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(54) Title: CHIMERIC ANTIGEN RECEPTORS WITH MYD88 AND CD40 COSTIMULATORY DOMAINS

(57) Abstract: The application relates to a chimeric antigen receptor that targets Eph receptors and allows activation of co-stimulatory pathways. The application also relates to polynucleotides that encode the chimeric antigen receptor, vectors, and host cells comprising the chimeric antigen receptor. The application also relates to methods for preparing host cells comprising a chimeric antigen receptor in order to improve the *in vivo* effector function of the chimeric antigen receptor host cells.

CHIMERIC ANTIGEN RECEPTORS WITH MYD88 AND CD40 COSTIMULATORY DOMAINS

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0001] This invention was made with government support under grant number CA173750 awarded by the National Institutes of Health. The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority to U.S. Provisional Application No. 62/672,819, filed May 17, 2018, the disclosure of which is herein incorporated by reference in its entirety.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 16, 2019, is named 243734_000120_SL.txt and is 231,265 bytes in size.

FIELD

[0004] The application relates to chimeric antigen receptors (CARs), particularly CARs with MyD88 and CD40 costimulatory domains. The application further relates to methods for activating T cells and other lymphocytes resulting in an immune response against a target antigen. The application further relates to therapeutic cells that express said CARs and methods for treating patients using the modified therapeutic cells.

BACKGROUND

[0005] Chimeric antigen receptors (CARs) co-express a ligand recognition domain specific for a disease (*e.g.*, tumor) antigen with signaling components that trigger T cell activation and killing of the target cell. Initially, CARs incorporated an anti-tumor specific single chain variable fragment (scFv) linked to the CD3ζ endodomain, and showed limited *in vivo* longevity and anti-tumor efficacy. CARs, incorporating cytoplasmic signaling domains from T cell co-stimulatory molecules such as CD28 and 4-1BB, to improve the longevity and tumoricidal activity in preclinical models and clinical trials. Despite this improvement, the adoptive transfer of CAR T cells still have limited antitumor activity to treat, for example, solid tumors, thus leading unfortunately to limited clinical success (Park et. al., *Blood*, 2016;

127:3312-20; Beatty et al., *Pharmacol. Ther.* 2016; 166:30-39). This lack of efficacy is most likely due to several factors, including suboptimal CAR signaling, which may result in limited T cell activation in the solid tumor microenvironment. The majority of CARs encode an endodomain that consists of zeta (ζ) signaling domain and a canonical CD28 and/or 4-1BB co-stimulatory signaling domain.

[0006] The Eph receptor tyrosine kinases have been studied extensively for their roles in developmental processes. In humans, nine EphA (EphA1–8 and EphA10) and five EphB (EphB1–4 and EphB6) receptors have been identified. *See* Pitulescu and Adams, *Genes Dev.* 2010, 24(22):2480-2492, incorporated by reference herein in its entirety of all purposes. In recent years, Eph receptors have been found to play a widespread role in normal physiology and disease pathogenesis and to be integral players in cancer formation and progression. *See* Pasquale *Nat. Rev. Cancer* (2010) 10(3):165-180 and Xi et al., *J. Cell Mol. Med.* (2012) 16(12): 2894-2909, each incorporated by reference herein in its entirety of all purposes. In fact, multiple Eph receptors and/or ephrins are expressed in cancer cells and the tumor microenvironment, wherein they influence tumor properties and promote aberrant cell to cell communication within the tumor and beyond. *Id.* As an example, EphA2 is involved in the development and maintenance of many different types of solid tumors. *See,* Wykosky et al., *Mol. Cancer Res.* 2008 6(12): 1795-1806, which is incorporated herein by reference in its entirety for all purposes. In particular, EphA2 is overexpressed in at least breast, prostate, urinary bladder, skin, lung, ovary, sarcoma and brain cancers. *See,* Tandon et al., *Expert Opin. Ther. Targets* 2011 15(1):31-51, which is incorporated herein by reference in its entirety for all purposes. In preclinical models of breast cancer, EphA2 expression leads to increased tumor formation, progression, angiogenesis, and therapeutic resistance. *See,* Amato et al., *Cancer Discov.* 2014 4(6):OF14, which is incorporated herein by reference in its entirety for all purposes. Notably, EphA2 is commonly overexpressed in numerous cancers, including non–small cell lung cancer (NSCLC), in which EphA2 overexpression is associated with relapse and poor patient survival. As such, EphA2 cancers are associated with increased malignancy and high mortality rates. *See* Pasquale *Nat. Rev. Cancer* (2010) 10(3):165-180.

[0007] Thus, there exists a need to for effective therapy against solid tumors (e.g., those expressing Eph receptors). This need can be met with a CAR with enhanced ability to activate T cells as disclosed herein.

SUMMARY OF THE INVENTION

[0008] As specified in the Background section above, there is a great need in the art for CARs with enhanced ability to activate lymphocytes (e.g., induce greater and/or longer T cell activity) to allow for, for example, increased tumoricidal activity. The present application addresses these and other needs.

[0009] In one aspect, the invention provides a polynucleotide encoding a chimeric antigen receptor (CAR) comprising (a) an extracellular target-binding domain comprising an Eph receptor-binding moiety, (b) a transmembrane domain, and (c) a cytoplasmic domain comprising a lymphocyte activation domain, a MyD88 polypeptide or a functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof. In some embodiments, the MyD88 polypeptide comprises amino acid sequence SEQ ID NO:25, SEQ ID NO:30, SEQ ID NO: 31, or SEQ ID NO:33, or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 25, SEQ ID NO: 30, SEQ ID NO: 31, or SEQ ID NO: 33. In some embodiments, the MyD88 functional fragment comprises a MyD88 polypeptide lacking the Toll/Il-1 receptor domain. In some embodiments, the MyD88 functional fragment comprises amino acid sequence SEQ ID NO:15, SEQ ID NO:28, or SEQ ID NO:60 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:15, SEQ ID NO:28, or SEQ ID NO:60. In some embodiments, the nucleotide sequence encoding the MyD88 functional fragment comprises SEQ ID NO:16, SEQ ID NO:29, or SEQ ID NO: 61 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:16, SEQ ID NO:29, or SEQ ID NO:61. In some embodiments, the CD40 cytoplasmic polypeptide region or functional fragment thereof is derived from a CD40 polypeptide comprising the amino acid sequence SEQ ID NO:32 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:32. In some embodiments, the CD40 cytoplasmic polypeptide region comprises the amino acid sequence SEQ ID NO:17 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:17. In some embodiments, the nucleotide sequence encoding the CD40 cytoplasmic polypeptide region comprises SEQ ID NO:18 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:18. In some embodiments, the transmembrane domain is derived from CD8, CD8α, CD4, CD3ζ, CD28, CD40, CD134 (OX-40), or CD7. In some embodiments, the CAR lacks the CD40 transmembrane domain, CD40 extracellular domain, or both the CD40 transmembrane domain and the CD40 extracellular domain. In some embodiments, the transmembrane domain is derived from CD28 or CD8α. In some embodiments, the transmembrane domain derived from CD28 comprises the amino acid sequence SEQ ID NO:13 or an amino acid

sequence that has at least 50% sequence identity to SEQ ID NO:13. In some embodiments, the nucleotide sequence encoding the transmembrane domain derived from CD28 comprises SEQ ID NO:14 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:14. In some embodiments, the transmembrane domain derived from CD8α comprises the amino acid sequence SEQ ID NO:48, 94, or 96 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 48, 94, or 96. In some embodiments, the nucleotide sequence encoding the transmembrane domain derived from CD8α comprises SEQ ID NO:49, 95, or 97 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO: 49, 95, or 97. In some embodiments, the lymphocyte activation domain is a natural killer (NK) cell activation domain. In some embodiments, the lymphocyte activation domain is a T cell activation domain. In some embodiments, the lymphocyte activation domain is derived from DAP10, DAP12, Fc epsilon receptor I γ chain (FCER1G), CD3δ, CD3ε, CD3γ, CD3ζ, CD226, or CD79A. In some embodiments, the lymphocyte activation domain is derived from CD3ζ. In some embodiments, the lymphocyte activation domain derived from CD3ζ comprises the amino acid sequence SEQ ID NO:19 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:19. In some embodiments, the nucleotide sequence encoding the lymphocyte activation domain derived from CD3ζ comprises SEQ ID NO:20 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:20. In some embodiments, wherein the extracellular target binding domain further comprises a linker domain between the Eph receptor-binding moiety and the transmembrane domain. In some embodiments, the linker domain comprises an immunoglobulin IgG hinge or functional fragment thereof, or a linker domain derived from CD8, CD8α, or CD28. In some embodiments, the linker domain comprises an immunoglobulin IgG hinge or functional fragment thereof. In some embodiments, the immunoglobulin IgG hinge is an IgG1 core hinge region comprising the amino acid sequence SEQ ID NO:11 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:11. In some embodiments, the nucleotide sequence encoding the IgG1 core hinge region comprises SEQ ID NO:12 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:12. In some embodiments, the linker comprises the amino acid sequence SEQ ID NO:11, 35, 81, 83, 84, or 86 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 11, 35, 81, 83, 84, or 86. In some embodiments, the nucleotide sequence encoding the linker comprises SEQ ID NO: 12, 82, 85, or 87 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO: 12, 82, 85, or 87. In some embodiments, the Eph receptor-binding moiety comprises an Eph receptor-binding polypeptide or a ligand for the Eph

receptor. In some embodiments, the Eph receptor-binding moiety is an Eph receptor-binding polypeptide. In some embodiments, the Eph receptor-binding polypeptide is an antibody or an antibody fragment. In some embodiments, the Eph receptor-binding polypeptide is a murine antibody, rabbit antibody, human antibody, humanized antibody, fully humanized antibody, single chain variable fragment (scFv), camelid antibody variable domain and humanized versions, shark antibody variable domain and humanized versions, single domain antibody variable domain, nanobody (VHH), or camelized antibody variable domain. In some embodiments, the antigen-binding polypeptide is a single chain variable fragments (scFv). In some embodiments, the Eph receptor is an EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6 receptor or a variant thereof. In some embodiments, the Eph receptor is an EphA2 receptor or variant thereof. In some embodiments, the Eph receptor-binding moiety comprises anti-EphA2 scFv 4H5. In some embodiments, the anti-EphA2 scFv 4H5 comprises the amino acid sequence SEQ ID NO:9 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:9. In some embodiments, the nucleotide sequence encoding the anti-EphA2 scFv 4H5 comprises SEQ ID NO:10 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:10. In some embodiments, the ligand for EphA2 is Ephrin-A1, Ephrin-A2, Ephrin-A3, Ephrin-A4, Ephrin-A5 or Ephrin-A6. In some embodiments, the extracellular target-binding domain further comprises a leader sequence. In some embodiments, the leader sequence comprises the amino acid sequence of SEQ ID NO:7 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:7. In some embodiments, the nucleotide sequence encoding the leader sequence comprises SEQ ID NO:8 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:8. In some embodiments, the polynucleotide comprises at least one additional polypeptide sequence. In some embodiments, the at least one polynucleotide sequence encodes a transduced host cell selection marker, an *in vivo* tracking marker, a cytokine, or a suicide gene. In some embodiments, the transduced host cell selection marker is a truncated CD19 (tCD19) polypeptide. In some embodiments, the tCD19 comprises the amino acid sequence SEQ ID NO:23 or an amino acid sequence having at least 50% sequence identity to SEQ ID NO:23. In some embodiments, the nucleotide sequence encoding the tCD19 comprises SEQ ID NO:24 or a nucleotide sequence having at least 50% sequence identity to SEQ ID NO:24. In some embodiments, the CAR comprises the amino acid sequence SEQ ID NO:1, 26, 50, 52, 54, 56, or 58 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 1, 26, 50, 52, 54, 56, or 58. In some embodiments, the CAR is encoded by the

nucleotide sequence SEQ ID NO:2, 27, 51, 53, 55, 57, or 59 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:2, 27, 51, 53, 55, 57, or 59. In some embodiments, the polynucleotide is operatively linked to at least one regulatory element for expression of the CAR. In some embodiments, the at least one regulatory element is a promoter (e.g., a T lymphocyte-specific promoter or an NK cell-specific promoter). In certain embodiments, the polynucleotide is a DNA molecule. In certain embodiments, the polynucleotide is an RNA molecule or derivative thereof.

[0010] In one aspect, the invention provides a recombinant vector comprising the polynucleotide as described herein, wherein the polynucleotide is operatively linked to at least one regulatory element for expression of the CAR.

[0011] In one aspect, the invention provides a recombinant vector comprising a polynucleotide encoding a CAR comprising (a) an extracellular target-binding domain comprising an Eph receptor-binding moiety, (b) a transmembrane domain, and (c) a cytoplasmic domain comprising a lymphocyte activation domain, a MyD88 polypeptide or a functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof, wherein the polynucleotide is operatively linked to at least one regulatory element for expression of the CAR. In some embodiments, the vector is a viral vector. In some embodiments, the viral vector is a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated virus vector, an alphaviral vector, a herpes virus vector, or a vaccinia virus vector. In some embodiments, the vector is a non-viral vector.

[0012] In one aspect, the invention provides a CAR encoded by any of the polynucleotides disclosed herein.

[0013] In one aspect, the invention provides a CAR comprising (a) an extracellular target-binding domain comprising an Eph receptor-binding moiety, (b) a transmembrane domain, and (c) a cytoplasmic domain comprising a lymphocyte activation domain, a MyD88 polypeptide or functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof. In some embodiments, the MyD88 polypeptide comprises amino acid sequence SEQ ID NO:25, 30, 31, or 33 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 25, 30, 31, or 33. In some embodiments, the MyD88 functional fragment comprises the amino acid sequence SEQ ID NO:15, 28, or 60, or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:15, 28, or 60. In some embodiments, the CD40 polypeptide comprises the amino acid sequence SEQ ID NO:32 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:32. In some embodiments, the CD40 cytoplasmic polypeptide region comprises the amino acid

sequence SEQ ID NO:17 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:17. In some embodiments, the transmembrane domain is derived from CD8, CD8α, CD4, CD3ζ, CD28, CD40, CD134 (OX-40), or CD7. In some embodiments, the CAR lacks the CD40 transmembrane domain, CD40 extracellular domain, or both CD40 transmembrane and CD40 extracellular domain. In some embodiments, the transmembrane domain is derived from CD28 or CD8α. In some embodiments, the transmembrane domain derived from CD28 comprises the amino acid sequence SEQ ID NO:13 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:13. In some embodiments, the transmembrane domain derived from CD8α comprises the amino acid sequence SEQ ID NO:48, 94, or 96 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 48, 94, or 96. In some embodiments, the lymphocyte activation domain is a T cell activation domain. In some embodiments, the lymphocyte activation domain is a natural killer cell activation domain. In some embodiments, the lymphocyte activation domain is derived from DAP10, DAP12, Fc epsilon receptor I γ chain (FCER1G), CD3δ, CD3ε, CD3γ, CD3ζ, CD226, or CD79A. In some embodiments, the lymphocyte activation domain is derived from CD3ζ. In some embodiments, the lymphocyte activation domain derived from CD3ζ comprises the amino acid sequence SEQ ID NO:19 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:19. In some embodiments, the CAR comprises a linker domain between the extracellular target-binding domain and the transmembrane domain. In some embodiments, the linker domain comprises an immunoglobulin IgG hinge, or functional fragment thereof, or a linker domain derived from CD8, CD8α, or CD28. In some embodiments, the linker domain comprises an immunoglobulin IgG hinge or functional fragment thereof. In some embodiments, the immunoglobulin IgG hinge comprises an IgG1 core hinge region comprising the amino acid sequence SEQ ID NO:11 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:11. In some embodiments, the linker comprises the amino acid sequence SEQ ID NO:11, 35, 81, 83, 84, or 86 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 11, 35, 81, 83, 84, or 86. In some embodiments, the Eph receptor-binding moiety comprises an Eph receptor-binding polypeptide or a ligand for the Eph receptor. In some embodiments, the Eph receptor-binding moiety is an Eph receptor-binding polypeptide. In some embodiments, the Eph receptor-binding polypeptide is an antibody or an antibody fragment. In some embodiments, the Eph receptor-binding polypeptide is a murine antibody, rabbit antibody, human antibody, humanized antibody, fully humanized antibody, single chain variable fragment (scFv), camelid antibody variable domain and humanized versions, shark antibody

variable domain and humanized versions, single domain antibody variable domain, nanobody (VHH), or camelized antibody variable domain. In some embodiments, wherein the antigen-binding polypeptide is a single chain variable fragments (scFv). In some embodiments, the Eph receptor is a EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6 receptor or a variant thereof. In some embodiments, the Eph receptor is a EphA2 receptor or variant thereof. In some embodiments, the Eph receptor-binding moiety comprises anti-EphA2 scFv 4H5. In some embodiments, the anti-EphA2 scFv 4H5 comprises the amino acid sequence SEQ ID NO:9 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:9. In some embodiments, the ligand for EphA2 is Ephrin-A1, Ephrin-A2, Ephrin-A3, Ephrin-A4, Ephrin-A5 or Ephrin-A6. In some embodiments, the CAR comprises at least one additional polypeptide sequence (e.g., at least one of a transduced host cell selection marker, an *in vivo* tracking marker, a cytokine, or a suicide protein, co-stimulatory endodomains, signal sequences, epitope tags, or polypeptides that produce a detectable signal). In some embodiments, the CAR comprises amino acid sequence SEQ ID NO:126, 50, 52, 54, 56, or 58 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: SEQ ID NO:1, 26, 50, 52, 54, 56, or 58.

[0014] In one aspect, the invention provides an isolated host cell comprising any one of the CARs as disclosed herein. In some embodiments, the host cell further comprising a co-stimulatory fusion protein. In some embodiments, the host cell comprises any of the polynucleotides as disclosed herein. In some embodiments, the polynucleotide is operatively linked to at least one regulatory element which is capable of mediating expression of the chimeric antigen receptor in the host cell. In some embodiments, the host cell comprises any of the vectors disclosed herein. In some embodiments, the host cell is a mammalian cell. In some embodiments, the host cell is a cytotoxic cell, a T cell, a stem cell, a progenitor cell, or a cell derived from a stem cell or a progenitor cell. In some embodiments, wherein the T cell is at least one of T-helper cells, cytotoxic T-cells, T-regulatory cells (Treg), invariant natural killer T (NKT) cells, or gamma-delta T cells. In some embodiments, the cytotoxic cell is a cytotoxic T cell or a natural killer (NK) cell. In some embodiments, the host cell has been activated and/or expanded *ex vivo*. In some embodiments, the host cell is an allogeneic cell. In some embodiments, the host cell is an autologous cell. In some embodiments, the host cell is isolated from a subject having a disease, wherein EphA2 is expressed. In some embodiments, the cancer is a solid tumor. In some embodiments, the disease is a cancer that expresses EphA2. In some embodiments, the cancer is breast, prostate, urinary bladder, skin, lung, ovary, sarcoma, or brain cancer. In some embodiments, the skin cancer is melanoma. In

some embodiments, the lung cancer is non–small cell lung cancer (NSCLC). In some embodiments, the sarcoma is osteosarcoma or rhabdomyosarcoma. In some embodiments, the brain cancer is a high grade glioma, diffuse pontine glioma, medulloblastoma, or ependymoma. In some embodiments, the host cell is derived from a blood, marrow, tissue, or a tumor sample. In some embodiments, the cell surface expression of the endogenous TCR of the cell has been decreased or eliminated. In some embodiments, the host cell is an allogeneic cell.

[0015] In one aspect, the invention provides a pharmaceutical composition comprising any one of the host cells described herein and a pharmaceutically acceptable carrier and/or excipient.

[0016] In one aspect, the invention provides a method of enhancing activity of a T lymphocyte or a natural killer (NK) cell towards cells expressing EphA2 in a subject in need thereof comprising administering to the subject an effective amount of any one of the polynucleotides disclosed herein, any one of vectors disclosed herein, any one of host cells disclosed herein, and/or any one of pharmaceutical compositions disclosed herein. In another aspect, the invention provides a method of treating a disease in a subject in need thereof comprising administering to the subject an effective amount of any one of the polynucleotides disclosed herein, any one of vectors disclosed herein, any one of host cells disclosed herein, and/or any one of pharmaceutical compositions disclosed herein. In some embodiments, the host cell is an autologous cell. In some embodiments, the subject has a disease, wherein EphA2 is expressed. In some embodiments, the cancer is a solid tumor. In some embodiments, the disease is a cancer that expresses EphA2. In some embodiments, the cancer is breast, prostate, urinary bladder, skin, lung, ovary, sarcoma, or brain cancer. In some embodiments, the skin cancer is melanoma. In some embodiments, the lung cancer is non–small cell lung cancer (NSCLC). In some embodiments, the sarcoma is osteosarcoma or rhabdomyosarcoma. In some embodiments, the brain cancer is a high grade glioma, diffuse pontine glioma, medulloblastoma, or ependymoma.

[0017] In one aspect, the invention provides a method for producing any of the host cells disclosed herein, comprising genetically modifying the host cells with any of the polynucleotides disclosed herein or any of the vectors disclosed herein. In some embodiments, the genetic modifying step is conducted via viral gene delivery. In some embodiments, the genetic modifying step is conducted via non-viral gene delivery. In some embodiments, the genetic modifying step is conducted *ex vivo*. In some embodiments, the method further comprises activation and/or expansion of the cell *ex vivo*.

[0018] In one aspect, the invention provides a method for stimulating elimination of a cell comprising an EphA2 antigen in a subject in need thereof, said method comprising administering to the subject an effective amount of cytotoxic T cells or natural killer (NK) cells comprising any of the CARs disclosed herein.

[0019] In one aspect, the invention provides a method for treating a cancer in a subject in need thereof, wherein cancer cells express EphA2, said method comprising administering to the subject a therapeutically effective amount of a cytotoxic T cells or natural killer (NK) cells comprising any one of the CARs disclosed herein. In some embodiments, the cancer is a solid tumor. In some embodiments, the disease is a cancer that expresses EphA2. In some embodiments, the cancer is breast, prostate, urinary bladder, skin, lung, ovary, sarcoma, or brain cancer. In some embodiments, the skin cancer is melanoma. In some embodiments, the lung cancer is non–small cell lung cancer (NSCLC). In some embodiments, the sarcoma is osteosarcoma or rhabdomyosarcoma. In some embodiments, the brain cancer is a high grade glioma, diffuse pontine glioma, medulloblastoma, or ependymoma.

[0020] In certain embodiments, any of the methods include a) isolating T cells or NK cells from the subject; b) genetically modifying said T cells or NK cells *ex vivo* with any of the polynucleotides disclosed herein or any one of the vectors disclosed herein; c) optionally, expanding and/or activating said T cells or NK cells before, after or during step (b); and d) introducing the genetically modified T cells or NK cells into the subject.

[0021] In certain embodiments, the cell surface expression of the endogenous TCR has been decreased or eliminated in the administered cells.

[0022] These and other aspects of the present invention will be apparent to those of ordinary skill in the art in the following description, claims and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **Figures 1A-1C** show the EphA2-targeting constructs evaluated. **(Figure 1A)** Schematic of EphA2-targeting CD28.ζ.CAR (SEQ ID NOs: 3-4), 4-1BB.ζ.CAR (SEQ ID NOs: 5-6), and Myd88.CD40.ζ.CAR (SEQ ID NOs: 1-2) constructs. Each consists of the same humanized EphA2-recognizing scFv (single chain variable fragment) 4H5, an IgG core hinge, a transmembrane domain, a costimulatory domain, CD3ζ, and a truncated CD19 tag for detection separated from the CAR by a 2A sequence. **(Figure 1B)** Transduction efficiency of EphA2-CAR T cells. Flow cytometry was used to determine the expression of the truncated CD19 tag one week after transduction. Non-transduced (NT) T cells served as controls. There was no significant difference between the transduction efficiency of

MyD88.C40.ζ.CAR- and 4-1BB.ζ.CAR-encoding retroviral vectors. However, the transduction efficiency of the CD28.ζ.CAR-encoding retroviral vector was significantly higher (**p<0.01). **(Figure 1C)** CAR expression does not significantly change T-cell phenotype. Phenotype was determined 7-12 days after transduction using flow cytometry. N: Naïve, CCR7+, CD45RA+; CM: Central Memory, CCR7+, CD45RA-; EM: Effector Memory, CCR7-, CD45RA-; TE: Terminal Effector, CCR7-, CD45RA+. Anti-human CCR7-AF488 (BioLegend; clone G043H7). Anti-human CD45RA-APC-H7 (BD Pharmigen; clone HI100).

[0024] **Figures 2A-2C** demonstrate that all three EphA2-targeting CARs effectively recognize and kill EphA2+ U373 glioma cells. **(Figures 2A-2B)** CAR T cells were cocultured with EphA2+ U373 cells or U373 cells in which EphA2 had been knocked out (k/o U373). After 24 hours media was collected and the concentration of IFNγ and IL2 was determined. Myd88.CD40.ζ.CAR T cells produced significant amount of IFNγ (*p<0.05) and IL2 (*p<0.01) only in the presence of EphA2+ U373 cells. While there were no significant differences in IFNγ production between different CAR T-cell populations, CD28.ζ.CAR T cells produced significantly higher (**p<0.01) and 4-1BB.ζ.CAR T cells significantly lower (*p<0.05) levels of IL2. **(Figure 2C)** T cells were co-cultured with tumor cells at multiple effector:target ratios with U373 or k/o U373 cells. After 24 hours, viable tumor cells were quantified with a MTS assay. NT cells had no antitumor activity. CD28.CAR, 4-1BB.CAR, and Myd88.CD40.ζ.CAR T cells killed EphA2+ positive target cells (*p<0.05; ***p<0.001). While CD28.ζ.CAR and 4-1BB.ζ.CAR T cells exhibited background killing of k/o U373 cells, Myd88.CD40.ζ.CAR T cells did not.

[0025] **Figures 3A-3C** demonstrate that MyD88.CD40.CAR T cells are able to proliferate after repeat stimulation with tumor cells *in vitro*. **(Figure 3A)** Experimental setup for repeat stimulation assay. 10^6 T cells were stimulated against $5x10^5$ U373 or k/o U373 cells. 7 days later, T cells were counted and 10^6 were re-stimulated against $5x10^5$ fresh tumor cells. T cells were re-stimulated weekly until they no longer killed tumor cells. **(Figure 3B)** MyD88.CD40.ζ.CAR T cells (n=8) expanded more after restimulation with U373 cells than CD28.ζ.CAR (p<0.05; n=8) or 4-1BB.ζ.CAR (p<0.05; n=4) T cells. In addition, MyD88.CD40.ζ.CAR T cells were able to kill U372 cells at least 6 times, whereas the effector function of CD28.ζ.CAR or 4-1BB.ζ.CAR T cells rapidly eroded being able to kill U373 cells no more than 5 times (p<0.005). No expansion was observed in the presence of k/o U373 cells, confirming antigen specificity. **(Figure 3C)** Phenotype of CAR T cells 7 days after co-culture with U373. MyD88.ζ.CD40 CAR T cells had a significantly larger

proportion of naïve (CCR7+, CD45RA+) cells than the CD28 and 4-1BB CAR T cells (n=4; Naïve MyD88.ζ.CD40 vs. CD28 or 4-1BB: $p<0.001$).

[0026] **Figures 4A-4D** demonstrate that MyD88.CD40 CAR T cells exhibit superior anti-tumor activity compared with 4-1BB.CAR T cells in an intraperitoneal LM7 xenograft model. **(Figure 4A)** 8-week old female NSG mice were injected intraperitoneally (i.p.) with 10^6 LM7 tumor cells, which were genetically modified to express firefly luciferase (ffluc) to enable non-invasive bioluminescence imaging to track tumor cells. On day +7, mice received a single i.p. injection of 10^5 or 10^4 CAR T cells. Tumor burden was monitored by bioluminescence imaging. **(Figure 4B)** IVIS images of individual mice. **(Figure 4C)** Quantitative bioluminescence imaging and survival curve for 10^5 CAR T cells. After injection of 4-1BB.ζ.CAR or MyD88.CD40.ζ.CAR T cells there was a rapid reduction in tumor size as judged by bioluminescence imaging. While tumors after 4-1BB.ζ.CAR T-cell therapy recurred earlier, there was no significant difference in overall survival between both mice during the first 83 days post T-cell infection. **(Figure 4D)** Quantitative bioluminescence imaging and survival curve for 10^4 CAR T cells. After injection of 4-1BB.ζ.CAR or MyD88.CD40.ζ.CAR T cells only MyD88.CD40.ζ.CAR T-cell treated mice exhibited prolonged antitumor responses, which translated in a significant ($p<0.05$) survival advantage.

[0027] **Figures 5A-5D** demonstrate that MyD88.CD40.ζ.CAR T cells expand better compared with CD28.ζ.CAR or 4-1BB.ζ.CAR T cells in an intraperitoneal LM7 xenograft model. **(Figure 5A)** 8-week old female NSG mice were injected i.p. with 10^6 LM7 tumor cells on day -7. On day 0, mice received a single i.p. injection of CAR T cells that were also genetically-modified to express ffluc. CAR T cells expressing a nonfunctional CAR (Δ.CAR) served as controls. *In vivo* T-cell expansion was monitored by bioluminescence imaging. **(Figure 5B)** IVIS images of individual mice. **(Figure 5C)** Quantitative bioluminescence imaging. **(Figure 5D)** To quantitate T-cell expansion, area under the curve (AUC) was plotted. MyD88.CD40.ζ.CAR T cells expanded significantly more than Δ.CAR, CD28.ζ.CAR, or 4-1BB.ζ.CAR T cells.

[0028] **Figures 6A-6C** depict the amino acid and nucleic acid sequences of representative EphA2-targeting CAR constructs, including scFv4H5. CD28.ζ.CAR (SEQ ID NOs: 3-4) **(Figure 6A)**, scFv4H5.4-1BB.ζ.CAR (SEQ ID NOs: 5-6) **(Figure 6B)**, and scFv4H5.Myd88.CD40.ζ.CAR (SEQ ID NOs: 1-2) **(Figure 6C)** constructs.

[0029] **Figures 7A-7G** show the IL13Rα2-specific CAR constructs evaluated. **(Figure 7A)** Schematic of IL13Rα2-specific CD28.CAR, MyD88.CD40.CAR, and MyD88.CAR constructs, including m47.MyD88.CD40.ζ (SEQ ID NOs: 63-64) **(Figure 7B)**, m47.MyD88.ζ

(SEQ ID NOs: 65-66) **(Figure 7C)**, and m47.CD28.ζ (SEQ ID NOs: 67-68) **(Figure 7D)**. **(Figure 7E)** Transduction efficiency of IL13Rα2-CAR T cells. Flow cytometry was used to determine the expression of CARs 7 to 10 days after transduction. **(Figure 7F)** Western blot analysis of CAR T cells revealed equal expression of all three CARs, indicating that MyD88.CAR and MyD88.CD40.CARs are inefficiently transported to the cell surface and/or are rapidly internalized once they reach the cell surface. **(Figure 7G)** CAR T cells were incubated with IL13Rα2+ U373 cells and their ability to kill tumor cells was scored by microscopic examination after 48 to 72 hours.

[0030] **Figures 8A-8H** show the HER2-specific CAR constructs evaluated. **(Figure 8A)** Schematic of HER2-specific CD28.CAR, MyD88.CD40.CAR, MyD88.CAR, and CD40.CAR constructs, FRP5.MyD88.CD40.ζ (SEQ ID NOs: 69-70) **(Figure 8B)**, FRP5.MyD88.ζ (SEQ ID NOs: 71-72) **(Figure 8C)**, FRP5.CD40.ζ (SEQ ID NOs: 73-74) **(Figure 8D)**, and FRP5.CD28.ζ (SEQ ID NOs: 75-76) **(Figure 8E)**. **(Figure 8F, Figure 8G)** Flow cytometry was used to determine the expression of CARs 7 to 10 days after transduction. **(Figure 8F)** Individual FACS plot; **(Figure 8G)** Summary data. **(Figure 8H)** CAR T cells were incubated with HER2+ U373 cells and their ability to kill tumor cells was scored by microscopic examination after 48 to 72 hours.

[0031] **Figures 9A-9F** show the HER2-specific CAR constructs evaluated. **(Figure 9A)** Schematic of HER2-specific MyD88.CD40.CARs, including FRP5.MyD88.CD40.CAR (SH.TMCD28) (SEQ ID NOs: 69-70), FRP5.MyD88.CD40.CAR (SH.CH3.TMCD28) (SEQ ID NOs: 77-78) **(Figure 9B)**, and FRP5.MyD88.CD40.CAR (SH.CH2.CH3.TMCD28) (SEQ ID NOs: 79-80) **(Figure 9C)**. **(Figure 9D, Figure 9E)** Transduction efficiency of HER2-CAR T cells. Flow cytometry was used to determine the expression of CARs 7 to 10 days after transduction. **(Figure 9D)** Individual FACS plot; **(Figure 9E)** Summary data. **(Figure 9F)** CAR T cells were incubated with HER2+ U373 cells and their ability to kill tumor cells was scored by microscopic examination after 48 to 72 hours.

[0032] **Figures 10A-10G** show the HER2-specific CAR constructs evaluated. **(Figure 10A)** Schematic of HER2-specific MyD88.CD40.CARs, including FRP5.MyD88.CD40.CAR (SH.TMCD28) (SEQ ID NOs: 69-70), FRP5.MyD88.CD40.CAR (SH.TMCD8α) (SEQ ID NOs: 88-89) **(Figure 10B)**, FRP5.MyD88.CD40.CAR (8a.TMCD8α) (SEQ ID NOs: 90-91) **(Figure 10C)**, and FRP5.MyD88.CD40.CAR (8a.TMCD8α+8aa) (SEQ ID NOs: 92-93) **(Figure 10D)**. **(Figure 10E, Figure 10F)** Transduction efficiency of HER2-CAR T cells. Flow cytometry was used to determine the expression of CARs 7 to 10 days after transduction. **(Figure 10E)** Individual FACS plot; **(Figure 10F)** Summary data. **(Figure**

10G) CAR T cells were incubated with HER2+ U373 cells and their ability to kill tumor cells was scored by microscopic examination after 48 to 72 hours.

[0033] **Figure 11** shows examples of co-stimulatory fusion proteins.

[0034] **Figures 12A-E** show an updated experiment from Figure 4 with additional animals per group and longer follow up. In addition, it included one additional group (CD28.CAR T cells). **(Figure 12A)** 8-week old female NSG mice were injected intraperitoneally (i.p.) with 10^6 LM7 tumor cells, which were genetically modified to express firefly luciferase (ffluc) to enable non-invasive bioluminescence imaging to track tumor cells. On day +7, mice received a single i.p. injection of 10^5 **(Figures 12A-12C)** or 10^4 **(Figures 12D-12F)** CAR T cells. Tumor burden was monitored by bioluminescence imaging. **(Figure 12A, Figure 12D)** IVIS images of individual mice. **(Figure 12B, Figure 12C)** Quantitative bioluminescence imaging and survival curve for 10^5 CAR T cells. After injection of CD28.CAR, 41BB.CAR or MyD88.CD40.CAR (MC-CAR) T cells there was a rapid reduction in tumor size as judged by bioluminescence imaging. While tumors after CD28.CAR and 41BB.CAR T-cell therapy recurred earlier, there was no significant difference in overall survival between both mice during the first 83 days post T-cell infection. However, long term follow-up revealed a significant survival advantage ($p<0.05$). **(Figure 12E, Figure 12F)** Quantitative bioluminescence imaging and survival curve for 10^4 CAR T cells. After injection of CD28.CAR, 41BB.CAR or MyD88.CD40.CAR T cells only MyD88.CD40.CAR T-cell treated mice exhibited prolonged antitumor responses, which translated in a significant ($p<0.05$) survival advantage.

[0035] **Figures 13A-13C** demonstrate that the function of MyD88.CD40-CAR T cells depends on the presence of a functional zeta signaling domain within the CAR and not on the presence of endogenous αβ TCRs. **(Figure 13A)** MyD88.CD40-CAR (MC-CAR) T cells were generated in which the TRAC locus was knocked out (MC TRAC KO-CAR) resulting in the absence of cell surface expression of αβ TCRs. **(Figure 13B)** In a cytotoxicity assay, MC-CAR T cells and MC TRAC KO-CAR T cells killed EphA2+ tumor cells (U373, LM7). In contrast non-transduced T cells (NT) did not kill target cells. In addition, T cells were generated by expressing a non-functional MyD88.CD40 CAR in which the immunoreceptor tyrosine-based activation motif (ITAM) was mutated (MC-CAR.muITAM). MC-CAR.muITAM T cells also did not kill tumor cells indicating that killing depends on an active CAR. **(Figure 13C)** The antitumor activity of CAR T cells was evaluated in the intraperitoneal LM7 xenograft model described in Figure 4 with a T cell dose of 10^5. MC-

CAR T cells and MC TRAC KO-CAR T cells had similar antitumor activity whereas T cells expressing the non-functional CAR had no antitumor activity.

[0036] **Figures 14A-14B** demonstrate the antitumor activity of MC-CAR T cells and MC TRAC KO-CAR T cells in a glioma model. Luciferase-expressing EphA2+ U373 cells were injected into the brain of NSG mice and on day 7, MC-CAR T cells or MC TRAC KO-CAR T cells were injected intravenously. Within 4 weeks of intravenous injection there was a dramatic reduction in tumor size as judged by bioluminescence imaging. **(Figure 14A)** IVIS images of individual mice. **(Figure 14B)** Quantitative bioluminescence imaging.

DETAILED DESCRIPTION

[0037] The present invention provides chimeric antigen receptors (CARs), particularly CARs with enhanced ability to activate lymphocytes (e.g., T cells or natural killer (NK) cells) to allow for, for example, increased tumoricidal activity.

[0038] CARs are primarily comprised of 1) an antigen-binding moiety, such as a single-chain variable fragment (scFv) derived from an antigen-specific monoclonal antibody, and 2) a lymphocyte activation domain, such as the ζ-chain from the T cell receptor CD3. These two regions are fused together via a transmembrane domain. Upon transduction, the lymphocyte expresses the CAR on its surface, and upon contact and ligation with the target antigen, it signals through the lymphocyte activation domain (e.g., CD3ζ chain) inducing cytotoxicity and cellular activation.

[0039] Constructs with only the antigen-specific binding region together with the lymphocyte activation domain are termed first-generation CARs. While activation of lymphocytes through a lymphocyte activation domain such as CD3ζ is sufficient to induce tumor-specific killing, such CARs fail to optimally induce T cell proliferation and survival *in vivo*. The second-generation CARs added co-stimulatory polypeptides to boost the CAR-induced immune response. For example, the co-stimulating polypeptide CD28 signaling domain was added to the CAR construct. This region generally contains the transmembrane region of the co-stimulatory peptide (in place of the CD3ζ transmembrane domain) with motifs for binding other molecules such as PI3K and Lck. T cells expressing CARs with only CD3ζ vs CARs with both CD3ζ and a co-stimulatory domain (e.g., CD28) demonstrated the CARs expressing both domains achieve greater activity. The most commonly used co-stimulating molecules include CD28 and 4-1BB, which promotes both T cell proliferation and cell survival. The third-generation CAR includes three signaling domains (e.g., CD3ζ, CD28, and 4-1BB), which further improves lymphocyte cell survival and efficacy. Examples

of third-generation CARs include CD19 CARs, most notably for the treatment of chronic lymphocytic leukemia (Milone, M. C., et al., (2009) Mol. Ther. 17:1453-1464; Kalos, M., et al., Sci. Transl. Med. (2011) 3:95ra73; Porter, D., et al., (2011) N. Engl. J. Med. 365: 725-533; each of which is incorporated herein by reference in its entirety for all purposes). Studies in three patients showed impressive function, expanding more than a 1000-fold *in vivo*, and resulted in sustained remission in all three patients.

[0040] Investigators then turned to the use of auxiliary molecules to further enhance lymphocyte cell survival and efficacy. Such an example includes the use of a signaling molecule that includes a multimerization region that binds to a chemical inducer of dimerization (CID). CIDs can be small or large molecules that can bind together two separate CAR monomers, resulting in a dimer that can induce activation. For example, lymphocytes were engineered to express the CD3ζ chain linked to 1, 2, or 3 FKBP fragments. Lymphocytes expressing these CARs demonstrated CID-dependent T cell activation (Spencer, D. M., et al., *Science*, 1993. 262: p. 1019-1024, which is incorporated herein by reference in its entirety for all purposes).

[0041] Another example of an auxiliary molecule approach is the use of an inducible MyD88/CD40 molecule for the activation of CAR-modified lymphocytes, in which the CARs were co-expressed with the inducible MyD88/CD40 molecule. AP1930 (rimiducid) results in the dimerization of certain inducible MyD88/CD40 molecules, thus, leading to a powerful co-stimulation that increases lymphocyte survival, proliferation, activation and cancer cell death. An example of this system includes the HER2ζ-CAR MyD88/CD40 system in which T cells expressing both constructs had superior T cell proliferation, cytokine production, and the ability to kill target expressing cells when CID was present. Repeated injections of CID was able to further enhance the tumor clearance. *See* Mata et al., *Cancer Discovery* November 2017, OF1-OF14, which his incorporated herein by reference in its entirety for all purposes. The drawback of this system is that not only do you need to co-express the CAR and the inducible molecules, a CID must also be administered to enhance lymphocyte activity.

[0042] Here, a MyD88/CD40 molecule was assayed to determine whether it could also be used to physically replace CD28 and/or 4-1BB co-stimulation in CAR constructs. It was surprisingly and unexpectedly found that not only could MyD88/CD40 physically replace the common co-stimulatory domains in an EphA2-CAR, but it could also do so without the use of a separate dimerization agent. It is particularly surprising that such a construct is functional because numerous other MyD88/CD40-CAR constructs were not functional. See

for example Example 4, which demonstrates that the inclusion of MyD88/CD40 within certain CAR constructs results in non-functioning CARs.

Definitions

[0043] The term "chimeric antigen receptor" or "CAR" as used herein is defined as a cell-surface receptor comprising an extracellular target-binding domain, a transmembrane domain and a cytoplasmic domain, comprising a lymphocyte activation domain and optionally at least one co-stimulatory signaling domain, all in a combination that is not naturally found together on a single protein. This particularly includes receptors wherein the extracellular domain and the cytoplasmic domain are not naturally found together on a single receptor protein. The chimeric antigen receptors of the present invention are intended primarily for use with lymphocyte such as T cells and natural killer (NK) cells.

[0044] The terms "T cell" and "T lymphocyte" are interchangeable and used synonymously herein. As used herein, T cell includes thymocytes, naive T lymphocytes, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, or activated T lymphocytes. A T cell can be a T helper (Th) cell, for example a T helper 1 (Thl) or a T helper 2 (Th2) cell. The T cell can be a helper T cell (HTL; CD4+ T cell) CD4+ T cell, a cytotoxic T cell (CTL; CD8+ T cell), a tumor infiltrating cytotoxic T cell (TIL; CD8+ T cell), CD4+CD8+ T cell, or any other subset of T cells. Other illustrative populations of T cells suitable for use in particular embodiments include naive T cells and memory T cells. Also included are "NKT cells", which refer to a specialized population of T cells that express a semi-invariant αβ T-cell receptor, but also express a variety of molecular markers that are typically associated with NK cells, such as NK1.1. NKT cells include NK1.1+ and NK1.1-, as well as CD4+, CD4-, CD8+ and CD8- cells. The TCR on NKT cells is unique in that it recognizes glycolipid antigens presented by the MHC I-like molecule CD Id. NKT cells can have either protective or deleterious effects due to their abilities to produce cytokines that promote either inflammation or immune tolerance. Also included are "gamma-delta T cells (γδ T cells)," which refer to a specialized population that to a small subset of T cells possessing a distinct TCR on their surface, and unlike the majority of T cells in which the TCR is composed of two glycoprotein chains designated α- and β-TCR chains, the TCR in γδ T cells is made up of a γ-chain and a δ-chain. γδ T cells can play a role in immunosurveillance and immunoregulation, and were found to be an important source of IL-17 and to induce robust CD8+ cytotoxic T cell response. Also included are "regulatory T cells" or "Tregs" refers to T cells that suppress an abnormal or excessive immune response and play a role in immune tolerance. Tregs cells are typically transcription factor Foxp3-positive CD4+T cells and can

also include transcription factor Foxp3-negative regulatory T cells that are IL-10-producing CD4+T cells.

[0045] The terms "natural killer cell" and "NK cell" are used interchangeable and used synonymously herein. As used herein, NK cell refers to a differentiated lymphocyte with a CD 16+ CD56+ and/or CD57+ TCR- phenotype. NKs are characterized by their ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic enzymes, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response.

[0046] As used herein, the term "antigen" refers to any agent (e.g., protein, peptide, polysaccharide, glycoprotein, glycolipid, nucleic acid, portions thereof, or combinations thereof) molecule capable of being bound by a T-cell receptor. An antigen is also able to provoke an immune response. An example of an immune response may involve, without limitation, antibody production, or the activation of specific immunologically competent cells, or both. A skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components, organisms, subunits of proteins/antigens, killed or inactivated whole cells or lysates.

[0047] The term "antigen-binding moiety" refers to a target-specific binding element that may be any ligand that binds to the antigen of interest or a polypeptide or fragment thereof, wherein the ligand is either naturally derived or synthetic. Examples of antigen-binding moieties include, but are not limited to, antibodies; polypeptides derived from antibodies, such as, for example, single chain variable fragments (scFv), Fab, Fab′, F(ab′)2, and Fv fragments; polypeptides derived from T Cell receptors, such as, for example, TCR variable domains; secreted factors (e.g., cytokines, growth factors) that can be artificially fused to signaling domains (e.g., "zytokines"); and any ligand or receptor fragment (e.g., CD27, NKG2D) that binds to the antigen of interest. Combinatorial libraries could also be used to identify peptides binding with high affinity to the therapeutic target.

[0001] Terms "antibody" and "antibodies" refer to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv), single chain antibodies, Fab fragments, F(ab′) fragments, disulfide-linked Fvs (sdFv), intrabodies, minibodies, diabodies and anti-idiotypic (anti-Id) antibodies (including, e.g.,

anti-Id antibodies to antigen-specific TCR), and epitope-binding fragments of any of the above. The terms "antibody" and "antibodies" also refer to covalent diabodies such as those disclosed in U.S. Pat. Appl. Pub. 2007/0004909 and Ig-DARTS such as those disclosed in U.S. Pat. Appl. Pub. 2009/0060910. Antibodies useful as a TCR-binding molecule include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, i.e., molecules that contain an antigen-binding site. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgM1, IgM2, IgA1 and IgA2) or subclass.

[0048] The term "host cell" means any cell that contains a heterologous nucleic acid. The heterologous nucleic acid can be a vector (*e.g.*, an expression vector). For example, a host cell can be a cell from any organism that is selected, modified, transformed, grown, used or manipulated in any way, for the production of a substance by the cell, for example the expression by the cell of a gene, a DNA or RNA sequence, a protein or an enzyme. An appropriate host may be determined. For example, the host cell may be selected based on the vector backbone and the desired result. By way of example, a plasmid or cosmid can be introduced into a prokaryote host cell for replication of several types of vectors. Bacterial cells such as, but not limited to DH5α, JM109, and KCB, SURE® Competent Cells, and SOLOPACK Gold Cells, can be used as host cells for vector replication and/or expression. Additionally, bacterial cells such as E. coli LE392 could be used as host cells for phage viruses. Eukaryotic cells that can be used as host cells include, but are not limited to yeast (*e.g.*, YPH499, YPH500 and YPH501), insects and mammals. Examples of mammalian eukaryotic host cells for replication and/or expression of a vector include, but are not limited to, HeLa, NIH3T3, Jurkat, 293, COS, CHO, Saos, and PC12.

[0049] Host cells of the present invention include T cells and natural killer cells that contain the DNA or RNA sequences encoding the CAR and express the CAR on the cell surface. Host cells may be used for enhancing T cell activity, natural killer cell activity, treatment of cancer, and treatment of autoimmune disease.

[0050] The terms "activation" or "stimulation" means to induce a change in their biologic state by which the cells (*e.g.*, T cells and NK cells) express activation markers, produce cytokines, proliferate and/or become cytotoxic to target cells. All these changes can be produced by primary stimulatory signals. Co-stimulatory signals can amplify the magnitude of the primary signals and suppress cell death following initial stimulation resulting in a more durable activation state and thus a higher cytotoxic capacity. A "co-stimulatory signal" refers

to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to T cell and/or NK cell proliferation and/or upregulation or downregulation of key molecules.

[0051] The term "proliferation" refers to an increase in cell division, either symmetric or asymmetric division of cells. The term "expansion" refers to the outcome of cell division and cell death.

[0052] The term "differentiation" refers to a method of decreasing the potency or proliferation of a cell or moving the cell to a more developmentally restricted state.

[0053] The terms "express" and "expression" mean allowing or causing the information in a gene or DNA sequence to become produced, for example producing a protein by activating the cellular functions involved in transcription and translation of a corresponding gene or DNA sequence. A DNA sequence is expressed in or by a cell to form an "expression product" such as a protein. The expression product itself, e.g., the resulting protein, may also be said to be "expressed" by the cell. An expression product can be characterized as intracellular, extracellular or transmembrane.

[0054] The term "transfection" means the introduction of a "foreign" (*i.e.*, extrinsic or extracellular) nucleic acid into a cell using recombinant DNA technology. The term "genetic modification" means the introduction of a "foreign" (*i.e.*, extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. The introduced gene or sequence may also be called a "cloned" or "foreign" gene or sequence, may include regulatory or control sequences operably linked to polynucleotide encoding the chimeric antigen receptor, such as start, stop, promoter, signal, secretion, or other sequences used by a cell's genetic machinery. The gene or sequence may include nonfunctional sequences or sequences with no known function. A host cell that receives and expresses introduced DNA or RNA has been "genetically engineered." The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell, or from a different genus or species.

[0055] The term "transduction" means the introduction of a foreign nucleic acid into a cell using a viral vector.

[0056] The terms "genetically modified" or "genetically engineered" refers to the addition of extra genetic material in the form of DNA or RNA into a cell.

[0057] As used herein, the term "derivative" in the context of proteins or polypeptides (e.g., CAR constructs or domains thereof) refer to: (a) a polypeptide that has at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to

the polypeptide it is a derivative of; (b) a polypeptide encoded by a nucleotide sequence that has at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to a nucleotide sequence encoding the polypeptide it is a derivative of; (c) a polypeptide that contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acid mutations (*i.e.*, additions, deletions and/or substitutions) relative to the polypeptide it is a derivative of; (d) a polypeptide encoded by nucleic acids can hybridize under high, moderate or typical stringency hybridization conditions to nucleic acids encoding the polypeptide it is a derivative of; (e) a polypeptide encoded by a nucleotide sequence that can hybridize under high, moderate or typical stringency hybridization conditions to a nucleotide sequence encoding a fragment of the polypeptide, it is a derivative of, of at least 20 contiguous amino acids, at least 30 contiguous amino acids, at least 40 contiguous amino acids, at least 50 contiguous amino acids, at least 75 contiguous amino acids, at least 100 contiguous amino acids, at least 125 contiguous amino acids, or at least 150 contiguous amino acids; or (f) a fragment of the polypeptide it is a derivative of.

[0058] Percent sequence identity can be determined using any method known to one of skill in the art. In a specific embodiment, the percent identity is determined using the "Best Fit" or "Gap" program of the Sequence Analysis Software Package (Version 10; Genetics Computer Group, Inc., University of Wisconsin Biotechnology Center, Madison, Wisconsin). Information regarding hybridization conditions (*e.g.*, high, moderate, and typical stringency conditions) have been described, see, *e.g.*, U.S. Patent Application Publication No. US 2005/0048549 (*e.g.*, paragraphs 72-73).

[0059] The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (*e.g.*, a foreign gene) can be introduced into a host cell, so as to genetically modify the host and promote expression (*e.g.*, transcription and translation) of the introduced sequence. Vectors include plasmids, synthesized RNA and DNA molecules, phages, viruses, etc. In certain embodiments, the vector is a viral vector such as, but not limited to, viral vector is an adenoviral, adeno-associated, alphaviral, herpes, lentiviral, retroviral, or vaccinia vector.

[0060] The term "regulatory element" refers to any cis-acting genetic element that controls some aspect of the expression of nucleic acid sequences. In some embodiments, the term "promoter" comprises essentially the minimal sequences required to initiate transcription. In some embodiments, the term "promoter" includes the sequences to start transcription, and in addition, also include sequences that can upregulate or downregulate transcription, commonly termed "enhancer elements" and "repressor elements", respectively.

[0061] As used herein, the term "operatively linked," and similar phrases, when used in reference to nucleic acids or amino acids, refer to the operational linkage of nucleic acid sequences or amino acid sequence, respectively, placed in functional relationships with each other. For example, an operatively linked promoter, enhancer elements, open reading frame, 5' and 3' UTR, and terminator sequences result in the accurate production of a nucleic acid molecule (e.g., RNA). In some embodiments, operatively linked nucleic acid elements result in the transcription of an open reading frame and ultimately the production of a polypeptide (i.e., expression of the open reading frame). As another example, an operatively linked peptide is one in which the functional domains are placed with appropriate distance from each other to impart the intended function of each domain.

[0062] By "enhance" or "promote," or "increase" or "expand" or "improve" refers generally to the ability of a composition contemplated herein to produce, elicit, or cause a greater physiological response (i.e., downstream effects) compared to the response caused by either vehicle or a control molecule/composition. A measurable physiological response may include an increase in T cell expansion, activation, effector function, persistence, and/or an increase in cancer cell death killing ability, among others apparent from the understanding in the art and the description herein. In certain embodiments, an "increased" or "enhanced" amount can be a "statistically significant" amount, and may include an increase that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7. 1.8, etc.) the response produced by vehicle or a control composition.

[0063] By "decrease" or "lower," or "lessen," or "reduce," or "abate" refers generally to the ability of composition contemplated herein to produce, elicit, or cause a lesser physiological response (i.e., downstream effects) compared to the response caused by either vehicle or a control molecule/composition. In certain embodiments, a "decrease" or "reduced" amount can be a "statistically significant" amount, and may include a decrease that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7. 1.8, etc.) the response (reference response) produced by vehicle, a control composition, or the response in a particular cell lineage.

[0064] The terms "treat" or "treatment" of a state, disorder or condition include: (1) preventing, delaying, or reducing the incidence and/or likelihood of the appearance of at least one clinical or sub-clinical symptom of the state, disorder or condition developing in a subject that may be afflicted with or predisposed to the state, disorder or condition, but does

not yet experience or display clinical or subclinical symptoms of the state, disorder or condition; or (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof or at least one clinical or sub-clinical symptom thereof; or (3) relieving the disease, i.e., causing regression of the state, disorder or condition or at least one of its clinical or sub-clinical symptoms. The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

[0065] The term "effective" applied to dose or amount refers to that quantity of a compound or pharmaceutical composition that is sufficient to result in a desired activity upon administration to a subject in need thereof. Note that when a combination of active ingredients is administered, the effective amount of the combination may or may not include amounts of each ingredient that would have been effective if administered individually. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition being treated, the particular drug or drugs employed, the mode of administration, and the like.

[0066] The phrase "pharmaceutically acceptable", as used in connection with compositions described herein, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a mammal (*e.g.*, a human). Preferably, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans.

[0067] The term "protein" is used herein encompasses all kinds of naturally occurring and synthetic proteins, including protein fragments of all lengths, fusion proteins and modified proteins, including without limitation, glycoproteins, as well as all other types of modified proteins (e.g., proteins resulting from phosphorylation, acetylation, myristoylation, palmitoylation, glycosylation, oxidation, formylation, amidation, polyglutamylation, ADP-ribosylation, pegylation, biotinylation, etc.).

[0068] The terms "nucleic acid", "nucleotide", and "polynucleotide" encompass both DNA and RNA unless specified otherwise. By a "nucleic acid sequence" or "nucleotide sequence" is meant the nucleic acid sequence encoding an amino acid, the term may also refer to the nucleic acid sequence including the portion coding for any amino acids added as an artifact of cloning, including any amino acids coded for by linkers

[0069] The terms "patient", "individual", "subject", and "animal" are used interchangeably herein and refer to mammals, including, without limitation, human and veterinary animals (*e.g.*, cats, dogs, cows, horses, sheep, pigs, etc.) and experimental animal models. In a preferred embodiment, the subject is a human.

[0070] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Alternatively, the carrier can be a solid dosage form carrier, including but not limited to one or more of a binder (for compressed pills), a glidant, an encapsulating agent, a flavorant, and a colorant. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[0071] Singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure.

[0072] The term "about" or "approximately" includes being within a statistically meaningful range of a value. Such a range can be within an order of magnitude, preferably within 50%, more preferably within 20%, still more preferably within 10%, and even more preferably within 5% of a given value or range. The allowable variation encompassed by the term "about" or "approximately" depends on the particular system under study, and can be readily appreciated by one of ordinary skill in the art.

[0073] The practice of the present invention employs, unless otherwise indicated, conventional techniques of statistical analysis, molecular biology (including recombinant techniques), microbiology, cell biology, and biochemistry, which are within the skill of the art. Such tools and techniques are described in detail in e.g., Sambrook et al. (2001) Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York; Ausubel et al. eds. (2005) Current Protocols in Molecular Biology. John Wiley and Sons, Inc.: Hoboken, NJ; Bonifacino et al. eds. (2005) Current Protocols in Cell Biology. John Wiley and Sons, Inc.: Hoboken, NJ; Coligan et al. eds. (2005) Current Protocols in Immunology, John Wiley and Sons, Inc.: Hoboken, NJ; Coico et al. eds. (2005) Current Protocols in Microbiology, John Wiley and Sons, Inc.: Hoboken, NJ; Coligan et al. eds. (2005) Current Protocols in Protein Science, John Wiley and Sons, Inc.:

Hoboken, NJ; and Enna et al. eds. (2005) Current Protocols in Pharmacology, John Wiley and Sons, Inc.: Hoboken, NJ. Additional techniques are explained, e.g., in U.S. Patent No. 7,912,698 and U.S. Patent Appl. Pub. Nos. 2011/0202322 and 2011/0307437.

[0074] The technology illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein.

[0075] The terms and expressions which have been employed are used as terms of description and not of limitation, and use of such terms and expressions do not exclude any equivalents of the features shown and described or portions thereof, and various modifications are possible within the scope of the technology claimed.

Chimeric Antigen Receptors of the Invention

[0076] In one aspect, the invention provides a CAR with enhanced ability to activate, for example, lymphocytes (e.g., T cells or natural killer (NK) cells), to allow for increased tumoricidal activity (e.g., greater and/or longer activity). In particular, the CARs comprise both MyD88 and CD40 co-stimulatory domains.

[0077] The CARs of the present invention comprise an extracellular target-binding domain, a transmembrane domain and a cytoplasmic domain, wherein the cytoplasmic domain comprises a lymphocyte activation domain, a MyD88 polypeptide or functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof. In certain embodiments, the CAR lacks the transmembrane and/or extracellular domains naturally associated with lymphocyte activation domain. In certain embodiments, the CD40 cytoplasmic polypeptide region or functional fragment thereof lacks the transmembrane and/or extracellular domains. In certain embodiments, the CAR includes the CD40 transmembrane domain. In certain embodiments, the CAR includes the CD40 transmembrane domain and a portion of the CD40 extracellular domain, wherein the CD40 extracellular domain does not interact with natural or synthetic ligands of CD40. Each domain is fused in frame.

[0078] Extracellular Target-Binding Domain

[0079] In certain aspects, the CARs of the present invention comprise an extracellular target-binding domain, wherein the extracellular target-binding domain comprises an antigen-binding moiety. In certain embodiments, the extracellular target-binding domain further comprises a linker between the antigen-binding moiety and the transmembrane domain.

[0080] The choice of antigen-binding moiety depends upon the type and number of antigens that define the surface of a target cell. For example, the antigen-binding moiety may

be chosen to recognize an antigen that acts as a cell surface marker on target cells associated with a particular disease state. In certain embodiments, the CARs of the present invention can be genetically modified to target a tumor antigen of interest by way of engineering a desired antigen-binding moiety that specifically binds to an antigen (e.g., on a cancer cell). Non-limiting examples of cell surface markers that may act as targets for the antigen-binding moiety in the CAR of the invention include those associated with viral, bacterial and parasitic infections, autoimmune disease, and cancer cells.

[0081] Examples of antigens that may be targeted by the extracellular target-binding domains include, but are not limited to, carbonic anhydrase EX, alpha-fetoprotein, A3, antigen specific for A33 antibody, Ba 733, BrE3-antigen, CA125, CDl, CD1a, CD3, CD5, CD15, CD16, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD38, CD45, CD74, CD79a, CD80, CD 138, colon-specific antigen-p (CSAp), CEA (CEACAM5), CEACAM6, CSAp, EGFR, EGP-I, EGP-2, Ep-CAM, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, FIt-I, Flt-3, folate receptor, HLA-DR, human chorionic gonadotropin (HCG) and its subunits, HER2/neu, hypoxia inducible factor (HIF-I), Ia, IL-2, IL-6, IL-8, insulin growth factor-1 (IGF-I), KC4-antigen, KS-1-antigen, KS1-4, Le-Y, macrophage inhibition factor (MIF), MAGE, MUCl , MUC2, MUC3, MUC4, NCA66, NCA95, NCA90, antigen specific for PAM-4 antibody, placental growth factor, p53, prostatic acid phosphatase, PSA, PSMA, RS5, S100, TAC, TAG-72, tenascin, TRAIL receptors, Tn antigen, Thomson-Friedenreich antigens, tumor necrosis antigens, VEGF, ED-B fibronectin, 17-lA-antigen, an angiogenesis marker, an oncogene marker or an oncogene product.

[0082] In a preferred embodiment, the antigen that is targeted by the extracellular target-binding domain is EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, or a variant of any of these Eph receptors. In another preferred embodiment, the antigen that is targeted by the extracellular target-binding domain is EphA2 or a variant thereof.

[0083] In certain embodiments, the extracellular target-binding domain comprises an antigen-binding moiety that can include an antigen-binding polypeptide or a ligand that binds the target antigen (e.g., EphA2 or a variant thereof). In certain embodiments, the antigen-binding domain is specific for an antigen (e.g., EphA2 or a variant thereof) or can non-specifically bind to related family members (e.g., EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, or a variant of any of these Eph receptors).

[0084] In certain embodiments, the antigen-binding polypeptide can be monomeric or multimeric (*e.g.*, homodimeric or heterodimeric), or associated with multiple proteins in a non-covalent complex. In certain embodiments, the extracellular target-binding domain may consist of an Ig heavy chain. In certain embodiments, the Ig heavy chain can be covalently associated with Ig light chain (e.g., via the hinge and optionally the CH1 region). In certain embodiments, the Ig heavy chain may become covalently associated with other Ig heavy/light chain complexes (e.g., by the presence of hinge, CH2, and/or CH3 domains). In the latter case, the heavy/light chain complex that becomes joined to the chimeric construct may constitute an antibody with a specificity distinct from the antibody specificity of the chimeric construct. In certain embodiments, the entire chain may be used. In certain embodiments, a truncated chain may be used, where all or a part of the CH1, CH2, or CH3 domains may be removed or all or part of the hinge region may be removed. Non-limiting examples of antigen-binding polypeptides include antibodies and antibody fragments such as e.g., murine antibodies, rabbit antibodies, human antibodies, fully humanized antibodies, single chain variable fragments (scFv), camelid antibody variable domains and humanized versions, shark antibody variable domains and humanized versions, single domain antibody variable domains, nanobodies (VHHs), and camelized antibody variable domains.

[0085] In certain embodiments, the antigen-binding moiety comprises an antigen-binding polypeptide or functional variant thereof, as described above, that binds to the antigen. In certain embodiments, the antigen-binding polypeptide is an antibody or an antibody fragment, as described above, that binds to the antigen.

[0086] In certain embodiments, the antigen-binding moiety comprises an Eph receptor-binding polypeptide or functional variant thereof. In certain embodiments, the antigen-binding polypeptide is an antibody or an antibody fragment that binds to an Eph receptor. In certain embodiments, the Eph receptor is EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, and/or a variant of any of these Eph receptors. In certain embodiments, the Eph receptor is an EphA2 or a variant thereof.

[0087] In certain embodiments, the Eph receptor-binding moiety is an anti-Eph receptor scFv. In certain embodiments, the Eph receptor-binding moiety is an anti-EphA2 scFv. In certain embodiments, the Eph receptor-binding moiety is an anti-EphA2 scFv comprising or consisting essentially of the amino acid sequence set forth in SEQ ID NO: 9 or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ

ID NO:9. In certain embodiments, the nucleotide sequence encoding the anti-EphA2 scFV comprises or consists essentially of the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 9, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:9. In certain embodiments, the nucleotide sequence encoding the anti-EphA2 scFV comprises or consists essentially of the sequence set forth in SEQ ID: 10, or a nucleotide sequence having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:10. In certain embodiments, the anti-EphA2 scFV comprises or consists essentially of the amino acid sequence of SEQ ID NO: 9. In certain embodiments, the nucleotide sequence encoding the anti-EphA2 scFV comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 10. In certain embodiments, the extracellular target-binding domain of the CAR is a ligand for a target cell antigen or receptor. In certain embodiments, the ligand is a natural ligand of the antigen or receptor.

[0088] In certain embodiments, the Eph receptor-binding moiety comprises an Eph receptor ligand. In certain embodiments, the Eph receptor-binding moiety comprises a natural Eph receptor ligand. In certain embodiments, the Eph receptor is EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, and/or a variant of any of these Eph receptors. In certain embodiments, the Eph receptor is an EphA2 or a variant thereof.

[0089] In certain embodiments, the Eph receptor-binding moiety is an EphA2-binding moiety. In certain embodiments, the EphA2-binding moiety comprises an EphA2 ligand. In certain embodiments, the EphA2-binding moiety comprises a natural EphA2 ligand. In certain embodiments, the natural ligand for a target cell antigen or receptor is a natural ligand for EphA2 such as, but not limited to, Ephrin-A1, Ephrin-A2, Ephrin-A3, Ephrin-A4, Ephrin-A5 or Ephrin-A6.

[0090] Additional examples of EphA2-binding moieties can be found in US20040028685, US20050049176, US20050147593, US20050153923, US20050152899, US20080044413, US20100183618, US20150343081, US20170267768, US20170306032, US20170349663, US20180021294, US7101976, US7326552 (includes crystals relating to EphA2), WO2001012840, WO2005051307, WO2005056766, WO2006084226, WO2007075706, WO2008010101, WO2011039724, WO2012008397, WO2017161069, WO2017161071,

WO2018034332, and WO2018053649, each of which is incorporated herein by reference in its entirety for all purposes.

[0091] In certain embodiments, the extracellular target-binding domain comprises an optional leader sequence at the amino-terminus of the extracellular target-binding domain. In certain embodiments, the extracellular target-binding domain comprises a leader sequence at the amino-terminus of antigen-binding moiety, wherein the leader sequence is optionally cleaved from the antigen-binding moiety during cellular processing and localization of the CAR to the cellular membrane.

[0092] In certain embodiments, the leader sequence comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO:7 or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:7. In certain embodiments, the nucleotide sequence encoding the leader sequence comprises or consists essentially of the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO:7, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:7. In certain embodiments, the nucleotide sequence encoding the leader sequence comprises or consists essentially of the sequence set forth in SEQ ID:8, or a nucleotide sequence having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:8. In certain embodiments, the leader sequence comprises or consists essentially of the amino acid sequence of SEQ ID NO:7. In certain embodiments, the nucleotide sequence encoding the leader sequence comprises or consists essentially of the nucleotide sequence set forth in SEQ ID:8.

[0093] In certain embodiments, the extracellular target-binding domain further comprises a linker domain between the antigen-binding moiety and the transmembrane domain, wherein the antigen-binding moiety, linker, and the transmembrane domain are in frame with each other.

[0094] The term "linker domain" as used herein generally means any oligo- or polypeptide that functions to link the antigen-binding moiety to the transmembrane domain. A linker domain can be used to provide more flexibility and accessibility for the antigen-binding moiety. A linker domain may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. A linker domain may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of

CD8, CD4 or CD28, or from all or part of an antibody constant region. Alternatively, the linker domain may be a synthetic sequence that corresponds to a naturally occurring linker domain sequence, or may be an entirely synthetic linker domain sequence. Non-limiting examples of linker domains which may be used in accordance to the invention include a part of human CD8 α chain, partial extracellular domain of CD28, FcγRIIIa receptor, IgG, IgM, IgA, IgD, IgE, an Ig hinge, or functional fragment thereof. In certain embodiments, additional linking amino acids are added to the linker domain to ensure that the antigen-binding moiety is an optimal distance from the transmembrane domain. In certain embodiments, when the linker is derived from an Ig, the linker may be mutated to prevent Fc receptor binding.

[0095] In certain embodiments, the linker domain comprises an immunoglobulin IgG hinge or functional fragment thereof. In certain embodiments, the IgG hinge is from IgG1, IgG2, IgG3, IgG4, IgM1, IgM2, IgA1, IgA2, IgD, IgE, or a chimera thereof. In certain embodiments, the linker domain comprises the CH1, CH2, CH3 and/or hinge region of the immunoglobulin. In certain embodiments, the linker domain comprises the core hinge region of the immunoglobulin. The term "core hinge" can be used interchangeably with the term "short hinge" (a.k.a "SH"). Non-limiting examples of suitable linker domains are the core immunoglobulin hinge regions listed in Table 1 (*see also* Wypych et al., *JBC* 2008 283(23): 16194-16205, which is incorporated herein by reference in its entirety for all purposes). In certain embodiments, the linker domain is a fragment of the immunoglobulin hinge.

Table 1. Amino Acid Sequence of Core Hinge Regions of IgG Immunoglobulins

IgG Subtype	Core Hinge Sequence	SEQ ID NO
IgG1	EPKSCDKTHTCPPCP	SEQ ID NO:11
IgG2	ERKCCVECPPCP	SEQ ID NO:35
IgG3	ELKTPLGDTTHTCPRCP(EPKSCDTPPPCPRCP)$_3$	SEQ ID NO:36
IgG4	ESKYGPPCPSCP	SEQ ID NO:37

[0096] In certain embodiments, the linker domain comprises an IgG1 hinge, or a variant thereof. In certain embodiments, the linker domain comprises the core hinge structure of IgG1 or a variant thereof. In certain embodiments, linker domain comprises an IgG1 core hinge region and comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 11, 83, 84, or 86, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or

at least 99%, sequence identity with SEQ ID NO:11, 83, 84, or 86. In certain embodiments, the nucleotide sequence encoding the linker comprising the IgG1 core hinge region comprises or consists essentially of the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 11, 83, 84, or 86, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:11, 83, 84, or 86. In certain embodiments, the nucleotide sequence encoding the linker comprising the IgG1 core hinge region comprises or consists essentially of the nucleotide sequence of SEQ ID: 12, 85, or 87, or a nucleotide sequence having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:12, 85, or 87. In certain embodiments, linker domain comprises an IgG1 core hinge region and comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 11, 83, 84, or 86. In certain embodiments, the nucleotide sequence encoding the linker comprising the IgG1 core hinge region comprises or consists essentially of the nucleotide sequence of SEQ ID: 12, 85, or 87. In certain embodiments, linker domain is an IgG1 core hinge region comprising or consisting essentially of the amino acid sequence set forth in SEQ ID NO: 11 or a variant thereof. In certain embodiments, the nucleotide sequence encoding the IgG1 core hinge region comprises or consists essentially of the nucleotide sequence of SEQ ID: 12 or a variant thereof.

[0097] In certain embodiments, the linker domain comprises an IgG2 hinge, or a variant thereof. In certain embodiments, the linker domain comprises the core hinge structure of IgG2 or a variant thereof. In certain embodiments, linker domain comprises an IgG2 core hinge region and comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 35 or 81, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 35 or 81. In certain embodiments, the nucleotide sequence encoding the linker comprising the IgG2 core hinge region comprises or consists essentially of the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 35 or 81, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 35 or 81. In certain embodiments, the nucleotide sequence encoding the linker comprising the IgG2 core hinge region comprises or consists essentially of the nucleotide sequence of SEQ ID: 82, or a nucleotide sequence having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at

least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:82. In certain embodiments, linker domain comprises an IgG2 core hinge region and comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 35 or 81. In certain embodiments, the nucleotide sequence encoding the linker comprising the IgG2 core hinge region comprises or consists essentially of the nucleotide sequence of SEQ ID: 82.

[0098] Transmembrane Domain

[0099] In certain aspects, the CARs of the present invention comprise a transmembrane domain, fused in frame between the extracellular target-binding domain and the cytoplasmic domain.

[00100] The transmembrane domain may be derived from the protein contributing to the extracellular target-binding domain, the protein contributing the signaling or co-signaling domain, or by a totally different protein. In some instances, the transmembrane domain can be selected or modified by amino acid substitution, deletions, or insertions to minimize interactions with other members of the CAR complex. In some instances, the transmembrane domain can be selected or modified by amino acid substitution, deletions, or insertions to avoid-binding of proteins naturally associated with the transmembrane domain. In certain embodiments, the transmembrane domain includes additional amino acids to allow for flexibility and/or optimal distance between the domains connected to the transmembrane domain.

[00101] The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Non-limiting examples of transmembrane domains of particular use in this invention may be derived from (i.e. comprise at least the transmembrane region(s) of) the α, β or ζ chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD40, CD64, CD80, CD86, CD134, CD137, CD154. Alternatively, the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. For example, a triplet of phenylalanine, tryptophan and/or valine can be found at each end of a synthetic transmembrane domain.

[00102] In certain embodiments, it will be desirable to utilize the transmembrane domain of the ζ, η or FcεR1γ chains which contain a cysteine residue capable of disulfide bonding, so that the resulting chimeric protein will be able to form disulfide linked dimers with itself, or with unmodified versions of the ζ, η or FcεR1γ chains or related proteins. In some instances, the transmembrane domain will be selected or modified by amino acid substitution to avoid-

binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. In other cases, it will be desirable to employ the transmembrane domain of ζ, η or FcεR1γ and -β, MB1 (Igα.), B29 or CD3- γ, ζ, or η, in order to retain physical association with other members of the receptor complex.

[00103] In certain embodiments, the transmembrane domain in the CAR of the invention is derived from the CD28 transmembrane domain. In certain embodiments, the CD28 transmembrane domain comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 13, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:13. In certain embodiments, the nucleotide sequence that encodes the CD28 transmembrane domain comprises or consists essentially of the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 13, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:13. In certain embodiments, the nucleotide sequence that encodes the CD28 transmembrane domain comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 14, or a nucleotide sequence having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:14. In certain embodiments, the CD28 transmembrane domain comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 13. In certain embodiments, the nucleotide sequence that encodes the CD28 transmembrane domain comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 14.

[00104] In certain embodiments, the transmembrane domain in the CAR of the invention is derived from the CD8α transmembrane domain. In certain embodiments, the CD8α transmembrane domain comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 48, 94, or 96, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 48, 94, or 96. In certain embodiments, the nucleotide sequence that encodes the CD8α transmembrane domain comprises or consists essentially of the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 48, 94, or 96, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%,

sequence identity with SEQ ID NO: 48, 94, or 96. In certain embodiments, the nucleotide sequence that encodes the CD8α transmembrane domain comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 49, 95, or 97, or a nucleotide sequence having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 49, 95, or 97. In certain embodiments, the CD8α transmembrane domain comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 48, 94, or 96. In certain embodiments, the nucleotide sequence that encodes the CD8α transmembrane domain comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 49, 95, or 97. In certain embodiments, the CD8α transmembrane domain comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 48 or variant thereof. In certain embodiments, the nucleotide sequence that encodes the CD8α transmembrane domain comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 49 or variant thereof.

[00105] Cytoplasmic Domain

[00106] In certain aspects, the CARs of the present invention comprise a cytoplasmic domain, which comprises a lymphocyte activation domain, a MyD88 polypeptide or functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof. In certain embodiments, the CAR lacks the CD40 transmembrane and/or CD40 extracellular domains. In certain embodiments, the CAR includes the CD40 transmembrane domain. In certain embodiments, the CAR includes the CD40 transmembrane domain and a portion of the CD40 extracellular domain, wherein the CD40 extracellular domain does not interact with natural or synthetic ligands of CD40.

[00107] In certain embodiments, the lymphocyte activation domain is separated from the MyD88 polypeptide or functional fragment thereof and/or the CD40 cytoplasmic polypeptide region or a functional fragment thereof. In certain embodiments, the lymphocyte activation domain is separated from the MyD88 polypeptide or functional fragment thereof and/or the CD40 cytoplasmic polypeptide region or a functional fragment thereof by at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids.

[00108] In certain embodiments, the lymphocyte activation domain and co-stimulatory domains can be in any order. The MyD88 polypeptide or functional fragment thereof and/or the CD40 cytoplasmic polypeptide region or a functional fragment thereof can be in any order. In certain embodiments, the MyD88 polypeptide or functional fragment thereof is upstream of the CD40 cytoplasmic polypeptide region or a functional fragment thereof. In

certain embodiments, the MyD88 polypeptide or functional fragment thereof is downstream from the CD40 cytoplasmic polypeptide region or a functional fragment thereof. In certain embodiments, the MyD88 polypeptide or functional fragment thereof and/or the CD40 cytoplasmic polypeptide region or a functional fragment thereof are next to each other. In certain embodiments, the lymphocyte activation domain is upstream of the co-stimulatory genes. In certain embodiments, the lymphocyte activation domain is downstream from the co-stimulatory genes.

[00109] The cytoplasmic domain, which comprises the lymphocyte activation domain of the CAR of the invention, is responsible for activation of at least one of the normal effector functions of the lymphocyte in which the CAR has been placed in. The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus, the term "lymphocyte activation domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire lymphocyte activation domain is present, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the lymphocyte activation domain sufficient to transduce the effector function signal.

[00110] Non-limiting examples of lymphocyte activation domains which can be used in the CARs of the invention include, e.g., lymphocyte activation domains derived from DAP10, DAP12, Fc epsilon receptor I gamma chain (FCER1G), FcR β, CD3δ, CD3ε, CD3γ, CD3ζ, CD5, CD22, CD226, CD66d, CD79A, and CD79B.

[00111] In certain embodiments, the lymphocyte activation domain in the CAR of the invention is designed to comprise the signaling domain of CD3ζ. In certain embodiments, the CD3ζ signaling domain comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 19 or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:19. In certain embodiments, the nucleotide sequence that encodes the CD3ζ signaling domain comprises or consists essentially of the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 19, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ

ID NO:19. In certain embodiments, the nucleotide sequence that encodes the CD3ζ signaling domain comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 20, or a nucleotide sequence having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO:20. In certain embodiments, the CD3ζ signaling domain comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 19. In certain embodiments, the nucleotide sequence that encodes the CD3ζ signaling domain comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 20.

[00112] It is known that signals generated through the TCR alone are insufficient for full activation of lymphocytes and that a secondary or co-stimulatory signal is also required. Thus, lymphocyte activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary lymphocyte activation sequences (as discussed above)) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences).

[00113] In certain aspects, the cytoplasmic domain comprises a MyD88 polypeptide or functional fragment thereof and a CD40 cytoplasmic polypeptide region or a functional fragment thereof.

[00114] Myeloid differentiation primary response gene 88 (MyD88) plays a central role in the innate and adaptive immune response. It functions as an essential signal transducer in the interleukin-1 and Toll-like receptor signaling pathways, which regulate that activation of numerous proinflammatory genes. *See e.g.,* Deguine and Barton *F1000Prime Reports* 2014, 6:97; Xie *Journal of Molecular Signaling* 2013, 8:7; Ngo et al., *Nature* 2011, 470:115-119 each of which are incorporated by reference herein in their entirety for all purposes. The encoded protein consists of an N-terminal death domain and a C-terminal Toll-interleukin1 receptor domain. By MyD88 polypeptide or functional fragment thereof is meant the polypeptide product of MyD88. An example of a MyD88 polypeptide, includes, but not limited to the human MyD88 (NCBI Gene ID 4615; U84408.1; or NP_00245932). Non-limiting examples of a MyD88 polypeptide is presented as SEQ ID NO: 25, 30, 31, or 33 or an amino acid sequence having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity thereto. Non-limiting example of a MyD88 functional fragment includes a MyD88 polypeptide that is capable of amplifying the lymphocyte-mediated immune response. In certain embodiments, the MyD88 is a functional fragment (i.e., the protein is not full length

and may lack, for example, a domain, but still functions as a co-stimulatory domain). For example, a MyD88 functional fragment may be missing the Toll/Il-1 receptor domain (TIR) domain. In certain embodiments, a MyD88 functional fragment may be missing a portion of the TIR domain. In certain embodiments, the MyD88 polypeptide or functional fragment thereof comprises at least a portion of the TIR domain. In certain embodiments, the MyD88 polypeptide or functional fragment thereof comprises a gain of function mutation such as L to P mutation (*e.g.,* corresponding to L265 of SEQ ID NO: 31). In certain embodiments, the MyD88 polypeptide or functional fragment thereof comprises a gain of function mutation such as R to C mutation (*e.g.,* corresponding to R193 of SEQ ID NO: 31). In certain embodiments, the MyD88 functional fragment comprises the death domain. In certain embodiments, the MyD88 functional fragment comprises the death domain and the intermediate domain. In certain embodiments, the MyD88 interacts with IRAK-1 and/or IRAK-4. In certain embodiments, the MyD88 polypeptide or functional fragment thereof brings IRAK-1 and IRAK-4 signaling molecules together. An example of a MyD88 functional fragment is presented as SEQ ID NOS: 15, 28, or 60 (amino acid sequence) and 16 or 29 (nucleic acid sequence). By a nucleotide sequence coding for a MyD88 functional fragment is meant the nucleotide sequence coding for the MyD88 functional fragment peptide, the term may also refer to the nucleotide sequence including the portion coding for any amino acids added as an artifact of cloning, including any amino acids coded for by the linkers. It is understood that where a method or construct refers to a MyD88 functional fragment polypeptide, the method may also be used, or the construct designed to refer to another MyD88 polypeptide, such as a full length MyD88 polypeptide. Where a method or construct refers to a full length MyD88 polypeptide, the method may also be used, or the construct designed to refer to a MyD88 functional fragment polypeptide.

[00115] In certain embodiments, the MyD88 polypeptide or functional fragment thereof in the CAR of the invention is designed to comprise the co-stimulatory domain of MyD88, or variant thereof. In certain embodiments, the MyD88 functional fragment comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 15, 28, or 60, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 15, 28, or 60. In certain embodiments, the nucleotide sequence encoding the MyD88 functional fragment comprises or consists essentially of the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 15, 28, or 60, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at

least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 15, 28, or 60. In certain embodiments, the nucleotide sequence encoding the MyD88 functional fragment comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 16, 29, or 61, or a nucleotide sequence having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 16, 29, or 61. In certain embodiments, the MyD88 functional fragment comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 15, 28, or 60. In certain embodiments, the nucleotide sequence that encodes the MyD88 functional fragment comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 16, 29, or 61.

[00116] Cluster of differentiation 40 (CD40) is a co-stimulatory protein found on antigen presenting cells. The protein receptor encoded by the CD40 gene is a member of the TNF-receptor superfamily and is found to be essential in mediating a broad variety of immune and inflammatory responses including T cell-dependent immunoglobulin class switching, memory B cell development, and germinal center formation. *See e.g.,* Grewal, IS; Flavell, RA (1998). *Annual Review of Immunology.* 16: 111–35; An et al., JBC 2011 286(13):11226-11235; and Chen et. al., *Cellular & Molecular Immunology*, 2006 3(3):163-169, each of which are incorporated by reference herein in their entirety for all purposes. By CD40 polypeptide or functional fragment thereof is meant the polypeptide product of CD40. An example of CD40 polypeptide, includes, but not limited to the human CD40 (e.g., NCBI Gene ID 958; X60592.1). A non-limiting example of a CD40 polypeptide is presented as SEQ ID NO: 32. A functional fragment of CD40, refers to a CD40 nucleic acid fragment, variant, or analog, refers to a nucleic acid that codes for a CD40 polypeptide, or a CD40 polypeptide, that stimulates an immune response. A non-limiting example of a CD40 functional fragment includes a CD40 polypeptide that is lacking the extracellular domain, but is capable of amplifying the lymphocyte immune response. In certain embodiments, the CD40 is a functional fragment (i.e., the protein is not full length and may lack, for example, a domain, but still functions as a co-stimulatory domain). For example, a CD40 functional fragment may lack its transmembrane and/or extracellular domain but is capable of amplifying the lymphocyte immune response. In certain embodiments, the CD40 functional fragment includes the transmembrane domain. In certain embodiments, the CD40 functional fragment includes the transmembrane domain and a portion of the extracellular domain, wherein the extracellular domain does not interact with natural or synthetic ligands of CD40. In certain embodiments, the CD40 functional fragment interacts with Jak3, TRAF2, TRAF3,

and/or TRAF6. By a nucleotide sequence coding for a CD40 functional fragment is meant the nucleotide sequence coding for the CD40 functional fragment peptide, the term may also refer to the nucleic acid sequence including the portion coding for any amino acids added as an artifact of cloning, including any amino acids coded for by the linkers. It is understood that where a method or construct refers to a CD40 functional fragment polypeptide, the method may also be used, or the construct designed to refer to another CD40 polypeptide, such as a full length CD40 polypeptide. Where a method or construct refers to a full length CD40 polypeptide, the method may also be used, or the construct designed to refer to a CD40 functional fragment polypeptide.

[00117] In certain embodiments, the CD40 polypeptide or functional fragment thereof in the CAR of the invention is designed to comprise the CD40 cytoplasmic polypeptide region. In certain embodiments, the CD40 cytoplasmic polypeptide region comprises or consists essentially of the amino acid sequence set forth in SEQ ID NO: 17 or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 17. In certain embodiments, the nucleotide sequence encoding the CD40 cytoplasmic polypeptide region comprises or consists essentially of the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 17, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 17. In certain embodiments, the nucleotide sequence encoding the CD40 cytoplasmic polypeptide region comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 18, or a nucleotide sequence having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 18. In certain embodiments, the CD40 cytoplasmic polypeptide region comprises or consists essentially of the amino acid sequence of SEQ ID NO: 17. In certain embodiments, the nucleotide sequence encoding the CD40 cytoplasmic polypeptide region comprises or consists essentially of the nucleotide sequence set forth in SEQ ID: 18.

[00118] In certain embodiments, the CARs of the invention can include additional co-stimulatory domains. Non-limiting co-stimulatory domains include, but are not limited to, 4-1BB (CD137), CD28, ICOS, CD134 (OX-40), BTLA, CD27, CD30, GITR, CD226, and HVEM.

[00119] Accessory Genes

[00120] In addition to the CAR construct, the CAR may further comprise an accessory gene that encodes an accessory peptide. Examples of accessory genes can include a transduced host cell selection marker, an *in vivo* tracking marker, a cytokine, a suicide gene, or some other functional gene. For example, the constructs depicted in Figure 1A comprise the EphA2-CAR, a 2A sequence, and the accessory gene for truncated CD19 (tCD19). In certain embodiments, the tCD19 can be used as a tag. For example, expression of tCD19 can help determine transduction efficiency. In certain embodiments, the CAR comprises the tCD19 construct. In certain embodiments, the CAR does not include the tCD19 construct. In certain embodiments, the tCD19 can be replaced with a functional accessory gene to enhance the effector function of the CAR (e.g., EphA2-CAR) containing host cells. In certain embodiments, the functional accessory gene can increase the safety of the CAR. In certain embodiments, the CAR comprises at least one accessory gene. In certain embodiments, the CAR comprises one accessory gene. In other embodiments, the CAR comprises two accessory genes. In yet another embodiment, the CAR comprises three accessory genes.

[00121] Non-limiting examples of classes of accessory genes that can be used to increase the effector function of CAR containing host cells, include i) sectretable cytokines (e.g., but not limited to, IL-7, IL-12, IL-15, IL-18), ii) membrane bound cytokines (e.g., but not limited to, IL-15), iii) chimeric cytokine receptors (e.g., but not limited to, IL-2/IL-7, IL-4/IL-7), iv) constitutive active cytokine receptors (e.g., but not limited to, C7R), v) dominant negative receptors (DNR; e.g., but not limited to TGFRII DNR), vi) ligands of costimulatory molecules (e.g., but not limited to, CD80, 4-1BBL), vii) antibodies, including fragments thereof and bispecific antibodies (e.g., but not limited to, bispecific T-cell engagers (BiTEs)), or vii) a second CAR.

[00122] In certain embodiments, the functional accessory gene can be a suicide gene. A suicide gene is a recombinant gene that will cause the host cell that the gene is expressed in to undergo programmed cell death or antibody mediated clearance at a desired time. Suicide genes can function to increase the safety of the CAR. In another embodiment, the accessory gene is an inducible suicide gene. Non-limiting examples of suicide genes include i) molecules that are expressed on the cell surface and can be targeted with a clinical grade monoclonal antibody including CD20, EGFR or a fragment thereof, HER2 or a fragment thereof, and ii) inducible suicide genes (e.g., but not limited to inducible caspase 9 (*see* Straathof et al. (2005) *Blood.* 105(11): 4247-4254; US Publ. No. 2011/0286980, each of which are incorporated herein by reference in their entirety for all purposes)).

[00123] tCD19 could also be replaced with two accessory genes separated by a separation sequence (e.g., a 2A sequence) using a combination of the classes of molecules listed above (e.g., CAR-2A-CD20-2A-IL15). In addition, the use of two separation sequences (e.g., 2A sequences) would allow the expression of TCR (e.g., CAR-2A-TCRα-2A-TCRβ). In the constructs with a CAR and two or three accessory genes, the order of the CAR and the 2nd or 3rd transgene could be switched.

[00124] A "separation sequence" refers to a peptide sequence that causes a ribosome to release the growing polypeptide chain that it is being synthesizes without dissociation from the mRNA. In this respect, the ribosome continues translating and therefore produces a second polypeptide. Non-limiting examples of separation sequences includes T2A (EGRGSLLTCGDVEENPGP, SEQ ID NO: 21 or GSGEGRGSLLTCGDVEENPGP, SEQ ID NO: 38) the foot and mouth disease virus (FMDV) 2A sequence (GSGSRVTELLYRMKRAETYCPRPLLAIHPTEARHKQKIVAPVK QLLNFDLLKLAGDVESNPGP, SEQ ID NO: 39); Sponge (*Amphimedon queenslandica*) 2A sequence (LLCFLLLLLSGDVELNPGP, SEQ ID NO: 40; or HHFMFLLLLLAGDIELNPGP, SEQ ID NO: 41); acorn worm (*Saccoglossus kowalevskii*) 2A sequence (WFLVLLSFILSGDIEVNPGP, SEQ ID NO: 42); amphioxus (*Branchiostoma floridae*) 2A sequence (KNCAMYMLLLSGDVETNPGP, SEQ ID NO: 43; or MVISQLMLKLAGDVEENPGP, SEQ ID NO: 44); porcine teschovirus-1 2A sequence (GSGATNFSLLKQAGDVEENPGP, SEQ ID NO: 45); and equine rhinitis A virus 2A sequence (GSGQCTNYALLKLAGDVESNPGP, SEQ ID NO: 46). In some embodiments, the separation sequence is a naturally occurring or synthetic sequence. In certain embodiments, the separation sequence includes the 2A consensus sequence D-X-E-X-NPGP (SEQ ID NO: 47), in which X is any amino acid residue.

[00125] Non-Limited Examples of CARs of the Invention

[00126] In certain embodiments, the CAR can be encoded by one polypeptide chain. In certain embodiments, the CAR of the invention is encoded by a nucleotide sequence comprising the nucleotides sequence of SEQ ID NO: 2, 27, 51, 53, 55, 57, or 59, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 2, 27, 51, 53, 55, 57, or 59. In certain embodiments, the CAR is encoded by the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 1, 26, 50, 52, 54, 56, or 58. In another embodiment, the CAR comprises the amino acid sequence of SEQ ID NO: 1, 26, 50, 52, 54, 56, or 58, or a variant thereof having at least 50, at least 55, at least 60,

at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 1, 26, 50, 52, 54, 56, or 58. In another embodiment, the CAR comprises the amino acid sequence of SEQ ID NO: 1, 26, 50, 52, 54, 56, or 58.

[00127] In certain embodiments, the CAR can be encoded by two polypeptide chains. For example, the first polypeptide chain can encode an extracellular target-binding domain comprising an Eph receptor-binding moiety, a transmembrane domain, and a short cytoplasmic tail, and the second polypeptide chain can encode a short extracellular domain, a transmembrane domain, and a cytoplasmic domain comprising a lymphocyte activation domain, a MyD88 polypeptide or functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof. Each domain is described in greater detail above. In certain embodiments, both polypeptides can interact via their respective transmembrane domain.

[00128] Co-stimulatory Fusion Proteins

[00129] In certain embodiments, the host cells can be genetically modified to express not only CARs as disclosed herein but to also express a co-stimulatory fusion protein. These co-stimulatory fusion proteins can improve host cell activation and responsiveness. In certain embodiments, the co-stimulatory fusion protein can enhance the host cell's response to the target antigen. In certain embodiments, the co-stimulatory fusion protein can impart resistance to suppression signals.

[00130] The co-stimulatory fusion proteins can comprise portions of CD4, CD8α, CD28, portions of a T cell receptor, or an antigen-binding moiety (e.g., scFv) linked to a MyD88, CD40, and/or other signaling molecules.

[00131] In certain embodiments, the co-stimulatory fusion protein comprises an extracellular target-binding domain (as disclosed above), a transmembrane domain (as described above) and a cytoplasmic domain, wherein the cytoplasmic domain comprises at least one co-stimulatory protein (as described above). In certain embodiments, the co-stimulatory fusion protein does not comprise a lymphocyte activation domain (e.g., Cd3ζ). In certain embodiments, the at least one co-stimulatory protein can be a MyD88 polypeptide or functional fragment thereof (as described above), and/or a CD40 cytoplasmic polypeptide region or a functional fragment thereof (as described above).

[00132] In certain embodiments, the co-stimulatory fusion protein comprises an extracellular domain (such as, but not limited to CD19, CD34), a transmembrane domain (as described above) and a cytoplasmic domain, wherein the cytoplasmic domain comprises at least one co-

stimulatory protein (as described above). In certain embodiments, the co-stimulatory fusion protein does not comprise a lymphocyte activation domain (e.g., Cd3ζ). In certain embodiments, the at least one co-stimulatory protein can be a MyD88 polypeptide or functional fragment thereof (as described above), and/or a CD40 cytoplasmic polypeptide region or a functional fragment thereof (as described above).

[00133] Non-limiting examples of such co-stimulatory fusion proteins include, but are not limited to, the constructs in Figure 11. *See also* WO2016073875, which is incorporated herein by reference in its entirety for all purposes.

[00134] In certain embodiments, the co-stimulatory fusion proteins are introduced into the host cell on a separate vector from the CAR. In certain embodiments, the co-stimulatory fusion proteins are introduced into the host cell on the same vector as the CAR. In certain embodiments, the co-stimulatory fusion proteins are introduced into the host cell on the same vector as the CAR but separated by a separation sequence such as 2A.

[00135] Vectors

[00136] The present invention provides recombinant vectors comprising a polynucleotide encoding a CAR comprising polynucleotides encoding the proteins disclosed above. In certain embodiments, the polynucleotide is operatively linked to at least one regulatory element for expression of the chimeric antigen receptor.

[00137] In certain embodiments, the recombinant vector comprises a polynucleotide encoding a CAR comprising (a) an extracellular target-binding domain, (b) a transmembrane domain, and (c) a cytoplasmic domain comprising a lymphocyte activation domain, a MyD88 polypeptide or functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof (all as described above), wherein the polynucleotide is operatively linked to at least one regulatory element for expression of the chimeric antigen receptor.

[00138] In certain embodiments, the recombinant vector comprises a polynucleotide encoding a CAR comprising (a) an extracellular target-binding domain comprising an Eph receptor-binding moiety, (b) a transmembrane domain, and (c) a cytoplasmic domain comprising a lymphocyte activation domain, a MyD88 polypeptide or functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof (all as described above), wherein the polynucleotide is operatively linked to at least one regulatory element for expression of the chimeric antigen receptor

[00139] In certain embodiments, the recombinant vector of the invention comprises the nucleotide sequence of SEQ ID NO: 2, 27, 51, 53, 55, 57, or 59, or a variant thereof having at

least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 2, 27, 51, 53, 55, 57, or 59. In certain embodiments, the recombinant vector comprises the nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 1, 26, 50, 52, 54, 56, or 58, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 98 or at least 99%, sequence identity with SEQ ID NO: 1, 26, 50, 52, 54, 56, or 58.

[00140] In certain embodiments, the vector is a viral vector. In certain embodiments, the viral vector can be, but is not limited to, adenoviral vector, an adeno-associated virus vector, an alphaviral vector, a herpes virus vector, a lentiviral vector, a retroviral vector, and vaccinia virus vector. In certain embodiments, the viral vector is a retroviral vector.

[00141] In certain embodiments, the polynucleotide encoding the CAR is operably linked to at least a regulatory element. The regulatory element can be capable of mediating expression of the CAR in the host cell. Regulatory elements include, but are not limited to, promoters, enhancers, initiation sites, polyadenylation (polyA) tails, IRES elements, response elements, and termination signals. In certain embodiments, the regulatory element regulates CAR expression. In certain embodiments, the regulatory element increased the expression of the CAR. In certain embodiments, the regulatory element increased the expression of the CAR once the host cell is activated. In certain embodiments, the regulatory element decreases expression of the CAR. In certain embodiments, the regulatory element decreases expression of the CAR once the host cell is activated.

Methods for Producing CAR-Modified Host Cells

[00142] In certain embodiments, the host cells can be T cells. T cells include, but are not limited to, thymocytes, naive T lymphocytes, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, or activated T lymphocytes. A T cell can be a T helper (Th) cell, for example a T helper 1 (Thl) or a T helper 2 (Th2) cell. The T cell can be a helper T cell (HTL; CD4+ T cell) CD4+ T cell, a cytotoxic T cell (CTL; CD8+ T cell), a tumor infiltrating cytotoxic T cell (TIL; CD8+ T cell), CD4+ CD8+ T cell, or any other subset of T cells. Other illustrative populations of T cells suitable for use in particular embodiments include naive T cells memory T cells, and NKT cells.

[00143] In certain embodiments, the host cells can be NK cells. NK cell refers to a differentiated lymphocyte with a CD3- CD16+, CD3- CD56+, CD16+ CD56+ and/or CD57+ TCR- phenotype.

[00144] CAR-modified host cells can be expanded *ex vivo* for use in adoptive cellular immunotherapy in which infusions of such cells have been shown to have anti-disease reactivity in a disease-bearing host. The compositions and methods of this invention can be used to generate a population of host cells (*e.g.*, T lymphocyte or natural killer cells) that deliver both primary and co-stimulatory signals for use in immunotherapy in the treatment of the disease. In certain embodiments, the disease is cancer. In certain embodiments, the disease is a cancer that expresses EphA2. In certain embodiments, the cancer is a solid tumor (e.g., a solid tumor expressing EphA2).

[00145] Isolation/Enrichment

[00146] The host cells may be autologous/autogeneic ("self") or non-autologous ("non-self," e.g., allogeneic, syngeneic or xenogeneic). In certain embodiments, the host cells are obtained from a mammalian subject. In other embodiments, the host cells are obtained from a primate subject. In certain embodiments, the host cells are obtained from a human subject.

[00147] Lymphocytes can be obtained from sources such as, but not limited to, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. Lymphocytes may also be generated by differentiation of stem cells. In certain embodiments, lymphocytes can be obtained from blood collected from a subject using techniques generally known to the skilled person, such as sedimentation, e.g., FICOLL™ separation.

[00148] In certain embodiments, cells from the circulating blood of a subject are obtained by apheresis. An apheresis device typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In certain embodiments, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing. The cells can be washed with PBS or with another suitable solution that lacks calcium, magnesium, and most, if not all other, divalent cations. A washing step may be accomplished by methods known to those in the art, such as, but not limited to, using a semiautomated flowthrough centrifuge (*e.g.*, Cobe 2991 cell processor, or the Baxter CytoMate). After washing, the cells may be resuspended in a variety of biocompatible buffers, cell culture medias, or other saline solution with or without buffer.

[00149] In certain embodiments, host cells can be isolated from peripheral blood mononuclear cells (PBMCs) by lysing the red blood cells and depleting the monocytes. As an example, the cells can be sorted by centrifugation through a PERCOLL™ gradient. In certain embodiments, after isolation of PBMC, both cytotoxic and helper T lymphocytes can

be sorted into naive, memory, and effector T cell subpopulations either before or after activation, expansion, and/or genetic modification.

[00150] In certain embodiments, T lymphocytes can be enriched. For example, a specific subpopulation of T lymphocytes, expressing one or more markers such as, but not limited to, CD3, CD4, CD8, CD14, CD15, CD16, CD19, CD27, CD28, CD34, CD36, CD45RA, CD45RO, CD56, CD62, CD62L, CD122, CD123, CD127, CD235a, CCR7, HLA-DR or a combination thereof using either positive or negative selection techniques. In certain embodiments, the T lymphocytes for use in the compositions of the invention do not express or do not substantially express one or more of the following markers: CD57, CD244, CD160, PD-1, CTLA4, TIM3, and LAG3.

[00151] In certain embodiments, NK cells can be enriched. For example, a specific subpopulation of T lymphocytes, expressing one or more markers such as, but not limited to, CD2, CD16, CD56, CD57, CD94, CD122 or a combination thereof using either positive or negative selection techniques.

[00152] Stimulation/Activation

[00153] In order to reach sufficient therapeutic doses of host cell compositions, host cells are often subjected to one or more rounds of stimulation/activation. In certain embodiments, a method of producing host cells for administration to a subject comprises stimulating the host cells to become activated in the presence of one or more stimulatory signals or agents (e.g., compound, small molecule, e.g., small organic molecule, nucleic acid, polypeptide, or a fragment, isoform, variant, analog, or derivative thereof). In certain embodiments, a method of producing host cells for administration to a subject comprises stimulating the host cells to become activated and to proliferate in the presence of one or more stimulatory signals or agents.

[00154] Host cells (e.g., T lymphocytes and NK cells) can be activated by inducing a change in their biologic state by which the cells express activation markers, produce cytokines, proliferate and/or become cytotoxic to target cells. All these changes can be produced by primary stimulatory signals. Co-stimulatory signals amplify the magnitude of the primary signals and suppress cell death following initial stimulation resulting in a more durable activation state and thus a higher cytotoxic capacity.

[00155] T cells can be activated generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; and 6,867,041, each of which is incorporated herein by reference in its entirety.

[00156] In certain embodiments, the T cell based host cells can be activated by binding to an agent that activates CD3ζ.

[00157] In other embodiments, a CD2-binding agent may be used to provide a primary stimulation signal to the T cells. For example, and not by limitation, CD2 agents include, but are not limited to, CD2 ligands and anti-CD2 antibodies, e.g., the Tl 1.3 antibody in combination with the Tl 1.1 or Tl 1.2 antibody (Meuer, S. C. et al. (1984) Cell 36:897-906) and the 9.6 antibody (which recognizes the same epitope as TI 1.1) in combination with the 9-1 antibody (Yang, S. Y. et al. (1986) J. Immunol. 137:1097-1100). Other antibodies which bind to the same epitopes as any of the above described antibodies can also be used.

[00158] In certain embodiments, the host cells are activated by administering phorbol myristate acetate (PMA) and ionomycine. In certain embodiments, the host cells are activated by administering an appropriate antigen that induces activation and then expansion. In certain embodiments, PMA, ionomycin, and/or appropriate antigen are administered with CD3 induce activation and/or expansion.

[00159] In general, the activating agents used in the present invention includes, but is not limited to, an antibody, a fragment thereof and a proteinaceous binding molecule with antibody-like functions. Examples of (recombinant) antibody fragments are Fab fragments, Fv fragments, single-chain Fv fragments (scFv), a divalent antibody fragment such as an (Fab)2′-fragment, diabodies, triabodies (Iliades, P., et al., FEBS Lett (1997) 409, 437-441), decabodies (Stone, E., et al., Journal of Immunological Methods (2007) 318, 88-94) and other domain antibodies (Holt, L. J., et al., Trends Biotechnol. (2003), 21, 11, 484-490). The divalent antibody fragment may be an (Fab)2′-fragment, or a divalent single-chain Fv fragment while the monovalent antibody fragment may be selected from the group consisting of a Fab fragment, a Fv fragment, and a single-chain Fv fragment (scFv).

[00160] In certain embodiments, one or more binding sites of the CD3ζ agents may be a bivalent proteinaceous artificial binding molecule such as a dimeric lipocalin mutein (*i.e.*, duocalin). In certain embodiments the receptor binding reagent may have a single second binding site, (*i.e.*, monovalent). Examples of monovalent agents include, but are not limited to, a monovalent antibody fragment, a proteinaceous binding molecule with antibody-like binding properties or an MHC molecule. Examples of monovalent antibody fragments include, but are not limited to a Fab fragment, a Fv fragment, and a single-chain Fv fragment (scFv), including a divalent single-chain Fv fragment.

[00161] The agent that specifically binds CD3 includes, but is not limited to, an anti-CD3-antibody, a divalent antibody fragment of an anti-CD3 antibody, a monovalent antibody

fragment of an anti-CD3-antibody, and a proteinaceous CD3-binding molecule with antibody-like binding properties. A proteinaceous CD3-binding molecule with antibody-like binding properties can be an aptamer, a mutein based on a polypeptide of the lipocalin family, a glubody, a protein based on the ankyrin scaffold, a protein based on the crystalline scaffold, an adnectin, and an avimer. It also can be coupled to a bead.

[00162] In certain embodiments, the activating agent (e.g., CD3-binding agents) can be present in a concentration of about 0.1 to about 10 μg/ml. In certain embodiments, the activating agent (e.g., CD3-binding agents) can be present in a concentration of about 0.2 μg/ml to about 9 μg/ml, about 0.3 μg/ml to about 8 μg/ml, about 0.4 μg/ml to about 7 μg/ml, about 0.5 μg/ml to about 6 μg/ml, about 0.6 μg/ml to about 5 μg/ml, about 0.7 μg/ml to about 4 μg/ml, about 0.8 μg/ml to about 3 μg/ml, or about 0.9 μg/ml to about 2 μg/ml. In certain embodiments, the activating agent (e.g., CD3-binding agents) is administered at a concentration of about 0.1 μg/ml, about 0.2 μg/ml, about 0.3 μg/ml, about 0.4 μg/ml, about 0.5 μg/ml, about 0.6 μg/ml, about 0.7 μg/ml, about 0.8 μM, about 0.9 μg/ml, about 1 μg/ml, about 2 μg/ml, about 3 μg/ml, about 4 μM, about 5 μg/ml, about 6 μg/ml, about 7 μg/ml, about 8 μg/ml, about 9 μg/ml, or about 10 μg/ml. In certain embodiments, the CD3-binding agents can be present in a concentration of 1 μg/ml.

[00163] NK cells can be activated generally using methods as described, for example, in U.S. Patents 7,803,376, 6,949,520, 6,693,086, 8,834,900, 9,404,083, 9,464,274, 7,435,596, 8,026,097, 8,877,182; U.S. Patent Applications US2004/0058445, US2007/0160578, US2013/0011376, US2015/0118207, US2015/0037887; and PCT Patent Application WO2016/122147, each of which is incorporated herein by reference in its entirety.

[00164] In certain embodiments, the NK based host cells can be activated by, for example and not limitation, inhibition of inhibitory receptors on NK cells (e.g., KIR2DL1, KIR2DL2/3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2, KIR3DL3, LILRB1, NKG2A, NKG2C, NKG2E or LILRB5 receptor).

[00165] In certain embodiments, the NK based host cells can be activated by, for example and not limitation, feeder cells (e.g., native K562 cells or K562 cells that are genetically modified to express 4-1BBL and cytokines such as IL15 or IL21).

[00166] In other embodiments, interferons or macrophage-derived cytokines can be used to activate NK cells. For example and not limitation, such interferons include but are not limited to interferon alpha and interferon gamma, and such cytokines include but are not limited to IL-15, IL-2, IL-21.

[00167] In certain embodiments, the NK activating agent can be present in a concentration of about 0.1 to about 10 μg/ml. In certain embodiments, the NK activating agent can be present in a concentration of about 0.2 μg/ml to about 9 μg/ml, about 0.3 μg/ml to about 8 μg/ml, about 0.4 μg/ml to about 7 μg/ml, about 0.5 μg/ml to about 6 μg/ml, about 0.6 μg/ml to about 5 μg/ml, about 0.7 μg/ml to about 4 μg/ml, about 0.8 μg/ml to about 3 μg/ml, or about 0.9 μg/ml to about 2 μg/ml. In certain embodiments, the NK activating agent is administered at a concentration of about 0.1 μg/ml, about 0.2 μg/ml, about 0.3 μg/ml, about 0.4 μg/ml, about 0.5 μg/ml, about 0.6 μg/ml, about 0.7 μg/ml, about 0.8 μM, about 0.9 μg/ml, about 1 μg/ml, about 2 μg/ml, about 3 μg/ml, about 4 μM, about 5 μg/ml, about 6 μg/ml, about 7 μg/ml, about 8 μg/ml, about 9 μg/ml, or about 10 μg/ml. In certain embodiments, the NK activating agent can be present in a concentration of 1 μg/ml.

[00168] In certain embodiments, the activating agent is attached to a solid support such as, but not limited to, a bead, an absorbent polymer present in culture plate or well or other matrices such as, but not limited to, Sepharose or glass; may be expressed (such as in native or recombinant forms) on cell surface of natural or recombinant cell line by means known to those skilled in the art.

[00169] Polynucleotide Transfer

[00170] In certain embodiments, the host cells are genetically modified to express a CAR. The host cells can be genetically modified after stimulation/activation. In certain embodiments, the CAR is that described above. In certain embodiments, the host cells are modified within 12 hours, 16 hours, 24 hours, 36 hours, or 48 hours of stimulation/activation. In certain embodiments, the cells are modified within 16 to 24 hours after stimulation/activation. In certain embodiments, the host cells are modified within 24 hours.

[00171] In order to genetically modify the host cell to express the CAR, the CAR polynucleotide construct must be transferred into the host cell. Polynucleotide transfer may be via viral or non-viral gene methods. Suitable methods for polynucleotide delivery for use with the current methods include any method known by those of skill in the art, by which a polynucleotide can be introduced into an organelle, cell, tissue or organism.

[00172] Nucleic acid vaccines can be used to transfer CAR polynucleotides into the host cells. Such vaccines include, but are not limited to non-viral polynucleotide vectors, "naked" DNA and RNA, and viral vectors. Methods of genetically modifying cells with these vaccines, and for optimizing the expression of genes included in these vaccines are known to those of skill in the art.

[00173] In certain embodiments, the host cells can be genetically modified by methods ordinarily used by one of skill in the art. In certain embodiments, the host cells can be transduced via retroviral transduction. References describing retroviral transduction of genes are Anderson et al., U.S. Pat. No. 5,399,346; Mann et al., Cell 33:153 (1983); Temin et al., U.S. Pat. No. 4,650,764; Temin et al., U.S. Pat. No. 4,980,289; Markowitz et al., J. Virol. 62:1120 (1988); Temin et al., U.S. Pat. No. 5,124,263; International Patent Publication No. WO 95/07358, published Mar. 16, 1995, by Dougherty et al.; and Kuo et al., Blood 82:845 (1993), each of which is incorporated herein by reference in its entirety.

[00174] One method of genetic modification includes *ex vivo* modification. Various methods are available for transfecting cells and tissues removed from a subject via *ex vivo* modification. For example, retroviral gene transfer *in vitro* can be used to genetically modified cells removed from the subject and the cell transferred back into the subject. See e.g., Wilson et al., *Science*, 244:1344-1346, 1989 and Nabel et al., *Science*, 244(4910):1342-1344, 1989, both of which are incorporated herein by reference in their entity. In certain embodiments, the host cells may be removed from the subject and transfected *ex vivo* using the polynucleotides (e.g., expression vectors) of the invention. In certain embodiments, the host cells obtained from the subject can be transfected or transduced with the polynucleotides (e.g., expression vectors) of the invention and then administered back to the subject.

[00175] Another method of gene transfer includes injection. In certain embodiments, a cell or a polynucleotide or viral vector may be delivered to a cell, tissue, or organism via one or more injections (e.g., a needle injection). Non-limiting methods of injection include injection of a composition (e.g., a saline based composition). Polynucleotides can also be introduced by direct microinjection. Non-limiting sites of injection include, subcutaneous, intradermal, intramuscular, intranodal (allows for direct delivery of antigen to lymphoid tissues). intravenous, intraprotatic, intratumor, intralymphatic (allows direct administration of DCs) and intraperitoneal. It is understood that proper site of injection preparation is necessary (e.g., shaving of the site of injection to observe proper needle placement).

[00176] Electroporation is another method of polynucleotide delivery. *See e.g.,* Potter et al., (1984) *Proc. Nat'l Acad. Sci. USA,* 81, 7161-7165 and Tur-Kaspa et al., (1986) *Mol. Cell Biol.,* 6, 716-718, both of which are incorporated herein in their entirety for all purposes. Electroporation involves the exposure of a suspension of cells and DNA to a high-voltage electric discharge. In certain embodiments, cell wall-degrading enzymes, such as pectin-degrading enzymes, can be employed to render the host cells more susceptible to genetic

modification by electroporation than untreated cells. See e.g., U.S. Pat. No. 5,384,253, incorporated herein by reference in its entirety for all purposes.

[00177] *In vivo* electroporation involves a basic injection technique in which a vector is injected intradermally in a subject. Electrodes then apply electrical pulses to the intradermal site causing the cells localized there (e.g., resident dermal dendritic cells), to take up the vector. These tumor antigen-expressing dendritic cells activated by local inflammation can then migrate to lymph-nodes.

[00178] Methods of electroporation for use with this invention include, for example, Sardesai, N. Y., and Weiner, D. B., *Current Opinion in Immunotherapy* 23:421-9 (2011) and Ferraro, B. et al., *Human Vaccines* 7:120-127 (2011), both of which are hereby incorporated by reference herein in their entirety for all purposes.

[00179] Additional methods of polynucleotide transfer include liposome-mediated transfection (e.g., polynucleotide entrapped in a lipid complex suspended in an excess of aqueous solution. *See e.g.,* Ghosh and Bachhawat, (1991) *In: Liver Diseases, Targeted Diagnosis and Therapy Using Specific Receptors and Ligands*. pp. 87-104). Also contemplated is a polynucleotide complexed with Lipofectamine, or Superfect); DEAE-dextran (e.g., a polynucleotide is delivered into a cell using DEAE-dextran followed by polyethylene glycol. See e.g., Gopal, T. V., *Mol Cell Biol.* 1985 May; 5(5):1188-90); calcium phosphate (e.g., polynucleotide is introduced to the cells using calcium phosphate precipitation. *See e.g.,* Graham and van der Eb, (1973) *Virology*, 52, 456-467; Chen and Okayama, *Mol. Cell Biol.,* 7(8):2745-2752, 1987), and Rippe et al., *Mol. Cell Biol.*, 10:689-695, 1990); sonication loading (introduction of a polynucleotide by direct sonic loading. *See e.g.,* Fechheimer et al., (1987) *Proc. Nat'l Acad. Sci. USA*, 84, 8463-8467); microprojectile bombardment (e.g., one or more particles may be coated with at least one polynucleotide and delivered into cells by a propelling force. *See e.g.,* U.S. Pat. No. 5,550,318; U.S. Pat. No. 5,538,880; U.S. Pat. No. 5,610,042; and PCT Application WO 94/09699; Klein et al., (1987) *Nature*, 327, 70-73, Yang et al., (1990) *Proc. Nat'l Acad. Sci. USA*, 87, 9568-9572); and receptor-mediated transfection (e.g., selective uptake of macromolecules by receptor-mediated endocytosis that will be occurring in a target cell using cell type-specific distribution of various receptors. *See e.g.,* Wu and Wu, (1987) *J. Biol. Chem.,* 262, 4429-4432; Wagner et al., *Proc. Natl. Acad. Sci. USA*, 87(9):3410-3414, 1990; Perales et al., *Proc. Natl. Acad. Sci. USA*, 91:4086-4090, 1994; Myers, EPO 0273085; Wu and Wu, *Adv. Drug Delivery Rev.*, 12:159-167, 1993; Nicolau et al., (1987) *Methods Enzymol.,* 149, 157-176), each reference cited here is incorporated by reference in their entirety for all purposes.

[00180] Expansion/Proliferation

[00181] After the host cells are activated and transduced, the cells are cultured to proliferate. T cells may be cultured for at least 1, 2, 3, 4, 5, 6, or 7 days, at least 2 weeks, at least 1, 2, 3, 4, 5, or 6 months or more with 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more rounds of expansion.

[00182] Agents that can be used for the expansion of T cells can include interleukins, such as IL-2, IL-7, IL-15, or IL-21 (see for example Cornish et al. 2006, Blood. 108(2):600-8, Bazdar and Sieg, 2007, Journal of Virology, 2007, 81(22):12670-12674, Battalia et al, 2013, Immunology, 139(1):109-120). Other illustrative examples for agents that may be used for the expansion of T cells are agents that bind to CD8, CD45 or CD90, such as αCD8, αCD45 or αCD90 antibodies. Illustrative examples of T cell population including antigen-specific T cells, T helper cells, cytotoxic T cells, memory T cell (an illustrative example of memory T-cells are CD62L|CD8| specific central memory T cells) or regulatory T cells (an illustrative example of Treg are CD4+CD25+CD45RA+ Treg cells).

[00183] Additional agents that can be used to expand T lymphocytes includes methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; and 6,867,041, each of which is incorporated herein by reference in its entirety.

[00184] In certain embodiments, the agent(s) used for expansion (e.g., IL-2) are administered at about 20 units/ml to about 200 units/ml. In certain embodiments, the agent(s) used for expansion (e.g., IL-2) are administered at about 25 units/ml to about 190 units/ml, about 30 units/ml to about 180 units/ml, about 35 units/ml to about 170 units/ml, about 40 units/ml to about 160 units/ml, about 45 units/ml to about 150 units/ml, about 50 units/ml to about 140 units/ml, about 55 units/ml to about 130 units/ml, about 60 units/ml to about 120 units/ml, about 65 units/ml to about 110 units/ml, about 70 units/ml to about 100 units/ml, about 75 units/ml to about 95 units/ml, or about 80 units/ml to about 90 units/ml. In certain embodiments, the agent(s) used for expansion (e.g., IL-2) are administered at about 20 units/ml, about 25 units/ml, about 30 units/ml, 35 units/ml, 40 units/ml, 45 units/ml, about 50 units/ml, about 55 units/ml, about 60 units/ml, about 65 units/ml, about 70 units/ml, about 75 units/ml, about 80 units/ml, about 85 units/ml, about 90 units/ml, about 95 units/ml, about 100 units/ml, about 105 units/ml, about 110 units/ml, about 115 units/ml, about 120 units/ml, about 125 units/ml, about 130 units/ml, about 135 units/ml, about 140 units/ml, about 145 units/ml, about 150 units/ml, about 155 units/ml, about 160 units/ml, about 165 units/ml, about 170 units/ml, about 175 units/ml, about 180 units/ml, about 185 units/ml, about 190

units/ml, about 195 units/ml, or about 200 units/ml. In certain embodiments, the agent(s) used for expansion (e.g., IL-2) are administered at about 5 mg/ml to about 10 ng/ml. In certain embodiments, the agent(s) used for expansion (e.g., IL-2) are administered at about 5.5 ng/ml to about 9.5 ng/ml, about 6 ng/ml to about 9 ng/ml, about 6.5 ng/ml to about 8.5 ng/ml, or about 7 ng/ml to about 8 ng/ml. In certain embodiments, the agent(s) used for expansion (e.g., IL-2) are administered at about 5 ng/ml, 6 ng/ml, 7 ng/ml, 8 ng/ml, 9, ng/ml, or 10 ng/ml.

[00185] After the host cells are activated and transduced, the cells are cultured to proliferate. NK cells may be cultured for at least 1, 2, 3, 4, 5, 6, or 7 days, at least 2 weeks, at least 1, 2, 3, 4, 5, or 6 months or more with 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more rounds of expansion.

[00186] Agents that can be used for the expansion of natural killer cells can include agents that bind to CD16 or CD56, such as for example αCD16 or αCD56 antibodies. In certain embodiments, the binding agent includes antibodies (see for example Hoshino et al, Blood. 1991 Dec. 15; 78(12):3232-40.). Other agents that may be used for expansion of NK cells may be IL-15 (see for example Vitale et al. 2002. The Anatomical Record. 266:87-92).

[00187] Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media (MEM), RPMI Media 1640, Lonza RPMI 1640, Advanced RPMI, Clicks, AIM-V, DMEM, a-MEM, F-12, TexMACS, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion).

[00188] Examples of other additives for host cell expansion include, but are not limited to, surfactant, piasmanate, pH buffers such as HEPES, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol, Antibiotics (e.g., penicillin and streptomycin), are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C) and atmosphere (e.g., air plus 5% CO_2).

[00189] In certain embodiments, host cells of the present invention may be modified such that the expression of an endogenous TCR, MHC molecule, or other immunogenic molecule is decreased or eliminated. When allogeneic cells are used, rejection of the therapeutic cells may be a concern as it may cause serious complications such as the graft-versus-host disease (GvHD). Although not wishing to be bound by theory, immunogenic molecules (e.g., endogenous TCRs and/or MHC molecules) are typically expressed on the cell surface and are

involved in self vs non-self discrimination. Decreasing or eliminating the expression of such molecules may reduce or eliminate the ability of the therapeutic cells to cause GvHD.

[00190] In certain embodiments, expression of an endogenous TCR in the host cells is decreased or eliminated. In a particular embodiment, expression of an endogenous TCR (e.g., αβ TCR) in the host cells is decreased or eliminated. Expression of the endogenous TCR may be decreased or eliminated by disrupting the TRAC locus, TCR beta constant locus, and/or CD3 locus. In certain embodiments, expression of an endogenous TCR may be decreased or eliminated by disrupting one or more of the TRAC, TRBC1, TRBC2, CD3E, CD3G, and/or CD3D locus.

[00191] In certain embodiments, expression of one or more endogenous MHC molecules in the host cells is decreased or eliminated. Modified MHC molecule may be an MHC class I or class II molecule. In certain embodiments, expression of an endogenous MHC molecule may be decreased or eliminated by disrupting one or more of the MHC, β2M, TAP1, TAP2, CIITA, RFX5, RFXAP and/or RFXANK locus.

[00192] Expression of the endogenous TCR, an MHC molecule, and/or any other immunogenic molecule in the host cell can be disrupted using genome editing techniques such as Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and Meganucleases. These genome editing methods may disrupt a target gene by entirely knocking out all of its output or partially knocking down its expression. In a particular embodiment, expression of the endogenous TCR, an MHC molecule and/or any other immunogenic molecule in the host cell is disrupted using the CRISPR/Cas technique.

Therapeutic Methods of the Invention

[00193] In certain embodiments, the compositions comprise one or more polypeptides, polynucleotides, vectors comprising same, and host cell compositions, as disclosed herein. Compositions include, but are not limited to pharmaceutical compositions. In certain embodiments, the compositions of the present invention comprise an amount of modified host cells manufactured by the methods disclosed herein.

[00194] In one aspect, the invention provides a method for treating a disease or disorder associated in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the host cells disclosed herein comprising a CAR as disclosed herein. In certain embodiments, the host cells expressing the CAR are prepared by the methods as disclosed above.

[00195] In certain embodiments, the disease or disorder is cancer. In certain embodiments, the cancer can be, but is not limited to, a solid tumor. In certain embodiments, the disease or disorder is cancer expressing an Eph receptor. Table 2 provides non-limiting examples of cancers associated with increased Eph receptors. In certain embodiments, the disease or disorder is cancer expressing EphA2.

Table 2. Non-limiting Examples of Eph Receptor Involvement in Cancers

Cancer type	Eph Receptor
Breast cancer	EphA2, EphB4
Colorectal cancer	EphA1, EphA2, EphA3, EphA8, EphB4
Prostate cancer	EphA2, EphA3, EphA5, EphA6, EphA7, EphA8, EphA10, EphB3,
Brain tumor, GBM	EphA2, EphA3, EphA4, EphA7, EphB2, EphB4
Melanoma	EphA2, EphA3, EphB3
Lung cancer	EphA2, EphB3
Hepatocellular cancer	EphA3
Gastric cancer	EphA1, EphA2, EphA3, EphB2

[00196] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. The term "cancer" includes, for example, the soft tissue tumors (e.g., lymphomas), and tumors of the blood and blood-forming organs (e.g., leukemias), and solid tumors, which is one that grows in an anatomical site outside the bloodstream (e.g., carcinomas). Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma (e.g., osteosarcoma or rhabdomyosarcoma), and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g., epithelial squamous cell cancer), adenosquamous cell carcinoma, lung cancer (e.g., including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (e.g., including gastrointestinal cancer, pancreatic cancer), cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile

carcinoma, primary or metastatic melanoma, multiple myeloma and B-cell lymphoma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, brain (e.g., high grade glioma, diffuse pontine glioma, ependymoma, neuroblastoma, or glioblastoma), as well as head and neck cancer, and associated metastases. Additional examples of cancer can be found in The Merck Manual of Diagnosis and Therapy, 19th Edition, § on Hematology and Oncology, published by Merck Sharp & Dohme Corp., 2011 (ISBN 978-0-911910-19-3); The Merck Manual of Diagnosis and Therapy, 20th Edition, § on Hematology and Oncology, published by Merck Sharp & Dohme Corp., 2018 (ISBN 978-0-911-91042-1) (2018 digital online edition at internet website of Merck Manuals); and SEER Program Coding and Staging Manual 2016, each of which are incorporated by reference in their entirety for all purposes.

[00197] Other hyperproliferative diseases, including solid tumors, that may be treated using the host cells discloser herein include, but are not limited to rheumatoid arthritis, inflammatory bowel disease, osteoarthritis, leiomyomas, adenomas, lipomas, hemangiomas, fibromas, vascular occlusion, restenosis, atherosclerosis, pre-neoplastic lesions (such as adenomatous hyperplasia and prostatic intraepithelial neoplasia), carcinoma in situ, oral hairy leukoplakia, or psoriasis.

[00198] In certain embodiments, composition comprising the host cells manufactured by the methods described herein may be administered at a dosage of 10^2 to 10^{10} cells/kg body weight, 10^5 to 10^9 cells/kg body weight, 10^5 to 10^8 cells/kg body weight, 10^5 to 10^7 cells/kg body weight, 10^7 to 10^9 cells/kg body weight, or 10^7 to $10^{8.}$ cells/kg body weight, including all integer values within those ranges. The number of host cells will depend on the therapeutic use for which the composition is intended for.

[00199] Host cells modified to express an engineered TCR or CAR may be administered multiple times at dosages listed above. The host cells may be allogeneic, syngeneic, xenogeneic, or autologous to the patient undergoing therapy. When allogeneic cells are used, the cell surface expression of the endogenous TCR, MHC molecule, and/or any other immunogenic molecule may be decreased or eliminated in the administered cells.

[00200] The compositions and methods described in the present invention may be utilized in conjunction with other types of therapy for cancer, such as chemotherapy, surgery, radiation, gene therapy, and so forth.

[00201] In certain embodiments of any of the above methods of the invention, the composition is administered in a therapeutically effective amount. The dosages of the composition administered in the methods of the invention will vary widely, depending upon the subject's physical parameters, the frequency of administration, the manner of

administration, the clearance rate, and the like. The initial dose may be larger, and might be followed by smaller maintenance doses. The dose may be administered as infrequently as weekly or biweekly, or fractionated into smaller doses and administered daily, semi-weekly, etc., to maintain an effective dosage level. It is contemplated that a variety of doses will be effective to achieve *in vivo* persistence of CAR containing host cells. It is also contemplated that a variety of doses will be effective to improve *in vivo* effector function of CAR containing host cells.

[00202] Compositions comprising modified host cells disclosed herein may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives.

[00203] Compositions comprising modified host cells disclosed herein may comprise one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. An injectable pharmaceutical composition is preferably sterile.

[00204] In certain embodiments, the compositions are formulated for parenteral administration, e.g., intravascular (intravenous or intraarterial), intraperitoneal, intratumoral, intraventricular, intrapleural or intramuscular administration. In certain embodiments, the composition is reconstituted from a lyophilized preparation prior to administration.

[00205] In certain embodiments, the CAR containing host cells may be mixed with substances that adhere or penetrate then prior to their administration, e.g., but not limited to, nanoparticles.

[00206] It is also contemplated that when used to treat various diseases/disorders, the compositions and methods of the present invention can be utilized with other therapeutic methods/agents suitable for the same or similar diseases/disorders. Such other therapeutic methods/agents can be co-administered (simultaneously or sequentially) to generate additive

or synergistic effects. Suitable therapeutically effective dosages for each agent may be lowered due to the additive action or synergy.

[00207] In one embodiment of any of the above methods of the invention, the method further comprises administering to the subject one or more additional compounds selected from the group consisting of immuno-suppressives, biologicals, probiotics, prebiotics, and cytokines (e.g., IFN or IL-2).

[00208] As a non-limiting example, the invention can be combined with other therapies that block inflammation (e.g., via blockage of IL1, INFα/β, IL6, TNF, IL23, etc.).

[00209] The methods and compositions of the invention can be combined with other immunomodulatory treatments such as, e.g., therapeutic vaccines (including but not limited to GVAX, DC-based vaccines, etc.), checkpoint inhibitors (including but not limited to agents that block CTLA4, PD1, LAG3, TIM3, etc.) or activators (including but not limited to agents that enhance 4-1BB, OX40, etc.). The methods of the invention can be also combined with other treatments that possess the ability to modulate NKT function or stability, including but not limited to CD1d, CD1d-fusion proteins, CD1d dimers or larger polymers of CD1d either unloaded or loaded with antigens, CD1d-chimeric antigen receptors (CD1d-CAR), or any other of the five known CD1 isomers existing in humans (CD1a, CD1b, CD1c, CD1e). The methods of the invention can also be combined with other treatments such as midostaurin, enasidenib, or a combination thereof.

[00210] Therapeutic methods of the invention can be combined with additional immunotherapies and therapies. For example, when used for treating cancer, the compositions of the invention can be used in combination with conventional cancer therapies, such as, e.g., surgery, radiotherapy, chemotherapy or combinations thereof, depending on type of the tumor, patient condition, other health issues, and a variety of factors. In certain aspects, other therapeutic agents useful for combination cancer therapy with the inhibitors of the invention include anti-angiogenic agents. Many anti-angiogenic agents have been identified and are known in the art, including, e.g., TNP-470, platelet factor 4, thrombospondin-1, tissue inhibitors of metalloproteases (TIMP1 and TIMP2), prolactin (16-Kd fragment), angiostatin (38-Kd fragment of plasminogen), endostatin, bFGF soluble receptor, transforming growth factor beta, interferon alpha, soluble KDR and FLT-1 receptors, placental proliferin-related protein, as well as those listed by Carmeliet and Jain (2000). In one embodiment, the host cells of the invention can be used in combination with a VEGF antagonist or a VEGF receptor antagonist such as anti-VEGF antibodies, VEGF variants, soluble VEGF receptor fragments, aptamers capable of blocking VEGF or VEGFR,

neutralizing anti-VEGFR antibodies, inhibitors of VEGFR tyrosine kinases and any combinations thereof (e.g., anti-hVEGF antibody A4.6.1, bevacizumab or ranibizumab).

[00211] Non-limiting examples of chemotherapeutic compounds which can be used in combination treatments of the present invention include, for example, aminoglutethimide, amsacrine, anastrozole, asparaginase, azacitidine, bcg, bicalutamide, bleomycin, buserelin, busulfan, campothecin, capecitabine, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, decitabine, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estradiol, estramnustine, etoposide, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, letrozole, leucovorin, leuprolide, levamisole, lomustine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, suramin, tamoxifen, temozolomide, teniposide, testosterone, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, and vinorelbine.

[00212] These chemotherapeutic compounds may be categorized by their mechanism of action into, for example, following groups: anti-metabolites/anti-cancer agents, such as pyrimidine analogs (5-fluorouracil, floxuridine, capecitabine, gemcitabine and cytarabine) and purine analogs, folate antagonists and related inhibitors (mercaptopurine, thioguanine, pentostatin and 2-chlorodeoxyadenosine (cladribine)); antiproliferative/antimitotic agents including natural products such as vinca alkaloids (vinblastine, vincristine, and vinorelbine), microtubule disruptors such as taxane (paclitaxel, docetaxel), vincristin, vinblastin, nocodazole, epothilones and navelbine, epidipodophyllotoxins (etoposide, teniposide), DNA damaging agents (actinomycin, amsacrine, anthracyclines, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin, cyclophosphamide, cytoxan, dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethyhnelamineoxaliplatin, iphosphamide, melphalan, merchlorehtamine, mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, teniposide, triethylenethiophosphoramide and etoposide (VP16)); antibiotics such as dactinomycin (actinomycin D), daunorubicin, doxorubicin (adriamycin), idarubicin, anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin) and mitomycin; enzymes (L-asparaginase which systemically metabolizes L-

asparagine and deprives cells which do not have the capacity to synthesize their own asparagine); antiplatelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa), alkyl sulfonates-busulfan, nitrosoureas (carmustine (BCNU) and analogs, streptozocin), trazenes-dacarbazinine (DTIC); antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate); platinum coordination complexes (cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide; hormones, hormone analogs (estrogen, tamoxifen, goserelin, bicalutamide, nilutamide) and aromatase inhibitors (letrozole, anastrozole); anticoagulants (heparin, synthetic heparin salts and other inhibitors of thrombin); fibrinolytic agents (such as tissue plasminogen activator, streptokinase and urokinase), aspirin, dipyridamole, ticlopidine, clopidogrel, abciximab; antimigratory agents; antisecretory agents (breveldin); immunosuppressives (cyclosporine, tacrolimus (FK-506), sirolimus (rapamycin), azathioprine, mycophenolate mofetil); anti-angiogenic compounds (e.g., TNP-470, genistein, bevacizumab) and growth factor inhibitors (e.g., fibroblast growth factor (FGF) inhibitors); angiotensin receptor blocker; nitric oxide donors; anti-sense oligonucleotides; antibodies (trastuzumab); cell cycle inhibitors and differentiation inducers (tretinoin); mTOR inhibitors, topoisomerase inhibitors (doxorubicin (adriamycin), amsacrine, camptothecin, daunorubicin, dactinomycin, eniposide, epirubicin, etoposide, idarubicin and mitoxantrone, topotecan, irinotecan), corticosteroids (cortisone, dexamethasone, hydrocortisone, methylpednisolone, prednisone, and prenisolone); growth factor signal transduction kinase inhibitors; mitochondrial dysfunction inducers and caspase activators; and chromatin disruptors.

[00213] In accordance with the present invention there may be numerous tools and techniques within the skill of the art, such as those commonly used in molecular biology, pharmacology, and microbiology. Such tools and techniques are described in detail in e.g., Sambrook et al. (2001) Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York; Ausubel et al. eds. (2005) Current Protocols in Molecular Biology. John Wiley and Sons, Inc.: Hoboken, NJ; Bonifacino et al. eds. (2005) Current Protocols in Cell Biology. John Wiley and Sons, Inc.: Hoboken, NJ; Coligan et al. eds. (2005) Current Protocols in Immunology, John Wiley and Sons, Inc.: Hoboken, NJ; Coico et al. eds. (2005) Current Protocols in Microbiology, John Wiley and Sons, Inc.: Hoboken, NJ; Coligan et al. eds. (2005) Current Protocols in Protein Science,

John Wiley and Sons, Inc.: Hoboken, NJ; and Enna et al. eds. (2005) Current Protocols in Pharmacology, John Wiley and Sons, Inc.: Hoboken, NJ.

EXAMPLES

[00214] The present invention is also described and demonstrated by way of the following examples. However, the use of these and other examples anywhere in the specification is illustrative only and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to any particular preferred embodiments described here. Indeed, many modifications and variations of the invention may be apparent to those skilled in the art upon reading this specification, and such variations can be made without departing from the invention in spirit or in scope. The invention is therefore to be limited only by the terms of the appended claims along with the full scope of equivalents to which those claims are entitled.

[00215] EXAMPLE 1. Generation of CARs

[00216] Retroviral vectors were generated encoding CARs comprising CD28.ζ (SEQ ID NOs: 3-4), 4-1BB.ζ (SEQ ID NOs: 5-6), or MyD88.CD40.ζ (SEQ ID NOs: 1-2) endodomains that recognize the solid tumor antigen EphA2 (**Figure 1A** and **Figure 6A-6C**). CAR T cells were generated by retroviral transduction, and all three CARs were expressed at similar levels as judged by FACS analysis (**Figures 1B-1C**).

[00217] EphA2-specific CD28.CAR and 41BB.CAR: A codon-optimized gene encoding the humanized version of EphA2 mAb EA2 was synthesized by GeneArt (Invitrogen, Carlsbad, CA) containing the immunoglobulin heavy-chain leader peptide and the 4H5 heavy and light chains connected by a short glycine-serine linker. The minigene was flanked by 5′ NcoI and 3′ BamHI sites and subcloned into an SFG retroviral vector containing a human IgG1 core hinge and CD28.ζ or 41BB.ζ endodomains. The retroviral vector with CD28.ζ contained a CD28 transmembrane domain and the vector with 41BB.ζ contained a CD8α transmembrane domain. In addition, all vectors encoded for a 2A sequence and truncated CD19.

[00218] EphA2-specific MyD88.CD40.CAR: In-Fusion cloning (Takara Bio USA, Mountain View, CA) was used to generate this CAR. First, an IL13Rα2-specific MyD88.CD40.CAR was generated by replacing CD28 of an IL13Rα2-specific CD28.CAR (Krenciute et al, Mol Ther 2016) with MyD88.CD40 amplified from plasmid FRP5.z.2A.iMyD88.CD40 (Mata et al; Cancer Discovery 2017). In-Fusion cloning was then used i) to replace the IL13Ra2-specific scFv with the EphA2-specific scFv 4H5 and ii) to add a 2A sequence and truncated CD19.

[00219] All cloning of CARs was verified by Sanger sequencing (Seqwright, Houston, TX and The Hartwell Center, SJCRH, Memphis, TN).

[00220] Generation of CAR T cells: Retroviral particles were generated by transient transfection of 293T cells with an EphA2 CAR encoding SFG retroviral vector, Peg-Pam-e plasmid containing the sequence for MoMLV gag-pol, and a plasmid containing the sequence for RD114 envelope proteins. Supernatants containing the retrovirus were collected after 48 hours, filtered, and snap frozen for later transduction of T cells. Human peripheral blood mononuclear cells (PBMCs) were obtained from whole blood of healthy donors under IRB-approved protocols at Baylor College of Medicine or St. Jude Children's Research Hospital. To generate EphA2-CAR T cells, PBMCs were isolated by Lymphoprep (Abbott Laboratories, Chicago, IL) gradient centrifugation and then stimulated on treated non-tissue culture 24-well plates, which were precoated with CD3 and CD28 (BD Biosciences, San Jose, CA,) antibodies. Recombinant human IL-7 and IL-15 (IL-7, 10 ng/mL; IL-15, 5 ng/mL; PeproTech, Rocky Hill, NJ) were added to cultures the next day. On day 2, CD3/CD28-stimulated T cells (2.5×10^5 cells/well) were transduced with RD114-pseudotyped retroviral particles on RetroNectin (Clontech, Mountain View, CA)-coated plates in the presence of IL-7 and IL-15. On day 5, transduced T cells were transferred into new tissue culture 24-well plates and subsequently expanded with IL-7 and IL-15. Non-transduced (NT) T cells were prepared in the same way minus the addition of retrovirus. CAR T cell expression was determined using flow cytometry at multiple time points post-transduction to ensure continued CAR expression. All *in vitro* experiments were performed 7-21 days post-transduction. Biological replicates were performed using PBMCs from different healthy donors.

[00221] Phenotypic analysis of CAR T cells: A FACSCanto II (BD Biosciences, San Jose, CA) instrument was used to acquire flow cytometry data, which was analyzed using FlowJo v10 (FlowJo, Ashland, OR). Samples were washed with and stained in PBS (Lonza, Basel, Switzerland) with 1% FBS (GE Healthcare Life Sciences HyClone). T cells were phenotyped with CD3-APC (clone UTCH1, Beckman Coulter, Brea, CA) CD4-Pacific Blue (clone SK3, BioLegend, San Diego, CA) CD8-PerCP-Cy5.5 (clone SK1, BioLegend), CD19-PE (clone J3-119, Beckman Coulter), CCR7-AF488 (clone G043H7, BioLegend), and CD45RA-APC-H7 (clone HI100, BD) at day 7 after transduction as well as 7 days after incubation with tumor cells. Samples were washed, stained with antibodies in the dark for 30 minutes at room temperature or 1 hour at 4C, and washed again before analysis.

[00222] EXAMPLE 2. *In Vitro* Characterization of CARs

[00223] Next, the CARs were evaluated *in vitro*. In coculture assays, CD28.ζ.CAR T cells secreted more cytokines (IFNγ and IL2, **Figures 2A-2B**, respectively) and killed EphA2+ target cells faster in comparison to MyD88.CD40.ζ.CAR T cells (**Figure 2C**). MyD88.CD40.ζ.CAR T cells, however, produced significant amounts of IFNγ (*$p<0.05$) and IL2 (*$p<0.01$) in the presence of EphA2+ U373 cells, and did not exhibit background killing of k/o U373 cells (**Figures 2A-2C**). Consistent with greater T-cell activation, CD28.ζ.CAR T-cell cultures had a higher percentage of terminally differentiated CAR T cells (CD45RA+/CCR7-) in comparison to MyD88.CD40.ζ.CAR T cells (**Figures 3B-3C**). Notably, in repeat stimulation assays (diagrammed in **Figure 3A**), in which fresh tumor cells were added to CAR T cells weekly, only MyD88.CD40.ζ.CAR T cells maintained their ability to expand and kill tumor cells in contrast to CD28.ζ.CAR or 4-1BB.ζ.CAR T cells (**Figures 3B-3C**).

[00224] The following cell lines were used for the *in vitro* characterization of CAR T cells: U373 (GBM), LM7 (osteosarcoma), and 293T (human embryonic kidney) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). LM7 cells expressing eGFP and firefly luciferase (LM7.eGFP.ffLuc) were generated by lentiviral transduction. U373 EphA2 KO cells were generated using CRISPR/Cas9. A guide RNA targeting the sequence GGGGGGCCGCTCACCCGCAA (SEQ ID NO:62) was selected using the online CRISPRscan scoring algorithm (Giraldez Lab, Yale University) to maximize cutting efficiency and minimize off-target effects. sgRNA was generated using the HiScribe T7 *in vitro* transcription kit (New England Biola bs, Ipswich, MA). U373 cells were electroporated with 1 μg Cas9 with NLS (PNA Bio, Newbury Park, CA) and 1 μg sgRNA using the Neon Transfection System (ThermoFisher Scientific, Waltham, MA). Flow cytometry was used to confirm EphA2 knockout. T cells and cell lines were grown in RPMI or DMEM (GE Healthcare Life Sciences HyClone Laboratories, Logan, UT) with 10% fetal bovine serum (FBS; GE Healthcare Life Sciences HyClone) and 2 mM GlutaMAX-I (Invitrogen, Carlsbad, CA). The "Characterized Cell Line Core Facility" at MD Anderson Cancer Center (Houston, TX) performed cell line validation.

[00225] Cytokine Production: In order to assess cytokine production, $1x10^6$ T cells were co-cultured with $5x10^5$ U373 cells without the provision of exogenous cytokines. 24 hours later, supernatant was collected and frozen for later analysis. Cytokines were measured using a MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore, Burlington, MA) kit on a FLEXMAP 3D® System (Luminex, Austin, TX).

[00226] Cytotoxicity Assay: A CellTiter96® AQ_{ueous} One Solution Cell Proliferation Assay (Promega, Madison, WI) was utilized to assess CAR T cell cytotoxicity. In a tissue culture-treated 96-well plate, 12,500 U373 cells were co-cultured with serial dilutions of T cells to give effector:target ratios of 2.5:1, 1.25:1, 0.6:1, and 0.3:1. Media or tumor cells alone served as controls for background and no T cell-mediated cytotoxicity, respectively. Each condition was plated in triplicate. After 24 hours, the media and T cells were removed by gently pipetting up and down to avoid disrupting adherent U373 cells. CellTiter96® AQ_{ueous} One Solution Reagent (MTS+phenazine ethosulfate, an electron coupling reagent) in RPMI-10% FBS was added to each well and incubated at 37C, 5% CO_2 for 2 hours. The absorbance at 492 nm was measured using an Infinite® 200 Pro MPlex plate reader (Tecan, Männedorf, Switzerland) to assess the number of viable cells in each well. %Live tumor cells was determined by the following formula: (sample-media alone)/(tumor alone-media alone)*100%.

[00227] Repeat Stimulation Assay: $1x10^6$ T cells were co-cultured with $5x10^5$ U373 cells in a 24-well plate without the provision of exogenous cytokines. Seven days later, the number of T cells was determined with a hemocytometer. If the T cells killed and expanded, $1x10^6$ T cells were re-plated against $5x10^5$ fresh U373 tumor cells. This process was repeated weekly until the T cells no longer killed the tumor cells or expanded.

[00228] Statistical analysis: All *in vitro* experiments were performed at least in triplicate, GraphPad Prism 7 software (GraphPad software, Inc., La Jolla, CA) was used for statistical analysis. Measurement data were presented as mean ± standard deviation (SD). The differences between means were tested by appropriate tests. The significance level used was $P < 0.05$.

[00229] EXAMPLE 3. *In Vivo* Characterization of CARs

[00230] Next, the CARs were evaluated *in vivo*. In *in vivo* experiments, a low dose of MyD88.CD40.ζ.CAR T cells ($1x10^4$) had superior antitumor activity in the EphA2+ LM7 osteosarcoma xenograft model in comparison to 4-1BB.ζ.CAR T cells and CD28.ζ.CAR T cells. MyD88.CD40.ζ.CAR T cells exhibited superior anti-tumor activity compared with 4-1BB.ζ.CAR T cells (**Figures 4A-4D**). After injection of 4-1BB.ζ.CAR or MyD88.CD40.ζ.CAR T cells there was a rapid reduction in tumor size as judged by bioluminescence imaging. While tumors after 4-1BB.ζ.CAR T-cell therapy recurred earlier, there was no significant difference in overall survival between both mice during the first 83 days post T-cell infection (**Figure 4C**). After injection of 4-1BB.ζ.CAR or MyD88.CD40.ζ.CAR T cells, only MyD88.CD40.ζ.CAR T-cell treated mice exhibited

prolonged antitumor responses, which translated in a significant ($p<0.05$) survival advantage (**Figure 4D**). Further, MyD88.CD40.ζ.CAR T cells expanded better compared with CD28.ζ.CAR or 4-1BB.ζ.CAR T cells in the intraperitoneal LM7 xenograft model (**Figures 5A-5D**).

[00231] Animal experiments followed a protocol approved by the St. Jude Children's Research Hospital Institutional Animal Care and Use Committee. All experiments utilized 8-10 week male NOD-*scid* IL2Rgamma^{null} (NSG) mice purchased from The Jackson Laboratory (Bar Harbor, ME). To assess anti-tumor activity, mice were injected intraperitoneally (i.p.) with $1x10^6$ LM7 cells genetically modified to express firefly-luciferase. Seven days later, $1x10^5$, $1x10^4$, or $1x10^3$ 41BB.CAR or MyD88.CD40.CAR T cells were injected i.p. Tumor burden was monitored weekly using bioluminescence imaging. Mice were injected i.p. with 150 mg/kg of D-luciferin 5-10 minutes before imaging, anesthetized with isoflurane (1.5-2% delivered in 100% O_2 at 1 l/min), and imaged with a Xenogen IVIS-200 (IVIS; Xenogen, Alameda, CA) imaging system. The photons emitted from the luciferase-expressing tumor cells were quantified using Living Image software (Caliper Life Sciences, Hopkinton, MA). To monitor T cell persistence, T cells were doubly transduced with eGFP-ffLuc and an EphA2.CAR. Mice were injected i.p. with $1x10^6$ unmodified LM7 cells. Seven days later, $1x10^5$ CD28.ζ-ffLuc, 41BB.ζ-ffLuc, or MyD88.CD40ζ-ffLuc T cells were injected i.p. The presence of T cells was monitored daily using bioluminescence imaging as described above. Survival determined from the time of tumor cell injection was analyzed by the Kaplan-Meier method, and area under the curve (AUC) analyses was performed using GraphPad Prism 7 software.

[00232] EXAMPLE 4. Difficulties in Generating MyD88.CD40 CARs

[00233] In an attempt to generate additional CARs with the MyD88.CD40 structure, additional CARs were generated and tested. This example demonstrates the difficulty of generating other MyD88.CD40 CARs, and potential solutions to overcome the issues encountered here.

[00234] Frist, the IL13Rα2-specific CAR constructs **(Figure 7A-7D)** were generated and evaluated. Each construct consists of the same IL13Rα2-specific scFv m47, an IgG core hinge, a transmembrane domain, a co-stimulatory domain, and CD3ζ. Flow cytometry was used to determine the expression of CARs 7 to 10 days after transduction **(Figure 7E)**. m47.MyD88.CARs were not expressed on the cell surface, and m47.MyD88.CD40.CARs were expressed at lower levels than CD28.CARs. Western blot analysis of CAR T cells revealed equal expression of all three CARs **(Figure 7F)**, indicating that MyD88.CAR and

MyD88.CD40.CARs are inefficiently transported to the cell surface and/or are rapidly internalized once they reach the cell surface. CAR T cells were incubated with IL13Rα2+ U373 cells and their ability to kill tumor cells was scored by microscopic examination after 48 to 72 hours **(Figure 7G)**. While CD28.CAR T cells readily killed U373 cells, no killing was induced by MyD88.CD40.CAR or MyD88.CAR T cells. These results demonstrate that MyD88 detrimentally affects the function of CARs containing the IL13Rα2-specific m47 scFv.

[00235] To confirm the finding that MyD88 can detrimentally affect CAR function, CAR using the HER2-specific scFv FRP5 **(Figure 8A-8E)** were generated and evaluated. Each consists of the same HER2-specific scFv FRP5, an IgG core hinge, a transmembrane domain, a costimulatory domain, and CD3ζ. For transduction efficiency of HER2-CAR T cells, flow cytometry was used to determine the expression of CARs 7 to 10 days after transduction **(Figure 8F** (Individual FACS plot) and **Figure 8G** (Summary data)**)**. The results demonstrate that MyD88.CARs and MyD88.CD40 CARs are expressed at lower levels than CD28.CARs. CD40.CAR expression was similar to CD28.CARs. CAR T cells were incubated with HER2+ U373 cells and their ability to kill tumor cells was scored by microscopic examination after 48 to 72 hours **(Figure 8H)**. While CD28.CAR and CD40.CAR T cells readily killed U373 cells, killing of MyD88.CD40.CAR or MyD88.CAR T cells was minimal. These results demonstrate that MyD88 also detrimentally affects the function of CARs containing the HER2-specific FRP5 scFv.

[00236] In **Figure 9** and **Figure 10** additional HER2-specific MyD88.CD40.CAR constructs are described and evaluated. In the experiments shown in **Figure 9**, it was first evaluated if increasing the hinge region could rescue CAR function. **Figures 8B** and **9A-9C** show the HER2-specific CAR constructs evaluated. Each consists of the same HER2-specific scFv FRP5, different hinges (IgG core hinge (SH), IgG.SH+IgG.CH3, or IgG.SH+IgG.CH2+IgG.CH3), transmembrane domain, MyD88.CD40, and CD3ζ. Transduction efficiency of HER2-CAR T cells are shown in **Figure 9D** and **Figure 9E**. Flow cytometry was used to determine the expression of CARs 7 to 10 days after transduction **(Figure 9D** (Individual FACS plot) and **Figure 9E** (Summary data)**)**. The results demonstrate no significant differences between CAR constructs. CAR T cells were incubated with HER2+ U373 cells and their ability to kill tumor cells was scored by microscopic examination after 48 to 72 hours. Only minimal killing was observed for all three constructs **(Figure 9F)**. These results demonstrate that increasing the hinge region does not improve HER2-specific MyD88.CD40.CAR function.

[00237] In the experiments shown in **Figure 10**, it was evaluated if substituting the IgG.SH hinge and/or CD28 transmembrane domain could rescue CAR function **(Figure 10A-10D)**. Each consists of the same HER2-specific scFv FRP5, different hinges (IgG.SH (core hinge) or CD8α stalk), different transmembrane domains (CD28, CD8α, or CD8α with 8 amino acids of its cytoplasmic tail), MyD88.CD40, and CD3ζ. Transduction efficiency of HER2-CAR T cells are shown in **Figure 10E** and **Figure 10F**. Flow cytometry was used to determine the expression of CARs 7 to 10 days after transduction **(Figure 10E** (Individual FACS plot) and **Figure 10F** (Summary data)**)**. The results demonstrate no significant differences between CAR constructs; however CARs with a CD8α stalk and a CD8α+8aa transmembrane domain (8α.TMCD8α+8aa) were expressed at slightly higher levels. CAR T cells were incubated with HER2+ U373 cells and their ability to kill tumor cells was scored by microscopic examination after 48 to 72 hours. Only minimal killing was observed for all four constructs **(Figure 10G)**. These results demonstrate that changing the transmembrane domain and hinge region to CD8α does not significantly improve HER2-specific MyD88.CD40.CAR function.

[00238] In summary, the experiments shown in **Figures 7** to **10** demonstrate that MyD88 interferes with CD3ζ activation. Thus, for MyD88.CD40.CARs, which are not functional, CD3ζ and MyD88.CD40 signaling might need to be separated. For example, T cells could be genetically modified with two molecules: the first encodes a CAR, and the second a chimeric molecule consisting of an extracellular domain, a transmembrane domain, and a MyD88.CD40 signaling domain. The extracellular domain may consist of a scFv, but if no antigen-specificity is desired, other amino acid sequences could be substituted (e.g., the extracellular domain of CD19, CD34 or a fragment there of). If needed, additional structures such as a hinge might also be included in the extracellular domain. The transmembrane domain is derived from CD8, CD8α, CD4, CD3ζ, CD28, CD40, CD134 (OX-40), or CD7. Examples of four such molecules are illustrated in **Figure 11**.

[00239] EXAMPLE 5. Additional *In Vivo* Experiments

[00240] An updated *in vivo* experiment from Figure 4 was carried out with additional animals per group and longer follow up. In addition, it included one additional group (CD28.CAR T cells). 8-week old female NSG mice were injected intraperitoneally (i.p.) with 10^6 LM7 tumor cells, which were genetically modified to express firefly luciferase (ffluc) to enable non-invasive bioluminescence imaging to track tumor cells. On day +7, mice received a single i.p. injection of 10^5 **(Figures 12A-12C)** or 10^4 **(Figures 12D-12F)** CAR T cells. Tumor burden was monitored by bioluminescence imaging. IVIS images of individual

mice are shown in **Figure 12A** and **Figure 12D**. Quantitative bioluminescence imaging and survival curve for 10^5 CAR T cells are shown in **Figure 12B** and **Figure 12C**, respectively. After injection of CD28.CAR, 41BB.CAR or MyD88.CD40.CAR (MC-CAR) T cells there was a rapid reduction in tumor size as judged by bioluminescence imaging. While tumors after CD28.CAR and 41BB.CAR T-cell therapy recurred earlier, there was no significant difference in overall survival between both mice during the first 83 days post T-cell infection. However, long term follow-up revealed a significant survival advantage ($p<0.05$). Quantitative bioluminescence imaging and survival curve for 10^4 CAR T cells are presented in **Figure 12E** and **Figure 12F**, respectively. After injection of CD28.CAR, 41BB.CAR or MyD88.CD40.CAR T cells only MyD88.CD40.CAR T-cell treated mice exhibited prolonged antitumor responses, which translated in a significant ($p<0.05$) survival advantage.

[00241] EXAMPLE 6. Knocking out Endogenous TCRs

[00242] In the experiments shown in **Figure 13**, it was demonstrated that the function of MyD88.CD40-CAR T cells depends on the presence of a functional zeta signaling domain within the CAR and not on the presence of endogenous αβ TCRs. MyD88.CD40-CAR (MC-CAR) T cells were generated in which the TRAC locus was knocked out (MC TRAC KO-CAR). The TRAC-KO CAR T cells were created by first transducing activated T cells with retroviral vectors encoding CARs, and then electroporating them with CAS9/TRAC-specific sgRNA ribonucleoprotein (RNP) complexes. The RNP complexes contained recombinant Cas9 (MacoLabs, Berkeley) and TRAC-specific sgRNA comprising the guide sequence CAGGGTTCTGGATATCTGT (SEQ ID NO: 98). Knocking out the TRAC locus resulted in the absence of cell surface expression of αβ TCRs **(Figure 13A)**. In a cytotoxicity assay, MC-CAR T cells and MC TRAC KO-CAR T cells killed EphA2+ tumor cells (U373, LM7) **(Figure 13B)**. In contrast non-transduced T cells (NT) did not kill target cells. In addition, T cells were generated by expressing a non-functional MyD88.CD40 CAR in which the immunoreceptor tyrosine-based activation motif (ITAM) was mutated (MC-CAR.muITAM). All six tyrosines within the cytoplasmic domain of CD3z (SEQ ID NO: 19) were mutated to phenylalanine. MC-CAR.muITAM T cells also did not kill tumor cells indicating that killing depends on an active CAR. The antitumor activity of CAR T cells was evaluated in the intraperitoneal LM7 xenograft model described in Figure 4 with a T cell dose of 10^5. MC-CAR T cells and MC TRAC KO-CAR T cells had similar antitumor activity whereas T cells expressing the non-functional CAR had no antitumor activity **(Figure 13C)**.

[00243] The antitumor activity of MC-CAR T cells and MC TRAC KO-CAR T cells was evaluated in a glioma model. Luciferase-expressing EphA2+ U373 cells were injected into

the brain of NSG mice and on day 7, MC-CAR T cells or MC TRAC KO-CAR T cells were injected intravenously. Within 4 weeks of intravenous injection there was a dramatic reduction in tumor size as judged by bioluminescence imaging. The IVIS images of individual mice and quantitative bioluminescence imaging are presented in **Figure 14A** and **Figure 14B**, respectively.

References

Park et. al., *Blood*, 2016; 127:3312-20

Beatty et al., *Pharmacol. Ther.* 2016; 166:30-39

* * *

[00244] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

[00245] All patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated by reference in their entirety as if physically present in this specification.

WHAT IS CLAIMED IS:

1. A polynucleotide encoding a chimeric antigen receptor (CAR) comprising (a) an extracellular target-binding domain comprising an Eph receptor-binding moiety, (b) a transmembrane domain, and (c) a cytoplasmic domain comprising a lymphocyte activation domain, a MyD88 polypeptide or a functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof.
2. The polynucleotide of claim 1, wherein the MyD88 polypeptide comprises amino acid sequence SEQ ID NO:25, SEQ ID NO:30, SEQ ID NO: 31, or SEQ ID NO:33, or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 25, SEQ ID NO: 30, SEQ ID NO: 31, or SEQ ID NO: 33.
3. The polynucleotide of claim 1 or claim 2, wherein the MyD88 functional fragment comprises a MyD88 polypeptide lacking the Toll/Il-1 receptor domain.
4. The polynucleotide of any one of claims 1-3, wherein the MyD88 functional fragment comprises amino acid sequence SEQ ID NO:15, SEQ ID NO:28, or SEQ ID NO:60 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:15, SEQ ID NO:28, or SEQ ID NO:60.
5. The polynucleotide of claim 4, wherein the nucleotide sequence encoding the MyD88 functional fragment comprises SEQ ID NO:16, SEQ ID NO:29, or SEQ ID NO: 61 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:16, SEQ ID NO:29, or SEQ ID NO:61.
6. The polynucleotide of any one of claims 1-5, wherein the CD40 cytoplasmic polypeptide region or functional fragment thereof is derived from a CD40 polypeptide comprising the amino acid sequence SEQ ID NO:32 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:32.
7. The polynucleotide of any one of claims 1-6, wherein the CD40 cytoplasmic polypeptide region comprises the amino acid sequence SEQ ID NO:17 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:17.
8. The polynucleotide of claim 7, wherein the nucleotide sequence encoding the CD40 cytoplasmic polypeptide region comprises SEQ ID NO:18 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:18.
9. The polynucleotide of any one of claims 1-8, wherein the transmembrane domain is derived from CD8, CD8α, CD4, CD3ζ, CD28, CD40, CD134 (OX-40), or CD7.

10. The polynucleotide of any one of claims 1-8, wherein the CAR lacks the CD40 transmembrane domain, CD40 extracellular domain, or both the CD40 transmembrane domain and the CD40 extracellular domain.
11. The polynucleotide of any one of claims 1-10, wherein the transmembrane domain is derived from CD28 or CD8α.
12. The polynucleotide of claim 11, wherein the transmembrane domain derived from CD28 comprises the amino acid sequence SEQ ID NO:13 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:13.
13. The polynucleotide of claim 12, wherein the nucleotide sequence encoding the transmembrane domain derived from CD28 comprises SEQ ID NO:14 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:14.
14. The polynucleotide of claim 11, wherein the transmembrane domain derived from CD8α comprises the amino acid sequence SEQ ID NO:48, 94, or 96 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 48, 94, or 96.
15. The polynucleotide of claim 12, wherein the nucleotide sequence encoding the transmembrane domain derived from CD8α comprises SEQ ID NO:49, 95, or 97 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO: 49, 95, or 97.
16. The polynucleotide of any one of claims 1-15, wherein the lymphocyte activation domain is a T cell activation domain.
17. The polynucleotide of any one of claims 1-15, wherein the lymphocyte activation domain is a natural killer (NK) cell activation domain.
18. The polynucleotide of any one of claims 1-17, wherein the lymphocyte activation domain is derived from DAP10, DAP12, Fc epsilon receptor I γ chain (FCER1G), CD3δ, CD3ε, CD3γ, CD3ζ, CD226, or CD79A.
19. The polynucleotide of claim 18, wherein the lymphocyte activation domain is derived from CD3ζ.
20. The polynucleotide of claim 19, wherein the lymphocyte activation domain derived from CD3ζ comprises the amino acid sequence SEQ ID NO:19 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:19.
21. The polynucleotide of claim 20, wherein the nucleotide sequence encoding the lymphocyte activation domain derived from CD3ζ comprises SEQ ID NO:20 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:20.

22. The polynucleotide of any one of claims 1-21, wherein the extracellular target binding domain further comprises a linker domain between the Eph receptor-binding moiety and the transmembrane domain.
23. The polynucleotide of claim 22, wherein the linker domain comprises an immunoglobulin IgG hinge or functional fragment thereof, or a linker domain derived from CD8, CD8α, or CD28.
24. The polynucleotide of claim 23, wherein the linker domain comprises an immunoglobulin IgG hinge or functional fragment thereof.
25. The polynucleotide of claim 24, wherein the immunoglobulin IgG hinge is an IgG1 core hinge region comprising the amino acid sequence SEQ ID NO:11 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:11.
26. The polynucleotide of claim 25, wherein the nucleotide sequence encoding the IgG1 core hinge region comprises SEQ ID NO:12 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:12.
27. The polynucleotide of claim 22, wherein the linker comprises the amino acid sequence SEQ ID NO:11, 35, 81, 83, 84, or 86 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 11, 35, 81, 83, 84, or 86.
28. The polynucleotide of claim 27, wherein the nucleotide sequence encoding the linker comprises SEQ ID NO: 12, 82, 85, or 87 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO: 12, 82, 85, or 87.
29. The polynucleotide of any one of claims 1-28, wherein the Eph receptor-binding moiety comprises an Eph receptor-binding polypeptide or a ligand for the Eph receptor.
30. The polynucleotide of claim 29, wherein the Eph receptor-binding moiety is an Eph receptor-binding polypeptide.
31. The polynucleotide of claim 30, wherein the Eph receptor-binding polypeptide is an antibody or an antibody fragment.
32. The polynucleotide of claim 30 or claim 31, wherein the Eph receptor-binding polypeptide is a murine antibody, rabbit antibody, human antibody, humanized antibody, fully humanized antibody, single chain variable fragment (scFv), camelid antibody variable domain and humanized versions, shark antibody variable domain and humanized versions, single domain antibody variable domain, nanobody (VHH), or camelized antibody variable domain.

33. The polynucleotide of claim 31 or claim 32, wherein the antigen-binding polypeptide is a single chain variable fragments (scFv).
34. The polynucleotide of any one of claims 1-33, wherein the Eph receptor is a EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6 receptor or a variant thereof.
35. The polynucleotide of claim 34, wherein the Eph receptor is a EphA2 receptor or variant thereof.
36. The polynucleotide of any one of claims 1-35, wherein the Eph receptor-binding moiety comprises anti-EphA2 scFv 4H5.
37. The polynucleotide of 36, wherein the anti-EphA2 scFv 4H5 comprises the amino acid sequence SEQ ID NO:9 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:9.
38. The polynucleotide of claim 37, wherein the nucleotide sequence encoding the anti-EphA2 scFv 4H5 comprises SEQ ID NO:10 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:10.
39. The polynucleotide of claim 29, wherein the ligand for EphA2 is Ephrin-A1, Ephrin-A2, Ephrin-A3, Ephrin-A4, Ephrin-A5 or Ephrin-A6.
40. The polynucleotides of any one of claims 1-39, wherein the extracellular target-binding domain further comprises a leader sequence.
41. The polynucleotide of claim 40, wherein the leader sequence comprises the amino acid sequence of SEQ ID NO:7 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:7.
42. The polynucleotide of claim 41, wherein the nucleotide sequence encoding the leader sequence comprises SEQ ID NO:8 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:8.
43. The polynucleotide of any one of claims 1-42, wherein the polynucleotide comprises at least one additional polypeptide sequence.
44. The polynucleotide of claim 43, wherein the at least one polynucleotide sequence encodes a transduced host cell selection marker, an *in vivo* tracking marker, a cytokine, or a suicide gene.
45. The polynucleotide of claim 44, wherein the transduced host cell selection marker is a truncated CD19 (tCD19) polypeptide.

46. The polynucleotide of claim 45, wherein the tCD19 comprises the amino acid sequence SEQ ID NO:23 or an amino acid sequence having at least 50% sequence identity to SEQ ID NO:23.
47. The polynucleotide of claim 46, wherein the nucleotide sequence encoding the tCD19 comprises SEQ ID NO:24 or a nucleotide sequence having at least 50% sequence identity to SEQ ID NO:24.
48. The polynucleotide of any one of claims 1-47, wherein the CAR comprises the amino acid sequence SEQ ID NO:1, 26, 50, 52, 54, 56, or 58 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 1, 26, 50, 52, 54, 56, or 58.
49. The polynucleotide of any one of claims 1-48, wherein the CAR is encoded by the nucleotide sequence SEQ ID NO:2, 27, 51, 53, 55, 57, or 59 or a nucleotide sequence that has at least 50% sequence identity to SEQ ID NO:2, 27, 51, 53, 55, 57, or 59.
50. The polynucleotide of any one of claims 1-49, wherein the polynucleotide is operatively linked to at least one regulatory element for expression of the CAR.
51. The polynucleotide of claim 50, wherein the at least one regulatory element is a promoter.
52. The polynucleotide of claim 51, wherein the promoter is a T lymphocyte-specific promoter or an NK cell-specific promoter.
53. The polynucleotide of any one of claims 1-52, which is a DNA molecule.
54. The polynucleotide of any one of claims 1-53, which is a RNA molecule or a derivative thereof.
55. A recombinant vector comprising the polynucleotide of any one of claims 1-54, wherein the polynucleotide is operatively linked to at least one regulatory element for expression of the CAR.
56. A recombinant vector comprising a polynucleotide encoding a CAR comprising (a) an extracellular target-binding domain comprising an Eph receptor-binding moiety, (b) a transmembrane domain, and (c) a cytoplasmic domain comprising a lymphocyte activation domain, a MyD88 polypeptide or a functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof, wherein the polynucleotide is operatively linked to at least one regulatory element for expression of the CAR.
57. The vector of claim 55 or claim 56, which is a viral vector.

58. The vector of claim 57, wherein the viral vector is a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated virus vector, an alphaviral vector, a herpes virus vector, or a vaccinia virus vector.
59. The vector of claim 55 or claim 56, wherein the vector is a non-viral vector.
60. A chimeric antigen receptor (CAR) encoded by the polynucleotide of any one of claims 1-54.
61. A chimeric antigen receptor (CAR) comprising (a) an extracellular target-binding domain comprising an Eph receptor-binding moiety, (b) a transmembrane domain, and (c) a cytoplasmic domain comprising a lymphocyte activation domain, a MyD88 polypeptide or functional fragment thereof, and a CD40 cytoplasmic polypeptide region or a functional fragment thereof.
62. The CAR of claim 61, wherein the MyD88 polypeptide comprises amino acid sequence SEQ ID NO:25, 30, 31, or 33 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 25, 30, 31, or 33.
63. The CAR of claim 62, wherein the MyD88 functional fragment comprises the amino acid sequence SEQ ID NO:15, 28, or 60, or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:15, 28, or 60.
64. The CAR of any one of claims 61-63, wherein the CD40 polypeptide comprises the amino acid sequence SEQ ID NO:32 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:32.
65. The CAR of any one of claims 61-63, wherein the CD40 cytoplasmic polypeptide region comprises the amino acid sequence SEQ ID NO:17 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:17.
66. The CAR of any one of claims 61-65, wherein the transmembrane domain is derived from CD8, CD8α, CD4, CD3ζ, CD28, CD40, CD134 (OX-40), or CD7.
67. The CAR of any one of claims 61-65, wherein the CAR lacks the CD40 transmembrane domain, CD40 extracellular domain, or both CD40 transmembrane and CD40 extracellular domain.
68. The CAR of claim 66 or claim 67, wherein the transmembrane domain is derived from CD28 or CD8α.
69. The CAR of claim 68, wherein the transmembrane domain derived from CD28 comprises the amino acid sequence SEQ ID NO:13 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:13.

70. The CAR of claim 68, wherein the transmembrane domain derived from CD8α comprises the amino acid sequence SEQ ID NO:48, 94, or 96 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 48, 94, or 96.
71. The CAR of any one of claims 61-70, wherein the lymphocyte activation domain is a T cell activation domain.
72. The CAR of any one of claims 61-70, wherein the lymphocyte activation domain is a natural killer cell activation domain.
73. The CAR of any one of claims 61-72, wherein the lymphocyte activation domain is derived from DAP10, DAP12, Fc epsilon receptor I γ chain (FCER1G), CD3δ, CD3ε, CD3γ, CD3ζ, CD226, or CD79A.
74. The CAR of claim 73, wherein the lymphocyte activation domain is derived from CD3ζ.
75. The CAR of claim 74, wherein the lymphocyte activation domain derived from CD3ζ comprises the amino acid sequence SEQ ID NO:19 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:19.
76. The CAR of any one of claims 61-75, wherein the CAR comprises a linker domain between the extracellular target-binding domain and the transmembrane domain.
77. The CAR of claim 76, wherein the linker domain comprises an immunoglobulin IgG hinge, or functional fragment thereof, or a linker domain derived from CD8, CD8α, or CD28.
78. The CAR of claim 77, wherein the linker domain comprises an immunoglobulin IgG hinge or functional fragment thereof.
79. The CAR of claim 78, wherein the immunoglobulin IgG hinge comprises an IgG1 core hinge region comprising the amino acid sequence SEQ ID NO:11 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:11.
80. The CAR of claim 76, wherein the linker comprises the amino acid sequence SEQ ID NO:11, 35, 81, 83, 84, or 86 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: 11, 35, 81, 83, 84, or 86.
81. The CAR of any one of claims 61-80, wherein the Eph receptor-binding moiety comprises an Eph receptor-binding polypeptide or a ligand for the Eph receptor.
82. The CAR of claim 81, wherein the Eph receptor-binding moiety is an Eph receptor-binding polypeptide.
83. The CAR of claim 82, wherein the Eph receptor-binding polypeptide is an antibody or an antibody fragment.

84. The CAR of claim 82 or claim 83, wherein the Eph receptor-binding polypeptide is a murine antibody, rabbit antibody, human antibody, humanized antibody, fully humanized antibody, single chain variable fragment (scFv), camelid antibody variable domain and humanized versions, shark antibody variable domain and humanized versions, single domain antibody variable domain, nanobody (VHH), or camelized antibody variable domain.
85. The CAR of claim 83 or claim 84, wherein the antigen-binding polypeptide is a single chain variable fragments (scFv).
86. The CAR of any one of claims 61-85, wherein the Eph receptor is a EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6 receptor or a variant thereof.
87. The CAR of any one of claims 61-86, wherein the Eph receptor is a EphA2 receptor or variant thereof.
88. The chimeric antigen receptor of any one of claims 61-87, wherein the Eph receptor-binding moiety comprises anti-EphA2 scFv 4H5.
89. The CAR of claim 88, wherein the anti-EphA2 scFv 4H5 comprises the amino acid sequence SEQ ID NO:9 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO:9.
90. The CAR of claim 81, wherein the ligand for EphA2 is Ephrin-A1, Ephrin-A2, Ephrin-A3, Ephrin-A4, Ephrin-A5 or Ephrin-A6.
91. The CAR of any one of claims 61-90, wherein the CAR comprises at least one additional polypeptide sequence.
92. The CAR of claim 91, wherein the at least one additional polypeptide sequence is at least one of a transduced host cell selection marker, an *in vivo* tracking marker, a cytokine, or a suicide protein, co-stimulatory endodomains, signal sequences, epitope tags, or polypeptides that produce a detectable signal.
93. The CAR of any one of claims 60-92, wherein the CAR comprises amino acid sequence SEQ ID NO:126, 50, 52, 54, 56, or 58 or an amino acid sequence that has at least 50% sequence identity to SEQ ID NO: SEQ ID NO:1, 26, 50, 52, 54, 56, or 58.
94. An isolated host cell comprising the chimeric antigen receptor (CAR) of any one of claims 60-93.
95. The host cell of claim 94, further comprising a co-stimulatory fusion protein.
96. An isolated host cell comprising the polynucleotide of any one of claims 1-54.

97. The isolated host cell of claim 96, wherein the polynucleotide is operatively linked to at least one regulatory element which is capable of mediating expression of the chimeric antigen receptor in the host cell.
98. An isolated host cell comprising the vectors of any one of claims 55-59.
99. The isolated host cell of any one of claims 94-98, wherein the host cell is a mammalian cell.
100. The isolated host cell of any one of claims 94-99, wherein the host cell is a cytotoxic cell, a T cell, a stem cell, a progenitor cell, or a cell derived from a stem cell or a progenitor cell.
101. The isolated host cell of claim 100, wherein the T cell is at least one of T-helper cells, cytotoxic T-cells, T-regulatory cells (Treg), invariant natural killer T (NKT) cells, or gamma-delta T cells.
102. The isolated host cell of claim 101, wherein the cytotoxic cell is a cytotoxic T cell or a natural killer (NK) cell.
103. The isolated host cell of any one of claims 94-102, wherein the host cell has been activated and/or expanded *ex vivo*.
104. The isolated host cell of any one of claims 94-103, wherein the host cell is an allogeneic cell.
105. The isolated host cell of any one of claims 94-103, wherein the host cell is an autologous cell.
106. The isolated autologous host cell of claim 105, wherein the host cell is isolated from a subject having a disease, wherein EphA2 is expressed.
107. The isolated autologous host cell of claim 106, wherein the disease is a cancer that expresses EphA2.
108. The isolated autologous host cell of claim 107, wherein the cancer is a solid tumor.
109. The isolated autologous host cell of claim 107 or claim 108, wherein the cancer is breast, prostate, urinary bladder, skin, lung, ovary, sarcoma, or brain cancer.
110. The isolated autologous host cell of claim 109, wherein the skin cancer is melanoma.
111. The isolated autologous host cell of claim 109, wherein the lung cancer is non–small cell lung cancer (NSCLC).
112. The isolated autologous host cell of claim 109, wherein the sarcoma is osteosarcoma or rhabdomyosarcoma.

113. The isolated autologous host cell of claim 109, wherein the brain cancer is a high grade glioma, diffuse pontine glioma, medulloblastoma, or ependymoma.
114. The isolated host cell of any one of claims 94-113, wherein the host cell is derived from a blood, marrow, tissue, or a tumor sample.
115. The host cell of any one of claims 94-114, wherein the cell surface expression of the endogenous TCR of the cell has been decreased or eliminated.
116. The host cell of claim 115, wherein the host cell is an allogeneic cell.
117. A pharmaceutical composition comprising the host cell of any one of claims 94-116 and a pharmaceutically acceptable carrier and/or excipient.
118. A method of enhancing activity of a T lymphocyte or a natural killer (NK) cell towards cells expressing EphA2 in a subject in need thereof comprising administering to the subject an effective amount of the polynucleotide of any one of claims 1-54, the vector of any one of claims 55-59, the host cell of any one of claims 94-116, or the pharmaceutical composition of claim 117.
119. The method of claim 118, wherein the host cell is an autologous cell.
120. The method of claim 118 or claim 119, wherein the subject has a disease, wherein EphA2 is expressed.
121. The method of claim 120, wherein the disease is a cancer expressing EphA2.
122. The method of claim 121, wherein the cancer is a solid tumor.
123. The method of claim 121 or claim 122, wherein the cancer is breast, prostate, urinary bladder, skin, lung, ovary, sarcoma, or brain cancer.
124. The method of claim 123, wherein the skin cancer is melanoma.
125. The method of claim 123, wherein the lung cancer is non–small cell lung cancer (NSCLC).
126. The method of claim 123, wherein the sarcoma is osteosarcoma or rhabdomyosarcoma.
127. The method of claim 123, wherein the brain cancer is a high grade glioma, diffuse pontine glioma, medulloblastoma, or ependymoma.
128. A method of treating a disease in a subject in need thereof comprising administering to the subject an effective amount of the polynucleotide of any one of claims 1-54, the vector of any one of claims 55-59, the host cell of any one of claims 94-116, or the pharmaceutical composition of claim 117.
129. The method of claim 128, wherein the host cell is an autologous cell.

130. The method of claim 128 or claim 129, wherein the subject has a disease, wherein EphA2 is expressed.
131. The method of claim 130, wherein the disease is a cancer expressing EphA2.
132. The method of claim 131, wherein the cancer is from a solid tumor.
133. The method of claim 131 or claim 132, wherein the cancer is breast, prostate, urinary bladder, skin, lung, ovary, sarcoma, or brain cancer.
134. The method of claim 133, wherein the skin cancer is melanoma.
135. The method of claim 133, wherein the lung cancer is non–small cell lung cancer (NSCLC).
136. The method of claim 133, wherein the sarcoma is osteosarcoma or rhabdomyosarcoma.
137. The method of claim 133, wherein the brain cancer is a high grade glioma, diffuse pontine glioma, medulloblastoma, or ependymoma.
138. A method for producing the host cell of any one of claims 94-116, comprising genetically modifying the host cell with the polynucleotides of any one of claims 1-54 or the vector of any one of claims 55-59.
139. The method of claim 138, wherein the genetic modifying step is conducted via viral gene delivery.
140. The method of claim 138, wherein the genetic modifying step is conducted via non-viral gene delivery.
141. The method of any one of claims claim 138-140, wherein the genetic modifying step is conducted *ex vivo*.
142. The method of any one of claims 138-141, wherein the method further comprises activation and/or expansion of the cell *ex vivo*.
143. A method for stimulating elimination of a cell comprising an EphA2 antigen in a subject in need thereof, said method comprising administering to the subject an effective amount of cytotoxic T cells or natural killer (NK) cells comprising the CAR of any one of claims 60-93.
144. A method for treating a cancer in a subject in need thereof, wherein cancer cells express EphA2, said method comprising administering to the subject a therapeutically effective amount of cytotoxic T cells or natural killer (NK) cells comprising the CAR of any one of claims 60-93.
145. The method of claim 144, wherein the cancer is from a solid tumor.

146. The method of claim 144 or claim 145, wherein the cancer is breast, prostate, urinary bladder, skin, lung, ovary, sarcoma, or brain cancer.
147. The method of claim 146, wherein the skin cancer is melanoma.
148. The method of claim 146, wherein the lung cancer is non–small cell lung cancer (NSCLC).
149. The method of claim 146, wherein the sarcoma is osteosarcoma or rhabdomyosarcoma.
150. The method of claim 146, wherein the brain cancer is a high grade glioma, diffuse pontine glioma, medulloblastoma, or ependymoma.
151. The method of any one of claims 128-150, the method comprising:
    a) isolating T cells or NK cells from the subject;
    b) genetically modifying said T cells or NK cells *ex vivo* with the polynucleotide of any one of claims 1-54 or the vector of any one of claims 55-59;
    c) optionally, expanding and/or activating said T cells or NK cells before, after or during step (b); and
    d) introducing the genetically modified T cells or NK cells into the subject.
152. The method of any one of claims 118-137 or 143-151, wherein the subject is human.
153. The method of any one of claims 143-152, wherein the cell surface expression of the endogenous TCR has been decreased or eliminated in the administered cells.

FIG. 1A

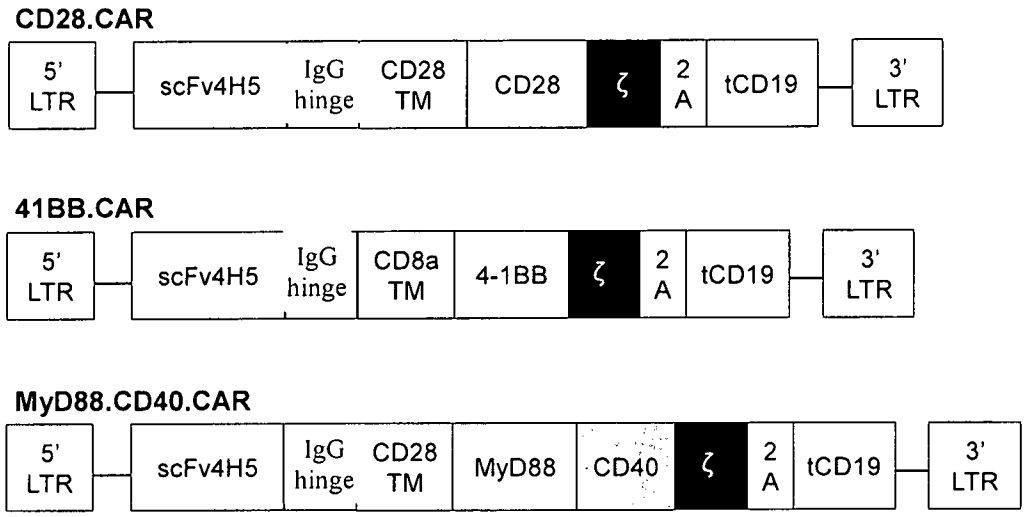


FIG. 1B

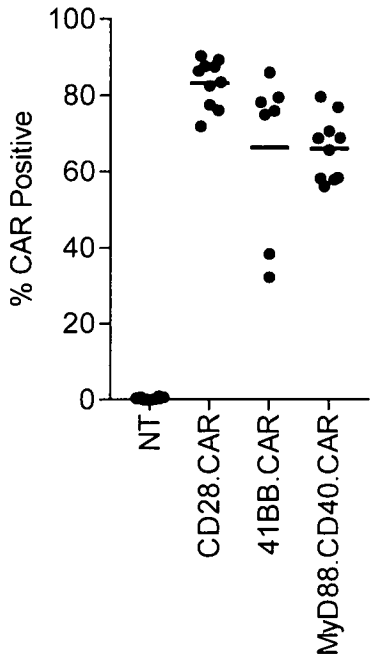


FIG. 1C

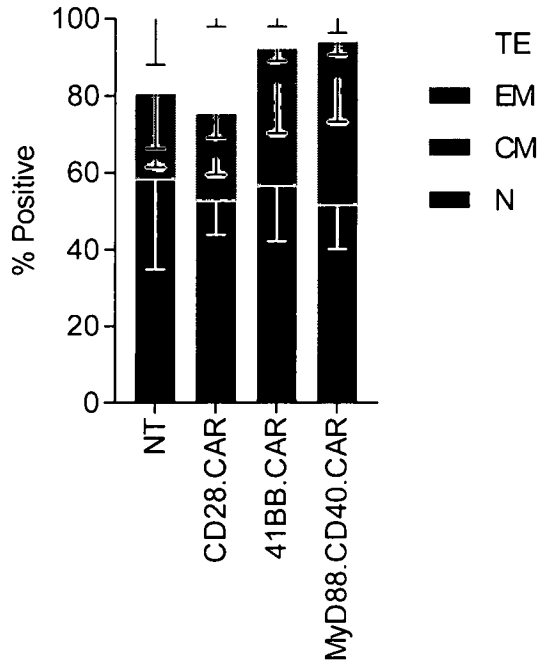


FIG. 2A

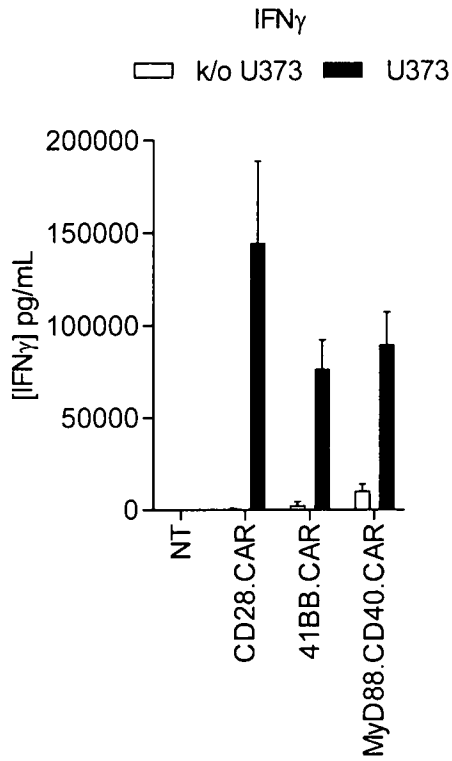


FIG. 2B

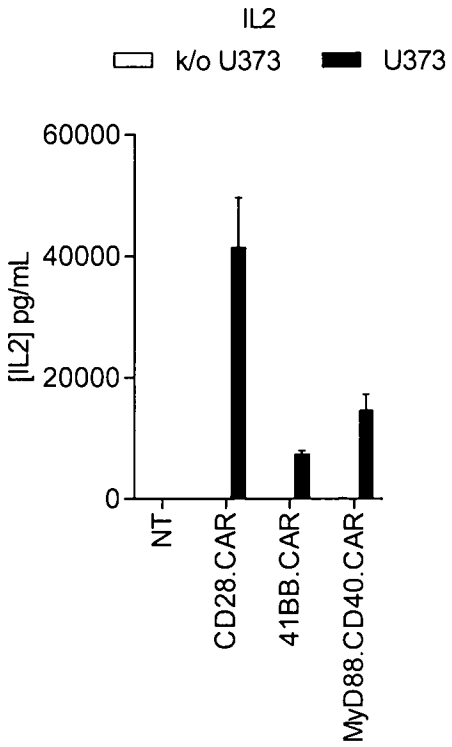


FIG. 2C

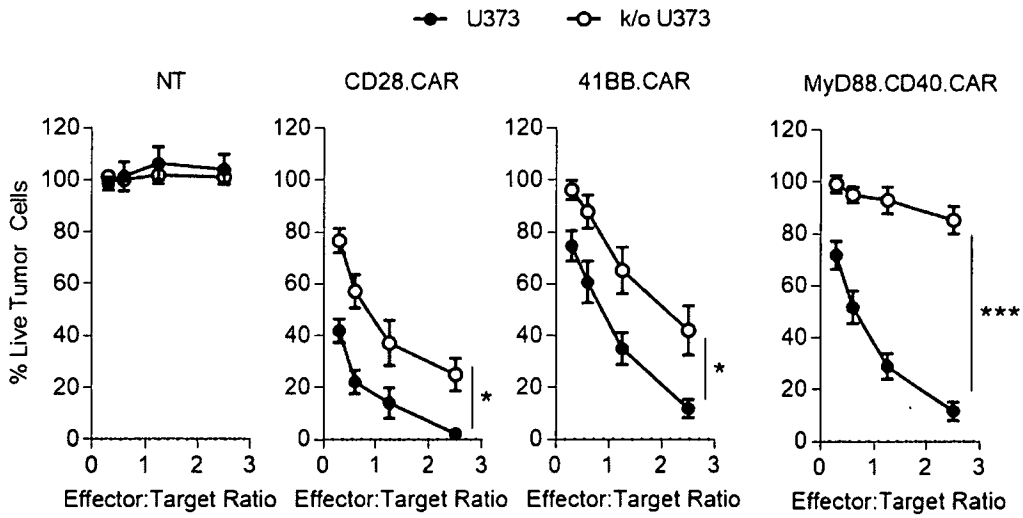


FIG. 3A

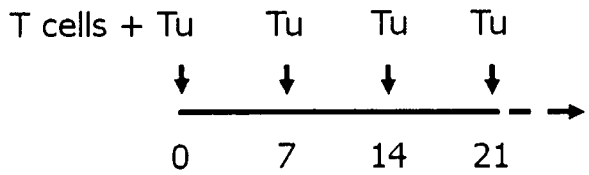


FIG. 3B

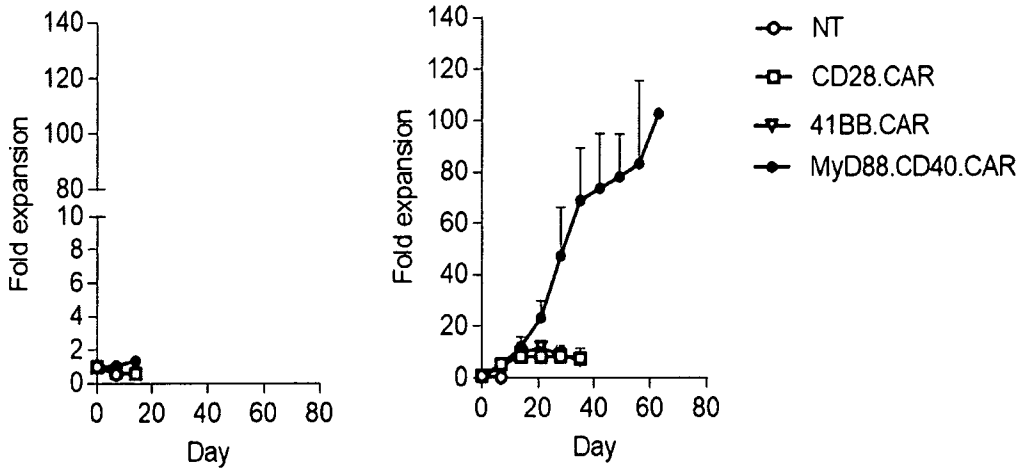


FIG. 3C

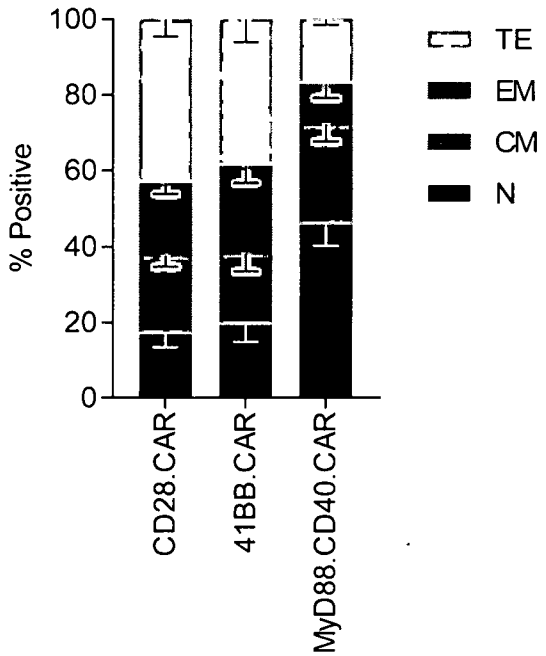


FIG. 4A

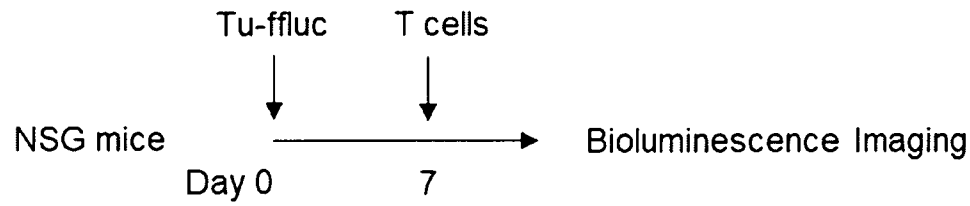

Tu-ffluc
T cells
NSG mice
Bioluminescence Imaging
Day 0
7

FIG. 4B

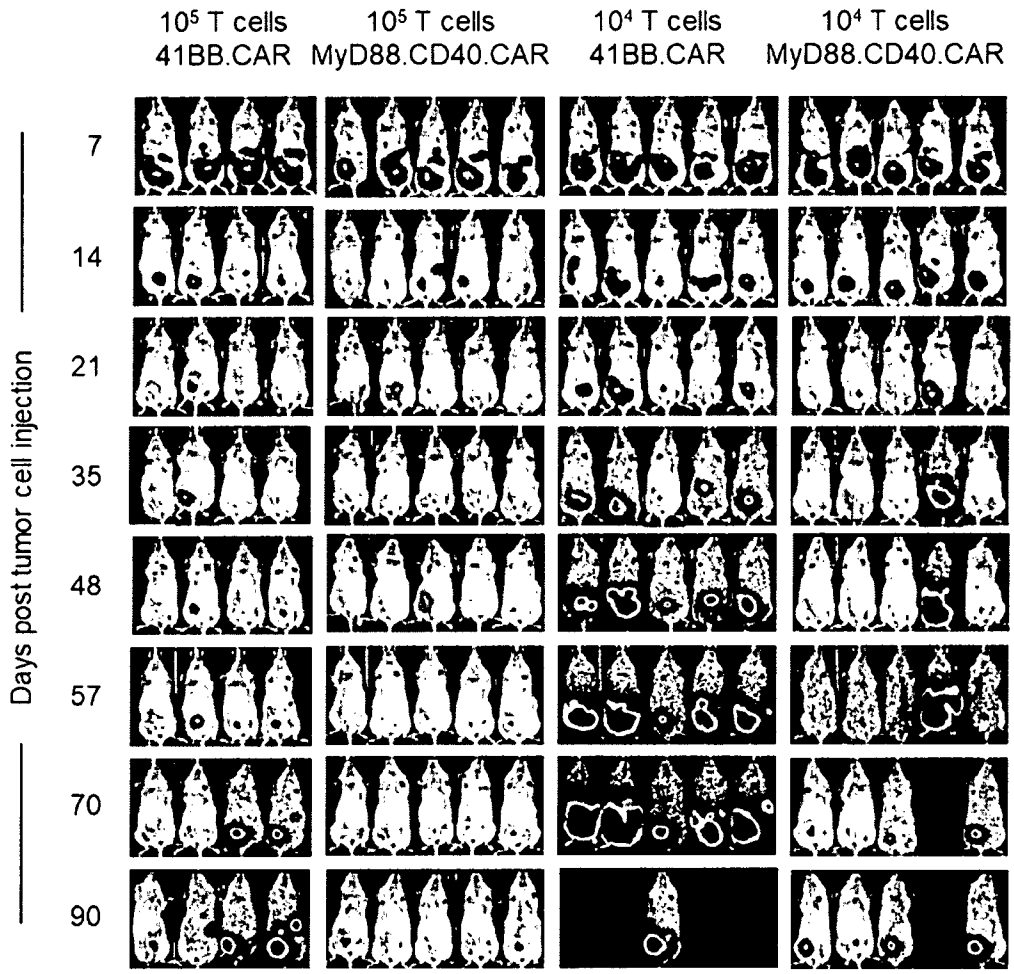
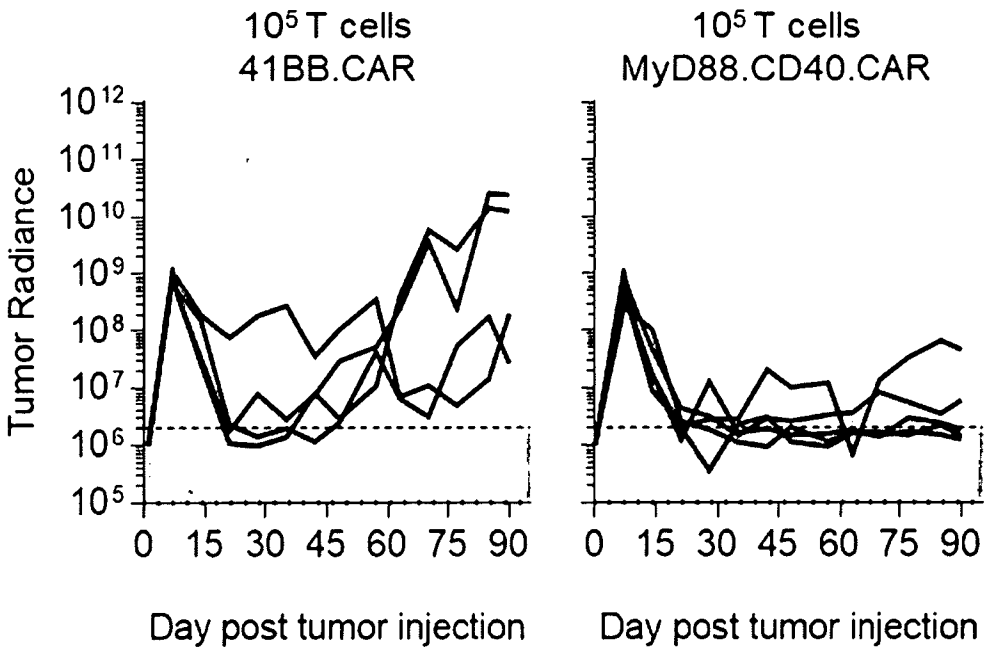

10⁵ T cells 41BB.CAR
10⁵ T cells MyD88.CD40.CAR
10⁴ T cells 41BB.CAR
10⁴ T cells MyD88.CD40.CAR
Days post tumor cell injection
7
14
21
35
48
57
70
90

FIG. 4C


10^5 T cells
41BB.CAR
10^5 T cells
MyD88.CD40.CAR
Tumor Radiance
10^12
10^11
10^10
10^9
10^8
10^7
10^6
10^5
0 15 30 45 60 75 90
0 15 30 45 60 75 90
Day post tumor injection
Day post tumor injection

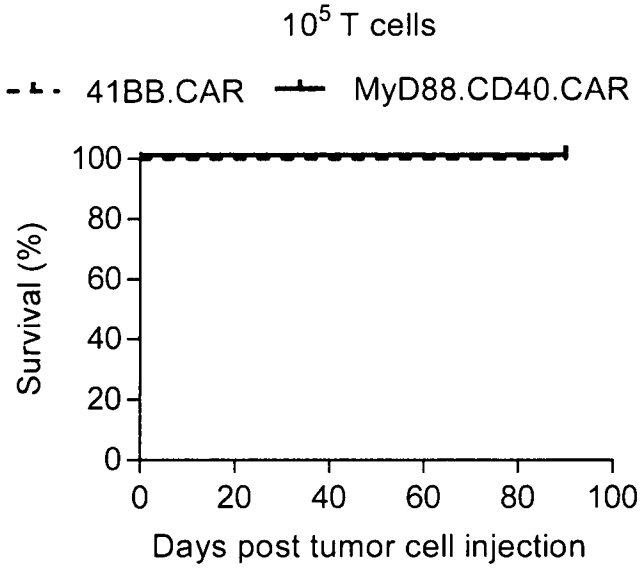
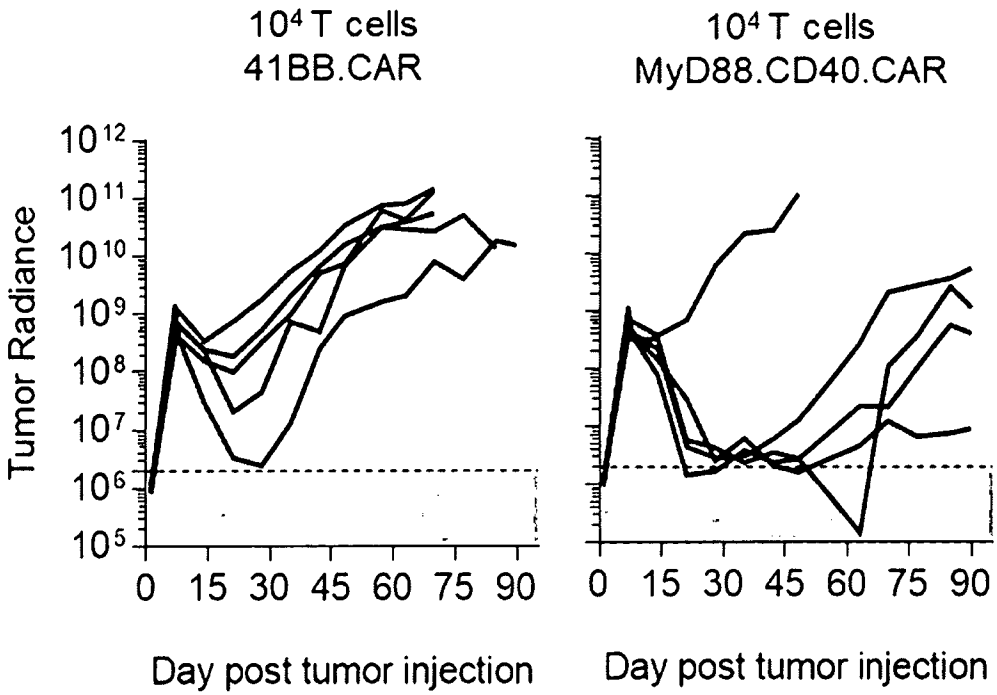

10^5 T cells
41BB.CAR
MyD88.CD40.CAR
Survival (%)
100
80
60
40
20
0
0 20 40 60 80 100
Days post tumor cell injection

FIG. 4D


10⁴ T cells
41BB.CAR
10⁴ T cells
MyD88.CD40.CAR
Tumor Radiance
10¹²
10¹¹
10¹⁰
10⁹
10⁸
10⁷
10⁶
10⁵
0 15 30 45 60 75 90
0 15 30 45 60 75 90
Day post tumor injection
Day post tumor injection

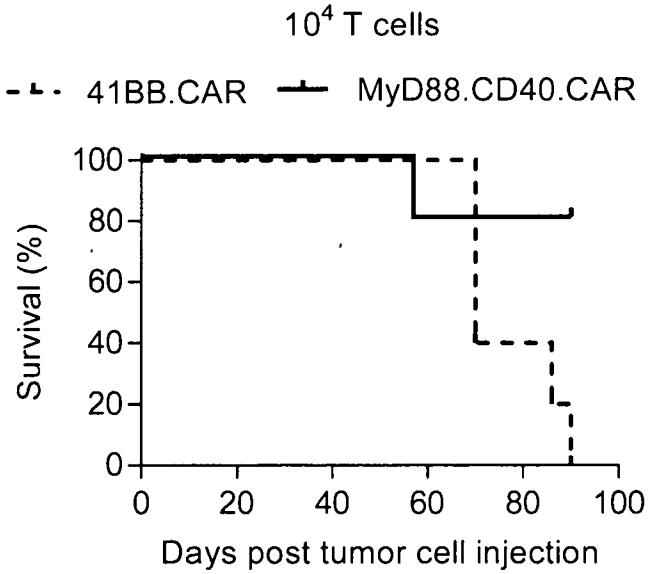

10⁴ T cells
41BB.CAR
MyD88.CD40.CAR
Survival (%)
100
80
60
40
20
0
0 20 40 60 80 100
Days post tumor cell injection

FIG. 5A

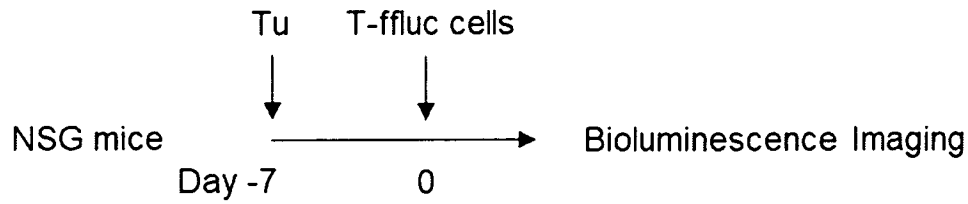

Tu
T-ffluc cells
NSG mice
Bioluminescence Imaging
Day -7
0

FIG. 5B

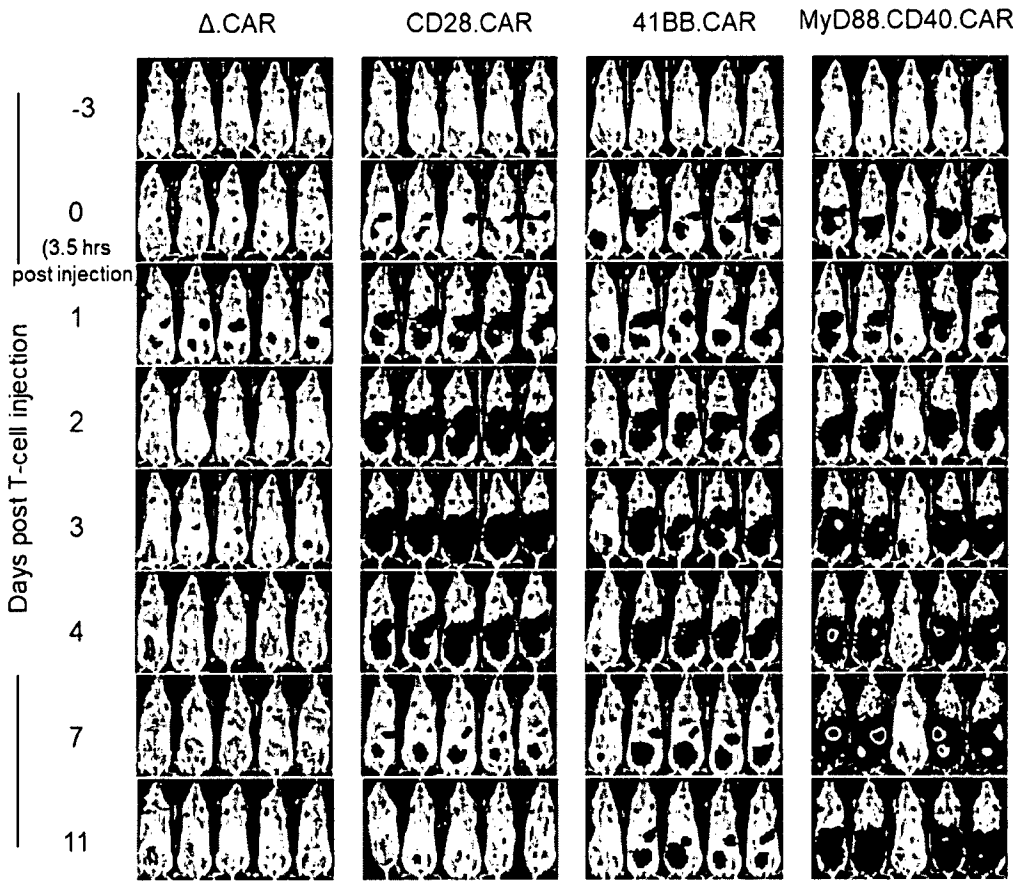

Δ.CAR
CD28.CAR
41BB.CAR
MyD88.CD40.CAR
Days post T-cell injection
-3
0
(3.5 hrs post injection)
1
2
3
4
7
11

FIG. 5C

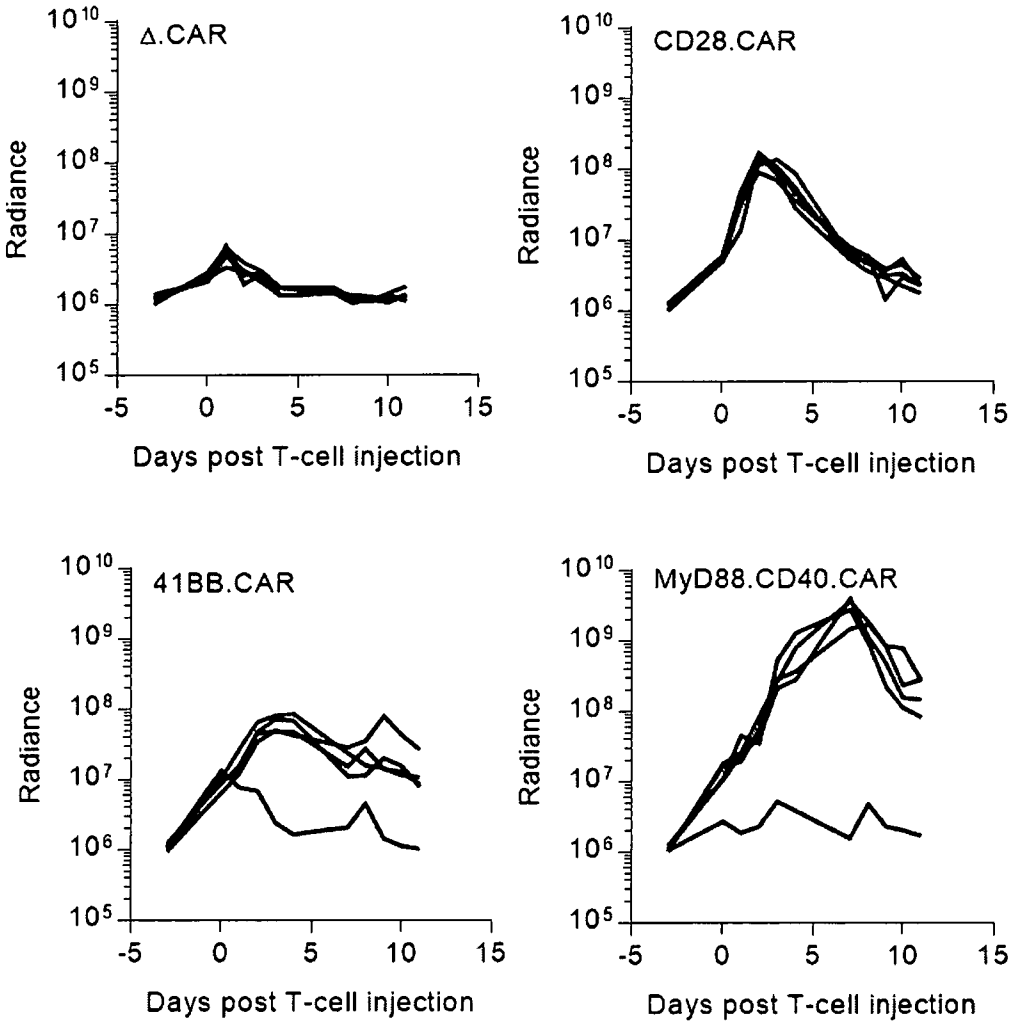

Δ.CAR
CD28.CAR
41BB.CAR
MyD88.CD40.CAR
Radiance
Days post T-cell injection
10^10
10^9
10^8
10^7
10^6
10^5
-5
0
5
10
15

FIG. 5D

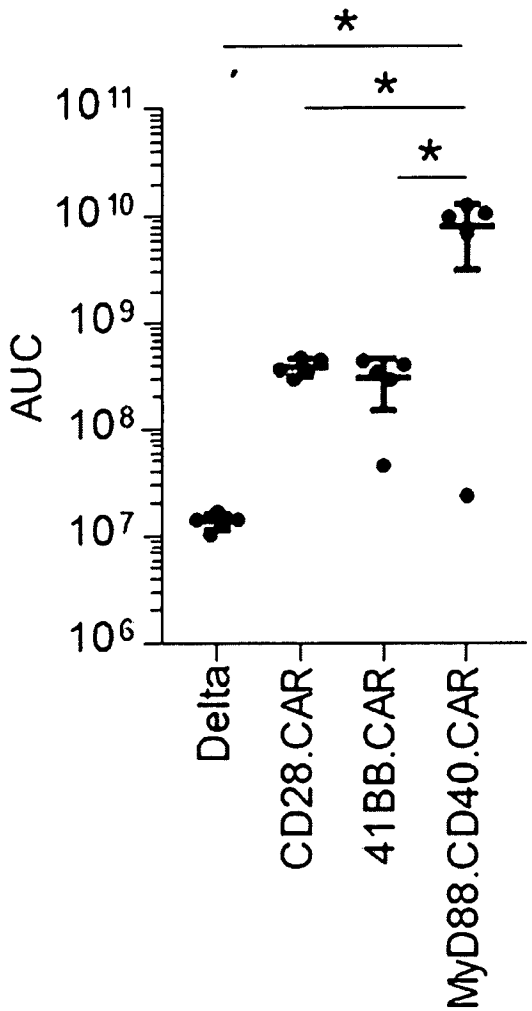

*
*
*
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
AUC
Delta
CD28.CAR
41BB.CAR
MyD88.CD40.CAR

FIG. 6A
CD28.ζ.CAR
Underline: Leader Sequence
Bold: scFv 4H5
Italic: IgG1 Core Hinge
Dashed underline: CD28 Transmembrane and Cytoplasmic Domain
Bold italic: CD3 Zeta
Italic and underline: T2A
Wave underline: Truncated CD19

Amino Acid Sequence (SEQ ID NO:3):
MDWIWRILFLVGAATGAHS**QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMS**
WVRQAPGQALEWMGTISSGGTYTYYPDSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCAREAIFTYWGRGTLVTSSGGGGSGGGGSGGGGSDIQLTQSPSSLSA
SVGDRVTITCKASQDINNYLSWYQQKPGQAPRLLIYRANRLVDGVPDRFSGSGY
GTDFTLTINNIESEDAAYYFCLKYDVFPYTFGQGTKVEIKDL*EPKSCDKTHTCPPC*
*P*DPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK
HYQPYAPPRDFAAYRS***RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG***
RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST
ATKDTYDALHMQALPPRASRA*EGRGSLLTCGDVEENPGP*MPPPRLLFFLLFLTPMEVR
PEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLKLSLGLPGLGIHMR
PLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRWNVSDLGGL
GCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSLNQSLSQDLT
MAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDMWVMET
GLLLPRATAQDAGKYYCHRGNLTMSFHLEITARPVLWHWLLRTGGWKVSAVTLAY
LIFCLCSLVGILHLQRALVLRRKRKRMTDPTRRF*

Nucleotide Sequence (SEQ ID NO:4):
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CA**CAGGTGCAGCTGCTGGAATCTGGCGGCGGACTGGTGCAGCCTGGCGGCA**
GCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACACCA
TGAGCTGGGTCCGGCAGGCTCCTGGACAGGCCCTGGAATGGATGGGCACCA
TCAGCAGCGGCGGCACCTACACCTACTACCCCGACAGCGTGAAGGGCCGGT
TCACCATCAGCCGGGACAACGCCAAGAACAGCCTGTACCTGCAGATGAACA
GCCTGAGAGCCGAGGACACAGCCGTGTACTACTGCGCCAGAGAGGCCATCT
TCACCTACTGGGGCAGAGGCACCCTGGTCACAAGCAGCGGAGGCGGAGGA
AGTGGAGGGGGAGGATCAGGCGGCGGAGGCAGCGATATCCAGCTGACCCA
GAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCACATG
CAAGGCCAGCCAGGACATCAACAACTACCTGAGCTGGTATCAGCAGAAGCC
CGGCCAGGCCCCCAGACTGCTGATCTACCGGGCCAACAGACTGGTGGACGG
CGTGCCCGATAGATTCAGCGGCAGCGGCTACGGCACCGACTTCACCCTGAC
CATCAACAACATCGAGTCCGAGGACGCCGCCTACTACTTCTGCCTGAAGTAC
GACGTGTTCCCCTACACCTTCGGCCAGGGCACCAAGGTGGAGATCAAGGAT
CTC*GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCG*GATCCCAAG
TTCTGGGTGCTGGTGGTCGTGGGCGGAGTGCTGGCCTGTTACAGCCTGCTCGTGA
CCGTGGCCTTCATCATCTTTTGGGTGCGCAGCAAGCGGAGCCGGCTGCTGCACAG
CGACTACATGAACATGACCCCCAGACGGCCTGGCCCCACCAGAAAGCACTACCA
GCCTTACGCCCCTCCCAGAGACTTCGCCGCCTACCGGTCC*AGAGTGAAGTTCAGC*
AGAAGCGCCGACGCCCCTGCCTATCAGCAGGGCCAGAACCAGCTGTACAACGAGC
TGAACCTGGGCAGACGGGAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGGG

ACCCTGAGATGGGCGGCAAGCCCAGAAGAAAGAACCCCCAGGAAGGCCTGTATAA
CGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGC
GAGCGGAGAAGAGGCAAGGGCCACGATGGCCTGTACCAGGGACTGAGCACCGCC
*ACCAAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCTCCAAGA*GCCTCTA
GAGCC*GAGGGCAGAGGCAGCCTGCTGACATGTGGCGACGTGGAAGAGAACCCAGGC*
*CCC*ATGCCTCCCCCCAGACTGCTGTTCTTCCTGCTGTTCCTGACCCCTATGGAAGT
GCGGCCCGAGGAACCCCTGGTCGTGAAAGTGGAAGAGGGCGACAACGCCGTGCT
GCAGTGTCTGAAGGGCACCTCCGATGGCCCTACCCAGCAGCTGACCTGGTCCAG
AGAGAGCCCCCTGAAGCCCTTCCTGAAGCTGTCTCTGGGCCTGCCTGGCCTGGGC
ATCCATATGAGGCCACTGGCCATCTGGCTGTTCATCTTCAACGTGTCCCAGCAGA
TGGGAGGCTTCTACCTGTGCCAGCCTGGCCCACCTTCTGAGAAGGCTTGGCAGCC
TGGCTGGACCGTGAACGTGGAAGGATCTGGCGAGCTGTTCCGGTGGAACGTGTC
CGATCTGGGCGGCCTGGGATGCGGCCTGAAGAACAGATCTAGCGAGGGCCCCAG
CAGCCCCAGCGGCAAACTGATGAGCCCCAAGCTGTACGTGTGGGCCAAGGACAG
ACCCGAGATTTGGGAGGGCGAGCCCCCTTGCCTGCCCCCTAGAGATAGCCTGAA
CCAGAGCCTGAGCCAGGACCTGACAATGGCCCCTGGCAGCACACTGTGGCTGAG
CTGTGGCGTGCCACCCGACTCTGTGTCTAGAGGCCCTCTGAGCTGGACCCACGTG
CACCCTAAGGGCCCTAAGAGCCTGCTGTCCCTGGAACTGAAGGACGACAGGCCC
GCCAGAGATATGTGGGTCATGGAAACCGGCCTGCTGCTGCCTAGAGCCACAGCC
CAGGATGCCGGCAAGTACTACTGCCACAGAGGCAACCTGACCATGAGCTTCCAC
CTGGAAATCACCGCCAGACCCGTGCTGTGGCACTGGCTGCTGAGAACCGGCGGA
TGGAAAGTGTCCGCCGTGACTCTGGCCTACCTGATCTTCTGCCTGTGCTCCCTCGT
GGGCATCCTGCATCTGCAGAGGGCTCTGGTGCTGCGGCGGAAGCGGAAGAGAAT
GACCGACCCTACCCGGCGGTTCTAA

FIG. 6B
4-1BB.ζ.CAR
Underline: Leader Sequence
Bold: scFv 4H5
Italic: IgG1 Core Hinge
Dashed underline: CD8a Transmembrane Domain
Bold and underline: 41BB
Bold italic: CD3 Zeta
Italic and underline: T2A
Wave underline: Truncated CD19

Amino Acid Sequence (SEQ ID NO:5):
MDWIWRILFLVGAATGAHS**QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMS**
WVRQAPGQALEWMGTISSGGTYTYYPDSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCAREAIFTYWGRGTLVTSSGGGGSGGGGSGGGGSDIQLTQSPSSLSA
SVGDRVTITCKASQDINNYLSWYQQKPGQAPRLLIYRANRLVDGVPDRFSGSGY
GTDFTLTINNIESEDAAYYFCLKYDVFPYTFGQGTKVEIKDL*EPKSCDKTHTCPPC*
*P*DPKCDIYIWAPLAGTCGVLLLSLVITLYCNHRN**KRGRKKLLYIFKQPFMRPVQTT**
QEEDGCSCRFPEEEEGGCEL*RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL*
DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLSTATKDTYDALHMQALPPRASRA*EGRGSLLTCGDVEENPGP*MPPPRLLFFLLFLT
PMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLKLSLGLPGL
GIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRWNVS
DLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSLNQSL
SQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDMW

VMETGLLLPRATAQDAGKYYCHRGNLTMSFHLEITARPVLWHWLLRTGGWKVSAV
TLAYLIFCLCSLVGILHLQRALVLRRKRKRMTDPTRRF*

Nucleotide Sequence (SEQ ID NO:6):
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CACAGGTGCAGCTGCTGGAATCTGGCGGCGGACTGGTGCAGCCTGGCGGCA
GCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACACCA
TGAGCTGGGTCCGGCAGGCTCCTGGACAGGCCCTGGAATGGATGGGCACCA
TCAGCAGCGGCGGCACCTACACCTACTACCCCGACAGCGTGAAGGGCCGGT
TCACCATCAGCCGGGACAACGCCAAGAACAGCCTGTACCTGCAGATGAACA
GCCTGAGAGCCGAGGACACAGCCGTGTACTACTGCGCCAGAGAGGCCATCT
TCACCTACTGGGGCAGAGGCACCCTGGTCACAAGCAGCGGAGGCGGAGGA
AGTGGAGGGGGAGGATCAGGCGGCGGAGGCAGCGATATCCAGCTGACCCA
GAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCACATG
CAAGGCCAGCCAGGACATCAACAACTACCTGAGCTGGTATCAGCAGAAGCC
CGGCCAGGCCCCCAGACTGCTGATCTACCGGGCCAACAGACTGGTGGACGG
CGTGCCCGATAGATTCAGCGGCAGCGGCTACGGCACCGACTTCACCCTGAC
CATCAACAACATCGAGTCCGAGGACGCCGCCTACTACTTCTGCCTGAAGTAC
GACGTGTTCCCCTACACCTTCGGCCAGGGCACCAAGGTGGAGATCAAGGAT
CTC*GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCG*GATCCCAAG
TGCGACATCTACATCTGGGCCCCTCTGGCCGGCACATGTGGCGTGCTGCTGCTGA
GCCTCGTGATCACCCTGTACTGCAACCACCGGAAC**AAGCGGGGCAGAAAGAAG**
CTGCTGTACATCTTCAAGCAGCCCTTCATGCGGCCCGTGCAGACCACCCAG
GAAGAGGACGGCTGCTCCTGCAGATTCCCCGAGGAAGAAGAAGGCGGCTGC
GAGCTG*AGAGTGAAGTTCAGCAGAAGCGCCGACGCCCCTGCCTATCAGCAGGGC*
CAGAACCAGCTGTACAACGAGCTGAACCTGGGCAGACGGGAAGAGTACGACGTGC
TGGACAAGCGGAGAGGCAGGGACCCTGAGATGGGCGGCAAGCCCAGAAGAAAGA
ACCCCCAGGAAGGCCTGTATAACGAACTGCAGAAAGACAAGATGGCCGAGGCCTA
CAGCGAGATCGGAATGAAGGGCGAGCGGAGAAGAGGCAAGGGCCACGATGGCCT
GTACCAGGGCCTGAGCACCGCCACCAAGGACACCTATGACGCCCTGCACATGCAG
GCCCTGCCCCCTAGAGCCTCTAGAGCC*GAGGGAAGAGGCAGCCTGCTGACCTGTGG*
*GGACGTGGAAGAGAACCCTGGCCCT*ATGCCCCCTCCCCGGCTGCTGTTCTTCCTGC
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AAGAGGGCGACAACGCCGTGCTGCAGTGTCTGAAGGGCACCTCCGATGGCCCTA
CCCAGCAGCTGACCTGGTCCAGAGAGAGCCCCCTGAAGCCCTTCCTGAAGCTGTC
TCTGGGCCTGCCTGGCCTGGGCATCCATATGAGGCCACTGGCCATCTGGCTGTTC
ATCTTTAACGTGTCCCAGCAGATGGGAGGCTTCTACCTGTGCCAGCCTGGCCCCC
CATCTGAGAAAGCTTGGCAGCCTGGCTGGACCGTGAACGTGGAAGGATCTGGCG
AGCTGTTCAGATGGAACGTGTCCGACCTGGGCGGCCTGGGCTGTGGCCTGAAGA
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GCCCTCTGAGCTGGACCCACGTGCACCCTAAGGGCCCTAAGAGCCTGCTGTCCCT
GGAACTGAAGGACGACAGGCCCGCCAGAGATATGTGGGTCATGGAAACCGGCCT
GCTGCTGCCTAGAGCCACTGCCCAGGATGCCGGCAAGTACTACTGCCACAGAGG
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TGGCTGCTGAGAACCGGCGGCTGGAAAGTGTCTGCCGTGACCCTGGCCTACCTG
ATCTTCTGCCTGTGCTCCCTCGTGGGCATCCTGCATCTGCAGAGGGCTCTGGTGCT
GCGGCGGAAGCGGAAGAGAATGACCGACCCCACCCGGCGGTTCTAA

FIG. 6C
Myd88.CD40.ζ.CAR
Underline: Leader Sequence (SEQ ID NO:7 (aa); SEQ ID NO:8 (na))
Bold: scFv 4H5 (SEQ ID NO:9 (aa); SEQ ID NO:10 (na))
Italic: IgG1 Core Hinge (SEQ ID NO:11 (aa); SEQ ID NO:12 (na))
Dashed underline: CD28 Transmembrane Domain (SEQ ID NO:13 (aa); SEQ ID NO:14(na))
Double underline: MyD88 (SEQ ID NO:15 (aa); SEQ ID NO:16 (na))
Bold and underline: CD40 (SEQ ID NO:17 (aa); SEQ ID NO:18 (na))
Bold italic: CD3 Zeta (SEQ ID NO:19 (aa); SEQ ID NO:20 (na))
Italic and underline: T2A (SEQ ID NO:21 (aa); SEQ ID NO:22 (na))
Wave underline: Truncated CD19 (SEQ ID NO:23 (aa); SEQ ID NO:24 (na))

Amino Acid Sequence (SEQ ID NO:1):
MDWIWRILFLVGAATGAHS**QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMS**
WVRQAPGQALEWMGTISSGGTYTYYPDSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCAREAIFTYWGRGTLVTSSGGGGSGGGGSGGGGSDIQLTQSPSSLSA
SVGDRVTITCKASQDINNYLSWYQQKPGQAPRLLIYRANRLVDGVPDRFSGSGY
GTDFTLTINNIESEDAAYYFCLKYDVFPYTFGQGTKVEIKDL*EPKSCDKTHTCPPC*
*P*DPKFWVLVVVGGVLACYSLLVTVAFIIFWVAAGGPGAGSAAPVSSTSSLPLAALN
MRVRRRLSLFLNVRTQVAADWTALAEEMDFEYLEIRQLETQADPTGRLLDAWQGR
PGASVGRLLELLTKLGRDDVLLELGPSIEEDCQKYILKQQQEEAEKPLQVAAVDSSV
PRTAELAGITTLDDPLGHMPERFDAFICYCPSDIVE**KKVAKKPTNKAPHPKQEPQEI**
NFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ*RVKFSRSADAPAYQ*
QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRASRA*EGRGSLLTC*
*GDVEENPGP*MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQ
QLTWSRESPLKPFLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKA
WQPGWTVNVEGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAK
DRPEIWEGEPPCLPPRDSLNQSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVH
PKGPKSLLSLELKDDRPARDMWVMETGLLLPRATAQDAGKYYCHRGNLTMSFHLEI
TARPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRRKRKRMTDPT
RRF*

Nucleotide Sequence (SEQ ID NO:2):
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CA**CAGGTGCAGCTGCTGGAATCTGGCGGCGGACTGGTGCAGCCTGGCGGCA**
GCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACACCA
TGAGCTGGGTCCGGCAGGCTCCTGGACAGGCCCTGGAATGGATGGGCACCA
TCAGCAGCGGCGGCACCTACACCTACTACCCCGACAGCGTGAAGGGCCGGT
TCACCATCAGCCGGGACAACGCCAAGAACAGCCTGTACCTGCAGATGAACA
GCCTGAGAGCCGAGGACACAGCCGTGTACTACTGCGCCAGAGAGGCCATCT
TCACCTACTGGGGCAGAGGCACCCTGGTCACAAGCAGCGGAGGCGGAGGA
AGTGGAGGGGGAGGATCAGGCGGCGGAGGCAGCGATATCCAGCTGACCCA
GAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCACATG
CAAGGCCAGCCAGGACATCAACAACTACCTGAGCTGGTATCAGCAGAAGCC
CGGCCAGGCCCCCAGACTGCTGATCTACCGGGCCAACAGACTGGTGGACGG
CGTGCCCGATAGATTCAGCGGCAGCGGCTACGGCACCGACTTCACCCTGAC
CATCAACAACATCGAGTCCGAGGACGCCGCCTACTACTTCTGCCTGAAGTAC
GACGTGTTCCCCTACACCTTCGGCCAGGGCACCAAGGTGGAGATCAAGGAT
CTC*GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCG*GATCCCAAA

TTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAA
CAGTGGCCTTTATTATTTTCTGGGTGGCTGCTGGCGGACCTGGCGCCGGATCTGC
TGCTCCTGTGTCTAGCACAAGCAGCCTGCCTCTGGCCGCCCTGAACATGAGAGTG
CGGAGAAGGCTGAGCCTGTTCCTGAACGTGCGGACACAGGTGGCCGCCGATTGG
ACAGCCCTGGCCGAGGAAATGGACTTCGAGTACCTGGAAATCCGGCAGCTGGAA
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CAGCAGCAGGAAGAGGCCGAGAAGCCTCTGCAGGTGGCAGCCGTGGATAGCAG
CGTGCCAAGAACAGCCGAGCTGGCCGGCATCACCACCCTGGATGATCCTCTGGG
CCACATGCCCGAGAGATTCGACGCCTTCATCTGCTACTGCCCCAGCGACATCGTG
GAA**AAGAAGGTGGCCAAGAAGCCCACCAACAAGGCCCCCCACCCCAAGCAG**
GAACCCCAGGAAATCAACTTCCCCGACGACCTGCCCGGCAGCAATACTGCT
GCACCCGTGCAGGAAACCCTGCACGGCTGTCAGCCTGTGACCCAGGAAGAT
GGCAAAGAAAGCCGGATCTCTGTGCAGGAACGCCAG*AGAGTGAAGTTCAGCA*
GGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCT
CAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGAC
CCTGAGATGGGCGGCAAGCCCAGAAGAAAGAACCCCCAGGAAGGCCTGTATAACG
AACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGA
GCGGAGAAGAGGCAAGGGCCACGATGGCCTGTACCAGGGACTGAGCACCGCCACC
AAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCTCCAAGAGCCTCTAGAG
CC*GAGGGCAGAGGCAGCCTGCTGACATGTGGCGACGTGGAAGAGAACCCAGGCCCC*
ATGCCTCCCCCCAGACTGCTGTTCTTCCTGCTGTTCCTGACCCCTATGGAAGTGCG
GCCCGAGGAACCCCTGGTCGTGAAAGTGGAAGAGGGCGACAACGCCGTGCTGCA
GTGTCTGAAGGGCACCTCCGATGGCCCTACCCAGCAGCTGACCTGGTCCAGAGA
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CATATGAGGCCACTGGCCATCTGGCTGTTCATCTTCAACGTGTCCCAGCAGATGG
GAGGCTTCTACCTGTGCCAGCCTGGCCCACCTTCTGAGAAGGCTTGGCAGCCTGG
CTGGACCGTGAACGTGGAAGGATCTGGCGAGCTGTTCCGGTGGAACGTGTCCGA
TCTGGGCGGCCTGGGATGCGGCCTGAAGAACAGATCTAGCGAGGGCCCCAGCAG
CCCCAGCGGCAAACTGATGAGCCCCAAGCTGTACGTGTGGGCCAAGGACAGACC
CGAGATTTGGGAGGGCGAGCCCCCTTGCCTGCCCCCTAGAGATAGCCTGAACCA
GAGCCTGAGCCAGGACCTGACAATGGCCCCTGGCAGCACACTGTGGCTGAGCTG
TGGCGTGCCACCCGACTCTGTGTCTAGAGGCCCTCTGAGCTGGACCCACGTGCAC
CCTAAGGGCCCTAAGAGCCTGCTGTCCCTGGAACTGAAGGACGACAGGCCCGCC
AGAGATATGTGGGTCATGGAAACCGGCCTGCTGCTGCCTAGAGCCACAGCCCAG
GATGCCGGCAAGTACTACTGCCACAGAGGCAACCTGACCATGAGCTTCCACCTG
GAAATCACCGCCAGACCCGTGCTGTGGCACTGGCTGCTGAGAACCGGCGGATGG
AAAGTGTCCGCCGTGACTCTGGCCTACCTGATCTTCTGCCTGTGCTCCCTCGTGG
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CCGACCCTACCCGGCGGTTCTAA

FIG. 7A

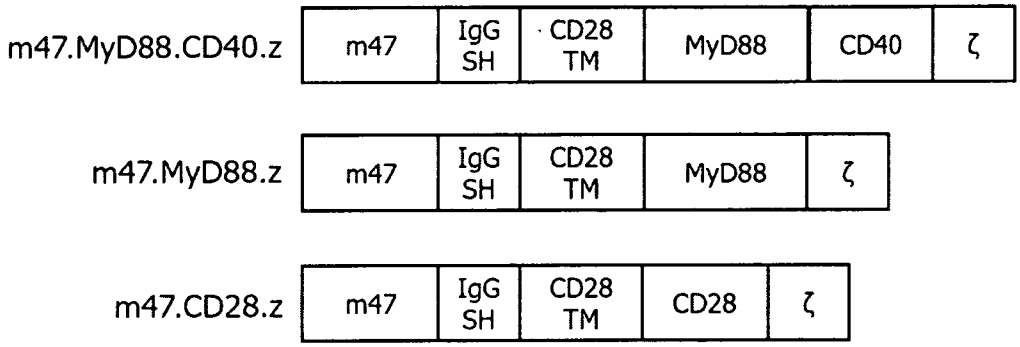


FIG. 7B

m47.MyD88.CD40.z
Underline: Leader Sequence
Bold: scFv m47
Italic: IgG1 Core Hinge
Dashed underline: CD28 Transmembrane Domain
Double underline: MyD88
Bold and underline: CD40
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 63):
MDWIWRILFLVGAATGAHS**QVQLQQPGAELVRPGASVKLSCKASGYTFSNYLMN**
WVKQRPEQDLDWIGRIDPYDGDIDYNQNFKDKAILTVDKSSSTAYMQLSSLTSED
SAVYYCARGYGTAYGVDYWGQGTSVTVSSAKTTPPKLEEGEFSEARVDIVLTQS
PASLAVSLGQRATISCRASESVDNYGISFMNWFQQKPGQPPKLLIYAASRQGSGV
PARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPWTFGGGTKLEIKDL*EPKS*
*CDKTHTCPPCP*DPKFWVLVVVGGVLACYSLLVTVAFIIFWVAAGGPGAGSAAPVSS
TSSLPLAALNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYLEIRQLETQADPTG
RLLDAWQGRPGASVGRLLELLTKLGRDDVLLELGPSIEEDCQKYILKQQQEEAEKP
LQVAAVDSSVPRTAELAGITTLDDPLGHMPERFDAFICYCPSDIVE**KKVAKKPTNKA**
PHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ*RVKF*
SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
PPR*

Nucleotide Sequence (SEQ ID NO: 64):
ATGGACTGGATCTGGCGCATCCTGTTTCTCGTGGGAGCCGCCACAGGCGCCCA
TTCT**CAGGTGCAGCTGCAGCAGCCTGGCGCTGAACTCGTGCGGCCAGGCGCT**
TCTGTGAAGCTGAGCTGTAAAGCCAGCGGCTACACCTTCAGCAACTACCTGAT
GAACTGGGTCAAGCAGCGGCCCGAGCAGGACCTGGATTGGATCGGCAGAAT
CGACCCCTACGACGGCGACATCGACTACAACCAGAACTTCAAGGACAAGGC
CATCCTGACCGTGGACAAGAGCAGCAGCACCGCCTACATGCAGCTGTCCAGC
CTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGAGGCTACGGCACA

GCCTACGGCGTGGACTATTGGGGCCAGGGCACAAGCGTGACCGTGTCCAGC
GCCAAGACCACCCCCCCTAAGCTGGAAGAGGGCGAGTTCTCCGAGGCCCGG
GTGGACATTGTGCTGACACAGTCTCCAGCCAGCCTGGCCGTGTCCCTGGGAC
AGAGAGCCACCATCAGCTGTAGGGCCAGCGAGAGCGTGGACAACTACGGCA
TCAGCTTCATGAATTGGTTCCAGCAGAAGCCCGGCCAGCCCCCCAAGCTGCT
GATCTATGCCGCCAGCAGACAGGGCAGCGGAGTGCCTGCCAGATTTTCTGGC
AGCGGCTCCGGCACCGACTTCAGCCTGAACATCCACCCTATGGAAGAGGACG
ACACCGCCATGTACTTTTGCCAGCAGAGCAAAGAGGTGCCCTGGACCTTTGG
CGGAGGCACCAAGCTGGAAATCAAGGATCTC*GAGCCCAAATCTTGTGACAAAA*
*CTCACACATGCCCACCGTGCCCG*GATCCCAAATTTTGGGTGCTGGTGGTGGTT
GGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCT
GGGTGGCTGCTGGCGGACCTGGCGCCGGATCTGCTGCTCCTGTGTCTAGCAC
AAGCAGCCTGCCTCTGGCCGCCCTGAACATGAGAGTGCGGAGAAGGCTGAGC
CTGTTCCTGAACGTGCGGACACAGGTGGCCGCCGATTGGACAGCCCTGGCCG
AGGAAATGGACTTCGAGTACCTGGAAATCCGGCAGCTGGAAACCCAGGCCGAC
CCTACAGGCAGACTGCTGGATGCTTGGCAGGGCAGACCAGGCGCTTCTGTGG
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ACTGGGCCCTAGCATCGAAGAGGACTGCCAGAAGTACATCCTGAAGCAGCAGC
AGGAAGAGGCCGAGAAGCCTCTGCAGGTGGCAGCCGTGGATAGCAGCGTGCC
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TGCCCGAGAGATTCGACGCCTTCATCTGCTACTGCCCCAGCGACATCGTGGAA
AAGAAGGTGGCCAAGAAGCCCACCAACAAGGCCCCCCACCCCAAGCAGGAA
CCCCAGGAAATCAACTTCCCCGACGACCTGCCCGGCAGCAATACTGCTGCAC
CCGTGCAGGAAACCCTGCACGGCTGTCAGCCTGTGACCCAGGAAGATGGCA
AAGAAAGCCGGATCTCTGTGCAGGAACGCCAG*AGAGTGAAGTTCAGCAGGA*
GCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGC
TCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCG
GGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCT
GTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGG
ATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGT
CTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGC
CCCCTCGCTAA

FIG. 7C

m47.MyD88.z
Underline: Leader Sequence
Bold: scFv m47
Italic: IgG1 Core Hinge
Dashed underline: CD28 Transmembrane Domain
Double underline: MyD88
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 65):
MDWIWRILFLVGAATGAHS**QVQLQQPGAELVRPGASVKLSCKASGYTFSNYLMN**
WVKQRPEQDLDWIGRIDPYDGDIDYNQNFKDKAILTVDKSSSTAYMQLSSLTSED
SAVYYCARGYGTAYGVDYWGQGTSVTVSSAKTTPPKLEEGEFSEARVDIVLTQS
PASLAVSLGQRATISCRASESVDNYGISFMNWFQQKPGQPPKLLIYAASRQGSGV
PARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPWTFGGGTKLEIKDL*EPKS*

*CDKTHTCPPCP*DPKFWVLVVVGGVLACYSLLVTVAFIIFWVAAGGPGAGSAAPVSS
TSSLPLAALNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYLEIRQLETQADPTG
RLLDAWQGRPGASVGRLLELLTKLGRDDVLLELGPSIEEDCQKYILKQQQEEAEKP
LQVAAVDSSVPRTAELAGITTLDDPLGHMPERFDAFICYCPSDI***RVKFSRSADAPAY***
QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*

Nucleotide Sequence(SEQ ID NO: 66):
ATGGACTGGATCTGGCGCATCCTGTTTCTCGTGGGAGCCGCCACAGGCGCCCA
TTCT**CAGGTGCAGCTGCAGCAGCCTGGCGCTGAACTCGTGCGGCCAGGCGCT**
TCTGTGAAGCTGAGCTGTAAAGCCAGCGGCTACACCTTCAGCAACTACCTGAT
GAACTGGGTCAAGCAGCGGCCCGAGCAGGACCTGGATTGGATCGGCAGAAT
CGACCCCTACGACGGCGACATCGACTACAACCAGAACTTCAAGGACAAGGC
CATCCTGACCGTGGACAAGAGCAGCAGCACCGCCTACATGCAGCTGTCCAGC
CTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGAGGCTACGGCACA
GCCTACGGCGTGGACTATTGGGGCCAGGGCACAAGCGTGACCGTGTCCAGC
GCCAAGACCACCCCCCCTAAGCTGGAAGAGGGCGAGTTCTCCGAGGCCCGG
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TCAGCTTCATGAATTGGTTCCAGCAGAAGCCCGGCCAGCCCCCCAAGCTGCT
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AGCGGCTCCGGCACCGACTTCAGCCTGAACATCCACCCTATGGAAGAGGACG
ACACCGCCATGTACTTTTGCCAGCAGAGCAAAGAGGTGCCCTGGACCTTTGG
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*CTCACACATGCCCACCGTGCCCG*GATCCCAAATTTTGGGTGCTGGTGGTGGTT
GGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCT
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AAGCAGCCTGCCTCTGGCCGCCCTGAACATGAGAGTGCGGAGAAGGCTGAGC
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AGGAAATGGACTTCGAGTACCTGGAAATCCGGCAGCTGGAAACCCAGGCCGAC
CCTACAGGCAGACTGCTGGATGCTTGGCAGGGCAGACCAGGCGCTTCTGTGG
GAAGGCTGCTGGAACTGCTGACCAAGCTGGGCAGGGACGACGTGCTGCTGGA
ACTGGGCCCTAGCATCGAAGAGGACTGCCAGAAGTACATCCTGAAGCAGCAGC
AGGAAGAGGCCGAGAAGCCTCTGCAGGTGGCAGCCGTGGATAGCAGCGTGCC
AAGAACAGCCGAGCTGGCCGGCATCACCACCCTGGATGATCCTCTGGGCCACA
TGCCCGAGAGATTCGACGCCTTCATCTGCTACTGCCCCAGCGACATC***AGAGTG***
AAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG
CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACA
AGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAAC
CCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCT
ACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGAT
GGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTC
ACATGCAGGCCCTGCCCCCTCGCTAA

FIG. 7D

m47.CD28.z
Underline: Leader Sequence
Bold: scFv m47
Italic: IgG1 Core Hinge
Dashed underline: CD28 Transmembrane and Cytoplasmic Domain
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 67):
MDWIWRILFLVGAATGAHS**QVQLQQPGAELVRPGASVKLSCKASGYTFSNYLMN**
WVKQRPEQDLDWIGRIDPYDGDIDYNQNFKDKAILTVDKSSSTAYMQLSSLTSED
SAVYYCARGYGTAYGVDYWGQGTSVTVSSAKTTPPKLEEGEFSEARVDIVLTQS
PASLAVSLGQRATISCRASESVDNYGISFMNWFQQKPGQPPKLLIYAASRQGSGV
PARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPWTFGGGTKLEIKDL*EPKS*
*CDKTHTCPPCP*DPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNM
TPRRPGPTRKHYQPYAPPRDFAAYRS***RVKFSRSADAPAYQQGQNQLYNELNLGR***
REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKGHDGLYQGLSTATKDTYDALHMQALPPR*

Nucleotide Sequence (SEQ ID NO: 68):
ATGGACTGGATCTGGCGCATCCTGTTTCTCGTGGGAGCCGCCACAGGCGCCCA
TTCT**CAGGTGCAGCTGCAGCAGCCTGGCGCTGAACTCGTGCGGCCAGGCGCT**
TCTGTGAAGCTGAGCTGTAAAGCCAGCGGCTACACCTTCAGCAACTACCTGAT
GAACTGGGTCAAGCAGCGGCCCGAGCAGGACCTGGATTGGATCGGCAGAAT
CGACCCCTACGACGGCGACATCGACTACAACCAGAACTTCAAGGACAAGGC
CATCCTGACCGTGGACAAGAGCAGCAGCACCGCCTACATGCAGCTGTCCAGC
CTGACCAGCGAGGACAGCGCCGTGTACTACTGCGCCAGAGGCTACGGCACA
GCCTACGGCGTGGACTATTGGGGCCAGGGCACAAGCGTGACCGTGTCCAGC
GCCAAGACCACCCCCCCTAAGCTGGAAGAGGGCGAGTTCTCCGAGGCCCGG
GTGGACATTGTGCTGACACAGTCTCCAGCCAGCCTGGCCGTGTCCCTGGGAC
AGAGAGCCACCATCAGCTGTAGGGCCAGCGAGAGCGTGGACAACTACGGCA
TCAGCTTCATGAATTGGTTCCAGCAGAAGCCCGGCCAGCCCCCCAAGCTGCT
GATCTATGCCGCCAGCAGACAGGGCAGCGGAGTGCCTGCCAGATTTTCTGGC
AGCGGCTCCGGCACCGACTTCAGCCTGAACATCCACCCTATGGAAGAGGACG
ACACCGCCATGTACTTTTGCCAGCAGAGCAAAGAGGTGCCCTGGACCTTTGG
CGGAGGCACCAAGCTGGAAATCAAGGATCTC*GAGCCCAAATCTTGTGACAAAA*
*CTCACACATGCCCACCGTGCCCG*GATCCCAAATTTTGGGTGCTGGTGGTGGTT
GGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCT
GGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACT
CCCCGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCAC
GCGACTTCGCAGCCTATCGCTCC***AGAGTGAAGTTCAGCAGGAGCGCAGACGC***
CCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGA
CGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAG
ATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAA
CTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGC
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AA

FIG. 7E

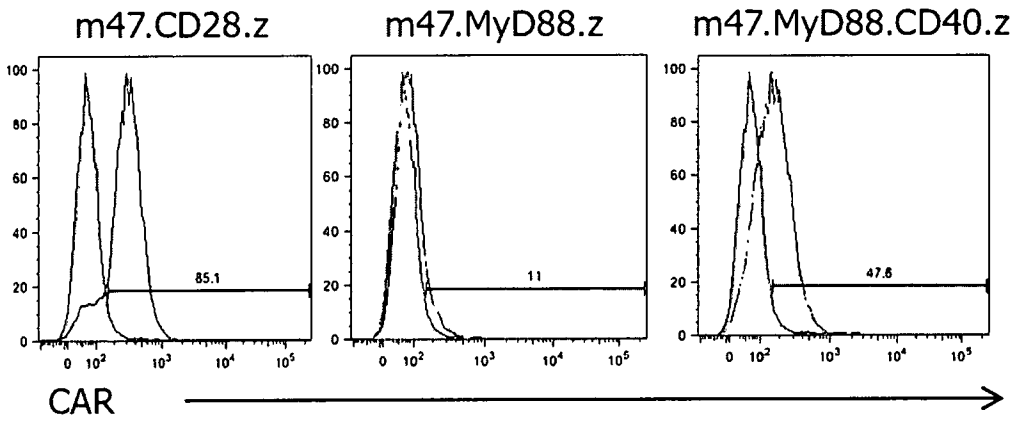
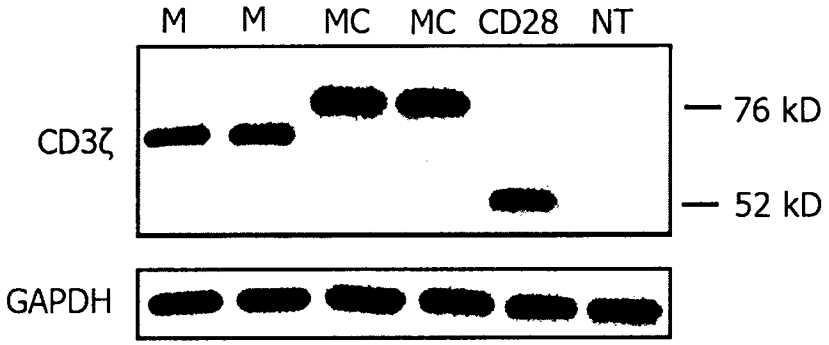


FIG. 7F



M: m47.SH.MyD88.z
MC: m47.SH.MyD88.CD40.z
CD28: m47.SH.CD28.z
NT: non-transduced

FIG. 7G

CARs	Cytotoxicity
m47.MyD88.CD40.z	-
m47.MyD88.z	-
m47.CD28.z	++

FIG. 8A

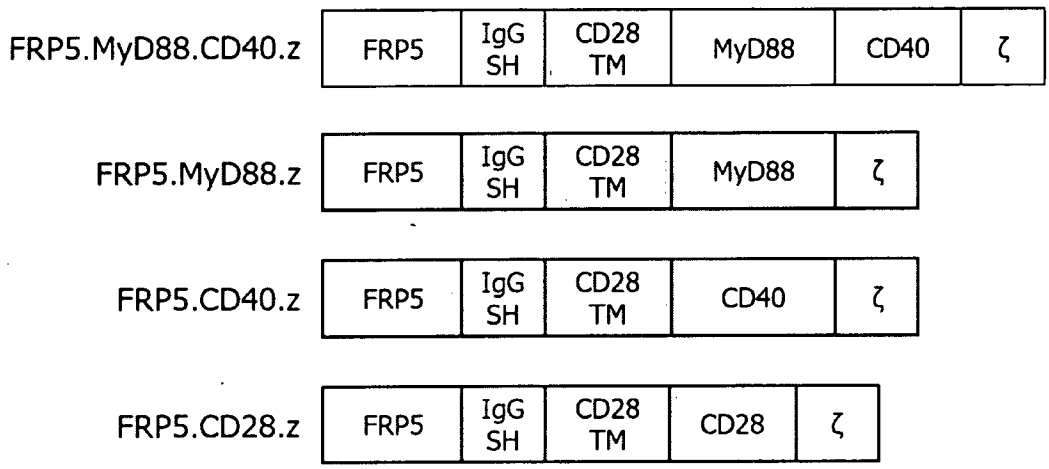


FIG. 8B

FRP5.MyD88.CD40.z (SH.TMCD28)
Underline: Leader Sequence
Bold: scFv FRP5
Italic: IgG1 Core Hinge
Dashed underline: CD28 Transmembrane Domain
Double underline: MyD88
Bold and underline: CD40
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 69):
MDWIWRILFLVGAATGAHS**EVQLQQSGPELKKPGETVKISCKASGYPFTNYGMN**
WVKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSE
DMATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQS
HKFLSTSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRF
TGSGSGPDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDL*EPKSCDK*
*THTCPPCP*DPKFWVLVVVGGVLACYSLLVTVAFIIFWVAAGGPGAGSAAPVSSTSS
LPLAALNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYLEIRQLETQADPTGRLL
DAWQGRPGASVGRLLELLTKLGRDDVLLELGPSIEEDCQKYILKQQQEEAEKPLQV
AAVDSSVPRTAELAGITTLDDPLGHMPERFDAFICYCPSDIVE**KKVAKKPTNKAPH**
PKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ*RVKFSR*
SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGL
YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP
R*

Nucleotide Sequence (SEQ ID NO: 70):
ATGGACTGGATCTGGCGCATCCTCTTCCTCGTCGGCGCTGCTACCGGCGCTCA
TTCT**GAGGTACAACTGCAGCAGTCTGGACCTGAACTGAAGAAGCCTGGAGAG**
ACAGTCAAGATCTCCTGCAAGGCCTCTGGGTATCCTTTCACAAACTATGGAAT
GAACTGGGTGAAGCAGGCTCCAGGACAGGGTTTAAAGTGGATGGGCTGGATT

AACACCTCCACTGGAGAGTCAACATTTGCTGATGACTTCAAGGGACGGTTTGA
CTTCTCTTTGGAAACCTCTGCCAACACTGCCTATTTGCAGATCAACAACCTCA
AAAGTGAAGACATGGCTACATATTTCTGTGCAAGATGGGAGGTTTACCACGG
CTACGTTCCTTACTGGGGCCAAGGGACCACGGTCACCGTTTCCTCTGGCGGT
GGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCTGACATCCAGCTGA
CCCAGTCTCACAAATTCCTGTCCACTTCAGTAGGAGACAGGGTCAGCATCACC
TGCAAGGCCAGTCAGGATGTGTATAATGCTGTTGCCTGGTATCAACAGAAACC
AGGACAATCTCCTAAACTTCTGATTTACTCGGCATCCTCCCGGTACACTGGAG
TCCCTTCTCGCTTCACTGGCAGTGGCTCTGGGCCGGATTTCACTTTCACCATC
AGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTTCTGTCAGCAACATTTTCG
TACTCCATTCACGTTCGGCTCGGGGACAAAATTGGAGATCAAAGCTCTAGATC
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ATCTGCTGCTCCTGTGTCTAGCACAAGCAGCCTGCCTCTGGCCGCCCTGAACA
TGAGAGTGCGGAGAAGGCTGAGCCTGTTCCTGAACGTGCGGACACAGGTGGC
CGCCGATTGGACAGCCCTGGCCGAGGAAATGGACTTCGAGTACCTGGAAATCC
GGCAGCTGGAAACCCAGGCCGACCCTACAGGCAGACTGCTGGATGCTTGGCA
GGGCAGACCAGGCGCTTCTGTGGGAAGGCTGCTGGAACTGCTGACCAAGCTG
GGCAGGGACGACGTGCTGCTGGAACTGGGCCCTAGCATCGAAGAGGACTGCC
AGAAGTACATCCTGAAGCAGCAGCAGGAAGAGGCCGAGAAGCCTCTGCAGGT
GGCAGCCGTGGATAGCAGCGTGCCAAGAACAGCCGAGCTGGCCGGCATCACC
ACCCTGGATGATCCTCTGGGCCACATGCCCGAGAGATTCGACGCCTTCATCTG
CTACTGCCCCAGCGACATCGTGGAA**AAGAAGGTGGCCAAGAAGCCCACCAAC**
AAGGCCCCCCACCCCAAGCAGGAACCCCAGGAAATCAACTTCCCCGACGAC
CTGCCCGGCAGCAATACTGCTGCACCCGTGCAGGAAACCCTGCACGGCTGTC
AGCCTGTGACCCAGGAAGATGGCAAAGAAAGCCGGATCTCTGTGCAGGAAC
GCCAG*AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGG*
GCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGA
TGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAG
AAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATG
GCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAA
GGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTAC
GACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA

FIG. 8C

FRP5.MyD88.z
Underline: Leader Sequence
Bold: scFv FRP5
Italic: IgG1 Core Hinge
Dashed underline: CD28 Transmembrane Domain
Double underline: MyD88
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 71):
MDWIWRILFLVGAATGAHS**EVQLQQSGPELKKPGETVKISCKASGYPFTNYGMN**
WVKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSE
DMATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQS

HKFLSTSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRF
TGSGSGPDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDL*EPKSCDK*
*THTCPPCP*DPKFWVLVVVGGVLACYSLLVTVAFIIFWVAAGGPGAGSAAPVSSTSS
LPLAALNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYLEIRQLETQADPTGRLL
DAWQGRPGASVGRLLELLTKLGRDDVLLELGPSIEEDCQKYILKQQQEEAEKPLQV
AAVDSSVPRTAELAGITTLDDPLGHMPERFDAFICYCPSDI***RVKFSRSADAPAYQQ***
GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*

Nucleotide Sequence (SEQ ID NO: 72):
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ACCCTGGATGATCCTCTGGGCCACATGCCCGAGAGATTCGACGCCTTCATCTG
CTACTGCCCCAGCGACATC***AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCC***
GCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAA
GAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGG
GGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGC
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GCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCA
CCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA

FIG. 8D

FRP5.CD40.z
Underline: Leader Sequence
Bold: scFv FRP5
Italic: IgG1 Core Hinge
Dashed underline: CD28 Transmembrane Domain
Bold and underline: CD40
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 73):
MDWIWRILFLVGAATGAHS**EVQLQQSGPELKKPGETVKISCKASGYPFTNYGMN**
WVKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSE
DMATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQS
HKFLSTSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRF
TGSGSGPDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKAL*DL*EPKSCDK*
*THTCPPCP*DPKFWVLVVVGGVLACYSLLVTVAFIIFWV**KKVAKKPTNKAPHPKQEP**
QEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ*RVKFSRSADAP*
AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ
KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*

Nucleotide Sequence (SEQ ID NO: 74):
ATGGACTGGATCTGGCGCATCCTCTTCCTCGTCGGCGCTGCTACCGGCGCTCA
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TGCAAGGCCAGTCAGGATGTGTATAATGCTGTTGCCTGGTATCAACAGAAACC
AGGACAATCTCCTAAACTTCTGATTTACTCGGCATCCTCCCGGTACACTGGAG
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TACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAG
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GGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGAC
*ACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC*TAA

FIG. 8E

FRP5.CD28.z
Underline: Leader Sequence
Bold: scFv FRP5
Italic: IgG1 Core Hinge
Dashed underline: CD28 Transmembrane and Cytoplasmic Domain
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 75):
MDWIWRILFLVGAATGAHS**EVQLQQSGPELKKPGETVKISCKASGYPFTNYGMN**
WVKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSE
DMATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQS
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*THTCPPCP*DPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPR
RPGPTRKHYQPYAPPRDFAAYRS***RVKFSRSADAPAYQQGQNQLYNELNLGRREE***
YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPR*

Nucleotide Sequence (SEQ ID NO: 76):
ATGGACTGGATCTGGCGCATCCTCTTCCTCGTCGGCGCTGCTACCGGCGCTCA
TTCT**GAGGTACAACTGCAGCAGTCTGGACCTGAACTGAAGAAGCCTGGAGAG**
ACAGTCAAGATCTCCTGCAAGGCCTCTGGGTATCCTTTCACAAACTATGGAAT
GAACTGGGTGAAGCAGGCTCCAGGACAGGGTTTAAAGTGGATGGGCTGGATT
AACACCTCCACTGGAGAGTCAACATTTGCTGATGACTTCAAGGGACGGTTTGA
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CCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCT
ACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGAT
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ACATGCAGGCCCTGCCCCCTCGCTAA

FIG. 8F

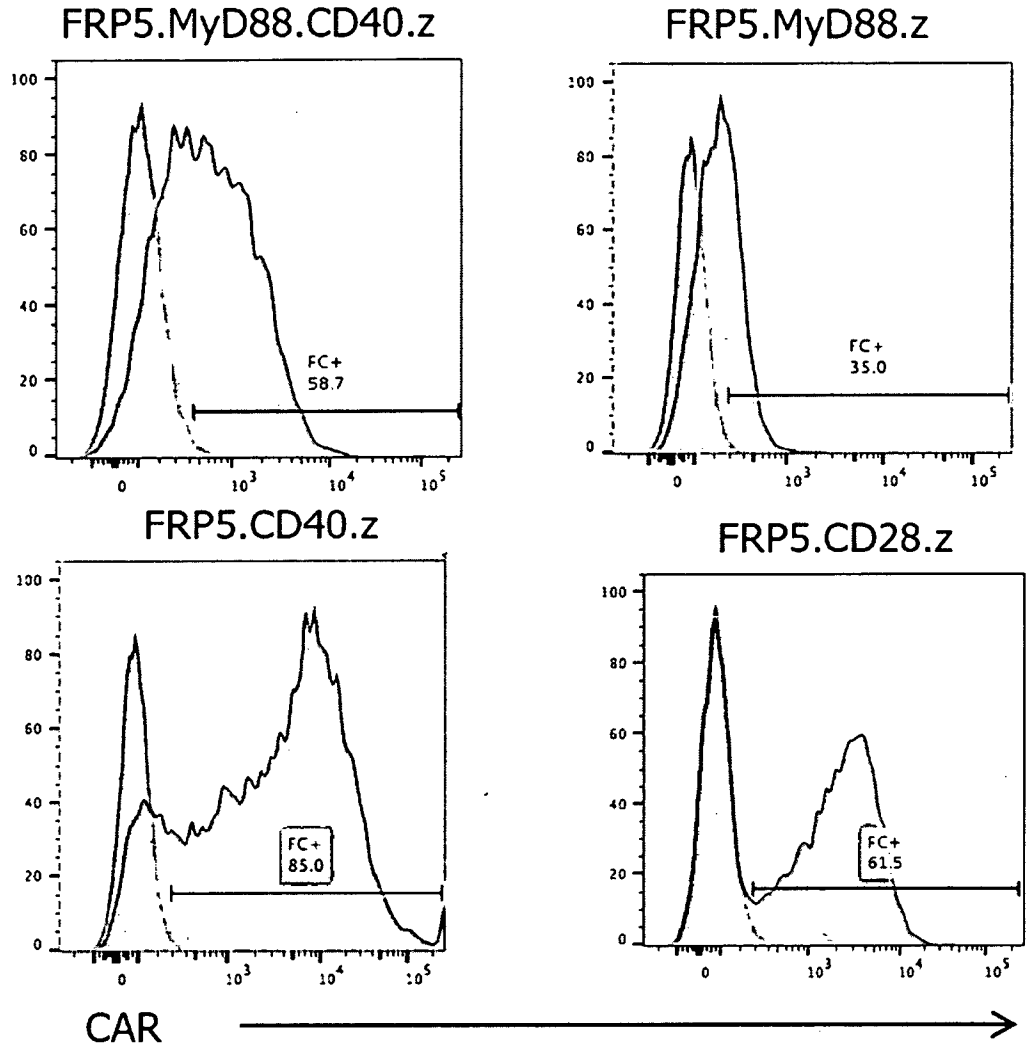

FRP5.MyD88.CD40.z
FC+
58.7
FRP5.MyD88.z
FC+
35.0
FRP5.CD40.z
FC+
85.0
FRP5.CD28.z
FC+
61.5
CAR

FIG. 8G

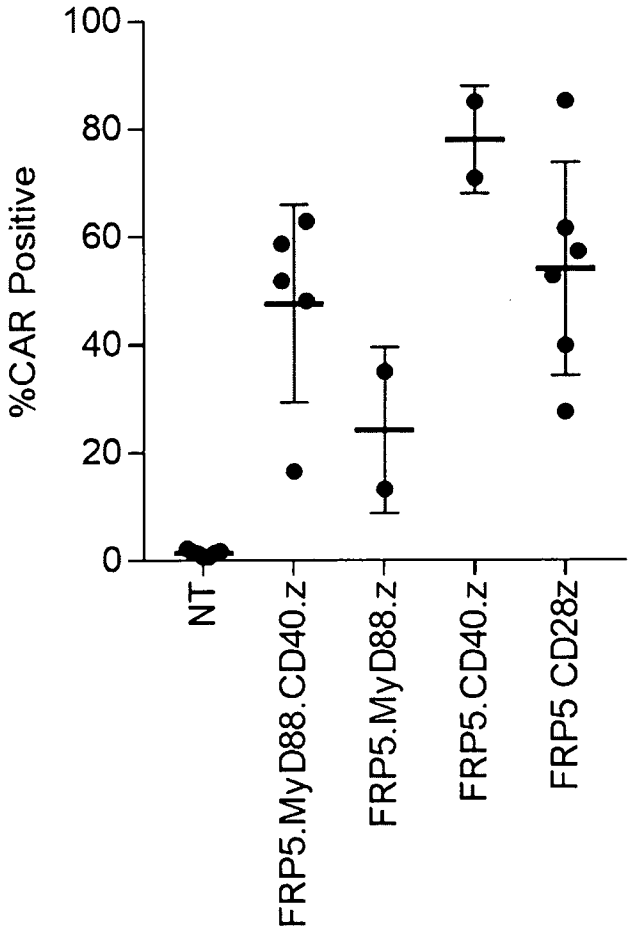


FIG. 8H

CARs	Cytotoxicity
FRP5.MyD88.CD40.z	+/-
FRP5.MyD88.z	+
FRP5.CD40.z	++
FRP5.CD28.z	+++

FIG. 9A

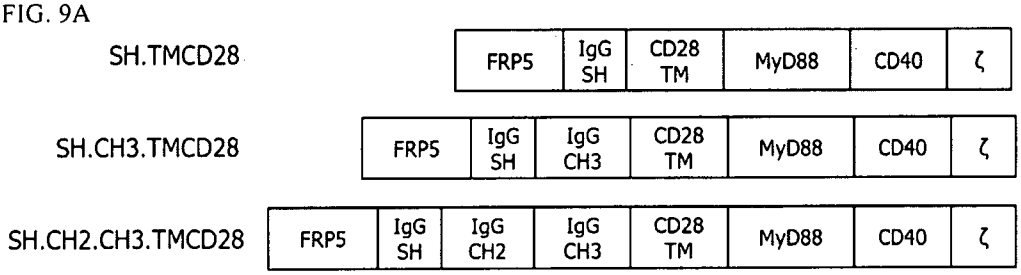


FIG. 9B

FRP5.MyD88.CD40.z (SH.CH3.TMCD28)
Underline: Leader Sequence
Bold: scFv FRP5
Italic: IgG2 Core Hinge and CH3 Domain
Dashed underline: CD28 Transmembrane Domain
Double underline: MyD88
Bold and underline: CD40
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 77):
MDWIWRILFLVGAATGAHS**EVQLQQSGPELKKPGETVKISCKASGYPFTNYGMN**
WVKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSE
DMATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQS
HKFLSTSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRF
TGSGSGPDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALDLE*EERKCCV*
ECPPCPGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPEN
NYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
*PGK*DPKFWVLVVVGGVLACYSLLVTVAFIIFWVAAGGPGAGSAAPVSSTSSLPLAA
LNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYLEIRQLETQADPTGRLLDAWQ
GRPGASVGRLLELLTKLGRDDVLLELGPSIEEDCQKYILKQQQEEAEKPLQVAAVD
SSVPRTAELAGITTLDDPLGHMPERFDAFICYCPSDIVE**KKVAKKPTNKAPHPKQE**
PQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ*RVKFSRSADA*
PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ
KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*

Nucleotide Sequence (SEQ ID NO: 78):
ATGGACTGGATCTGGCGCATCCTCTTCCTCGTCGGCGCTGCTACCGGCGCTCA
TTCT**GAGGTACAACTGCAGCAGTCTGGACCTGAACTGAAGAAGCCTGGAGAG**
ACAGTCAAGATCTCCTGCAAGGCCTCTGGGTATCCTTTCACAAACTATGGAAT
GAACTGGGTGAAGCAGGCTCCAGGACAGGGTTTAAAGTGGATGGGCTGGATT
AACACCTCCACTGGAGAGTCAACATTTGCTGATGACTTCAAGGGACGGTTTGA
CTTCTCTTTGGAAACCTCTGCCAACACTGCCTATTTGCAGATCAACAACCTCA
AAAGTGAAGACATGGCTACATATTTCTGTGCAAGATGGGAGGTTTACCACGG
CTACGTTCCTTACTGGGGCCAAGGGACCACGGTCACCGTTTCCTCTGGCGGT
GGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCTGACATCCAGCTGA
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TCGAG*GAGAGAAAATGTTGTGTGGAATGTCCACCATGCCCCGGCCAACCCCGG*
GAGCCGCAGGTGTACACACTCCCCCCTAGCAGGGAGGAGATGACAAAGAATCA
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GAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGA
ACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTT
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AGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGG
AGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGG
CACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACG
CCCTTCACATGCAGGCCCTGCCCCCTCGCTAA

FIG. 9C

FRP5.MyD88.CD40.z (SH.CH2.CH3.TMCD28)
Underline: Leader Sequence
Bold: scFv FRP5
Italic: IgG1 Core Hinge.CH2.CH3 (Shaded character indicates changes in the CH2CH3 domain to mutate the Fc receptor binding site)
Dashed underline: CD28 Transmembrane Domain
Double underline: MyD88
Bold and underline: CD40
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 79):
MDWIWRILFLVGAATGAHS**EVQLQQSGPELKKPGETVKISCKASGYPFTNYGMN**
WVKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSE

DMATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQS
HKFLSTSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRF
TGSGSGPDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKAL*DLEPKSPDK*
THTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
*GK*KDPKFWVLVVVGGVLACYSLLVTVAFIIFWVAAGGPGAGSAAPVSSTSSLPLAA
LNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYLEIRQLETQADPTGRLLDAWQ
GRPGASVGRLLELLTKLGRDDVLLELGPSIEEDCQKYILKQQQEEAEKPLQVAAVD
SSVPRTAELAGITTLDDPLGHMPERFDAFICYCPSDIVE**KKVAKKPTNKAPHPKQE**
PQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ*RVKFSRSADA*
PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ
KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*

Nucleotide Sequence (SEQ ID NO: 80):
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TTCT**GAGGTACAACTGCAGCAGTCTGGACCTGAACTGAAGAAGCCTGGAGAG**
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CTAA

FIG. 9D

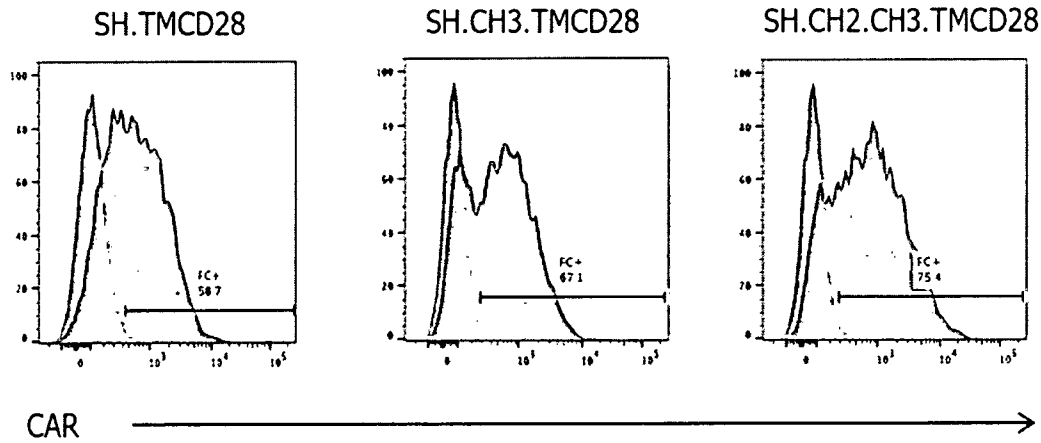


FIG. 9E

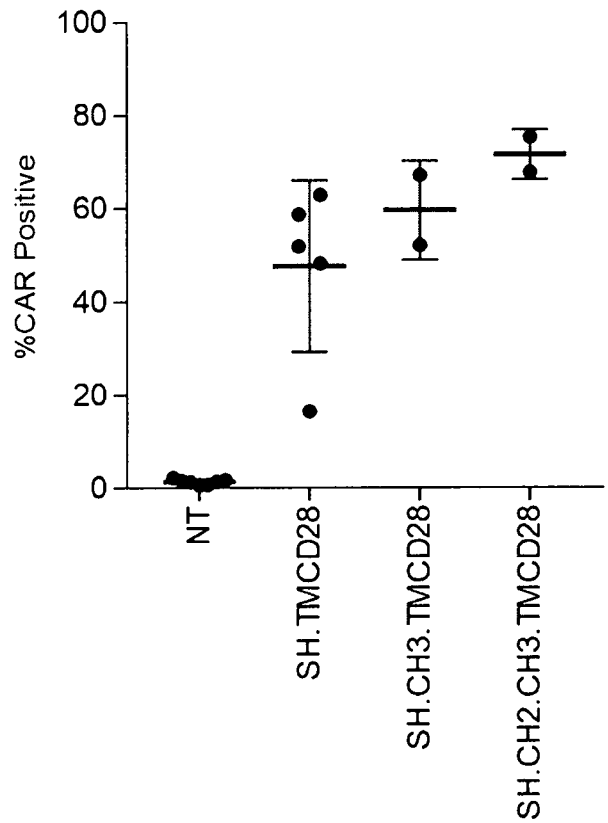


FIG. 9F

CARs	Cytotoxicity
SH.TMCD28	+/-
SH.CH3.TMCD28	+/-
SH.CH2.CH3.TMCD28	+/-

FIG. 10A

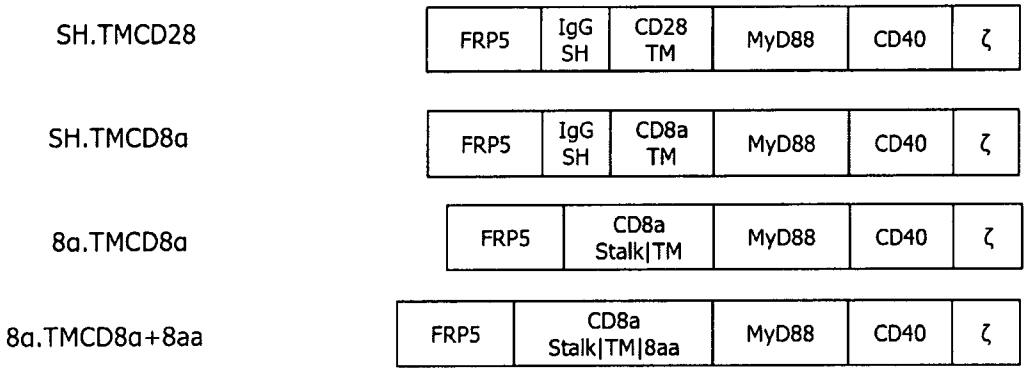


FIG. 10B

FRP5.MyD88.CD40.z (SH.TMCD8a)
Underline: Leader Sequence
Bold: scFv FRP5
Italic: IgG1 Short Hinge
Dashed underline: CD8a Transmembrane Domain
Double underline: MyD88
Bold and underline: CD40
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 88):
MDWIWRILFLVGAATGAHS**EVQLQQSGPELKKPGETVKISCKASGYPFTNYGMN WVKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSE DMATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQS HKFLSTSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRF TGSGSGPDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKAL**DL*EPKSCDK THTCPPCP*DPKCDIYIWAPLAGTCGVLLLSLVITLYCNHRNAAGGPGAGSAAPVSST SSLPLAALNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYLEIRQLETQADPTGR LLDAWQGRPGASVGRLLELLTKLGRDDVLLELGPSIEEDCQKYILKQQQEEAEKPL QVAAVDSSVPRTAELAGITTLDDPLGHMPERFDAFICYCPSDIVE**KKVAKKPTNKA PHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ*RVKF SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL PPR****

Nucleotide Sequence (SEQ ID NO: 89):
ATGGACTGGATCTGGCGCATCCTCTTCCTCGTCGGCGCTGCTACCGGCGCTCA TTCT**GAGGTACAACTGCAGCAGTCTGGACCTGAACTGAAGAAGCCTGGAGAG ACAGTCAAGATCTCCTGCAAGGCCTCTGGGTATCCTTTCACAAACTATGGAAT GAACTGGGTGAAGCAGGCTCCAGGACAGGGTTTAAAGTGGATGGGCTGGATT AACACCTCCACTGGAGAGTCAACATTTGCTGATGACTTCAAGGGACGGTTTGA CTTCTCTTTGGAAACCTCTGCCAACACTGCCTATTTGCAGATCAACAACCTCA**

AAAGTGAAGACATGGCTACATATTTCTGTGCAAGATGGGAGGTTTACCACGG
CTACGTTCCTTACTGGGGCCAAGGGACCACGGTCACCGTTTCCTCTGGCGGT
GGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCTGACATCCAGCTGA
CCCAGTCTCACAAATTCCTGTCCACTTCAGTAGGAGACAGGGTCAGCATCACC
TGCAAGGCCAGTCAGGATGTGTATAATGCTGTTGCCTGGTATCAACAGAAACC
AGGACAATCTCCTAAACTTCTGATTTACTCGGCATCCTCCCGGTACACTGGAG
TCCCTTCTCGCTTCACTGGCAGTGGCTCTGGGCCGGATTTCACTTTCACCATC
AGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTTCTGTCAGCAACATTTTCG
TACTCCATTCACGTTCGGCTCGGGGACAAAATTGGAGATCAAAGCTCTAGATC
*TCGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCG*GATCCC
AAGTGCGACATCTACATCTGGGCCCCTCTGGCCGGCACATGTGGCGTGCTGCT
GCTGAGCCTCGTGATCACCCTGTACTGCAACCACCGGAACGCTGCTGGCGGAC
CTGGCGCCGGATCTGCTGCTCCTGTGTCTAGCACAAGCAGCCTGCCTCTGGCC
GCCCTGAACATGAGAGTGCGGAGAAGGCTGAGCCTGTTCCTGAACGTGCGGA
CACAGGTGGCCGCCGATTGGACAGCCCTGGCCGAGGAAATGGACTTCGAGTA
CCTGGAAATCCGGCAGCTGGAAACCCAGGCCGACCCTACAGGCAGACTGCTG
GATGCTTGGCAGGGCAGACCAGGCGCTTCTGTGGGAAGGCTGCTGGAACTGC
TGACCAAGCTGGGCAGGGACGACGTGCTGCTGGAACTGGGCCCTAGCATCGA
AGAGGACTGCCAGAAGTACATCCTGAAGCAGCAGCAGGAAGAGGCCGAGAAG
CCTCTGCAGGTGGCAGCCGTGGATAGCAGCGTGCCAAGAACAGCCGAGCTGG
CCGGCATCACCACCCTGGATGATCCTCTGGGCCACATGCCCGAGAGATTCGAC
GCCTTCATCTGCTACTGCCCCAGCGACATCGTGGAA**AAGAAGGTGGCCAAGA**
AGCCCACCAACAAGGCCCCCCACCCCAAGCAGGAACCCCAGGAAATCAACT
TCCCCGACGACCTGCCCGGCAGCAATACTGCTGCACCCGTGCAGGAAACCCT
GCACGGCTGTCAGCCTGTGACCCAGGAAGATGGCAAAGAAAGCCGGATCTCT
GTGCAGGAACGCCAG*AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCG*
TACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAG
AGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGG
GAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGA
AAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCC
GGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCA
AGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA

FIG. 10C

FRP5.MyD88.CD40.z (8a.TMCD8a)
Underline: Leader Sequence
Bold: scFv FRP5
Dashed underline: CD8a Stalk and Transmembrane Domain
Double underline: MyD88
Bold and underline: CD40
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 90):
MDWIWRILFLVGAATGAHSE**VQLQQSGPELKKPGETVKISCKASGYPFTNYGMN**
WVKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSE
DMATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQS
HKFLSTSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRF
TGSGSGPDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALPAKPTTTPA
PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS

LVITLYCNHRNAAGGPGAGSAAPVSSTSSLPLAALNMRVRRRLSLFLNVRTQVAAD
WTALAEEMDFEYLEIRQLETQADPTGRLLDAWQGRPGASVGRLLELLTKLGRDDV
LLELGPSIEEDCQKYILKQQQEEAEKPLQVAAVDSSVPRTAELAGITTLDDPLGHMP
ERFDAFICYCPSDIVE**KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETL**
HGCQPVTQEDGKESRISVQERQ*RVKFSRSADAPAYQQGQNQLYNELNLGRREE*
YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPR*

Nucleotide Sequence (SEQ ID NO: 91):
ATGGACTGGATCTGGCGCATCCTCTTCCTCGTCGGCGCTGCTACCGGCGCTCA
TTCT**GAGGTACAACTGCAGCAGTCTGGACCTGAACTGAAGAAGCCTGGAGAG**
ACAGTCAAGATCTCCTGCAAGGCCTCTGGGTATCCTTTCACAAACTATGGAAT
GAACTGGGTGAAGCAGGCTCCAGGACAGGGTTTAAAGTGGATGGGCTGGATT
AACACCTCCACTGGAGAGTCAACATTTGCTGATGACTTCAAGGGACGGTTTGA
CTTCTCTTTGGAAACCTCTGCCAACACTGCCTATTTGCAGATCAACAACCTCA
AAAGTGAAGACATGGCTACATATTTCTGTGCAAGATGGGAGGTTTACCACGG
CTACGTTCCTTACTGGGGCCAAGGGACCACGGTCACCGTTTCCTCTGGCGGT
GGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCTGACATCCAGCTGA
CCCAGTCTCACAAATTCCTGTCCACTTCAGTAGGAGACAGGGTCAGCATCACC
TGCAAGGCCAGTCAGGATGTGTATAATGCTGTTGCCTGGTATCAACAGAAACC
AGGACAATCTCCTAAACTTCTGATTTACTCGGCATCCTCCCGGTACACTGGAG
TCCCTTCTCGCTTCACTGGCAGTGGCTCTGGGCCGGATTTCACTTTCACCATC
AGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTTCTGTCAGCAACATTTTCG
TACTCCATTCACGTTCGGCTCGGGGACAAAATTGGAGATCAAAGCTCTACCAG
CCAAGCCCACCACAACCCCTGCTCCTAGACCTCCTACCCCAGCCCCTACCATT
GCCTCCCAGCCACTGTCTCTGAGGCCCGAGGCTTGTAGACCTGCTGCAGGCG
GAGCCGTGCACACCAGAGGACTGGATTTCGCCTGCGACATCTATATCTGGGCC
CCTCTGGCCGGCACCTGTGGCGTGCTGCTGCTGTCACTCGTGATCACCCTGTA
CTGCAACCACCGGAACGCTGCTGGCGGACCTGGCGCCGGATCTGCTGCTCCT
GTGTCTAGCACAAGCAGCCTGCCTCTGGCCGCCCTGAACATGAGAGTGCGGA
GAAGGCTGAGCCTGTTCCTGAACGTGCGGACACAGGTGGCCGCCGATTGGAC
AGCCCTGGCCGAGGAAATGGACTTCGAGTACCTGGAAATCCGGCAGCTGGAAA
CCCAGGCCGACCCTACAGGCAGACTGCTGGATGCTTGGCAGGGCAGACCAGG
CGCTTCTGTGGGAAGGCTGCTGGAACTGCTGACCAAGCTGGGCAGGGACGAC
GTGCTGCTGGAACTGGGCCCTAGCATCGAAGAGGACTGCCAGAAGTACATCCT
GAAGCAGCAGCAGGAAGAGGCCGAGAAGCCTCTGCAGGTGGCAGCCGTGGAT
AGCAGCGTGCCAAGAACAGCCGAGCTGGCCGGCATCACCACCCTGGATGATC
CTCTGGGCCACATGCCCGAGAGATTCGACGCCTTCATCTGCTACTGCCCCAGC
GACATCGTGGAA**AAGAAGGTGGCCAAGAAGCCCACCAACAAGGCCCCCCAC**
CCCAAGCAGGAACCCCAGGAAATCAACTTCCCCGACGACCTGCCCGGCAGC
AATACTGCTGCACCCGTGCAGGAAACCCTGCACGGCTGTCAGCCTGTGACCC
AGGAAGATGGCAAAGAAAGCCGGATCTCTGTGCAGGAACGCCAG*AGAGTGA*
AGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGC
TCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAA
GAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACC
CTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTA
CAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATG
GCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCA
CATGCAGGCCCTGCCCCCTCGCTAA

FIG. 10D

FRP5.MyD88.CD40.z (8a.TMCD8a+8aa)
Underline: Leader Sequence
Bold: scFv FRP5
Dashed underline: CD8a Stalk and Transmembrane Domain+8aa
Double underline: MyD88
Bold and underline: CD40
Bold italic: CD3 Zeta

Amino Acid Sequence (SEQ ID NO: 92):
MDWIWRILFLVGAATGAHS**EVQLQQSGPELKKPGETVKISCKASGYPFTNYGMN**
WVKQAPGQGLKWMGWINTSTGESTFADDFKGRFDFSLETSANTAYLQINNLKSE
DMATYFCARWEVYHGYVPYWGQGTTVTVSSGGGGSGGGGSGGGGSDIQLTQS
HKFLSTSVGDRVSITCKASQDVYNAVAWYQQKPGQSPKLLIYSASSRYTGVPSRF
TGSGSGPDFTFTISSVQAEDLAVYFCQQHFRTPFTFGSGTKLEIKALPAKPTTTPA
PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
LVITLYCNHRNRRRVCKCPRPVV**AAGGPGAGSAAPVSSTSSLPLAALNMRVRRRL**
SLFLNVRTQVAADWTALAEEMDFEYLEIRQLETQADPTGRLLDAWQGRPGASVGR
LLELLTKLGRDDVLLELGPSIEEDCQKYILKQQQEEAEKPLQVAAVDSSVPRTAELA
GITTLDDPLGHMPERFDAFICYCPSDIVE**KKVAKKPTNKAPHPKQEPQEINFPDDL**
PGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ*RVKFSRSADAPAYQQGQN*
QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY
SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*

Nucleotide Sequence (SEQ ID NO: 93):
ATGGACTGGATCTGGCGCATCCTCTTCCTCGTCGGCGCTGCTACCGGCGCTCA
TTCT**GAGGTACAACTGCAGCAGTCTGGACCTGAACTGAAGAAGCCTGGAGAG**
ACAGTCAAGATCTCCTGCAAGGCCTCTGGGTATCCTTTCACAAACTATGGAAT
GAACTGGGTGAAGCAGGCTCCAGGACAGGGTTTAAAGTGGATGGGCTGGATT
AACACCTCCACTGGAGAGTCAACATTTGCTGATGACTTCAAGGGACGGTTTGA
CTTCTCTTTGGAAACCTCTGCCAACACTGCCTATTTGCAGATCAACAACCTCA
AAAGTGAAGACATGGCTACATATTTCTGTGCAAGATGGGAGGTTTACCACGG
CTACGTTCCTTACTGGGGCCAAGGGACCACGGTCACCGTTTCCTCTGGCGGT
GGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCTGACATCCAGCTGA
CCCAGTCTCACAAATTCCTGTCCACTTCAGTAGGAGACAGGGTCAGCATCACC
TGCAAGGCCAGTCAGGATGTGTATAATGCTGTTGCCTGGTATCAACAGAAACC
AGGACAATCTCCTAAACTTCTGATTTACTCGGCATCCTCCCGGTACACTGGAG
TCCCTTCTCGCTTCACTGGCAGTGGCTCTGGGCCGGATTTCACTTTCACCATC
AGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTTCTGTCAGCAACATTTTCG
TACTCCATTCACGTTCGGCTCGGGGACAAAATTGGAGATCAAAGCTCTACCAG
CCAAGCCCACCACAACCCCTGCTCCTAGACCTCCTACCCCAGCCCCTACCATT
GCCTCCCAGCCACTGTCTCTGAGGCCCGAGGCTTGTAGACCTGCTGCAGGCG
GAGCCGTGCACACCAGAGGACTGGATTTCGCCTGCGACATCTATATCTGGGCC
CCTCTGGCCGGCACCTGTGGCGTGCTGCTGCTGTCACTCGTGATCACCCTGTA
CTGCAACCACCGGAACCGGCGGAGAGTGTGCAAGTGCCCTAGACCCGTCGTG
GCTGCTGGCGGACCTGGCGCCGGATCTGCTGCTCCTGTGTCTAGCACAAGCA
GCCTGCCTCTGGCCGCCCTGAACATGAGAGTGCGGAGAAGGCTGAGCCTGTT
CCTGAACGTGCGGACACAGGTGGCCGCCGATTGGACAGCCCTGGCCGAGGAA
ATGGACTTCGAGTACCTGGAAATCCGGCAGCTGGAAACCCAGGCCGACCCTAC

AGGCAGACTGCTGGATGCTTGGCAGGGCAGACCAGGCGCTTCTGTGGGAAGG
CTGCTGGAACTGCTGACCAAGCTGGGCAGGGACGACGTGCTGCTGGAACTGG
GCCCTAGCATCGAAGAGGACTGCCAGAAGTACATCCTGAAGCAGCAGCAGGAA
GAGGCCGAGAAGCCTCTGCAGGTGGCAGCCGTGGATAGCAGCGTGCCAAGAA
CAGCCGAGCTGGCCGGCATCACCACCCTGGATGATCCTCTGGGCCACATGCC
CGAGAGATTCGACGCCTTCATCTGCTACTGCCCCAGCGACATCGTGGAA**AAGA**
AGGTGGCCAAGAAGCCCACCAACAAGGCCCCCCACCCCAAGCAGGAACCCC
AGGAAATCAACTTCCCCGACGACCTGCCCGGCAGCAATACTGCTGCACCCGT
GCAGGAAACCCTGCACGGCTGTCAGCCTGTGACCCAGGAAGATGGCAAAGA
AAGCCGGATCTCTGTGCAGGAACGCCAG*AGAGTGAAGTTCAGCAGGAGCGC*
AGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAAT
CTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGAC
CCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTAC
AATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGA
AAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCA
GTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCC
*TCGC*TAA

FIG. 10E

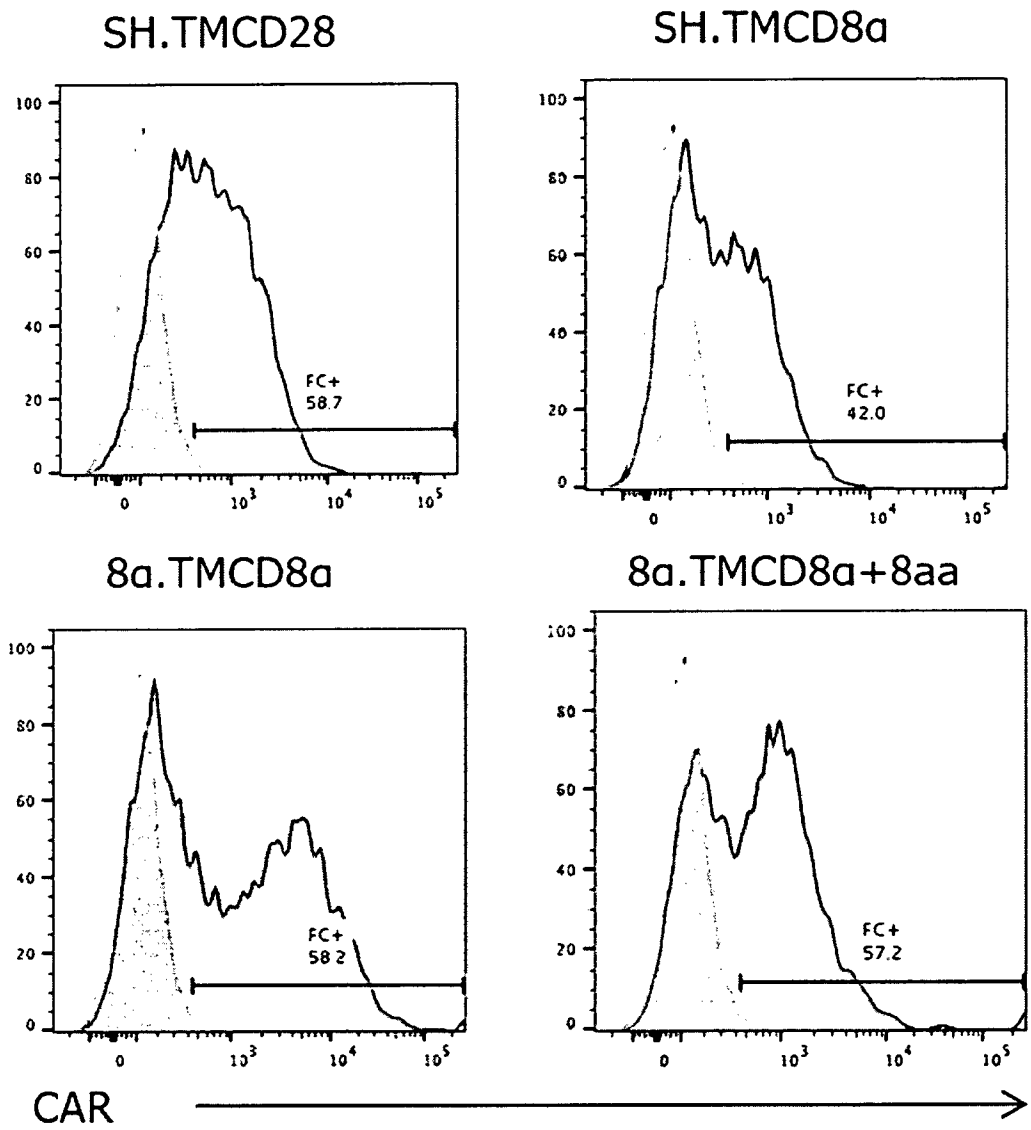

SH.TMCD28
FC+
58.7
SH.TMCD8a
FC+
42.0
8a.TMCD8a
FC+
58.2
8a.TMCD8a+8aa
FC+
57.2
CAR

FIG. 10F

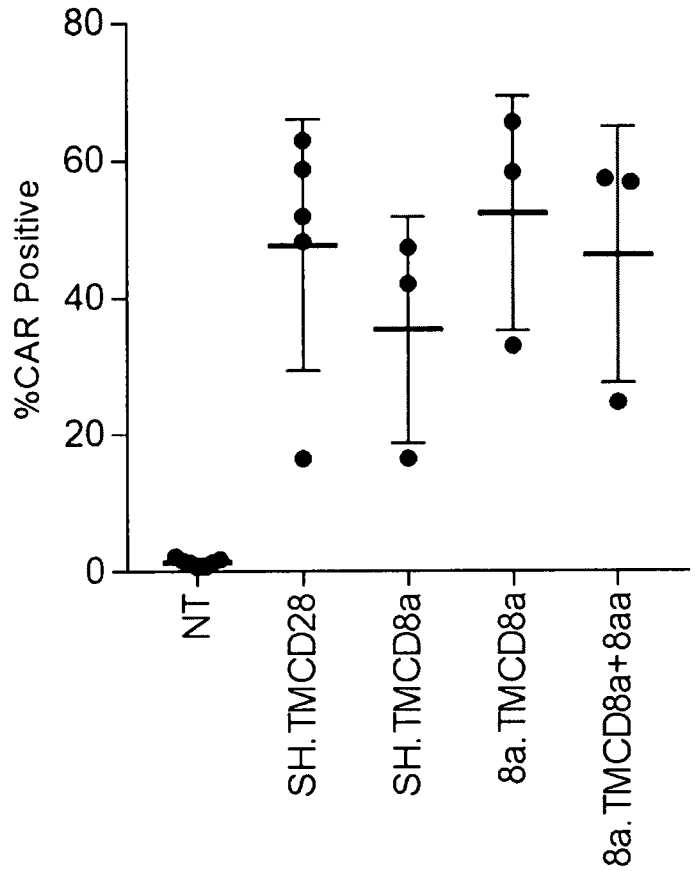


FIG. 10G

CARs	Cytotoxicity
SH.TMCD28	+/-
SH.TMCD8α	+/-
8α.TMCD8α	+/-
8α.TMCD8α+8aa	+

FIG. 11

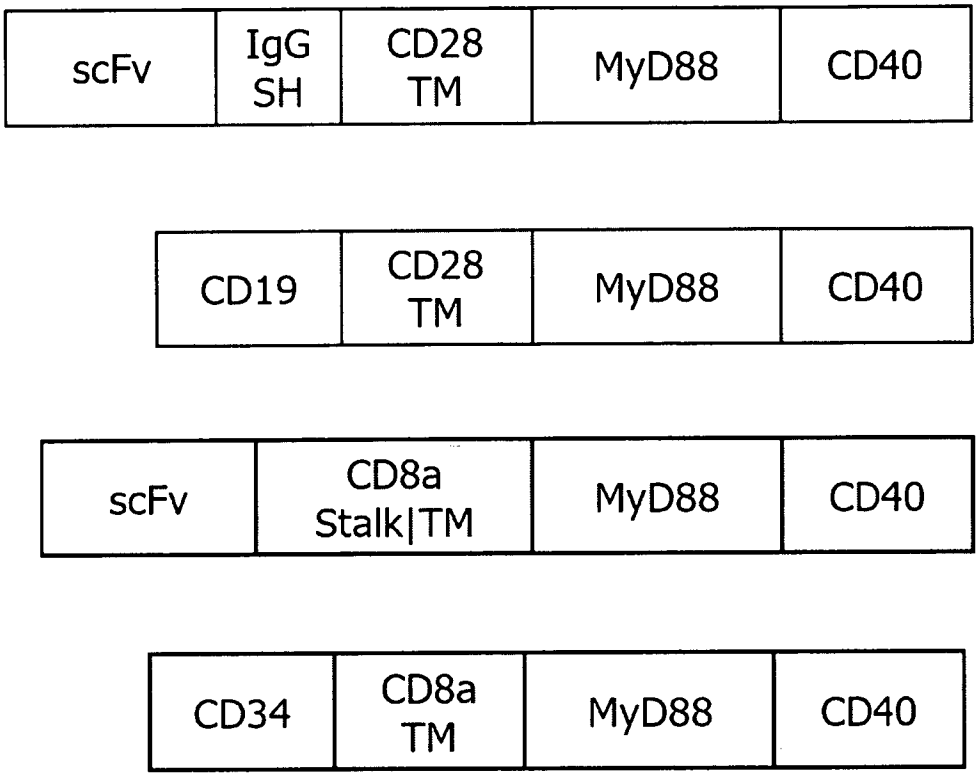
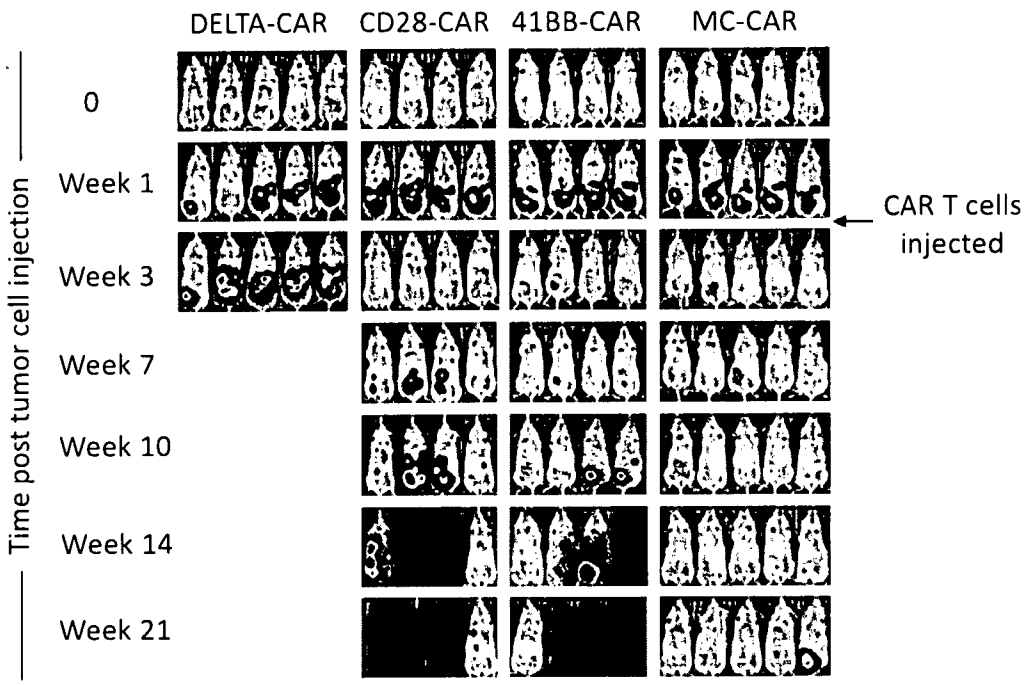

scFv
IgG SH
CD28 TM
MyD88
CD40
CD19
CD28 TM
MyD88
CD40
scFv
CD8a Stalk|TM
MyD88
CD40
CD34
CD8a TM
MyD88
CD40

FIG. 12A


DELTA-CAR
CD28-CAR
41BB-CAR
MC-CAR
Time post tumor cell injection
0
Week 1
Week 3
Week 7
Week 10
Week 14
Week 21
CAR T cells injected

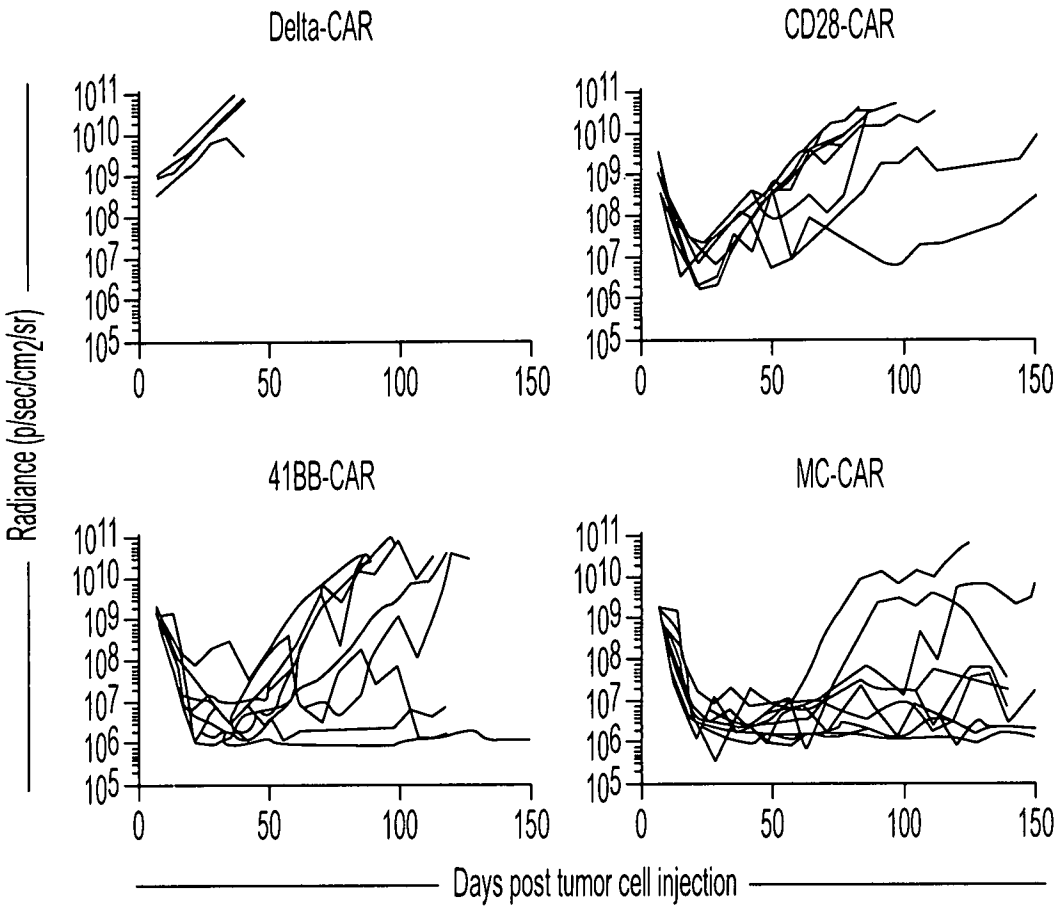


FIG. 12B

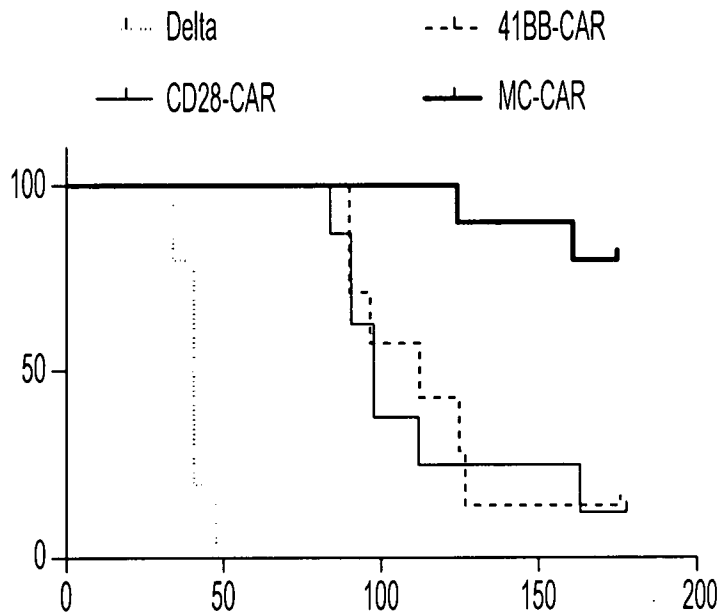


FIG. 12C

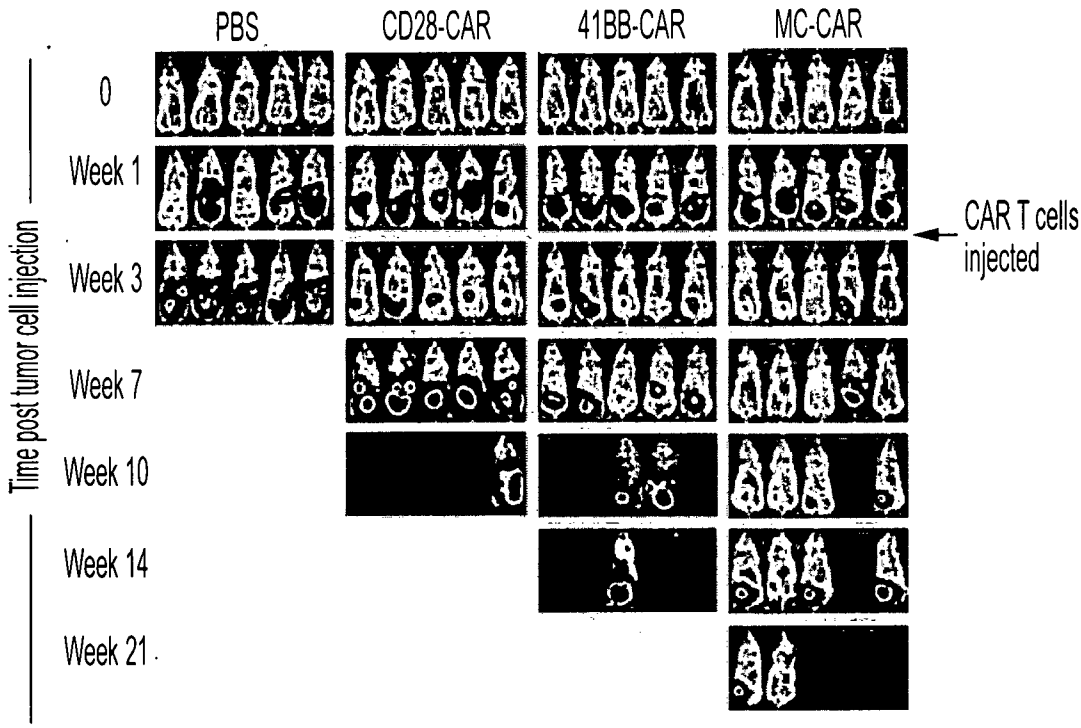

PBS
CD28-CAR
41BB-CAR
MC-CAR
0
Week 1
Week 3
Week 7
Week 10
Week 14
Week 21
Time post tumor cell injection
CAR T cells injected

FIG. 12D

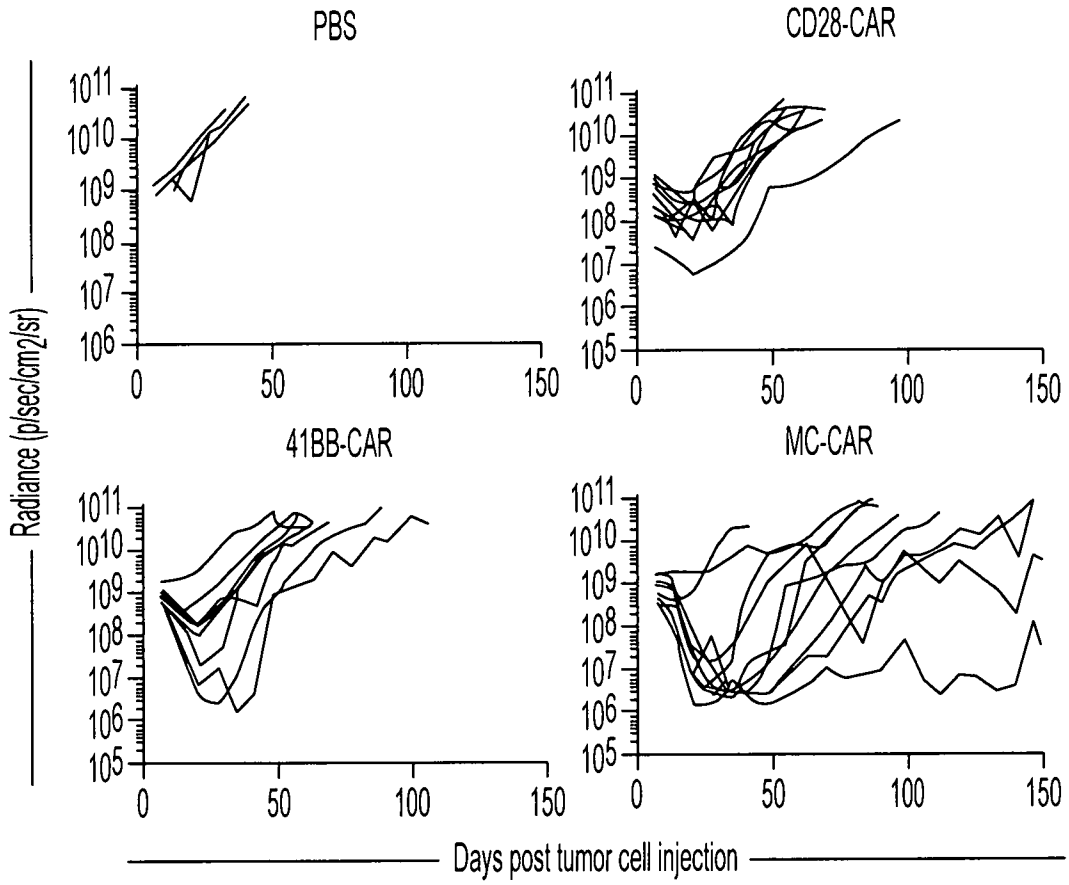
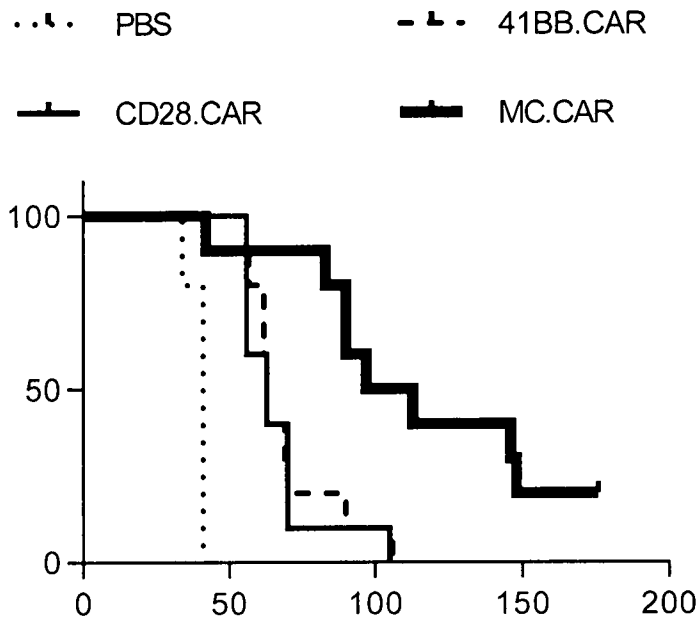

PBS
CD28-CAR
41BB-CAR
MC-CAR
Radiance (p/sec/cm2/sr)
Days post tumor cell injection
0
50
100
150

FIG. 12E

FIG. 12F



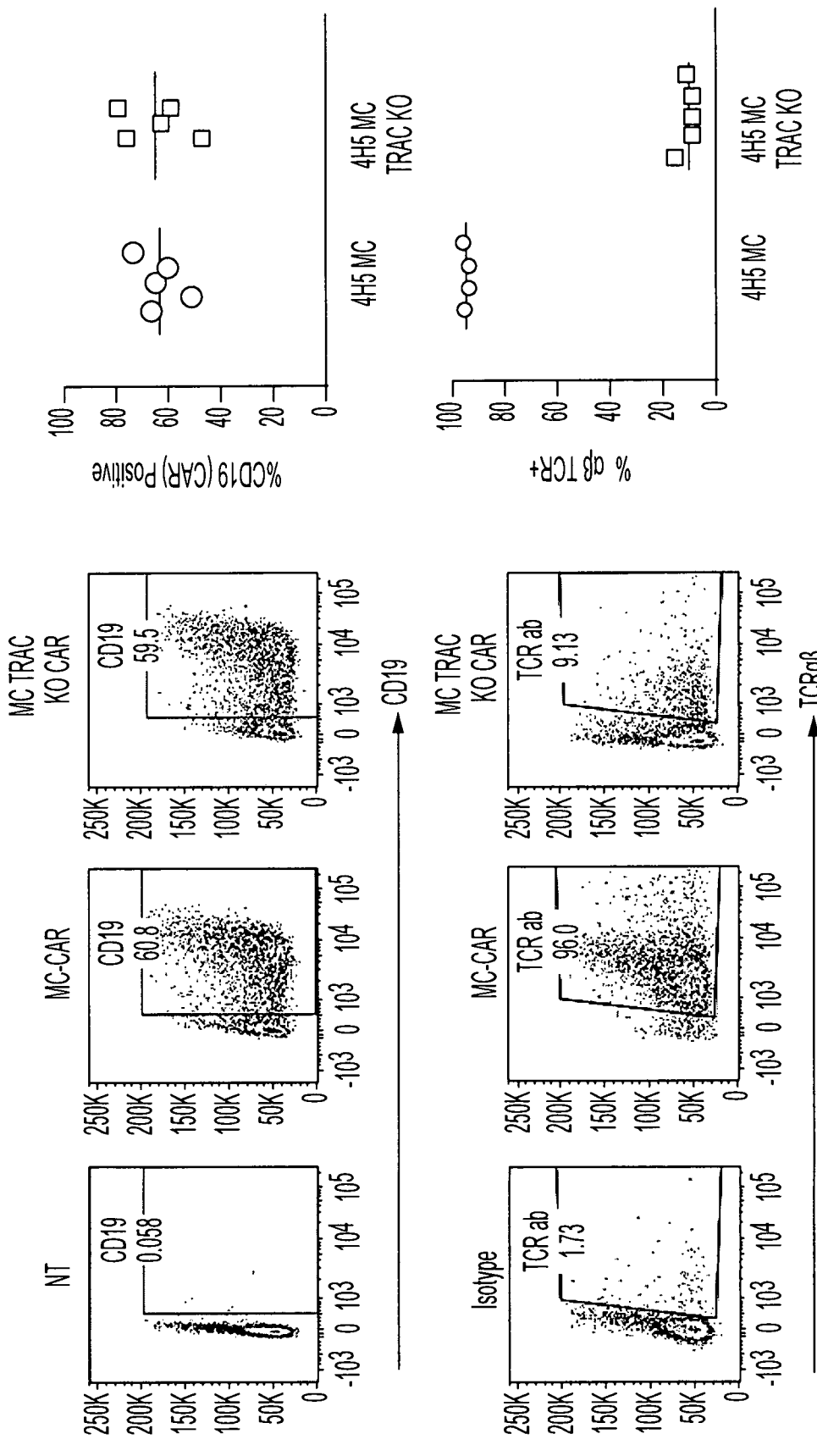

%CD19 (CAR) Positive
100
80
60
40
20
0
4H5 MC
4H5 MC TRAC KO
% αβ TCR+
100
80
60
40
20
0
4H5 MC
4H5 MC TRAC KO
NT
CD19
0.058
MC-CAR
CD19
60.8
MC TRAC KO CAR
CD19
59.5
CD19
250K
200K
150K
100K
50K
0
-10³ 0 10³ 10⁴ 10⁵
Isotype
TCR ab
1.73
MC-CAR
TCR ab
96.0
MC TRAC KO CAR
TCR ab
9.13
TCRαβ

FIG. 13A

FIG. 13B

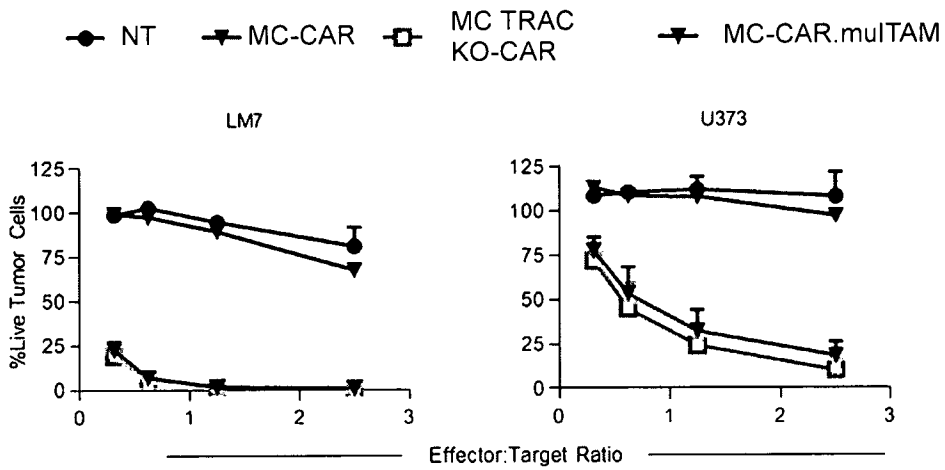
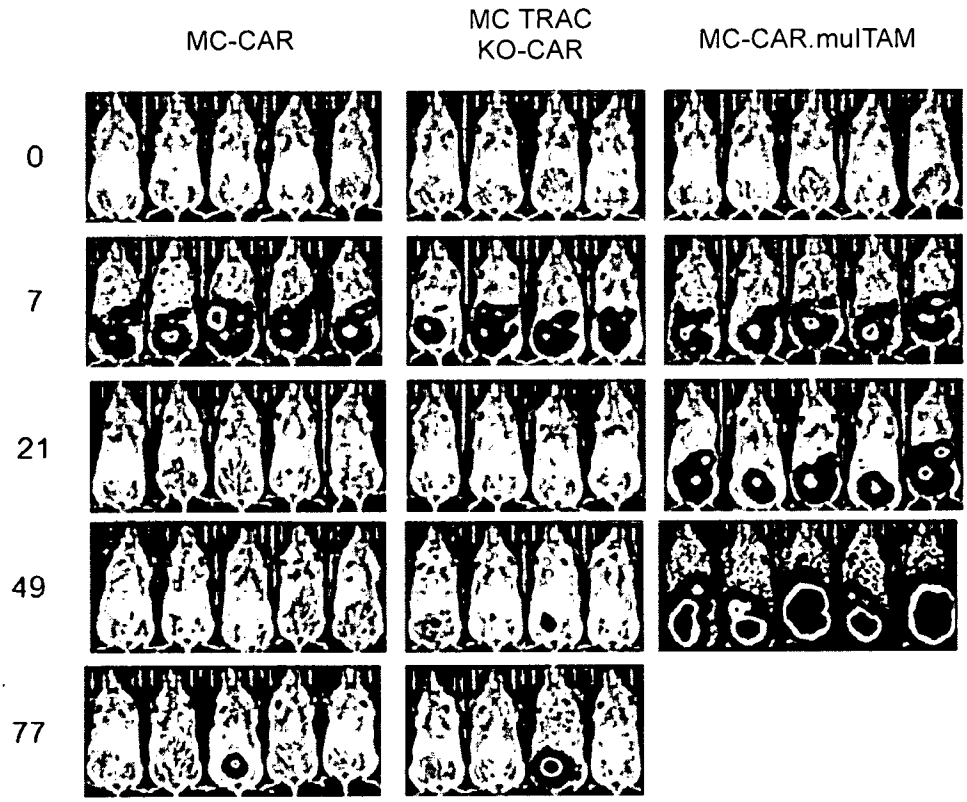


FIG. 13C



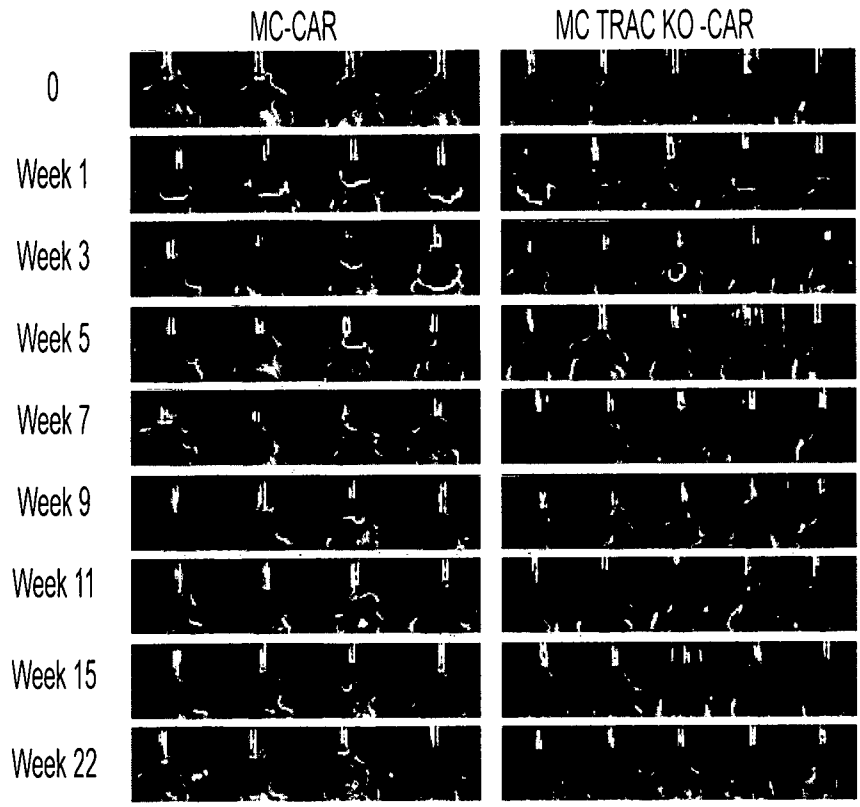


FIG. 14A

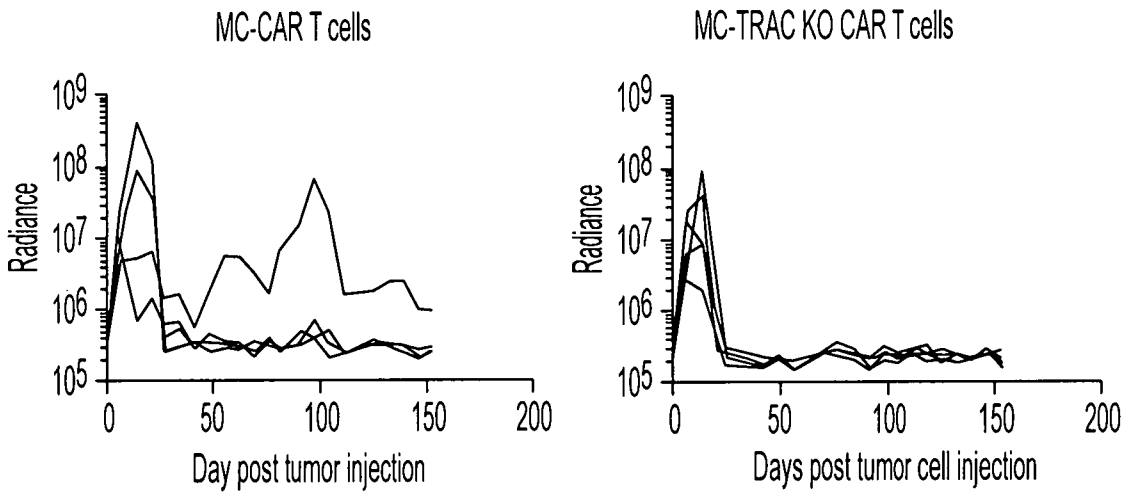


FIG. 14B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/32786

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/47, C07K 14/705, C07K 14/725, C07K 16/28 (2019.01)
CPC - C07K 14/4748, C12N 5/0638, C12N 15/62, C07K 14/70521

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2015/0307564 A1 (THE CALIFORNIA INSTITUTE FOR BIOMEDICAL RESEARCH) 29 October 2015 (29.10.2015) para [0070], [0084], [0087], [0097]	1-3, 56-59, 61-65
Y	US 2016/0175359 A1 (BELLICUM PHARMACEUTICALS, INC.) 23 June 2016 (23.06.2016) para [0004], [0012], [0099], [0187], [0343], [0674]; SEQ ID NOs: 214, 216, 282	1-3, 56-59, 61-65
Y	WO 2000/71150 A1 (HUMAN GENOME SCIENCES, INC.) 30 November 2000 (30.11.2000) Fig 2; SEQ ID NO 10; Y-64	64

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 July 2019	05 SEP 2019

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/32786

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-55, 60, 66-153
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.