chimeric antigen receptor or TcR specific for a B-cell specific cell surface molecule encoded by a nucleic acid of any one of Claims 1-2 or 7-56 or vector of any one of Claims 57 or 60-62.

64. A chimeric antigen receptor or TcR specific for a cell surface tumor specific molecule encoded by a nucleic acid of any one of Claims 3-4 or 7-56 or vector of any one of Claims 58 or 60-62.

65. A bi-specific chimeric antigen receptor specific for a B cell specific cell surface molecule and specific for a cell surface tumor specific molecule, encoded by a nucleic acid of any one of Claims 5-56 or vector of any one of Claims 59-62.

66. A cell comprising a first and second chimeric antigen receptor or TcR, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of an effector cell and, wherein the second chimeric antigen receptor or TcR is specific for a ligand on a tumor.

67. The cell of Claim 66, wherein the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147.

68. The cell of Claim 65 or 67, wherein the ligand on the tumor is a cancer antigen.

69. The cell of any one of Claims 66-68, wherein the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1.

70. The cell of Claim 69, wherein the cancer antigen is L1CAM.

71. The cell of Claim 69, wherein the cancer antigen is ROR1.

72. The cell of any one of Claims 66-71, wherein the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR are inducibly expressed in said cell.

73. The cell of any one of Claims 66-72, wherein expression of the first chimeric antigen receptor and/or the second chimeric antigen receptor or TcR is under the control of a regulatory element.

74. The cell of any one of Claims 66-73, wherein the first chimeric antigen receptor comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner.

75. The cell of any one of Claims 66-74, wherein the second chimeric antigen receptor or TcR comprises an antibody or binding fragment thereof or scFv, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner.

76. The cell of any one of Claims 66-75, wherein a first marker protein is co-expressed with the first chimeric antigen receptor and a second marker protein is co-expressed with the second chimeric antigen receptor or TcR.

77. The cell of Claim 76, wherein the first marker protein co-expressed with the first chimeric antigen receptor is EGFRt and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is Her2tg or first marker protein co-expressed with the first chimeric antigen receptor is Her2tg and the second marker protein co-expressed with the second chimeric antigen receptor or TcR is EGFRt.

78. A cell comprising a bi-specific chimeric antigen receptor, wherein the bi-specific chimeric antigen receptor comprises two binding domains, wherein a first binding domain is specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell and a second binding domain, wherein the second binding domain is specific for a ligand on a tumor.

79. The cell of Claim 78, wherein the ligand on a B cell is CD1d, CD5, CD19, CD20, CD21, CD22, CD23/Fc epsilon RII, CD24, CD25/IL-2 R alpha, CD27/TNFRSF7, CD32, CD34, CD35, CD38, CD40 (TNFRSF5), CD44, CD45, CD45.1, CD45.2, CD54 (ICAM-1), CD69, CD72, CD79, CD80, CD84/SLAMF5, LFA-1, CALLA, BCMA, B-cell receptor (BCR), IgMs, IgD, B220/CD45R, C1q R1/CD93, CD84/SLAMF5, BAFF R/ TNFRSF13C, B220/CD45R, B7-1/CD80, B7-2/CD86, TNFSF7, TNFRSF5, ENPP-1, HVEM/TNFRSF14, BLIMP1/PRDM1, CXCR4, DEP-1/CD148, or EMMPRIN/CD147.

80. The cell of Claim 78 or 79, wherein the ligand on the tumor is a cancer antigen.

81. The cell of Claim 78-80, wherein the cancer antigen is EGFR, HER2, Mesothelin, cancer testis antigens, L1CAM, o-acetylated GD2, GD2, neoantigens, Var2, glypican-2 (GPC2), HPV antigens, alphafetoprotein, carcinoembryonic antigen, CA-125, MUC-1, epithelial tumor antigen, abnormal products of ras or p53, EphA2, MAGE-A3, MAGE-A4, MAGE-C2, PRAME, SSX2, adipophilin, AIM2, ALDH1A1, BCLX, EpCAM, CS274, CPSF, cyclin D1, DKK1, ENAH, EpCAM, EphA3, EZH2, FGF5, glypican-3, G250, HLA-DOB, Hepsin, ID01, IGF2B3, IL13Ralpha2, Intestinal carboxylesterase, alpha-fetoprotein, kallikrein4, KIF20A, Lentsin, M-CSF, MCSP, mdm-2, Meloe, midkine, MMP-2, MMP-7, MUC1, MUC5AC, p53, PAX5, PBF, PRAME, PSMA, RAGE-1, RGS5, RhoC, RNF43, RUF43, FU2AS, secernin 1, SOX10, STEAP1, survivin, telomerase, TPBG, VEGF, WT1, NY-ESO-1 or ROR1.

82. The cell of Claim 80 or 81, wherein the cancer antigen is L1CAM.

83. The cell of Claim 80 or 81, wherein the cancer antigen is ROR1.

84. The cell of any one of Claims 78-83, wherein the first and second binding domain comprises an antibody or portion thereof, a receptor ligand or mutant thereof, peptide, and/or polypeptide affinity molecule or binding partner.

85. The cell of any one of Claims 66-84, wherein the cell further comprises a nucleic acid encoding a suicide gene system.

86. The cell of Claim 85, wherein the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system.

87. The cell of any one of Claims 66-86, wherein the cell expresses a soluble protein for therapy.

88. The cell of Claim 87, wherein the soluble protein is a homeostatic cytokine, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15.

89. The cell of any one of Claims 66-88, wherein the cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells.

90. The cell of any one of Claims 66-88, wherein the cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells.

91. The cell of any one of claims 66-77 or 85-90, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for L1CAM, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain.

92. The cell of any one of claims 66-77 or 85-90, wherein the first chimeric antigen receptor is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second chimeric antigen receptor is specific for ROR1, and wherein the chimeric antigen receptors further comprises a 4-1 BB and CD3-zeta signaling domain.

93. The cell of any one of Claims 78-90, wherein the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for L1CAM.

94. The cell of any one of Claims 78-90, wherein the first binding domain is specific for a ligand on a B cell, wherein the ligand on the B cell is CD19, and wherein the second binding domain is specific for ROR1.

95. A method of making a cell having a chimeric antigen receptor comprising:

introducing into a cell a first nucleic acid or a first vector comprising a polynucleotide sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which promotes the *in vivo* expansion and activation of the B cell;

introducing into the cell a second nucleic acid or a second vector comprising a polynucleotide sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor;

expanding the cell; and

isolating the cell.

96. The method of Claim 95, wherein the first nucleic acid and the second nucleic acid reside on separate viral vectors.

97. The method of Claim 96, wherein the viral vectors are retroviral vectors, gammaretroviral vectors, foamy viral vector and/or lentiviral vectors.

98. The method of Claim 96 or 97, wherein the viral vectors are co-introduced into the cell as a composition comprising the viral vectors.

99. The method of Claim 95, wherein the vectors are a transposon, integrase vector system, and/or an mRNA vector.

100. The method of any one of Claims 95-99, wherein expression of the first chimeric antigen receptor is linked to co-expression of EGFRt and expression of the second chimeric antigen receptor is linked to co-expression of Her2tg, or wherein expression of the first chimeric antigen receptor is linked to co-expression of Her2tg, and expression of the second chimeric antigen receptor is linked to co-expression of EGFRt.

101. The method of any one of Claims 95-100, further comprising introducing a vector comprising a sequence encoding a soluble protein into said cell.

102. The method of Claim 101, wherein the soluble protein is a homeostatic cytokine.

103. The method of claim 102, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15.

104. The method of any one of Claims 96-98 or 100-103, wherein the viral vectors further comprise a nucleic acid encoding a suicide gene system.

105. The method of Claim 104, wherein the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system.

106. The method of any one of Claims 95-105, further comprising introducing a vector comprising a sequence encoding a suicide gene system.

107. The method of Claim 106, wherein the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system.

108. A method of making a cell having a chimeric antigen receptor comprising:

co-delivering into a cell two vectors, wherein the first vector comprises a first nucleic acid sequence encoding a first chimeric antigen receptor that comprises a binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second vector wherein the second vector comprises a second polynucleotide

sequence encoding a second chimeric antigen receptor or TcR that comprises a binding domain specific for a ligand on a solid tumor;

expanding the cell; and

isolating the cell.

109. The method of Claim 108, wherein the vectors are plasmids and/or minicircle transposons.

110. The method of Claim 108 or 109, wherein the first nucleic acid and the second nucleic acid reside between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence.

111. The method of Claim 110, wherein the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons.

112. The method of Claim 111, wherein the method further comprises introducing a vector encoding the Sleeping Beauty transposase or PiggyBac transposase into the cell.

113. A method of making a cell having a bi-specific chimeric antigen receptor comprising:

introducing into a cell a nucleic acid comprising a polynucleotide sequence encoding a bi-specific chimeric antigen receptor that comprises a first binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second binding domain specific for a ligand on a solid tumor,

expanding the cells; and

isolating the cells.

114. The method of Claim 113, wherein the polynucleotide resides on a viral vector.

115. The method of Claim 110, wherein the viral vector is a lentiviral, retroviral vector, foamy viral vector or a gammaretroviral vector.

116. The method of Claim 113, wherein the polynucleotide resides on a transposon, integrase vector system, and/or an mRNA vector.

117. The method of any one of Claims 113-116, wherein the bi-specific chimeric antigen receptor is co-expressed with a marker protein.

118. The method of Claim 117, wherein the marker protein is EGFRt or Her2tg.

119. The method of any one of Claims 113-118, further comprising introducing a vector comprising a sequence encoding a soluble protein into said cell.

120. The method of Claim 119, wherein the soluble protein is a homeostatic cytokine.

121. The method of claim 120, wherein the homeostatic cytokine is IL2, IL7, IL12 or IL15.

122. The method of any one of Claims 114-115 or 117-121, wherein the viral vector further comprises a nucleic acid encoding a suicide gene system.

123. The method of Claim 122, wherein the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system.

124. The method of any one of Claims 113-121, further comprising introducing a vector comprising a sequence encoding a suicide gene system.

125. The method of Claim 124, wherein the suicide gene system is a Herpes Simplex Virus Thymidine Kinase (HSVTK)/Ganciclovir (GCV) suicide gene system or an inducible Caspase suicide gene system.

126. A method of making a cell having a bi-specific chimeric antigen receptor comprising:

introducing into a cell a vector, wherein the vector comprises a first nucleic acid encoding a bi-specific chimeric antigen receptor that comprises a first binding domain specific for a ligand on a B cell, which promotes the in vivo expansion and activation of the B cell, and a second binding domain wherein the second binding domain comprises a binding domain specific for a ligand on a solid tumor;

expanding the cell; and

isolating the cell.

127. The method of Claim 126, wherein the vector is a plasmid or minicircle transposon.

128. The method of Claim 126 or 127, wherein the first nucleic acid resides between a first inverted terminal repeat gene sequence and a second inverted terminal repeat gene sequence.

129. The method of Claim 128, wherein the inverted terminal repeat gene sequences are inverted repeats of a Sleeping Beauty transposon or PiggyBac transposons.

130. The method of Claim 129, wherein the method further comprises introducing a vector encoding a Sleeping Beauty transposase or PiggyBac transposase into the cell.

131. A composition comprising any one or more of the cells of Claims 66-94.

132. A method of treating, ameliorating, or inhibiting a non-B cell related disease in a subject comprising:

identifying a subject that does not have a B-cell related disease for therapy,

introducing, providing, or administering any one or more of the cells of Claims 66-94 or the cells made by any one or more of the methods of Claims 95-130 or the composition of Claim 131 into a subject for therapy.

133. The method of Claim 132, wherein the composition comprises any one or more of the cells of Claims 66-94 or the cells made by any one or more of the methods of Claims 95-130.

134. The method of Claim 132 or 133, wherein the composition comprises CD8⁺ T cytotoxic lymphocyte cells and/or CD4⁺ T helper lymphocyte cells, wherein the CD8⁺ T cytotoxic lymphocyte cells are selected from the group consisting of naïve CD8⁺ T-cells, CD8⁺ memory T-cells, central memory CD8⁺ T-cells, regulatory CD8⁺ T-cells, IPS derived CD8⁺ T-cells, effector memory CD8⁺ T-cells and bulk CD8⁺ T-cells and, wherein the CD4⁺ T helper lymphocyte cells are selected from the group consisting of naïve CD4⁺ T-cells, CD4⁺ memory T-cells, central memory CD4⁺ T-cells, regulatory CD4⁺ T-cells, IPS derived CD4⁺ T-cells, effector memory CD4⁺ T-cells and bulk CD4⁺ T-cells.

135. The method of any one of Claims 132-133, wherein the composition has a ratio of CD4⁺ T helper lymphocyte cells to CD8⁺ T lymphocytes of 1:10 to 10:1.

136. The method of any one of Claims 132-135, wherein the ratio of CD4⁺ T helper lymphocyte cells to CD8⁺ T lymphocytes is 1:1.

137. The method of any one of Claims 132-136, wherein the subject does not have a B-cell related disease.

138. The method of any one of Claims 132-137, wherein the subject does not have B-cell lymphoma, Hodgkin's lymphomas, non-Hodgkins lymphomas, Diffuse large B cell lymphoma, Follicular lymphoma, marginal zone lymphoma, Mucosa-Associated Lymphatic

Tissue lymphoma, small lymphocytic lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B cell lymphoma, Lymphoplasmacytic lymphoma, Waldenstrom macroglobulinemia, Nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B cell lymphoma, Intravascular large B-cell lymphoma, Primary effusion lymphoma, Lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, Primary central nervous system lymphoma, Primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, Diffuse large B-cell lymphoma associated with inflammation, Intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, ALK-positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-cell lymphoma arising in HHV8-associated multicentric Castleman's disease, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, or nodular lymphocyte predominant Hodgkin's lymphoma.

139. The method of any one of Claims 132-138, wherein the disease is a cancer.

140. The method of any one of Claims 132-138, wherein the disease is an infection, wherein the infection is a bacterial or viral infection.

141. The method of Claim 139, wherein the cancer is a solid tumor.

142. The method of Claim 141, wherein the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, liver cancer, stomach cancer, spleen cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, uterine cancer, skin cancer, head cancer, neck cancer, sarcomas, neuroblastomas and ovarian cancer.

143. The method of any one of Claims 132-142, wherein the subject has refractory and relapsed neuroblastoma.

144. The method of any one of Claims 132-143, wherein the subject is identified or selected to receive a non-B cell related disease therapy, anti-cancer therapy, anti-infection therapy, antibacterial therapy, anti-viral therapy, or anti-tumoral therapy.

145. The method of any one of Claims 132-144, further comprising measuring or evaluating an inhibition of said non-B cell related disease, cancer, infection, bacterial infection, viral infection, or tumor.

146. The method of any one or more of claims 132-145, further comprising introducing, providing, or administering to said subject an additional therapeutic agent, such as a chemotherapeutic agent, an antiviral agent, or an antibacterial agent or an adjunct therapy such as radiation therapy and/or surgery before, during, or after introducing, providing, or administering any one or more of the cells of 66-94 or the cells made by any one or more of the methods of Claims 95-130 or the composition of Claim 131 into the subject for therapy.

147. The method of Claim 146, wherein the composition comprises any one or more of the cells of Claims 66-94 or the cells made by any one or more of the methods of Claims 95-130.

148. The method of Claim 146 or 147, wherein the composition comprises CD8+ T cytotoxic lymphocyte cells and/or CD4+ T helper lymphocyte cells, wherein the CD8+ T cytotoxic lymphocyte cells are selected from the group consisting of naïve CD8+ T-cells, CD8+ memory T-cells, central memory CD8+ T-cells, regulatory CD8+ T-cells, IPS derived CD8+ T-cells, effector memory CD8+ T-cells and bulk CD8+ T-cells and, wherein the CD4+ T helper lymphocyte cells are selected from the group consisting of naïve CD4+ T-cells, CD4+ memory T-cells, central memory CD4+ T-cells, regulatory CD4+ T-cells, IPS derived CD4+ T-cells, effector memory CD4+ T-cells and bulk CD4+ T-cells.

149. The method of any one of Claims 146-148, wherein the composition has a ratio of CD4+ T helper lymphocyte cells to CD8+ T lymphocytes of 1:10 to 10:1.

150. The method of any one of Claims 146-148, wherein the ratio of CD4+ T helper lymphocyte cells to CD8+ T lymphocytes is 1:1.

151. The method of any one or more of claims 145-150, wherein the cells or compositions are introduced, provided, or administered to said subject by adoptive cell transfer.

152. The method of any one or more of Claims 132-151, further comprising introducing, providing, or administering a drug that induces expression of a chimeric antigen receptor or TcR.

153. The method of Claim 152, wherein the drug is a steroid.

154. The method of Claim 152 or 153, wherein the drug is tamoxifen and/or its metabolites.

155. The method of any one of Claims 132-154, wherein the subject is a mammalian species.
156. The method of any one of Claims 132-155, wherein the subject is a cow, sheep, pig, horse, dog, cat, primate or a human.
157. The method of any one of Claims 132-156, wherein the subject is human.
158. The method of any one of Claims 132-157, wherein the subject is of pediatric age.
159. The method of any one of Claims 132-158, wherein the method further comprises evaluating the subject for symptoms of cytokine storm or B-cell aplasia.
160. The method of any one of Claims 132-159, wherein the method further comprises administering to the subject a prodrug.
161. The method of Claim 160, wherein the prodrug is Erbitux, Herceptin, Ganciclovir, FK506 or a chemical inducer of dimerization.
162. The method of any one of claims 132-161, wherein the subject is suffering from refractory and relapsed neuroblastoma and wherein the method comprises administering the cell of any one of Claims 66-94.

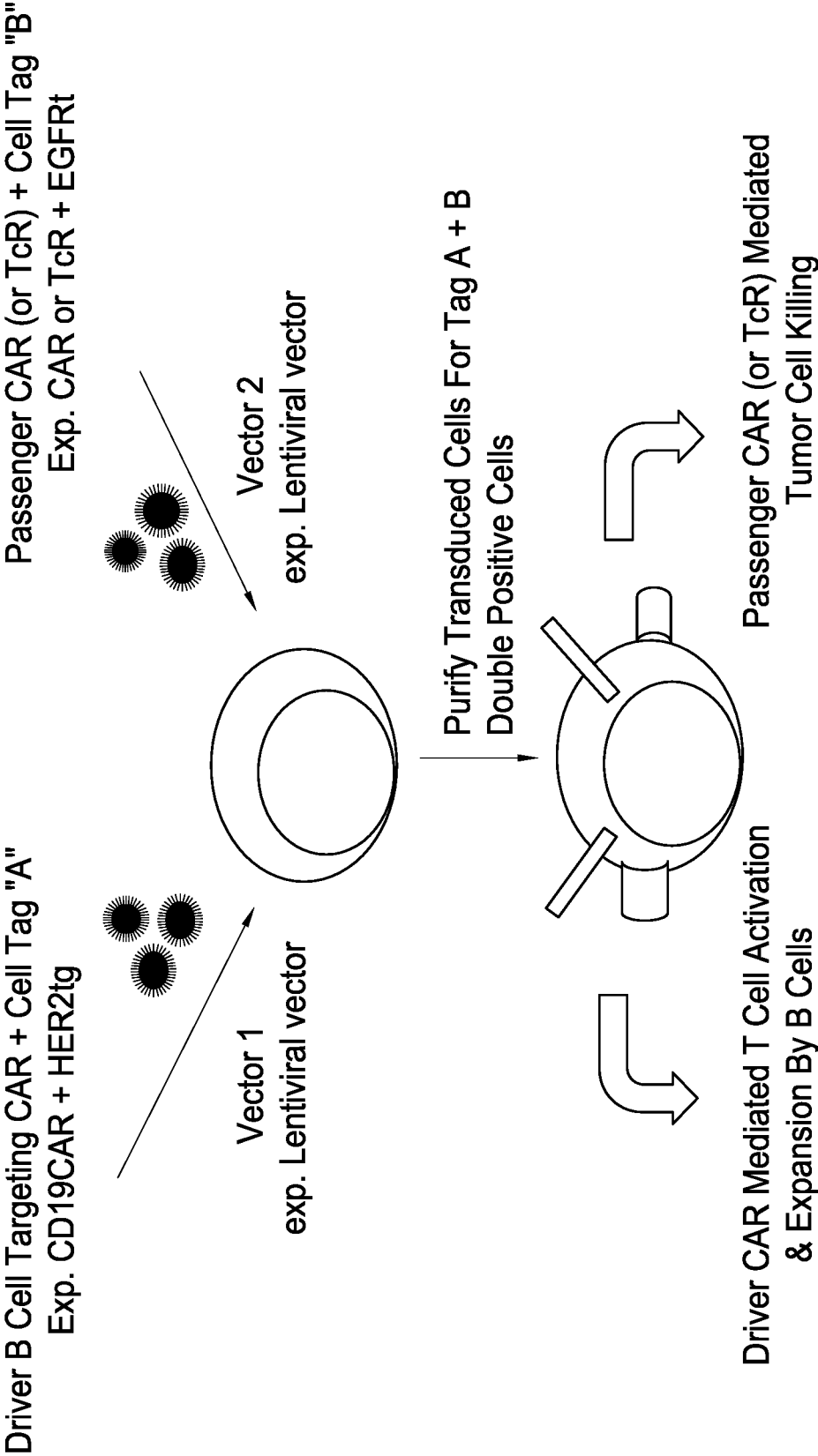


FIG. 1

Purpose: Render individual T cell bispecific

Model: CD19⁺ CD20⁺ malignancies

CD19CAR = short IgG4hinge ; CD20CAR = Medium IgG4hinge-CH3

CAR 941BB-ζ)-redirected CD4 or CD8 T cells

CD19CAR-T2A-Her2tG

CD19CAR-T2A-EGFRt

CD20CAR-T2A-EGFRt

CD19CAR-T2A-Her2tG/CD20CAR-T2A-EGFRt

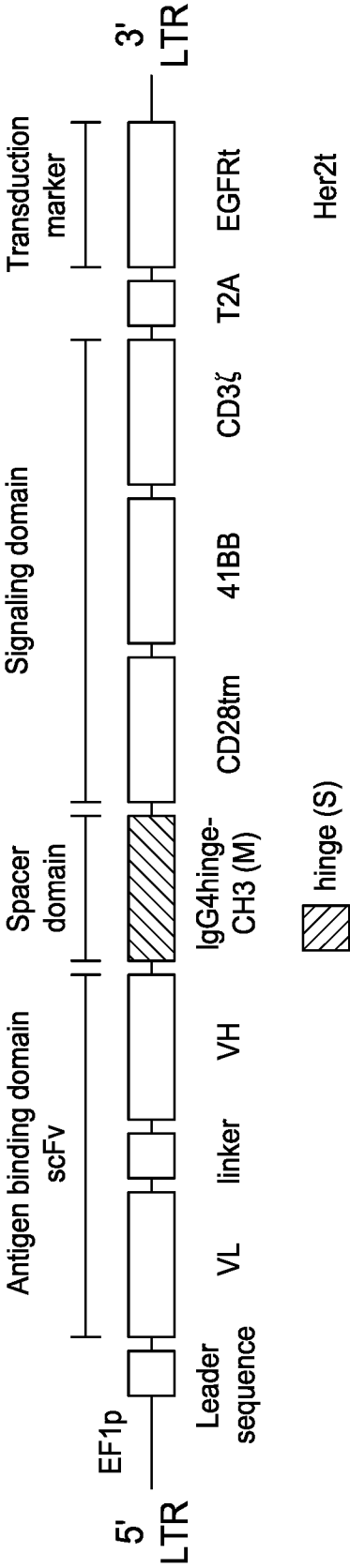
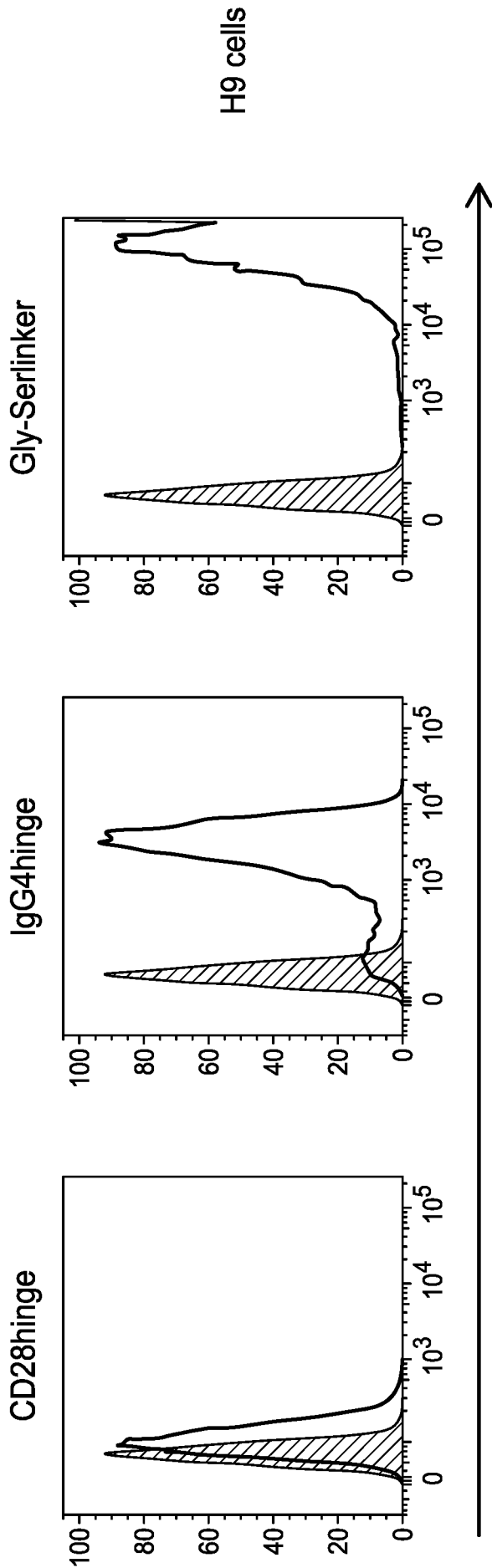


FIG. 2



CD4 or CD8 isolation
CD3/CD28 stimulation

FIG. 3A

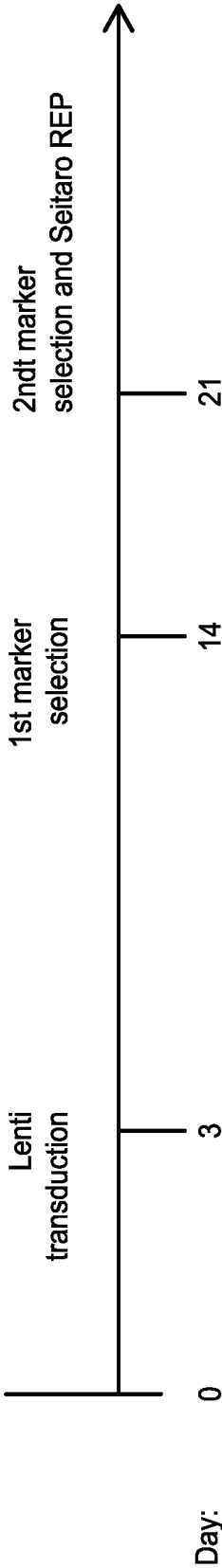


FIG. 3B

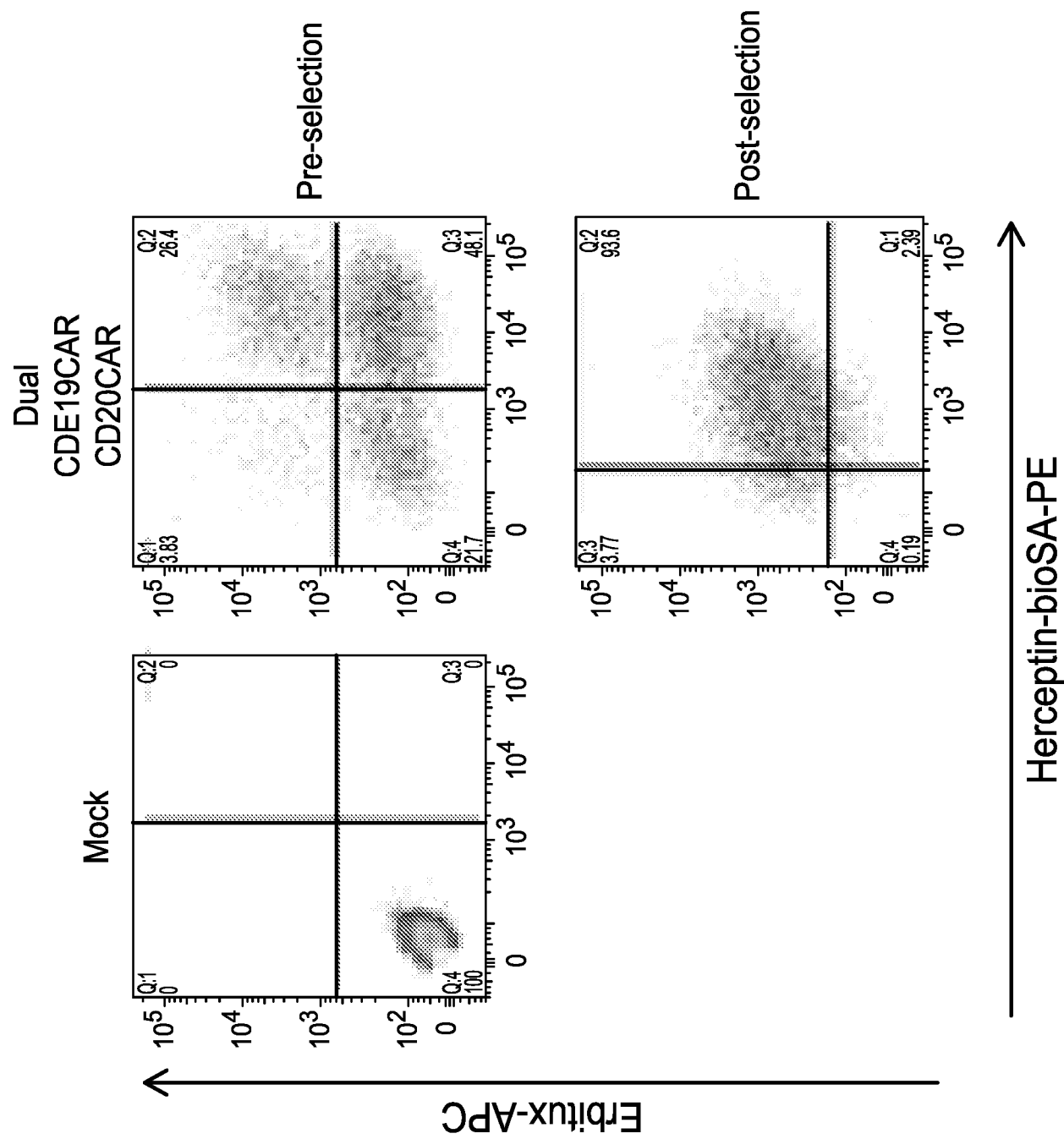


FIG. 3C

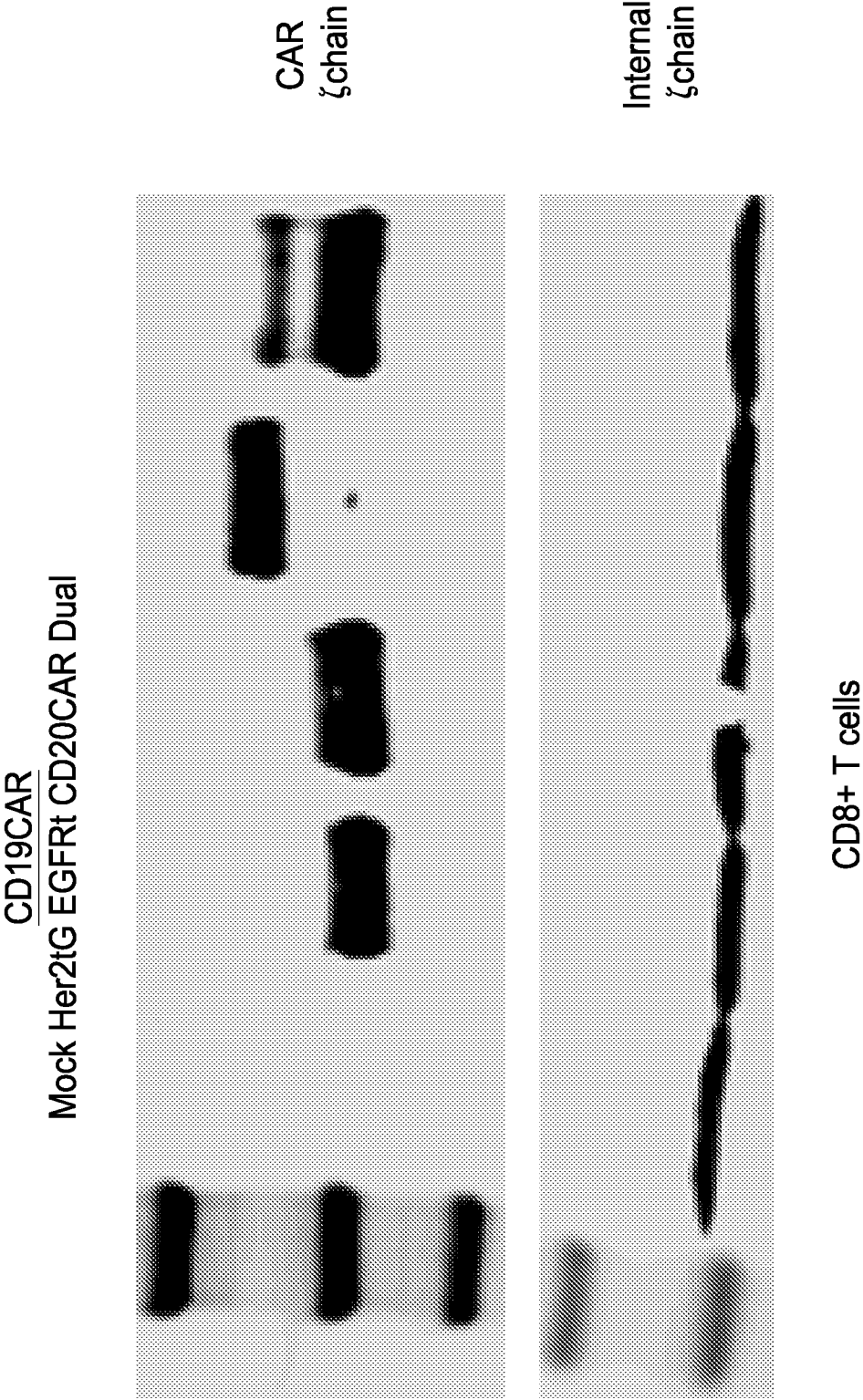


FIG. 3D

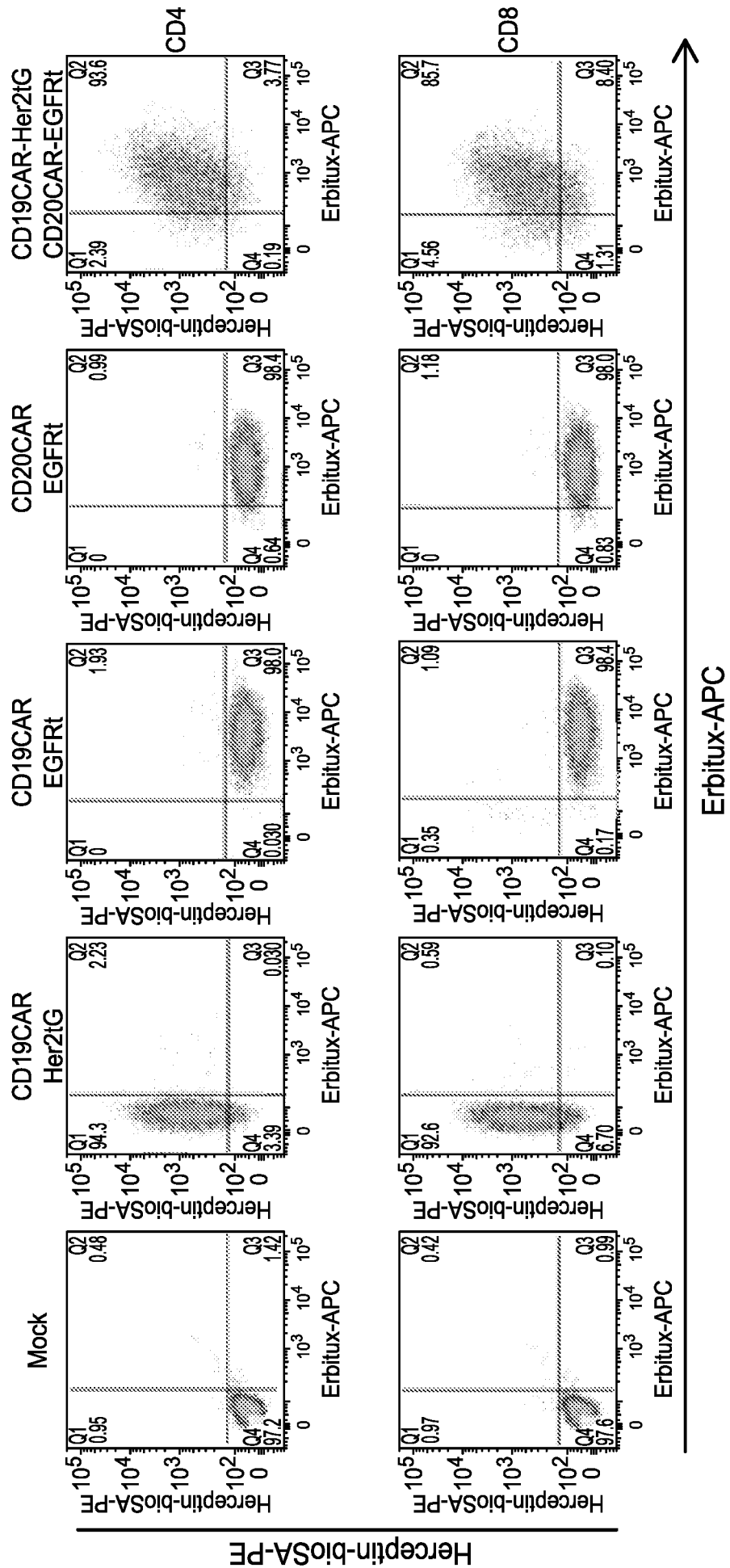


FIG. 4

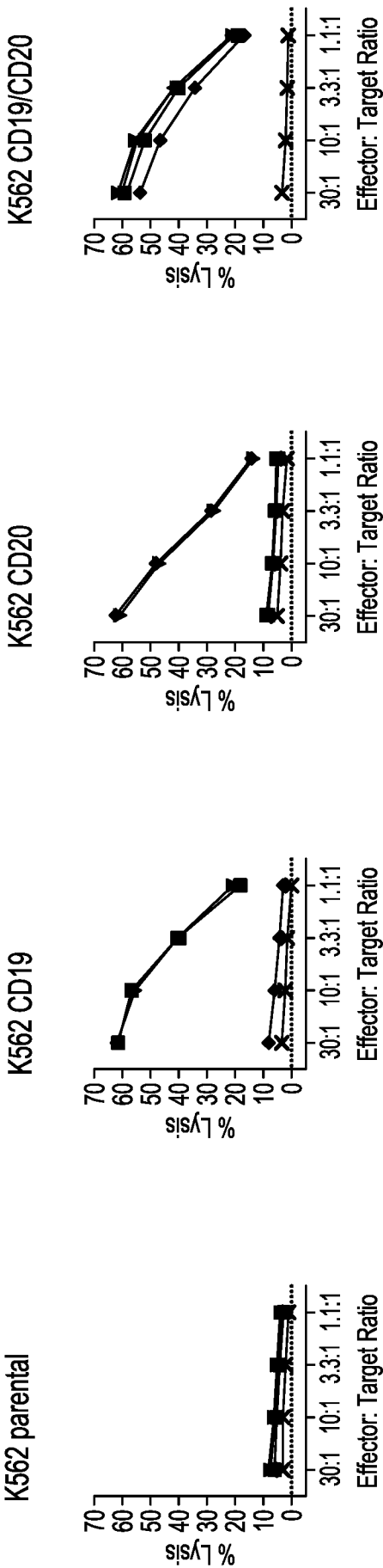


FIG. 5A

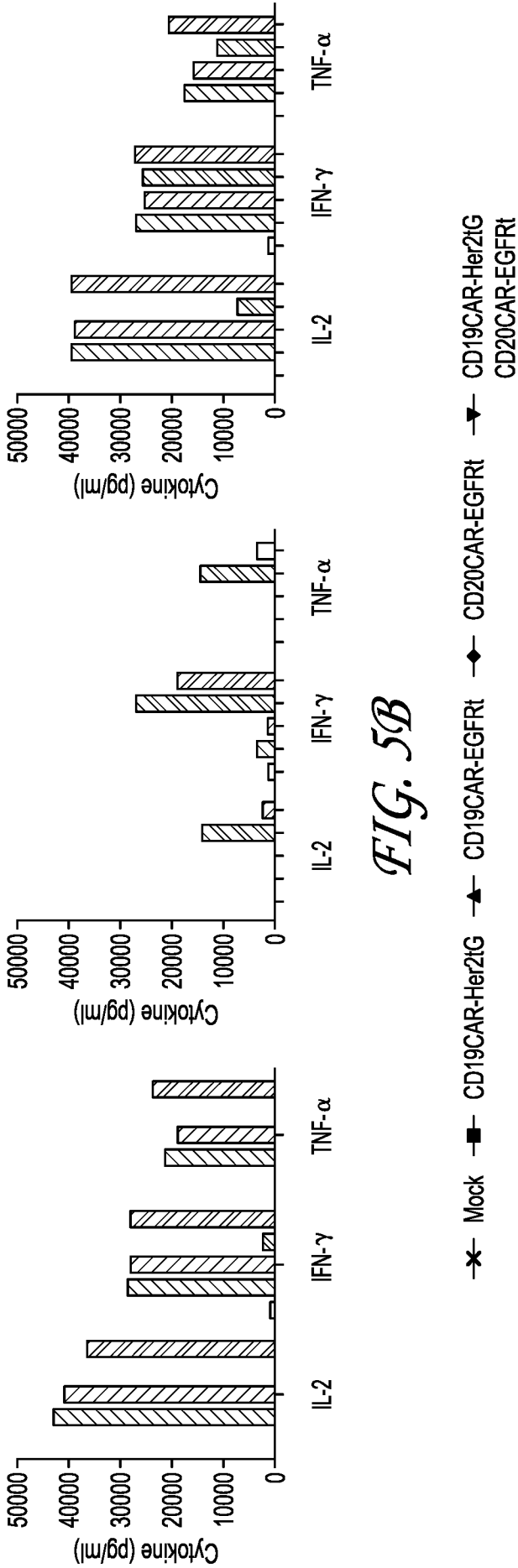
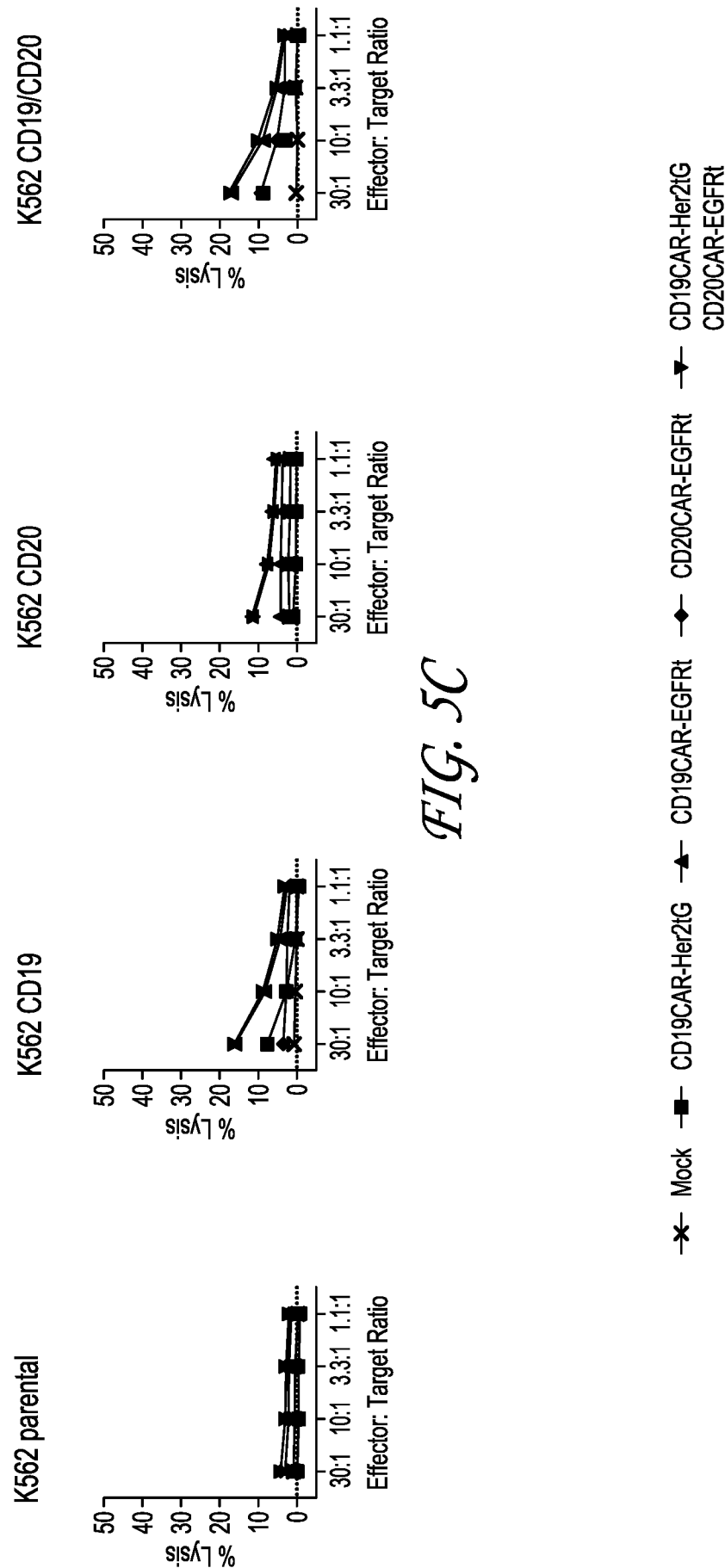


FIG. 5B



9/27

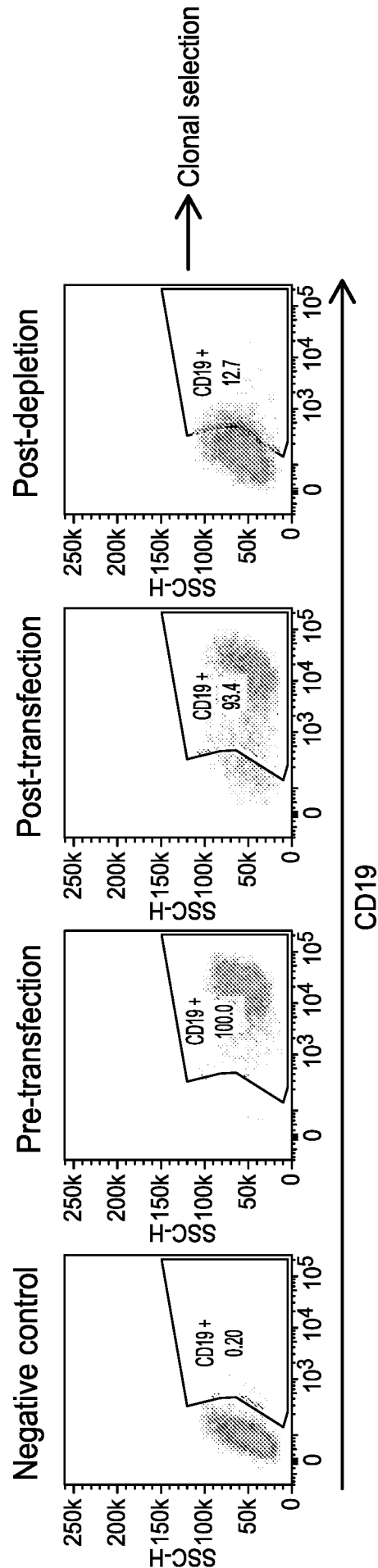
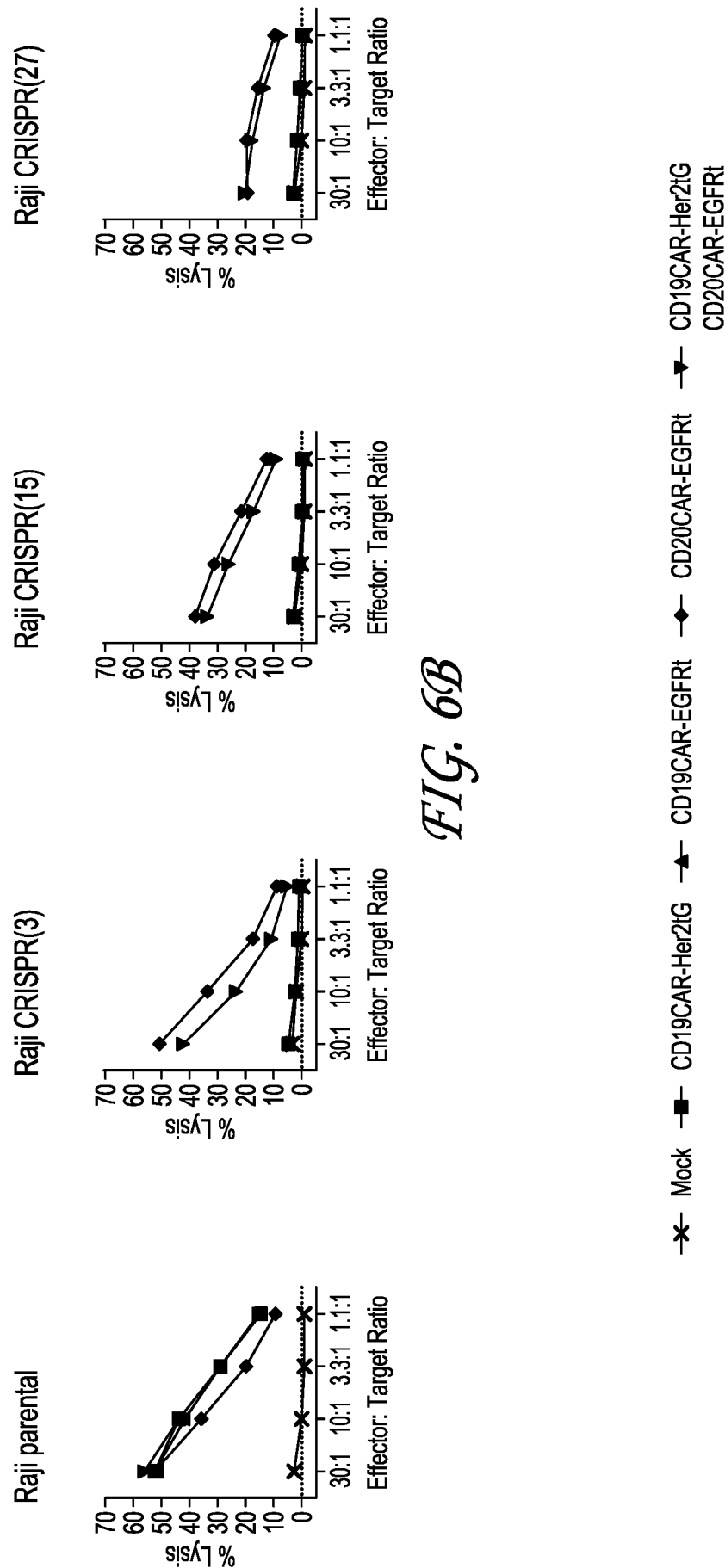


FIG. 6A



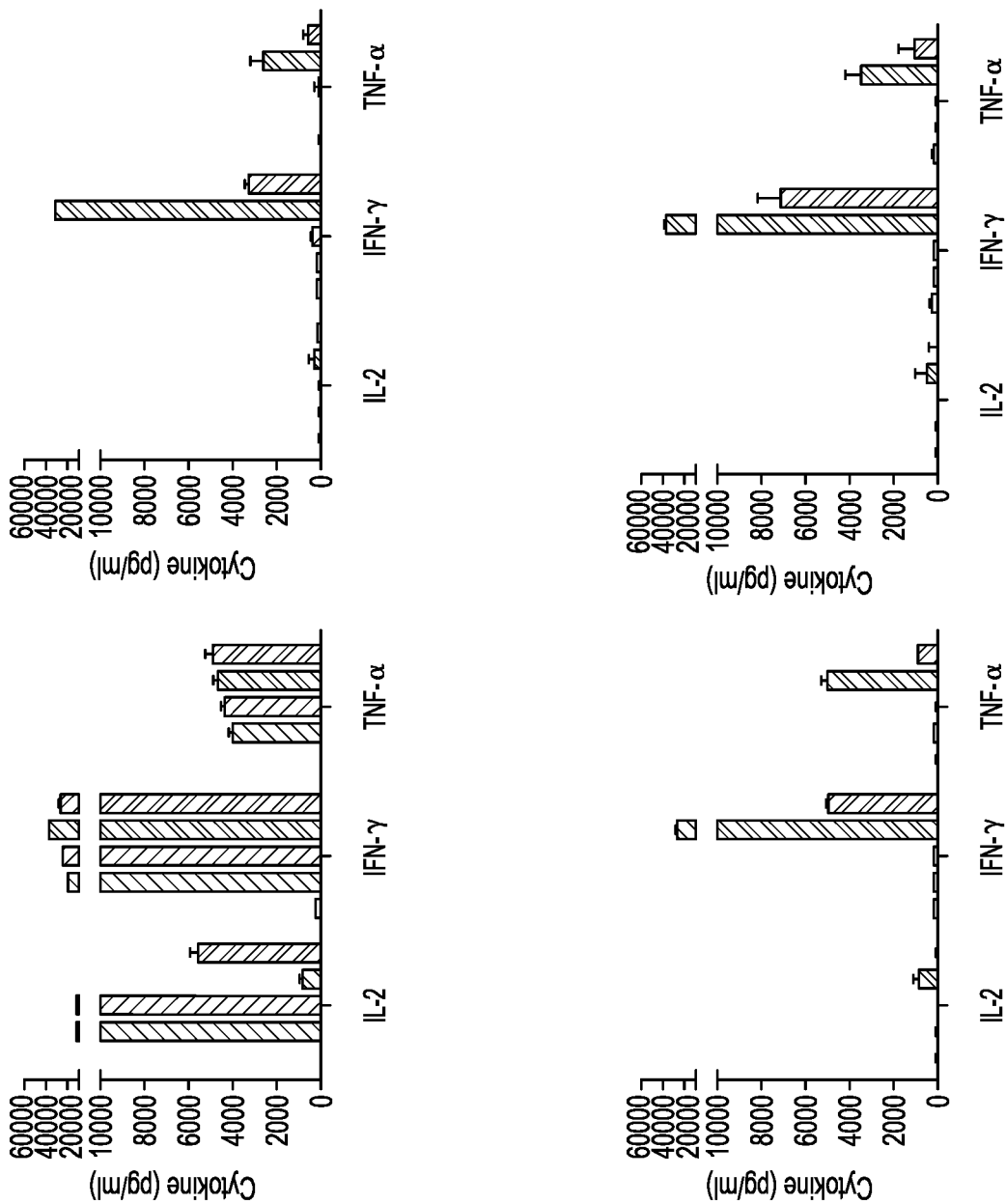


FIG. 6C

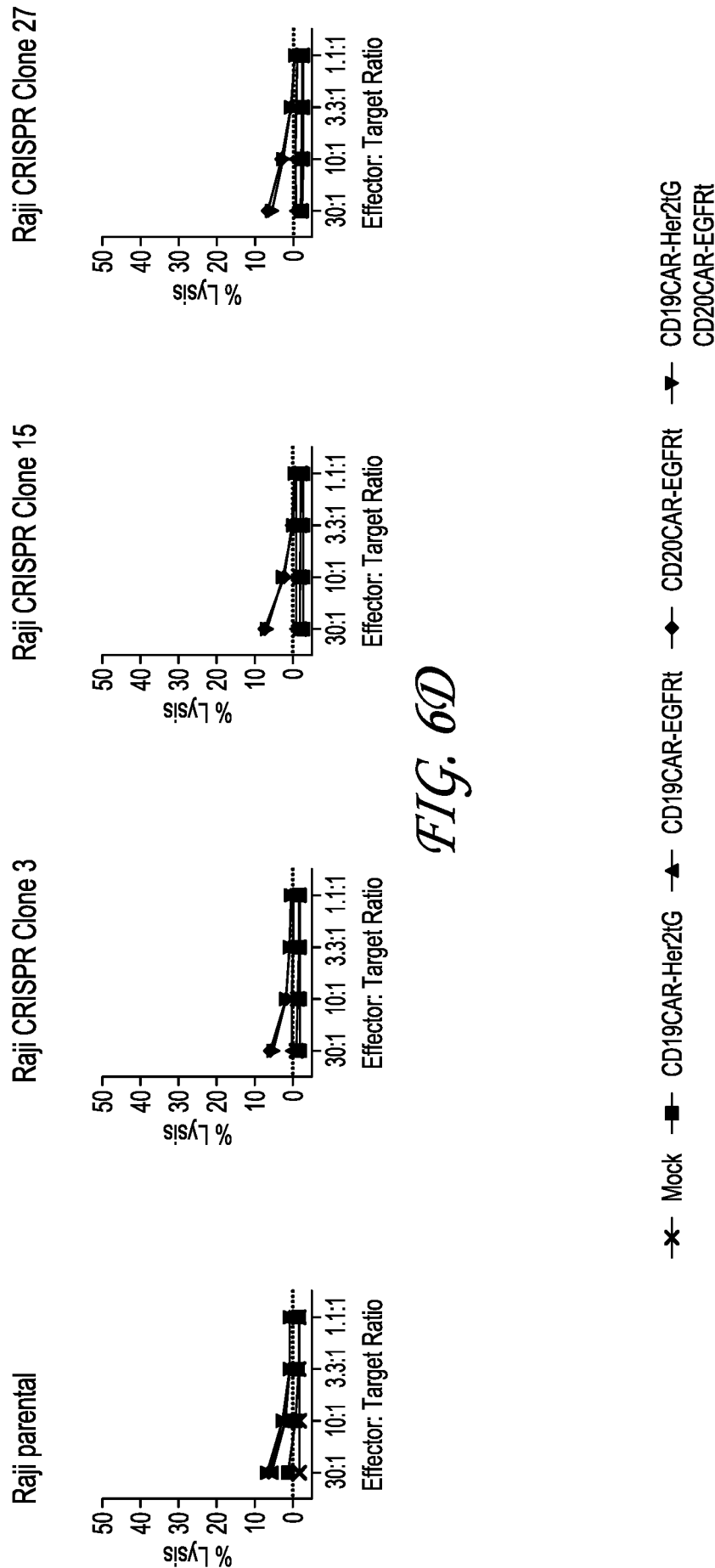


FIG. 6D

[illegible]

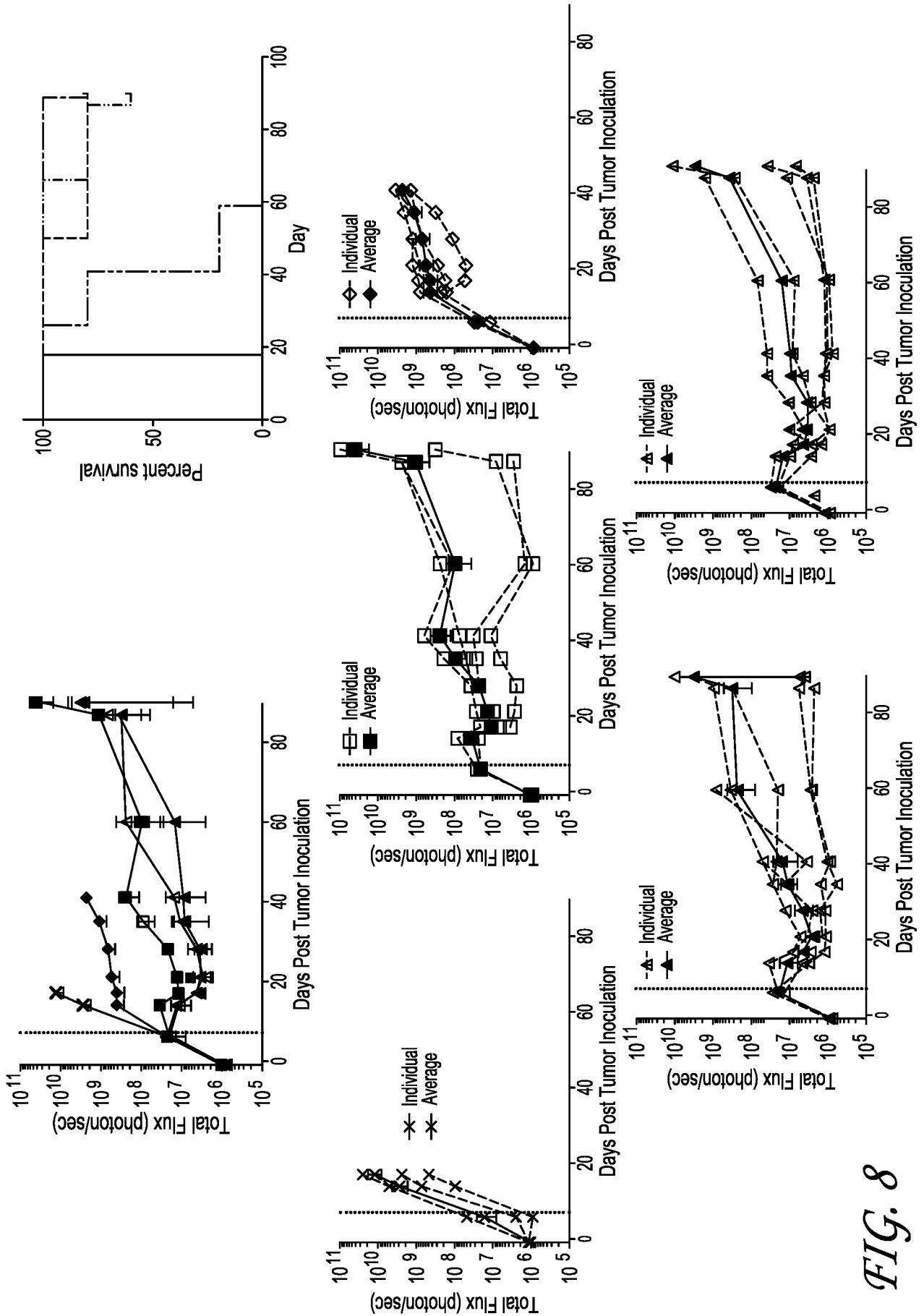


FIG. 8

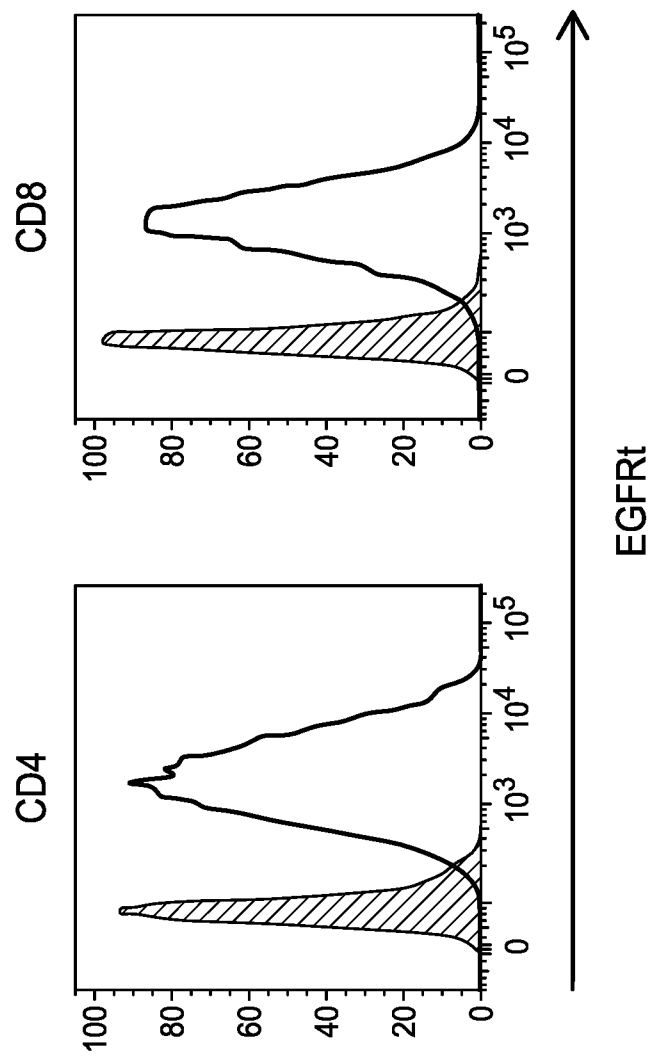


FIG. 9A

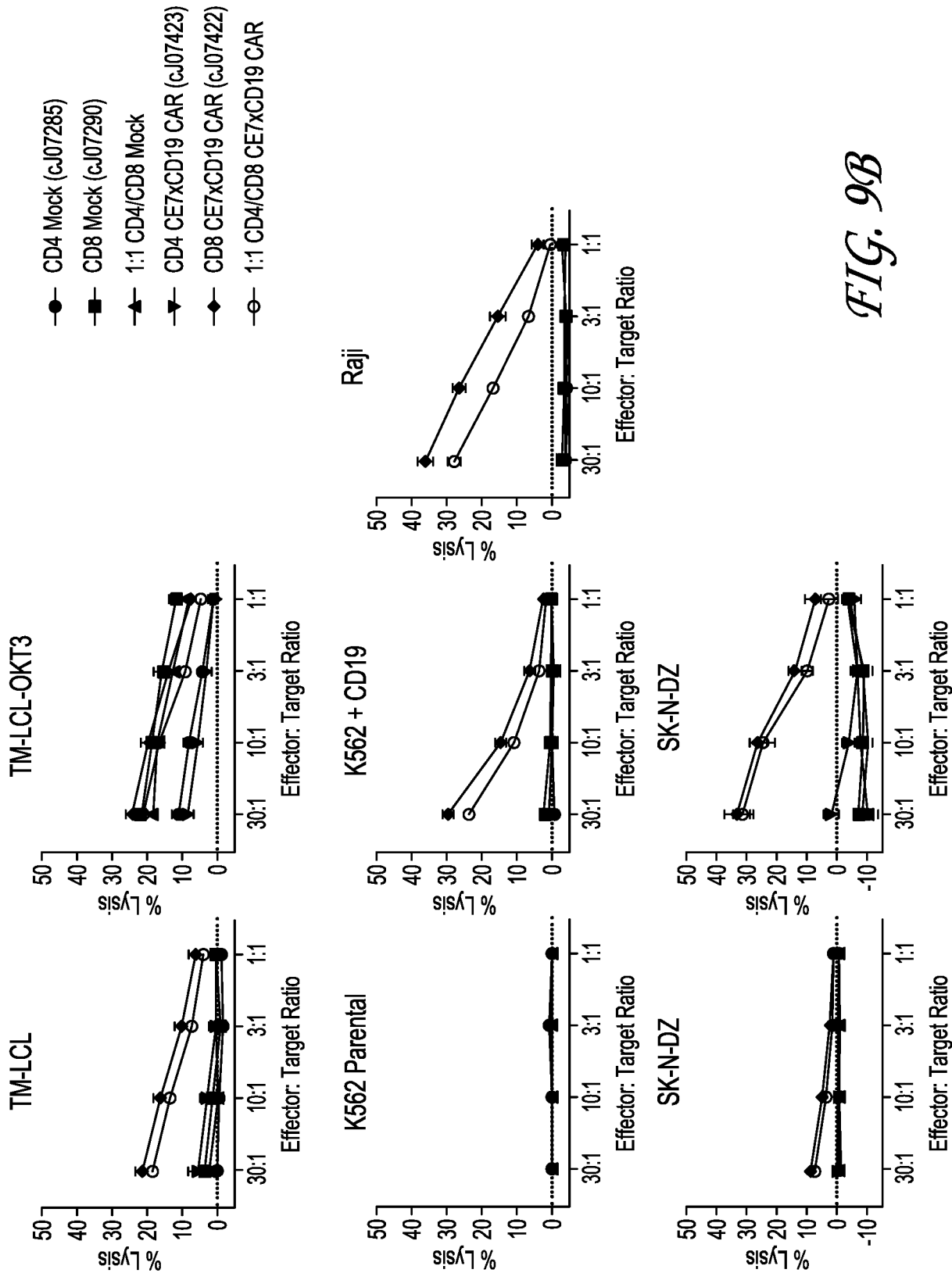
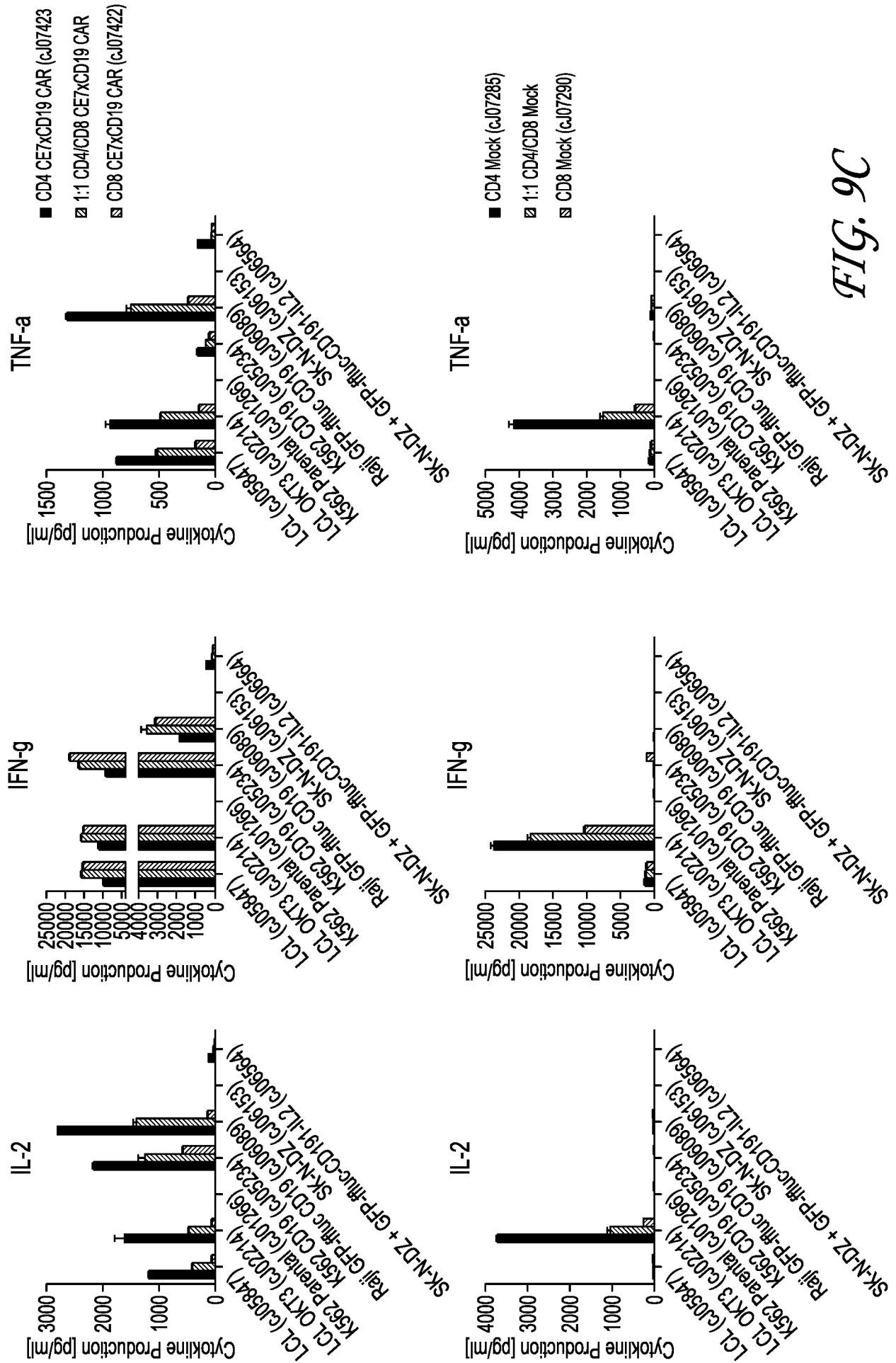


FIG. 9B



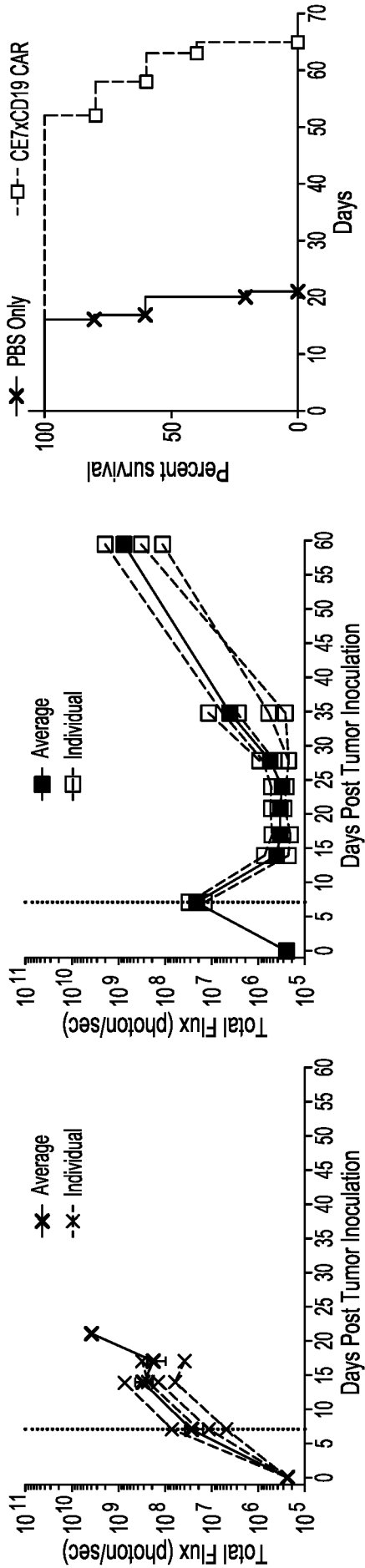


FIG. 9D

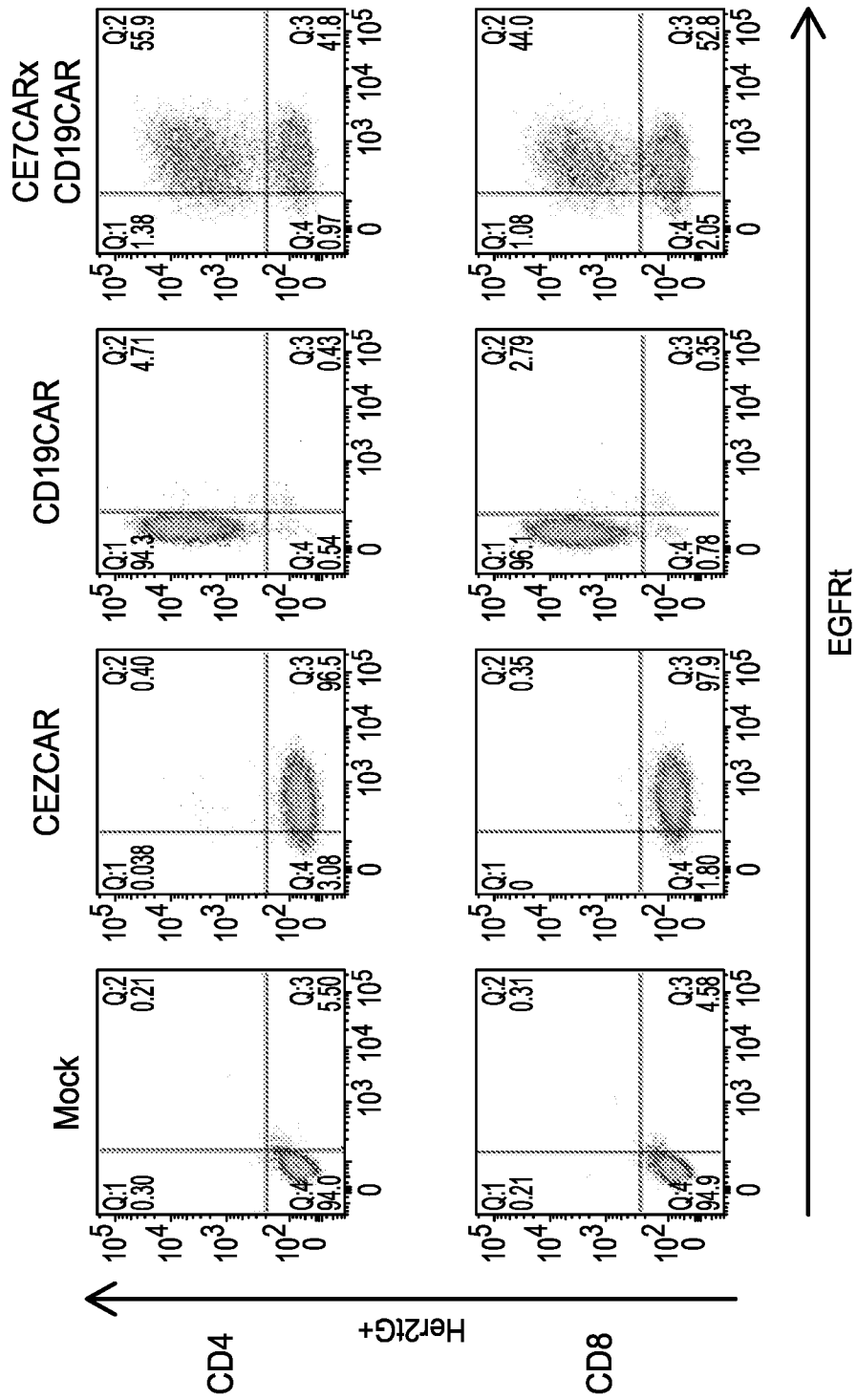


FIG. 10A

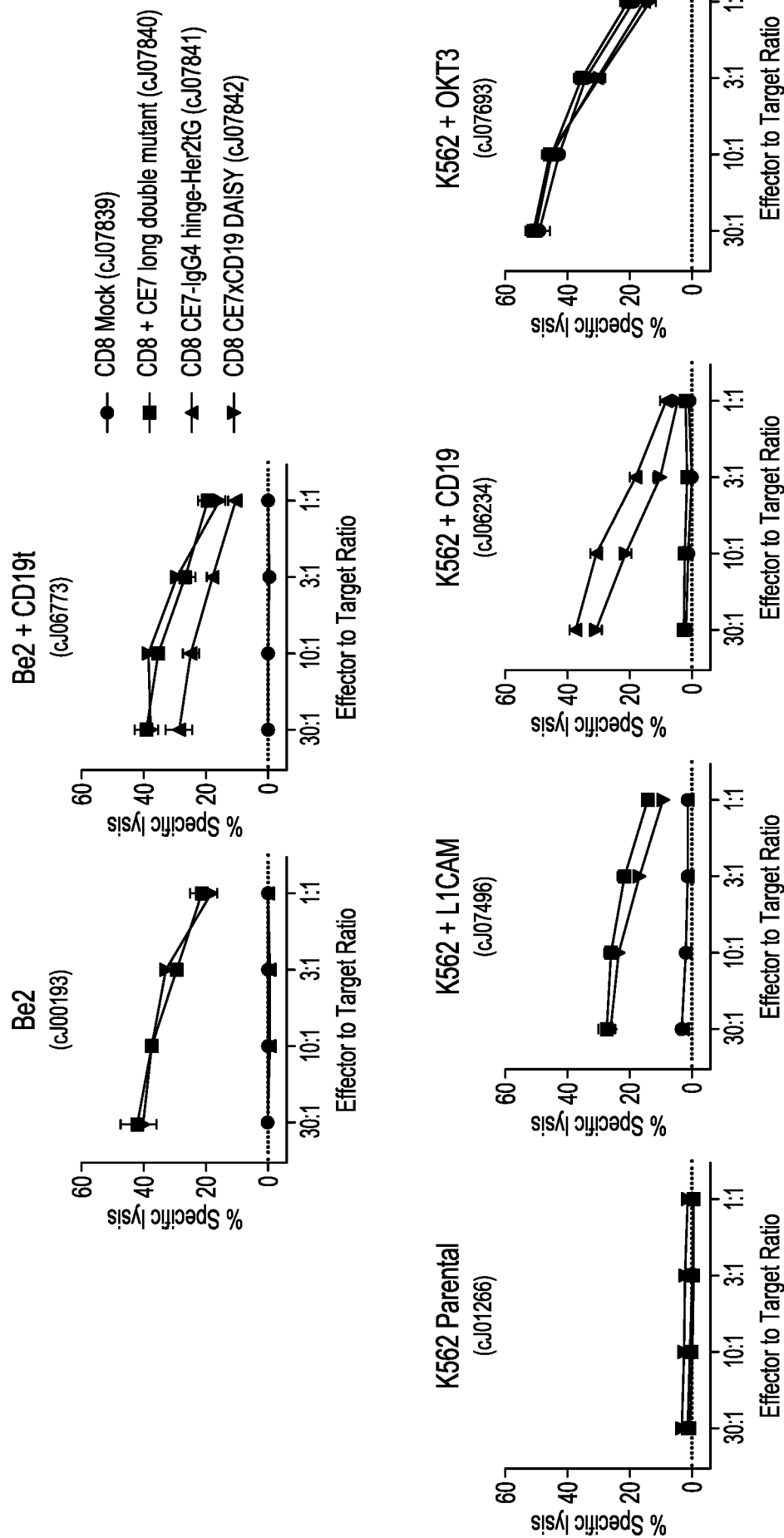


FIG. 10B

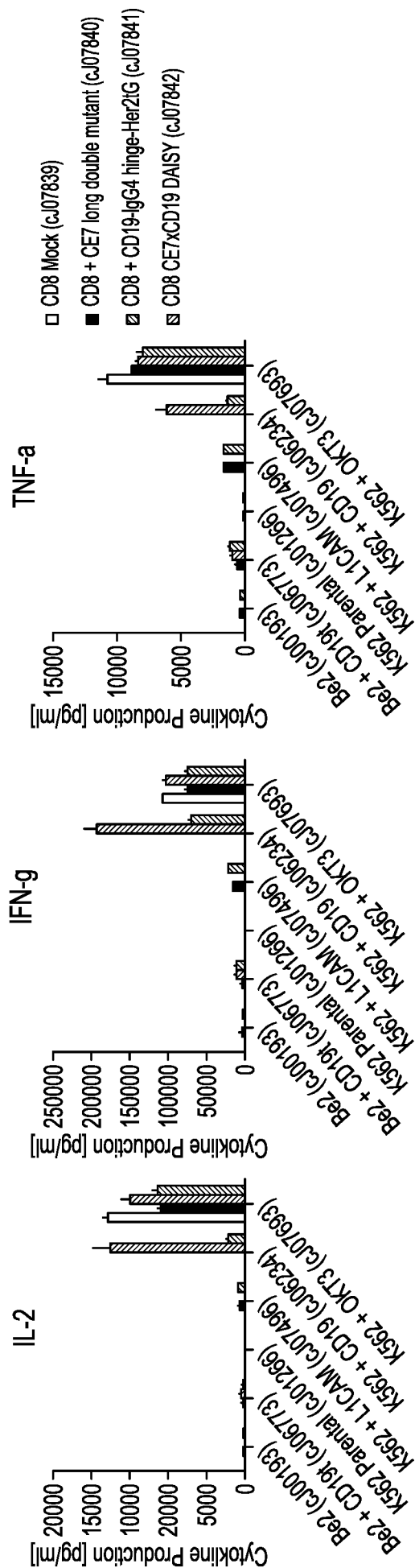
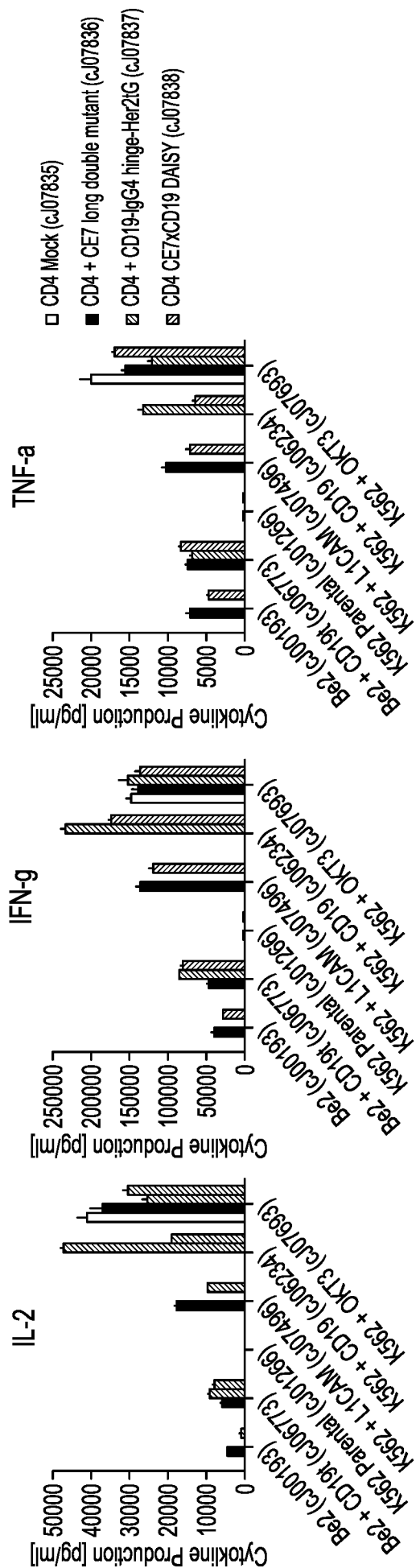


FIG. 10C

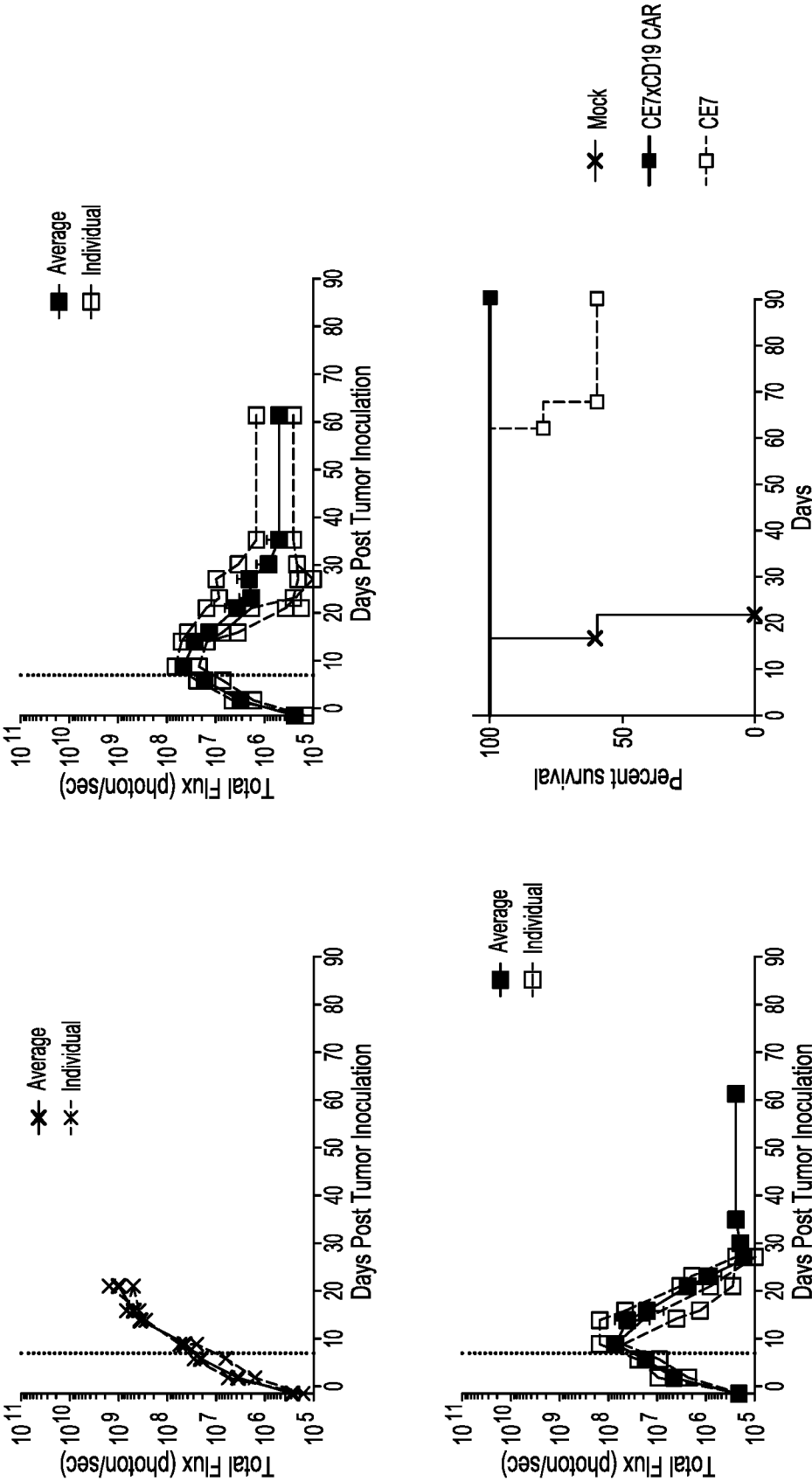


FIG. 10D

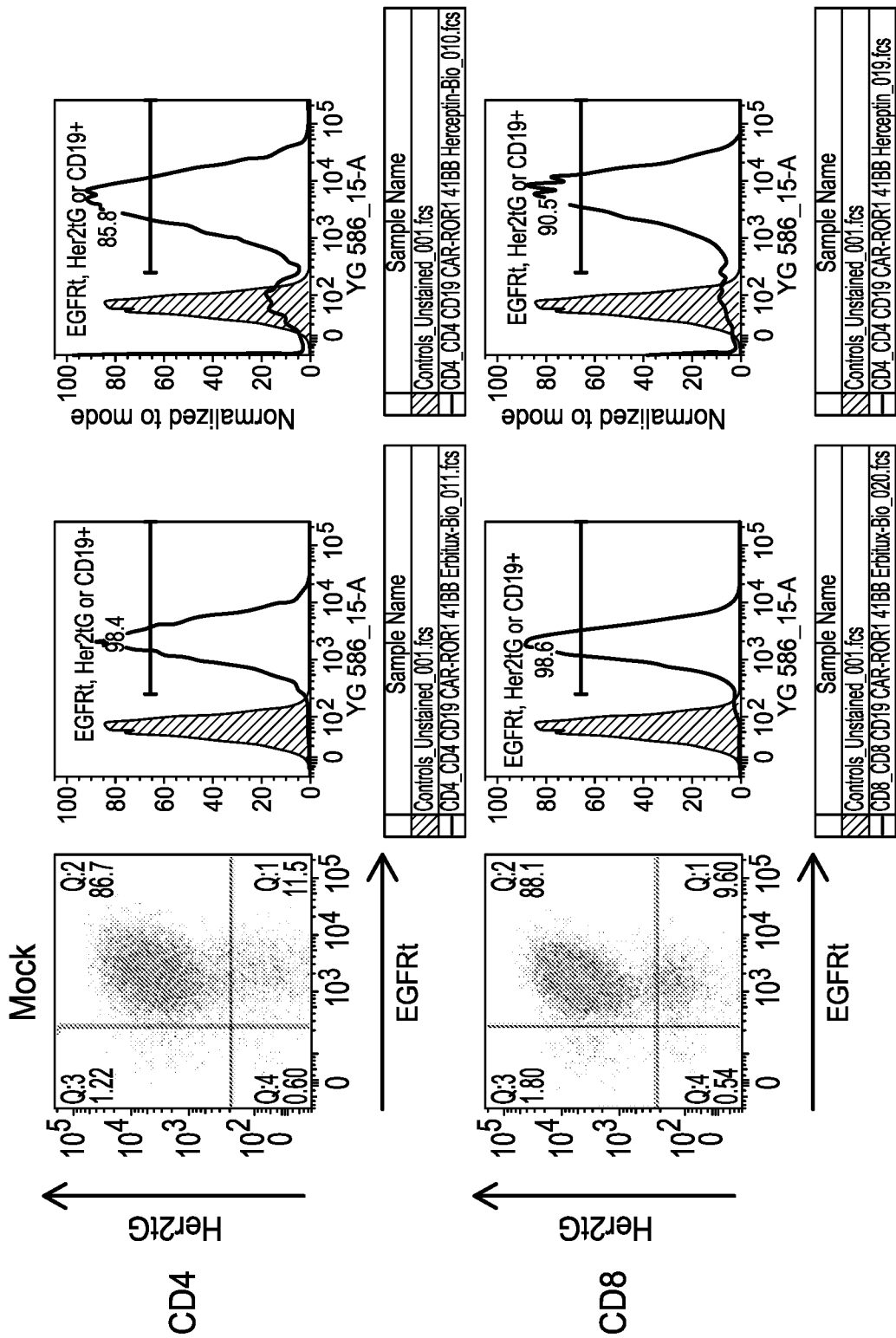


FIG. 11A

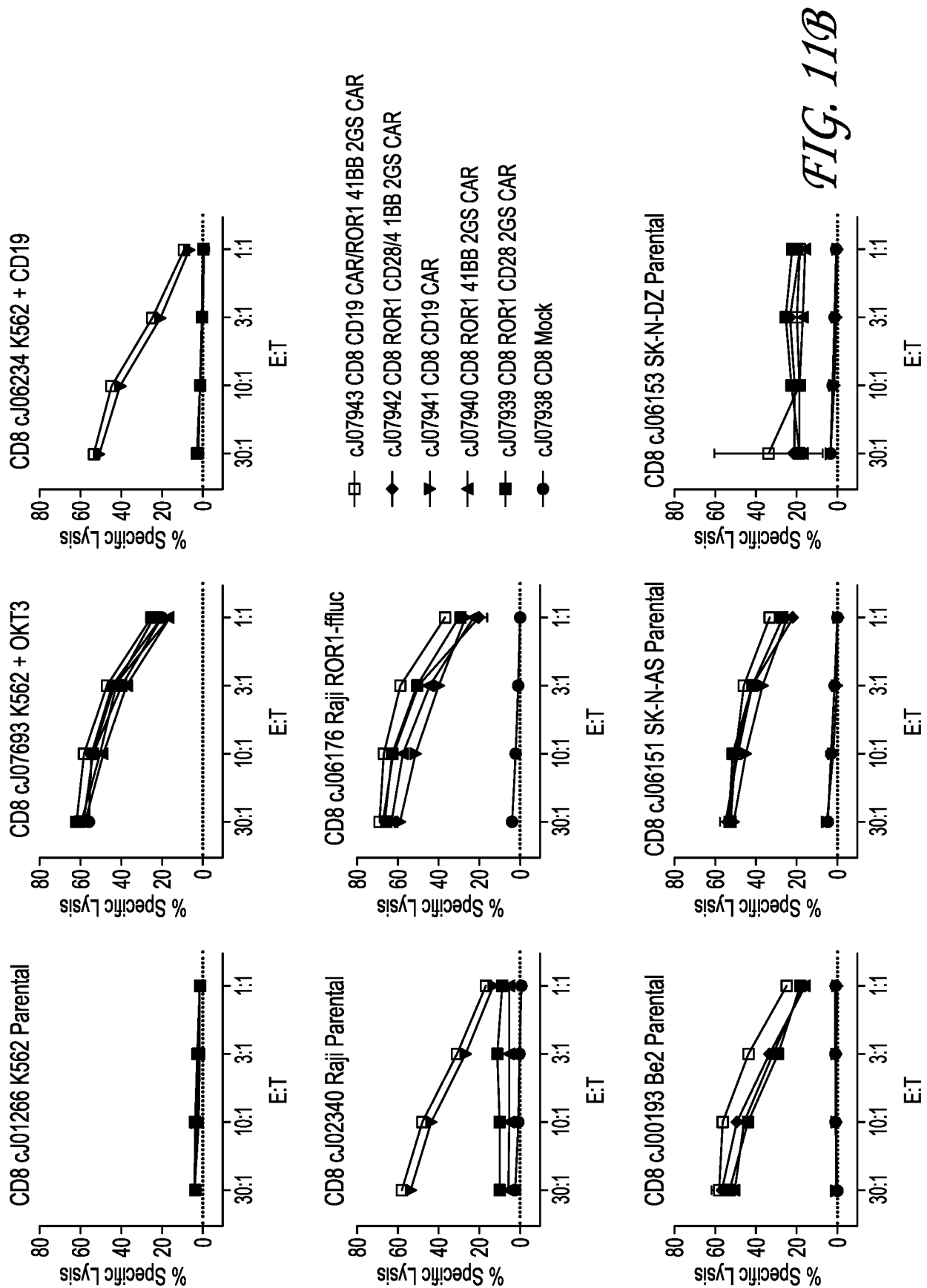
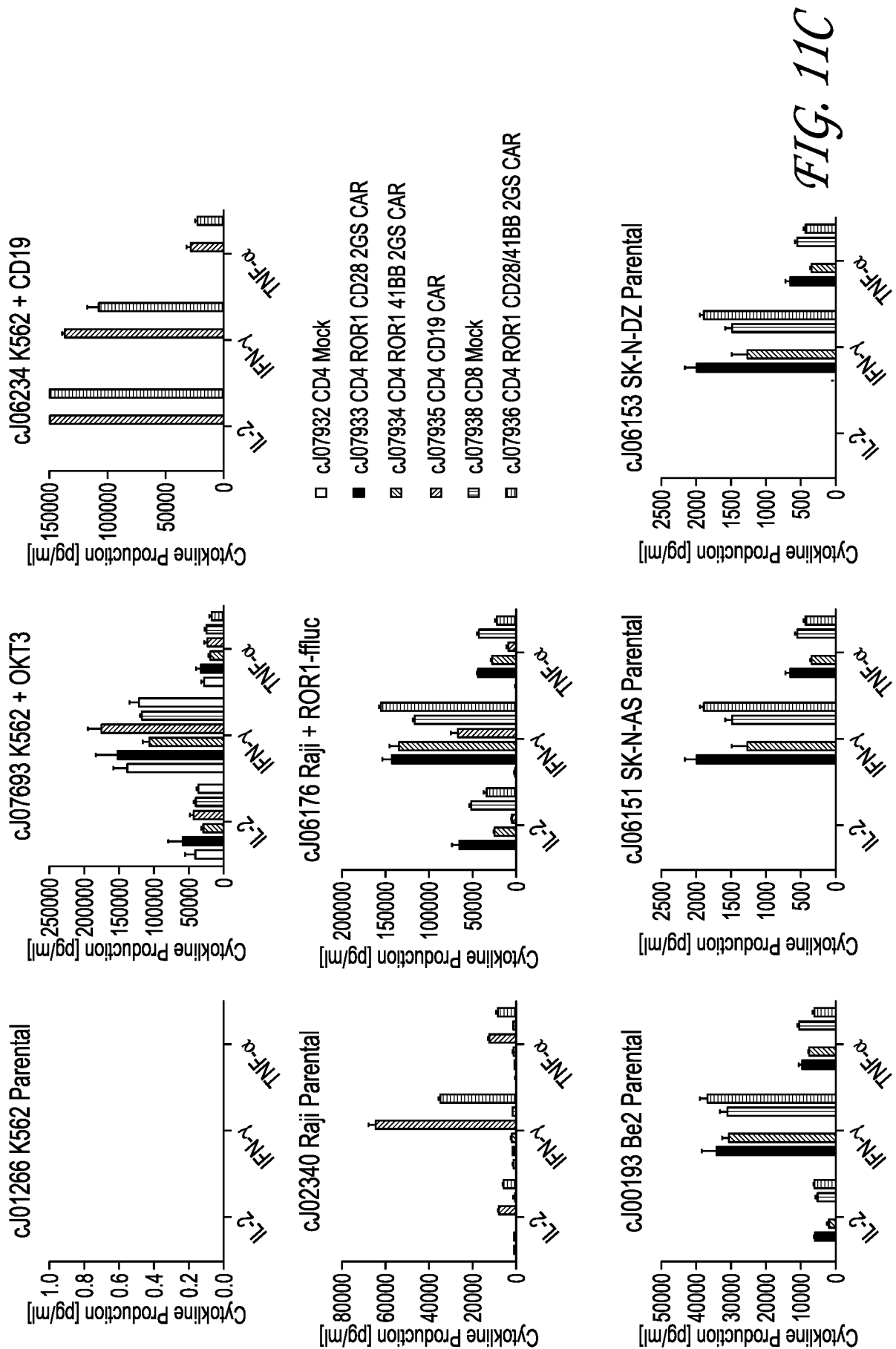
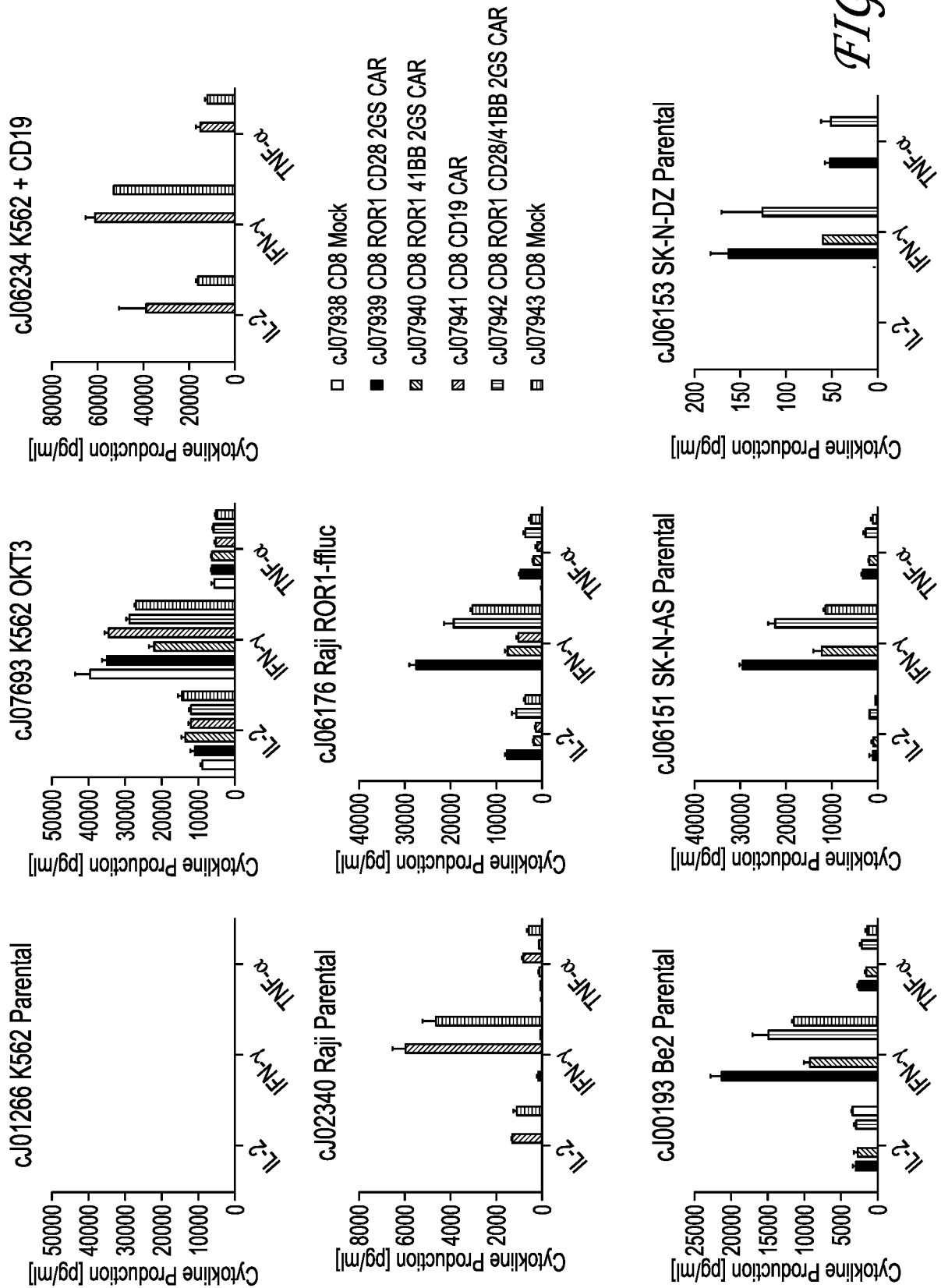


FIG. 11B





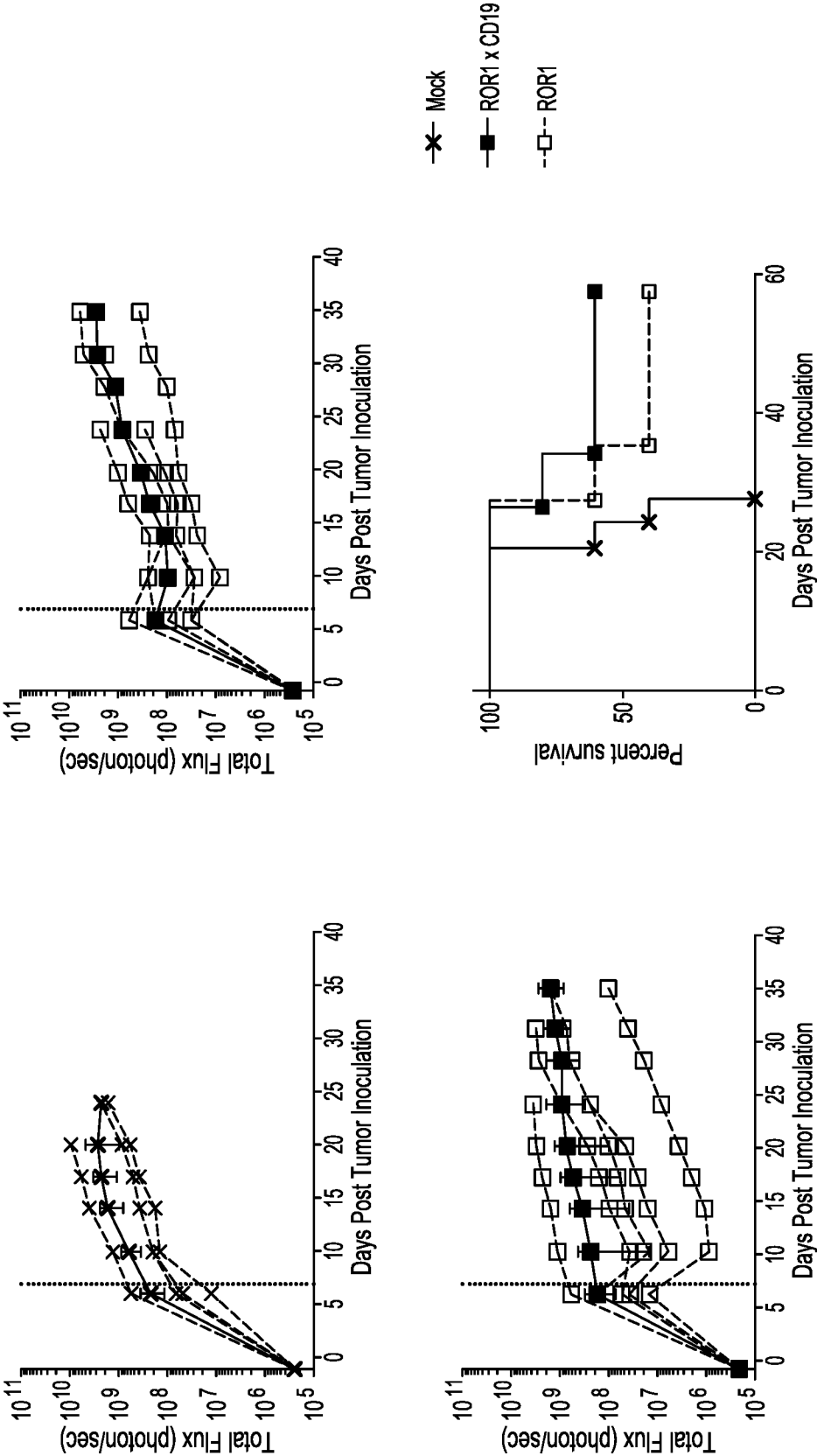


FIG. 11E

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/45360

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/30, C07K 14/705, A61K 38/17, A61K 35/12, A61K 39/395 (2016.01)

CPC - A61K 35/12, A61K 38/177, C07K 14/705, C07K 2317/565, C07K 2319/00, C07K 16/2878

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)

IPC(8) - C07K 16/30, C07K 14/705, A61K 38/17, A61K 35/12, A61K 39/395 (2016.01)

CPC - A61K 35/12, A61K 38/177, C07K 14/705, C07K 2317/565, C07K 2319/00, C07K 16/2878

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

CPC - C07K 2317/24, C07K 2317/622, C07K 2317/92, C07K 2319/30

USPC - 424/134.1, 435/320.1, 435/328, 435/455, 530/387.3, 536/23.4

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

PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: leader sequence, signal sequence, Bispecific T-cell engagers, BiTEs, B cell malignancies, chronic lymphocytic leukemia, CLL, B cell non-Hodgkin lymphoma, hairy cell leukemia (HCL), dual specificity, combinatorial/trans-signaling, inducible promoter. GenCore 6.4.1: SEQ ID NO: 33, 34

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2015/066551 A2 (FRED HUTCHINSON CANCER RESEARCH CENTER, et al.) 7 May 2015 (07.05.2015) claims 12, 67; para [0007], [0016], [0031], [0034], [0035], [0125], [0199]; SEQ ID NO:88, 89	1, 3, 5, 7/(1,3,5), (8-9)/7/(1,3,5), 10
Y	WO 2015/105522 A1 (FORMAN, et al.) 16 July 2015 (16.07.2015) claim 21	1-10
Y	US 2014/0271635 A1 (Brogdon, et al.) 18 September 2014 (18.09.2014) claims 1, 67; para [0012], [0059], [0067], [0199], [0257], [0258], [0291]	2, 4, 6, 7/(2,4,6), (8-10)/7/(2,4,6)
Y	Jensen, et al. Designing chimeric antigen receptors to effectively and safely target tumors. Curr Opin Immunol. April 2015, 33:9-15; Abstract, pg 2 to pg 3 [according to the posted document], pg 5, 1 st full para	5, 6, 7/(5,6), (8-10)/7/(5,6)

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

15 November 2016

Date of mailing of the international search report

08 DEC 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/45360

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 11-65, 69-77, 81-94, 100-107, 119-125, 131-162
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I: claims 1-10, directed to a nucleic acid encoding a CAR.

Group II: claims 00-08, 78-80, directed to a cell comprising a CAR.

Group III: claims 95-99, 108-118, 126-130, directed to a method of making a cell having a CAR.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

***** See Supplemental Sheet to continue *****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/45360

Box IV. Lack of unity of invention:

Special Technical Features

The inventions of Group I do not include the shared or common technical feature of a cell comprising a CAR, as required by Group II, or a method of making thereof, as required by Group III.

The inventions of Groups II and III do not include the shared or common technical feature of a nucleic acid encoding a de-immunized extracellular spacer, as required by Group I.

The inventions of Group II do not include the shared or common technical feature of a method of making a cell having a CAR, as required by Group III.

Common Technical Features

The inventions of Groups I-III share the technical feature of a CAR specific for a B-cell surface antigen. The inventions of Groups I and III share the technical feature of a nucleic acid encoding CAR specific for a B-cell surface antigen. The inventions of Groups II and III share the technical feature of a cell comprising a CAR. However, these shared technical features do not represent a contribution over prior art as being anticipated by WO 2015/066551 A2 to FRED HUTCHINSON CANCER RESEARCH CENTER, et al. (7 May 2015) (hereinafter "FHCRC").

FHCRC discloses a CAR specific for a CD19 antigen (para [0125], "each CD19 chimeric receptor can include a single chain variable fragment corresponding to the sequence of the CD19-specific mAb FMC63 (scFv: VLVH), a spacer derived from IgG4-Fc including either the 'Hinge-CH2-CH3' domain (229 AA, long spacer) or the 'Hinge' domain only (12 AA, short spacer), and a signaling module of CD3[zeta] with membrane proximal CD28 or 4-1BB costimulatory domains, either alone or in tandem (FIG. 13A). The transgene cassette can include a truncated EGFR (tEGFR) downstream from the chimeric receptor gene and be separated by a cleavable T2A element, to serve as a tag sequence for transduction, selection and in vivo tracking for chimeric receptor-modified cells"); a nucleic acid encoding thereof (para [0007], "FIG. 1. Nucleotide sequence of anti-CD19 short spacer chimeric receptor, GMCSFRss-CD19scFv-Ig G4hinge-CD28tm-41 BB-Zeta-T2A-EGFR1"; para [0034], "FIG. 28. CD19-chimeric receptor vectors. Design of lentiviral transgene inserts encoding a panel of CD19-specific chimeric receptors that differ in extracellular spacer length and intracellular co-stimulation. Each chimeric receptor encoded the CD19-specific single chain variable fragment derived from the FMC63 mAb in a VL-VH orientation, an IgG4-derived spacer domain of Hinge-CH2-CH3 (long spacer, 229 AA) or Hinge only (short spacer, 12 AA), and a signaling module containing CD3(with CD28 or 4-1BB alone or in tandem. Each chimeric receptor cassette contains a truncated EGFR marker encoded downstream of a cleavable 2A element"; para [0035], "FIG. 29A and 29B. Exemplary SIN lentiviral plasmids. FIG. 29A shows a SIN CD19 specific scFvFc-CD3 (CD28 CAR and huEGFRt lentiviral plasmid. FIG. 29B shows SIN CD19-specific scFv-4-1BB-CD3 (CAR and huEGFRt lentiviral plasmid"); and a cell comprising thereof (claim 12, "A non-T effector cell genetically modified to express a CAR of SEQ ID NO: 34").

FHCRC does not specifically disclose that CD19 is a B-cell surface antigen. However, said limitation is met by/inherently present in FHCRC (please see instant application, claim 67, "...wherein the ligand on B cell is.....CD19..."). As said technical features were known in the art at the time of the invention, they cannot be considered special technical feature that would otherwise unify the groups.

Some inventions of Groups I-III share the technical feature of a bi-specific CAR comprising a binding domain against a B-cell surface antigen. Some inventions of Groups II and III share the technical feature of a cell comprising a first and second CAR, wherein the first CAR is specific against a B-cell surface antigen, and a second CAR is specific for a ligand on a tumor. However, these shared technical features do not represent a contribution over prior art as being obvious over FHCRC, as above, in view of a paper titled "Designing chimeric antigen receptors to effectively and safely target tumors" by Jensen, et al. (Curr Opin Immunol. April 2015) (hereinafter "Jensen").

FHCRC discloses a CAR specific for a CD19 antigen(para [0125]) and that an antigen binding fragment of a CAR may include a bispecific antibody (para [0081], "Antibodies or antigen binding fragments can include ... bispecific antibodies..."), but does not provide a specific motivation to one of ordinary skill in the art to prepare a bi-specific CAR comprising a binding domain against a B-cell surface antigen or a cell comprising a first and second CAR, wherein the first CAR is specific against a B-cell surface antigen, and a second CAR is specific for a ligand on a tumor.

Such a motivation is provided by Jensen.

Jensen discloses that "[d]ual targeting with split receptor systems has been developed to achieve selective recognition of tumor cells where the target molecule on the tumor is also expressed on a subset of normal cells. One strategy is to co-express two CARs that operate as logic gates such that recognition of one target antigen is not sufficient to elicit a full T cell activation signal. This can be achieved by expressing a CAR specific for a molecule shared by the tumor and some normal cells but designed to have attenuated signaling by using an scFv of low affinity, an extracellular spacer that is suboptimal, or a CD3[zeta] construct that is mutated in one or more of the immune receptor tyrosine activation motifs (ITAMs). A second CAR specific for a target on the tumor but not the normal cells with the target for the activating CAR provides the full T cell activation signal and selective tumor recognition... These combinatorial systems, while not yet evaluated in the clinic, could allow for effective targeting of a greater number of targets that are expressed on solid tumors and a subset of normal tissues" (pg 5, 1st full para) and that "[t]umor escape from CAR-T cells can occur by the selective outgrowth of antigen loss variants and might be overcome by targeting two different tumor associated molecules. It is feasible to express two ligand binding domains in tandem separated by a flexible linker, or as single CAR constructs in the same or different T cells [15]. The expression of scFvs in tandem is appealing and has been shown to work in principle" (pg 2, last para to pg 3, 1st para; see also Abstract, "Clinical trials in patients with advanced B cell malignancies treated with CD19-specific CAR-modified T cells (CAR-T) have shown impressive antitumor efficacy, leading to optimism that this approach will be useful for treating common solid tumors... The ability to introduce or delete additional genes in T cells has the potential to provide therapeutic cell products with novel attributes that overcome impediments to immune mediated tumor elimination in immunosuppressive tumor microenvironments").

It would have been obvious to one of ordinary skill in the art at the time of the invention to combine, in the course of routine experimentation and with a reasonable expectation of success, FHCRC and Jensen by preparing a bi-specific CAR comprising a binding domain against a B-cell surface antigen and a cell comprising a first and second CAR, wherein the first CAR is specific against a B-cell surface antigen, and a second CAR is specific for a ligand on a tumor, to improve efficiency of anti-CD19 CAR immunotherapy disclosed by FHCRC, as suggested by Jensen (pg 5, 1st full para; pg 2, last para to pg 3, 1st para). As said technical features would have been obvious to one of ordinary skill in the time of the invention, they cannot be considered special technical feature that would otherwise unify the groups.

Groups I-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.