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(54) Titre : CAR DE SYNTHÈSE PERMETTANT DE TRAITER DES TUMEURS HUMAINE OU CANINE IL13R α 2 POSITIVES
(54) Title: SYNTHETIC CARS TO TREAT IL13R α 2 POSITIVE HUMAN AND CANINE TUMORS

(57) Abrégé/Abstract:

The present disclosure provides modified immune cells or precursors thereof (e.g. T cells) comprising chimeric antigen receptors (CARs) capable of binding human IL13 α 2. Also provided are bispecific CARs, parallel CARs, tandem CARs, BiTEs, BiTE/CARs, and BiTE/BiTEs. Compositions and methods of treatment are also provided.



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Abstract:

The present disclosure provides modified immune cells or precursors thereof (e.g. T cells) comprising chimeric antigen receptors (CARs) capable of binding human IL132. Also provided are bispecific CARs, parallel CARs, tandem CARs, BiTEs, BiTE/CARs, and BiTE/BiTEs. Compositions and methods of treatment are also provided.

SYNTHETIC CARS TO TREAT IL13R α 2 POSITIVE HUMAN AND CANINE TUMORS

CROSS REFERENCE TO RELATED APPLICATION

5 The present application is entitled to priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 62/892,114 filed August 27, 2019, which is hereby incorporated by reference in its entirety herein.

BACKGROUND OF THE INVENTION

10 Malignant gliomas, including Grade IV gliomas, also called glioblastomas (GBM), are the most common primary malignant brain tumors and are associated with high morbidity and mortality. The aggressive nature of glioma cell infiltrative growth in the central nervous system (CNS) makes total resection impossible to achieve. Despite best available therapy, including surgical resection, radiotherapy, chemotherapy and tumor treating field, the median
15 survival is only 12-17 months for patients with GBMs, and 2 to 5 years for patients with Grade III gliomas.

 Adoptive immunotherapy with redirected T cells is a feasible strategy to treat these malignant tumors. Long-term disease-free survival was achieved in a patient with refractory chronic lymphocytic leukemia after treatment with CD19 targeting chimeric antigen receptor
20 modified autologous T (CAR T) cells, and complete remission was achieved in 90% of patients with relapsed acute lymphoblastic leukemia (ALL) with this strategy. However, to date, the anti-tumor activity of CAR T cells in solid tumors has been much more modest. Humanized anti-EGFR variant III (EGFRvIII) CAR T cells (2173BBz) were previously utilized in a phase I clinical trial (NCT02209376) of 10 patients with recurrent GBM. There
25 were obvious changes in the tumor microenvironment after CAR T cell infusion, including reduction of the EGFRvIII target antigen associated with CAR T cell trafficking and *in situ* functional activation. However, the study was not powered to determine clinical response (median overall survival was 251 days). A recent report described the use of repeated intratumoral and intrathecal infusions of a redirected T cells expressing an IL13 zetakine, a
30 mutated IL13 cytokine, fused with a T cell signaling domain in a single patient with recurrent multifocal GBM, which led to complete tumor regression for 7.5 months.

 Interleukin 13 receptor α 2 (IL13R α 2) is expressed in different human tumor types but no expression is seen on normal human tissues, except adult testes (FIG. 7B). IL13 signaling through IL13R α 2 plays a critical role in cell migration and invasion. A previous study found

82% of GBM cases expressed IL13R α 2. Neutralizing antibody and drug conjugated antibody targeting IL13R α 2 inhibited tumor growth in xenograft mouse models. IL13R α 2 based tumor vaccine also benefitted pediatric glioma patients. Although IL13 zetakine redirected T cells bind IL13R α 2 and induced a limited clinical response, they also bind IL13R α 1 (FIG. 7A), which is expressed in some normal human tissues and have demonstrated adverse, off-target effects.

The tumor microenvironment of malignant gliomas is immunosuppressive, and this has been shown after CAR T cell infusion. Immune checkpoint receptors (*e.g.* PD-1, CTLA-4, TIM-3 and LAG-3) are a series of molecules that downregulate the stimulation of activated T cells with different temporal and spatial profiles to regulate T cell functions. Checkpoint inhibitors have been applied in cancer therapy to overcome T cell inhibition within the immunosuppressive tumor microenvironment and recruit the T cell repertoire to target tumor cells. To date, most combinatorial studies have used anti-PD-1 checkpoint blockade together with endogenous T-cell response to tumor antigens and a few selected reports on engineered T cells.

There is a need in the art for compositions and method for treating IL13R α 2 positive tumors. The present invention addresses and satisfies this need.

SUMMARY OF THE INVENTION

In one aspect, the invention provides a chimeric antigen receptor (CAR) comprising an antigen-binding domain capable of binding human IL13R α 2, a transmembrane domain, and an intracellular domain. The antigen-binding domain comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence VKWAGGSTDYNSALMS (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7).

In certain embodiments, the antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8. In certain embodiments, the antigen-binding domain comprises a light chain variable region comprising an amino acid sequence at least

80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9. In certain embodiments, the antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.

In certain embodiments, the antigen-binding domain is selected from the group consisting of a full length antibody or antigen-binding fragment thereof, a Fab, a single-chain variable fragment (scFv), or a single-domain antibody.

In certain embodiments, the antigen-binding domain is a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or 11.

In another aspect, the invention provides a chimeric antigen receptor (CAR) comprising an antigen-binding domain capable of binding IL13R α 2, a transmembrane domain, and an intracellular domain. The antigen-binding domain comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence QGTTALATRFFD (SEQ ID NO: 14); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence KASQDVGTA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence QHHYSAPWT (SEQ ID NO: 18).

In certain embodiments, the antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19. In certain embodiments, the antigen-binding domain comprises a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20. In certain embodiments, the antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20.

In certain embodiments, the antigen-binding domain is selected from the group consisting of a full length antibody or antigen-binding fragment thereof, a Fab, a single-chain variable fragment (scFv), or a single-domain antibody.

In certain embodiments, the antigen-binding domain is a single-chain variable
 5 fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 21 or 22.

In certain embodiments, the CAR is capable of binding IL13R α 2. In certain
 embodiments, the CAR is capable of binding human IL13R α 2. In certain embodiments, the
 CAR is capable of binding canine IL13R α 2. In certain embodiments, the CAR is capable of
 10 binding human and canine IL13R α 2.

In certain embodiments, the transmembrane domain is selected from the group
 consisting of an artificial hydrophobic sequence, and a transmembrane domain of a type I
 transmembrane protein, an alpha, beta, or zeta chain of a T cell receptor, CD28, CD3 epsilon,
 CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, OX40
 15 (CD134), 4-1BB (CD137), and CD154, or a transmembrane domain derived from a killer
 immunoglobulin-like receptor (KIR). In certain embodiments, the transmembrane domain
 comprises a transmembrane domain of CD8. In certain embodiments, the transmembrane
 domain of CD8 is a transmembrane domain of CD8 alpha.

In certain embodiments, the intracellular domain comprises a costimulatory signaling
 20 domain and an intracellular signaling domain. In certain embodiments, the intracellular
 domain comprises a costimulatory domain of a protein selected from the group consisting of
 proteins in the TNFR superfamily, CD28, 4-1BB (CD137), OX40 (CD134), PD-1, CD7,
 LIGHT, CD83L, DAP10, DAP12, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-
 II, Fas, CD30, CD40, ICOS, NKG2C, and B7-H3 (CD276), or a variant thereof, or an
 25 intracellular domain derived from a killer immunoglobulin-like receptor (KIR). In certain
 embodiments, the intracellular domain comprises a costimulatory domain of 4-1BB. In
 certain embodiments, the intracellular signaling domain comprises an intracellular domain
 selected from the group consisting of cytoplasmic signaling domains of a human CD3 zeta
 chain (CD3 ζ), Fc γ RIII, Fc ϵ RI, a cytoplasmic tail of an Fc receptor, an immunoreceptor
 30 tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, FcR
 gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or
 a variant thereof. In certain embodiments, the intracellular signaling domain comprises an
 intracellular domain of CD3 ζ .

In another aspect, the invention provides a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain. The antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or
5 100% identical to SEQ ID NO: 8; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.

In another aspect, the invention provides a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and
10 an intracellular domain, wherein the antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20.

In another aspect, the invention provides a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%,
15 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or SEQ ID NO: 24 or SEQ ID NO: 55 or SEQ ID NO: 56.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide
20 sequence encoding any of the CARs contemplated herein.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain. The antigen-binding domain comprises a heavy chain variable region that comprises
25 three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence VKWAGGSTDYNSALMS (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein
30 LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7).

In certain embodiments, the antigen-binding domain comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,

99%, or 100% identical to SEQ ID NO: 57. In certain embodiments, the antigen-binding domain comprises a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61. In certain embodiments, the antigen-binding domain comprises a heavy chain variable region
5 encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

In certain embodiments, the antigen-binding domain is a single-chain variable
10 fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 138 or 133.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular
15 domain. The antigen-binding domain comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence QGTTALATRFDD (SEQ ID NO: 14); and a light chain variable
20 region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence QHHYSAPWT (SEQ ID NO: 18).

In certain embodiments, the antigen-binding domain comprises a heavy chain variable
25 region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67. In certain embodiments, the antigen-binding domain comprises a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71. In certain embodiments, the antigen-binding domain comprises a heavy chain variable region
30 encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

In certain embodiments, the antigen-binding domain is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 134 or 135.

In certain embodiments, the transmembrane domain comprises a transmembrane domain of CD8 alpha. In certain embodiments, the intracellular domain comprises a costimulatory signaling domain and an intracellular signaling domain. In certain embodiments, the costimulatory signaling domain comprises a costimulatory domain of 4-1BB. In certain embodiments, the intracellular signaling domain comprises an intracellular domain of CD3ζ.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13Rα2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain. The antigen-binding domain comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13Rα2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or SEQ ID NO: 66 or SEQ ID NO: 75 or SEQ ID NO: 76.

In another aspect, the invention provides a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) capable of binding IL13Rα2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof,

wherein the first and second CAR each comprise an antigen-binding domain, a transmembrane domain, and an intracellular domain.

In certain embodiments, the antigen-binding domain of the first CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence VKWAGGSTDYNSALMS (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7).

In certain embodiments, the antigen-binding domain of the first CAR comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

In certain embodiments, the antigen-binding domain of the first CAR is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 138 or 133.

In certain embodiments, the antigen-binding domain of the first CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence QGTTALATRFFD (SEQ ID NO: 14); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence KASQDVGTA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence QHHYSAPWT (SEQ ID NO: 18).

In certain embodiments, the antigen-binding domain of the first CAR comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

In certain embodiments, the antigen-binding domain of the first CAR is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 134 or 135.

In certain embodiments, the first polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or SEQ ID NO: 66 or SEQ ID NO: 75 or SEQ ID NO: 76.

In certain embodiments, the antigen-binding domain of the second CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFPYW (SEQ ID NO: 27); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGTLNDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

In certain embodiments, the antigen-binding domain of the second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31. In certain embodiments, the antigen-binding domain of the second CAR comprises a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32. In certain embodiments, the antigen-binding domain of the second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32.

In certain embodiments, the antigen-binding domain of the second CAR is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 141.

In certain embodiments, the second polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35 or SEQ ID NO: 196.

In certain embodiments, the transmembrane domain of the first and/or second CAR is selected from the group consisting of an artificial hydrophobic sequence, and a

transmembrane domain of a type I transmembrane protein, an alpha, beta, or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, OX40 (CD134), 4-1BB (CD137), and CD154, or a transmembrane domain derived from a killer immunoglobulin-like receptor (KIR). In certain
 5 embodiments, the transmembrane domain of the first and/or second CAR comprises a transmembrane domain of CD8 alpha.

In certain embodiments, the intracellular domain of the first and/or second CAR comprises a costimulatory signaling domain and an intracellular signaling domain. In certain
 10 embodiments, the intracellular domain of the first and/or second CAR comprises a costimulatory domain of a protein selected from the group consisting of proteins in the TNFR superfamily, CD28, 4-1BB (CD137), OX40 (CD134), PD-1, CD7, LIGHT, CD83L, DAP10, DAP12, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40, ICOS, NKG2C, and B7-H3 (CD276), or a variant thereof, or an intracellular domain derived
 15 from a killer immunoglobulin-like receptor (KIR). In certain embodiments, the intracellular domain of the first and/or second CAR comprises a costimulatory domain of 4-1BB.

In certain embodiments, the intracellular signaling domain of the first and/or second CAR comprises an intracellular domain selected from the group consisting of cytoplasmic
 20 signaling domains of a human CD3 zeta chain (CD3 ζ), Fc γ RIII, Fc ϵ RI, a cytoplasmic tail of an Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or a variant thereof. In certain embodiments, the intracellular signaling domain of the first and/or second CAR comprises an intracellular
 domain of CD3 ζ .

25 In another aspect, the invention provides a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. The first CAR comprises a heavy chain variable region that comprises three heavy chain
 30 complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFDDV (SEQ ID NO: 15); and a

light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTA VA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18). The second CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFPYW (SEQ ID NO: 27); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGTNLDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

In another aspect, the invention provides a nucleic acid comprising a first polynucleotide sequence encoding a first CAR capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. The first CAR comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57 or 67; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61 or 71. The second CAR comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 139 or 194; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 140 or 195.

In another aspect, the invention provides a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. The first CAR comprises a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 133, 134, 135, or 138; and the second CAR comprises a single-

chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 141.

In another aspect, the invention provides a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or SEQ ID NO: 66 or SEQ ID NO: 75 or SEQ ID NO: 76; and the second polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35 or SEQ ID NO: 196.

In another aspect, the invention provides a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) capable of binding IL13R α 2, and a second polynucleotide sequence encoding an inhibitor of an immune checkpoint.

In certain embodiments, the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3. In certain embodiments, the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody. In certain embodiments, the inhibitor of the immune checkpoint is an anti-CTLA-4 antibody.

In another aspect, the invention provides a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) capable of binding IL13R α 2, and a second polynucleotide sequence encoding an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof.

In certain embodiments, the second polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence encoding SEQ ID NO: 53 or 54.

In certain embodiments, the BiTE is capable of binding wild type EGFR (wtEGFR).

In certain embodiments, the BiTE is capable of binding EGFR variant III (EGFRvIII).

In certain embodiments, the first polynucleotide sequence and the second polynucleotide sequence is separated by a linker. In certain embodiments, the linker comprises a nucleotide sequence encoding an internal ribosome entry site (IRES) or a self-cleaving peptide. In certain embodiments, the self-cleaving peptide is a 2A peptide. In certain

embodiments, the 2A peptide is selected from the group consisting of porcine teschovirus-1 2A (P2A), Thoseaasigna virus 2A (T2A), equine rhinitis A virus 2A (E2A), and foot-and-mouth disease virus 2A (F2A). In certain embodiments, the 2A peptide is T2A. In certain embodiments, the linker further comprises a furin cleavage site.

5 In certain embodiments, the nucleic acid comprises from 5' to 3' the first polynucleotide sequence, the linker, and the second polynucleotide sequence. In certain embodiments, the nucleic acid comprises from 5' to 3' the second polynucleotide sequence, the linker, and the first polynucleotide sequence.

10 In certain embodiments, the nucleic acid further comprises an inducible promoter, wherein the inducible promoter comprises a nucleotide sequence that is 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs. 161, 162, or 198.

 In another aspect, the invention provides a vector comprising any of the nucleic acids contemplated herein.

15 In certain embodiments, the vector is an expression vector. In certain embodiments, the vector is selected from the group consisting of a DNA vector, an RNA vector, a plasmid, a lentiviral vector, an adenoviral vector, an adeno- associated viral vector, and a retroviral vector. In certain embodiments, the vector further comprises an EF-1 a promoter. In certain embodiments, the vector further comprises a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE). In certain embodiments, the vector further comprises a rev response element (RRE). In certain embodiments, the vector further comprises a cPPT sequence. In certain embodiments, the vector is a self-inactivating vector.

20 In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising any of the CARs contemplated herein, any of the nucleic acids contemplated herein, or any of the vectors contemplated herein.

 In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2. The CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFDDV (SEQ ID NO: 15); and a light chain variable region that comprises three light chain complementarity determining

regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVAL (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18).

In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising CAR capable of binding IL13R α 2, wherein the CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20.

In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising a CAR capable of binding IL13R α 2, wherein the CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or 11.

In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising a CAR capable of binding IL13R α 2, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 21 or 22.

In certain embodiments, the CAR is capable of binding IL13R α 2. In certain embodiments, the CAR is capable of binding human IL13R α 2.

In certain embodiments, the modified cell further comprises an inhibitor of an immune checkpoint, wherein the modified cell secretes the inhibitor of the immune checkpoint. In certain embodiments, the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3. In certain embodiments, the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody.

In certain embodiments, the modified cell further comprises an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the modified cell secretes the BiTE. In certain embodiments, the inducible BiTE comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 53 or 54. In certain embodiments, the BiTE is capable of binding wild type EGFR (wtEGFR). In certain embodiments, the BiTE is capable of binding EGFR variant III (EGFRvIII).

In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising a first CAR comprising a first antigen-binding domain capable of binding IL13R α 2; and a second CAR comprising a second antigen-binding domain capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof.

5 In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. The first CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein
 10 HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFFDV (SEQ ID NO: 15); and a light chain variable region that comprises three light chain complementarity
 15 determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18). The second CAR comprises a heavy chain variable region
 20 that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFYV (SEQ ID NO: 27); and a light chain variable region that comprises three light chain complementarity determining regions
 25 (LCDRs), wherein LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGTNLDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, and a second CAR capable of
 30 binding epidermal growth factor receptor (EGFR) or an isoform thereof. The first CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20. The second CAR

comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32.

5 In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein: the first CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%,
10 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or 11; and the second CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34.

In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a
15 second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 24; and the second CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 36 or 197.

20 In certain embodiments, the modified cell further comprises an inhibitor of an immune checkpoint, wherein the modified cell secretes the inhibitor of the immune checkpoint. In certain embodiments, the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3. In certain embodiments, the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an
25 anti-PD-1 antibody, and an anti-TIM-3 antibody.

In certain embodiments, the CAR is capable of binding human IL13R α 2.

In certain embodiments of the modified cell, the second CAR is capable of binding an EGFR isoform selected from the group consisting of wild type EGFR (wtEGFR), mutated EGFR, EGFR^{A289V}, EGFR^{A289D}, EGFR^{A289T}, EGFR^{A289T}, EGFR^{R108K}, EGFR^{R108G},
30 EGFR^{G598V}, EGFR^{D126Y}, EGFR^{C628F}, EGFR^{R108K/A289V}, EGFR^{R108K/D126Y}, EGFR^{A289V/G598V}, EGFR^{A289V/C628F}, and EGFR variant II, or any combination thereof.

In certain embodiments, the modified cell is a modified immune cell. In certain embodiments, the modified cell is a modified T cell. In certain embodiments, the modified

cell is an autologous cell. In certain embodiments, the modified cell is an autologous cell obtained from a human subject.

In another aspect, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of any of the modified cells contemplated herein.

5 In another aspect, the invention provides a method of treating a disease in a subject in need thereof. The method comprises administering to the subject an effective amount of the any of the modified cells contemplated herein, or any of the pharmaceutical compositions contemplated herein.

10 In certain embodiments, the disease is a cancer. In certain embodiments, the cancer is a glioma. In certain embodiments, the cancer is an astrocytoma. In certain embodiments, the cancer is a high-grade astrocytoma. In certain embodiments, the cancer is glioblastoma.

In another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof. The method comprises administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of
15 binding IL13R α 2. The CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid
20 sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFFDV (SEQ ID NO: 15); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3
25 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18).

In another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2,
30 wherein the CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20.

In another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 21 or SEQ ID NO: 22.

In another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or SEQ ID NO: 24 or SEQ ID NO: 55 or SEQ ID NO: 56.

In certain embodiments, the method further comprises administering an inhibitor of an immune checkpoint, wherein the modified cell secretes the inhibitor of the immune checkpoint. In certain embodiments, the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3. In certain embodiments, the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody. In certain embodiments, the inhibitor of the immune checkpoint is co-administered with the modified T cell.

In certain embodiments, the method further comprises administering an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the modified cell secretes the BiTE.

In certain embodiments, the inducible BiTE comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 53 or 54. In certain embodiments, the BiTE is capable of binding wild type EGFR (wtEGFR). In certain embodiments, the BiTE is capable of binding EGFR variant III (EGFRvIII). In certain embodiments, the BiTE is co-administered with the modified T cell.

In certain embodiments, the method further comprises administering an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and an inhibitor of an immune checkpoint, wherein the modified cell secretes the BiTE and the inhibitor of the immune checkpoint. In certain embodiments, the inhibitor of the immune checkpoint is co-administered with the modified T cell.

In another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof. The method comprises administering to the subject an effective

amount of a modified T cell comprising a first chimeric antigen receptor (CAR) comprising a first antigen-binding domain capable of binding IL13R α 2; and a second chimeric antigen receptor (CAR) comprising a second antigen-binding domain capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof.

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In another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor
 10 receptor (EGFR) or an isoform thereof. The first CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYTYADSVKG (SEQ ID NO:
 15 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFFDV (SEQ ID NO: 15); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVAL (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or
 20 SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18). The second CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID
 25 NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFYV (SEQ ID NO: 27); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGTNLDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID
 30 NO: 30).

In another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor

receptor (EGFR) or an isoform thereof. The first CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20. The second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32.

In another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. The first CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or 11; and the second CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34.

In another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. The first CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 24; and the second CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 36 or 197.

In certain embodiments, the method further comprises administering an inhibitor of an immune checkpoint, wherein the modified cell secretes the inhibitor of the immune checkpoint. In certain embodiments, the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3. In certain embodiments, the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody. In certain embodiments, the inhibitor of the immune checkpoint is co-administered with the modified cell.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence encoding a CAR comprising a first antigen binding domain, a second antigen binding domain, a transmembrane domain, and an intracellular domain, wherein the first and second antigen binding domain are separate by a linker. In certain embodiments, the linker comprises 5, 10, 15, or 20 amino acids. In certain embodiments, the first antigen binding domain is capable of binding IL13R α 2, and the second antigen binding domain is capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. In certain embodiments, the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 163, 165, 167, or 169. In certain embodiments, the CAR is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 164, 166, 168, or 170.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence encoding a parallel CAR, wherein the parallel CAR comprises a first CAR and a second CAR, each comprising an antigen binding domain, a transmembrane domain, and an intracellular domain, and wherein the first CAR and the second CAR are separate by a cleavable linker. In certain embodiments, the parallel CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 171 and/or is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 172.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence encoding a BiTE and a CAR. In certain embodiments, the BiTE comprises an antigen binding domain capable of binding EGFR or an isoform thereof, and the CAR comprises an antigen binding domain capable of binding IL13R α 2. In certain embodiments, the BiTE comprises an antigen binding domain capable of binding IL13R α 2, and the CAR comprises an antigen binding domain capable of binding EGFR or an isoform thereof. In certain embodiments, the polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 176 or SEQ ID NO: 178. In certain embodiments, the polynucleotide sequence is encoded by an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 175 or SEQ ID NO: 177.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence encoding a first BiTE and a second BiTE. In certain embodiments, the first and/or

second BiTE comprises an antigen binding domain capable of binding IL13R α 2, and/or an antigen binding domain capable of binding EGFR or an isoform thereof. In certain embodiments, the polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 180. In certain embodiments, the polynucleotide sequence encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 179.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other features and advantages of the present invention will be more fully understood from the following detailed description of illustrative embodiments taken in conjunction with the accompanying drawings.

FIGs. 1A-1F illustrate humanized IL13R α 2 targeting CAR T cells. FIG. 1A depicts flow cytometric detection of CAR expression by human T cells, after mRNA electroporation of murine and humanized scFvs (07 and 08) based CAR constructs using rabbit anti-mouse or rabbit anti-human IgG antibodies. FIG. 1B shows vector maps of tested anti-IL13R α 2 CAR design based on the size of each components. FIG. 1C illustrates CAR expression staining of the humanized IL13R α 2 CAR transduced T cells used in the co-culture experiments. FIG. 1D depicts IL13R α 1 and IL13R α 2 expression analysis on the human tumor cell lines (Sup-T1, Jurkat, A549, U87, U251 and D270). FIG. 1E shows flow-based intracellular cytokine (IFN γ) staining of the humanized IL13R α 2 CAR T cells co-cultured with human tumor cell lines in FIG. 1D controlled with un-transduced T cells (UTD). Human CD8 was stained to distinguish the CD4 positive and CD8 positive subgroups of T cells along the x axis. FIG. 1F shows results from Chromium release assays of humanized IL13R α 2 CAR T cells co-cultured with tumor cell lines in FIG. 1D at different effector/target (E:T) ratios (1:1, 3:1, 10:1 and 30:1) compared with the un-transduced T cells (UTD) with one-way Analysis of Variance (ANOVA) post hoc Tukey test. ** P <0.01, *** P <0.001, **** P <0.0001.

FIGs. 2A-2E illustrate the finding that IL13R α 2 CAR T cells control tumor growth *in vivo*. FIG. 2A shows flow-based EGFRvIII and IL13R α 2 expression on the D270 tumor cell line controlled with control antibodies. FIG. 2B illustrates EGFRvIII targeting (2173BBz) and IL13R α 2 targeting (Hu08BBz) CAR T cells co-cultured with D270 tumor cell line. The stimulation of T cells was illustrated by FITC-conjugated anti-CD69 antibody staining, the median fluorescence intensity (MFI) was quantified on CD4 and CD8 CAR positive T cells after 24hrs or 48hrs co-culture, controlled with un-transduced T cells. Statistically significant differences were calculated by one-way ANOVA with post hoc Tukey test. FIG. 2C

illustrates human T cells enumerated in the spleens of D270 injected NSG mice (n=3), 11 days after i.v. transferring equal numbers of un-transduced T cells, EGFRvIII targeting (2173BBz) or IL13R α 2 targeting (Hu08BBz) CAR T cells. FIG. 2D illustrates five million CAR positive EGFRvIII targeting (2173BBz) or IL13R α 2 targeting (Hu07BBz and

5 Hu08BBz) CAR T cells or the same number of un-transduced T cells after i.v. infusion in a D270 subcutaneously implanted NSG mouse model (n=10 per group), 7 days after tumor implantation. Tumor volume measurements (left panel) and bioluminescence imaging (middle panel) were performed to evaluate the tumor growth. Linear regression was used to test for significant differences between the experimental groups. Endpoint was predefined by the mouse hunch, inability to ambulate, or tumor reaching 2 cm in any direction as
10 predetermined IACUC approved morbidity endpoint. Survival based on time to endpoint was plotted using a Kaplan-Meier curve (Prism software). Statistically significant differences were determined using log-rank test. FIG. 2E illustrates eight hundred thousand IL13R α 2 targeting CAR positive (Hu08BBz) CAR T cells or the same number of un-transduced T cells
15 were given by i.v. infusion in NSG mice (n=8 per group) orthotopically implanted with the D270 tumor, 8 days after tumor injection. Bioluminescence imaging were repeated every 3–4 days to evaluate the tumor growth. Endpoint was predefined and statistically significant differences were determined as described in FIG. 2D. * P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001.

20 FIGs. 3A-3D illustrate the finding that checkpoint blockades selectively enhances the function of CAR T cells. FIG. 3A illustrates EGFRvIII (2173BBz) targeting and IL13R α 2 (Hu08BBz) targeting CAR T cells as well as un-transduced T cells control were co-cultured with target positive D270 tumor cell line and target negative A549 tumor cell line. The expression of checkpoint receptors on the T cells was determined by flow-cytometry, by
25 staining with fluorochrome-conjugated anti-checkpoint receptor antibodies; the median fluorescence intensity (MFI) was quantified on CD4 and CD8 CAR positive T cells after 24hrs or 48hrs co-culture. Statistically significant differences were calculated by one-way ANOVA with post hoc Tukey test. FIG. 3B illustrates un-transduced (UTD) human T cells were i.v. infused into a D270 subcutaneously implanted mouse model (n=5 per group) seven
30 days after tumor implantation. From day six, PBS or the same volume of 200 μ g checkpoint blockade antibodies (anti-PD-1, anti-CTLA-4 and anti-TIM-3) were injected intraperitoneally every four days. Tumor size was measured and compared between the UTD plus PBS group and the UTD plus checkpoint blockade groups. FIG. 3C illustrates same numbers of EGFRvIII targeting (2173BBz) and IL13R α 2 targeting (Hu08BBz) CAR T cells infused and

combined with checkpoint blockade as described in (FIG. 3B). The tumor volume of checkpoint blockade combinational therapy groups were compared with PBS combined CAR T cell control group (n=5 per group). FIG. 3D illustrates different checkpoint blockade combinational therapies were compared in the EGFRvIII targeting (2173BBz) and IL13Rα2 targeting (Hu08BBz) CAR T cell groups based on the tumor size of mice. Survival curves were also compared in these two CAR T cell groups. Statistically significant differences of tumor growth between the experimental groups were determined by linear regression, and log-rank test was used for determining the statistically significant differences of survival curves. ns, not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

FIGs. 4A-4E illustrate the finding that IL13Rα2 CAR T cells are selectively enhanced by *in situ* secreted anti-CTLA-4 checkpoint blockade. FIG. 4A is a vector map of minibodies secreting anti-IL13Rα2 CAR design based on the size of each components. Minibodies were simplified as PD-1, CTLA-4 and TIM-3 targeting scFvs jointing with human IgG1 spacer and CH3 domain. A self-cleaving sequence (P2A) was used to express minibodies with anti-IL13Rα2 CAR in a same open reading frame. FIG. 4B illustrates CAR expression was detected on the minibodies secreting IL13Rα2 targeting CAR T cells as well as the no minibody secreting IL13Rα2 targeting CAR T cells. FIG. 4C illustrates supernatant of anti-PD-1 and anti-CTLA-4 minibodies secreting IL13Rα2 targeting CAR T cells was collected and concentrated separately. A standard direct ELISA was performed to evaluate the binding ability of anti-PD-1 and anti-CTLA-4 minibodies secreted by CAR T cells to recombinant hPD-1 and hCTLA-4. Statistically significant differences were calculated by unpaired *t* test. FIG. 4D illustrates un-transduced T cells, IL13Rα2 targeting (Hu08BBz) CAR T cells and minibody secreting Hu08BBz CAR T cells were co-cultured with D270 tumor cell line. Median fluorescence intensity (MFI) was quantified by BV605-conjugated anti-TIM-3 antibody staining on CD4 and CD8 subgroups of CAR positive T cells after 24hrs or 48hrs co-culture. Statistically significant differences were calculated by one-way ANOVA with post hoc Tukey test. FIG. 4E illustrates eight hundred thousand IL13Rα2 targeting (Hu08BBz) CAR T cells and minibodies secreting Hu08BBz CAR T cells or the same number of un-transduced T cells were injected i.v. eight days after D270 subcutaneous implantation (n=8). Tumor size was calipered and compared between each group. Statistically significant differences of tumor growth were determined by linear regression. ns, not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

FIGs. 5A-5D illustrate the finding that IL13Rα2 CAR T cells respond to canine tumors. FIG. 5A shows IL13Rα2 expression analysis on the patient derived glioma stem cell

lines (5077, 5430, 4860, 5377, 5560, 4806 and 4892). FIG. 5B illustrates CAR expression was detected on the mRNA electroporated IL13R α 2 targeting human CAR T cells (Hu07BBz and Hu08BBz). Intracellular cytokine (IFN γ) staining was performed after these CAR T cells co-cultured with human and canine IL13R α 2 protein controlled with bovine serum albumin (BSA). CD8 staining was used to distinguish CD4 and CD8 positive T cell groups on the x-axis. FIG. 5C illustrates the expression of canine IL13R α 1 and IL13R α 2 mRNA on various canine tumor cell lines (Camac2, CLBL-1, GL-1, Cacal3, Cacal5, BW-KOSA, CS-KOSA, MC-KOSA and SK-KOSA) was detected with reverse transcription polymerase chain reaction (RT-PCR), controlled with canine GAPDH. The percentage of cytokine (IFN γ , IL2 and TNF α) positive T cells in CD4 and CD8 positive T cell subgroups was analyzed for mRNA electroporated IL13R α 2 targeting (Hu07BBz and Hu08BBz) human CAR T cells and un-transduced T cells after co-culture with canine tumor cell lines mentioned before. FIG. 5D illustrates two million Hu08BBz transduced human CAR positive T cells were injected i.v. after seven days of five million MC-KOSA subcutaneous implantation (n=5 per group). Tumor size was calipered and compared with the same amount of un-transduced T cell control group. Statistically significant difference of tumor growth was determined by linear regression. **** P <0.0001.

FIGs. 6A-6E illustrate the finding that canine IL13R α 2 CAR T cells control canine tumor growth. FIG. 6A illustrates mRNA electroporated Hu08BBz canine CAR T cells were co-cultured with canine tumor cell lines (Camac2, CLBL-1, GL-1, Cacal3, Cacal5, BW-KOSA, CS-KOSA, MC-KOSA, SK-KPSA and J3T). Canine IFN γ secretion was detected with ELISA and compared the stimulation with un-transduced canine T cells. FIG. 6B shows vector maps of anti-IL13R α 2 human Hu08BBz CAR structure (Hu08HuBBz) and canine Hu08BBz CAR structure (Hu08CaBBz). FIG. 6C illustrates mRNA electroporated Hu07HuBBz, Hu08HuBBz and Hu08CaBBz canine CAR T cells co-cultured with CLBL1 and J3T tumor cell lines. Canine IFN γ secretion was detected with ELISA. Unpaired t test was used to determine the statistically significant difference of IFN γ secretion between Hu08HuBBz and Hu08CaBBz co-cultured with J3T glioma cells. FIG. 6D illustrates J3T canine glioma cell line orthotopically implanted into the NSG mouse brain. Twelve million electroporated Hu08HuBBz, Hu08CaBBz or un-transduced canine T cells were i.v. injected into the mice model (n=4 per group) on day 7, 10, 13 after tumor implantation. Tumor growth was evaluated by bioluminescence imaging every 3-4 days. Statistically significant differences of tumor growth were determined by linear regression. FIG. 6E illustrates the canine T cells used on the 2nd injection on day 10 analyzed for CAR expression and canine

IFN γ secretion after co-culture with J3T tumor cell line. Canine CD4 was stained to distinguish the canine CD4 and CD8 positive subgroups along x axis. ns, not significant; ** $P < 0.01$, **** $P < 0.0001$.

FIGs. 7A-7C illustrate IL13R α 1 and IL13R α 2 expression panels in the human normal or tumor tissues. FIGs. 7A-7B depict IL13R α 1 and IL13R α 2 expression in human normal tissues based on the Human Protein Atlas (HPA) (www.proteinatlas.org) RNA-seq data, which is reported as mean TPM (transcripts per million). FIG. 7C depicts IL13R α 2 expression in the human tumors listed as the median of the expression based on the cancer genome atlas (TCGA) data available on cBioPortal.

FIGs. 8A-8D illustrate murine scFv based IL13R α 2 targeting CAR T cells. FIG. 8A depicts vector maps of tested murine scFv based anti-IL13R α 2 CAR design. FIG. 8B illustrates expression of murine scFv (07 and 08) based IL13R α 2 targeting CAR constructs on electroporated human-T cells. FIG. 8C illustrates IL13R α 1 and IL13R α 2 expression analysis on the human tumor cell lines (Sup-T1, Jurkat, U87, U251 and D270). FIG. 8D illustrates flow-based intracellular cytokine (IFN γ) staining of the murine scFv based IL13R α 2 CAR T cells (Mu07BBz and Mu08BBz) co-cultured with human tumor cell lines in FIG. 8C controlled with un-transduced T cells (UTD). Human CD8 was stained to distinguish the CD4 positive and CD8 positive subgroups of T cells along the x axis.

FIGs. 9A-9C illustrate humanized IL13R α 2 targeting CAR T cells co-cultured with human normal cell types. FIG. 9A illustrates flow-based CAR expression staining of the humanized IL13R α 2 CAR transduced T cells used in the co-culture experiments. FIG. 9B illustrates flow cytometry of IL13R α 1 and IL13R α 2 expression analysis on the human normal cells (CD34 positive bone marrow cells, human pulmonary microvascular endothelial cells, human small airway epithelial cells, human renal epithelial cells, human keratinocytes, human neuronal progenitor cells, human aortic smooth muscle cells and human pulmonary artery smooth muscle cells). FIG. 9C illustrates flow-based intracellular cytokine (IFN γ) staining of the humanized IL13R α 2 CAR T cells co-cultured with human normal cells in FIG. 9B controlled with un-transduced T cells (UTD). Human CD3 and CD8 was stained to distinguish the CD4 positive and CD8 positive subgroups of T cells along the x axis.

FIGs. 10A-10E illustrate stimulation and expansion of IL13R α 2 targeting CAR T cells co-cultured *in vitro*. FIGs. 10A-10C illustrates flow-based intracellular cytokine (IFN γ , IL2 and TNF α) staining of murine IL13R α 2 CAR T cells co-cultured with human tumor cell lines (FIG. 10A), humanized IL13R α 2 CAR T cells co-cultured with human tumor cell lines (FIG. 10B) and humanized IL13R α 2 CAR T cells co-cultured with human normal cells (FIG.

10C). The percentage of cytokine positive T cells was illustrated in the CD4 and CD8 positive subgroups. FIG. 10D illustrates flow-based EGFRvIII and IL13R α 2 expression on the D270 tumor cell line of day 0, 1, 2, 3, 5 and 7 cultured *in vitro*, controlled with control antibodies. FIG. 10E illustrates flow cytometry determined T cell proliferation assay with CFSE staining performed on UTD T cells, 2173BBz and Hu08BBz CAR positive T cells on day 3, 5 and 8 co-culturing with D270 cell line controlled with A549 cell line.

FIGs. 11A-11B illustrate surface marker staining on CAR T cells co-cultured *in vitro*. FIG. 11A depicts a representative gating scheme illustrated with the samples of UTD T cells, 2173BBz and Hu08BBz CAR T cells co-cultured with D270 cell line for 48hrs. CD45+, CD3+ live lymphocytes were gated, expression of T cell surface markers was analyzed and compared among CAR+ T cells and UTD T cells. FIG. 11B illustrates the expression of CD69, PD-1, CTLA-4 and TIM-3 on the CD4+ and CD8+ T cells determined by flow-cytometry, by staining with fluorochrome-conjugated corresponding antibodies after 24hrs or 48hrs co-culture. Representative expression results were illustrated in D270 cell line co-cultured UTD T cells and CAR+ T cells.

FIGs. 12A-12C illustrate checkpoint receptor and ligand expression involved in the activity of CAR T cells *in vivo*. FIG. 12A illustrates flow based detection of checkpoint receptors (PD-1, CTLA-4 and TIM-3) and their ligands (PD-L1, CD80, CD86 and galectin-9) in CD4 and CD8 positive T cell subgroups during T cell *in vitro* expansion with anti-CD3 and anti-CD28 beads on day 0, 3, 7 and 13. FIG. 12B illustrates flow-based detection of checkpoint receptor ligand (PD-L1, CD80, CD86 and galectin-9) expression analysis on the D270 glioma cell line. FIG. 12C illustrates human PD-1, CD69, CD4 and CD8 staining on human CD3⁺ T cells in the mouse spleen *ex vivo* after 2173BBz CAR T cell infusion combined with anti-PD-1 checkpoint blockade in a D270 subcutaneously implanted NSG mouse model. Data shown as the percentage of positive cells. Statistically significant differences were calculated by unpaired *t* test. **P*<0.05, ***P*<0.01, ****P*<0.001.

FIGs. 13A-13C illustrates minibody secreting T-cells (MiST) was co-cultured with target cells and analyzed *in vitro*. FIG. 13A illustrates un-transduced T cells, IL13R α 2 targeting (Hu08BBz) CAR T cells and minibody secreting Hu08BBz CAR T cells (anti-PD1 and anti-CTLA4 MiST) were co-cultured with D270 tumor cell line. Median fluorescence intensity (MFI) was quantified by BV711-conjugated anti-PD1 antibody and PE-conjugated anti-CTLA-4 antibody staining on CD4 and CD8 subgroups of CAR positive T cells after 24hrs or 48hrs co-culture. FIG. 13B illustrates the stimulation of IL13R α 2 (Hu08BBz) targeting CAR T cells and minibody secretion ones evaluated after co-culture with D270

tumor cell line; median fluorescence intensity (MFI) was quantified by FITC-conjugated anti-CD69 antibody staining on CD4 and CD8 subgroups of CAR positive T cells after 24hrs or 48hrs co-culture. FIG. 13C illustrates the percentage of cytokine (IFN γ , IL2 and TNF α) staining positive T cells in CD4 and CD8 positive T cell subgroups was analyzed for IL13R α 2 targeting (Hu08BBz) CAR T cells and minibody secreted cells after co-culture with D270 target tumor cell lines. Statistically significant differences were calculated by one-way ANOVA with post hoc Tukey test. ns, not significant; * P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001.

FIGs. 14A-14B illustrates the amino acid sequence of IL13R α 2 and canine osteosarcoma mouse models. FIG. 14A illustrates the amino acid sequences of human and canine IL13R α 2 compared with the software of Geneious. FIG. 14B illustrates canine osteosarcoma tumor cell lines (BW-KOSA, CS-KOSA, MC-KOSA and SK-KOSA) were subcutaneously implanted into the right flank of NSG mice with different doses. Bioluminescence imaging was repeatedly performed to evaluate the tumor growth in each group.

FIGs. 15A-15B illustrate nucleotide sequences of an inducible promoter disclosed herein. FIG. 15A: DNA sequence for the inducible promoter which can promote expression after T-cell activation. This sequence can be partially repeated to enhance T-cell expression level. T cells/CAR T cells can be modified with this promoter to express designed RNA or amino acids. FIG. 15B: The underlined sequence (SEQ ID NO: 198) is shown repeated for enhanced activity.

FIGs. 16A-16B illustrate functional activity of the inducible promoter. FIG 16A is a schematic of a construct containing the inducible promoter, which includes a TDTomato gene for fluorescent expression. FIG 16B shows TD-Tomato expression in PMA/Ionomycin stimulated Jurkat cells (a T cell tumor line). When the cells were stimulated with PMA/Ionomycin, TD-Tomato expression was detected with flow cytometry, demonstrating promoter activation.

FIG. 17 illustrates Tandem (top) and Parallel (bottom) Bi-specific CARs. The tandem bi-specific CAR comprises IL13R α 2 antigen-binding domain (Hu08) linked to EGFR antigen-binding domain (806). The linker in the tandem CAR can be 5, 10, 15, or 20 amino acids (5AA/10AA/15AA/20AA) in length. The parallel CAR comprises a first CAR capable of binding IL13R α 2, and a second CAR capable of binding EGFR. A self-cleaving sequence (P2A) links the anti-IL13R α 2 CAR and the anti-EGFR CAR in the same open reading frame.

FIGs. 18A-18E show the amino acid and nucleic acid sequences for a Tandem CAR with 5AA linker ((G₄S); FIG. 18A), a Tandem CAR with 10AA linker (2(G₄S); FIG. 18B), a Tandem CAR with 15AA linker (3(G₄S); FIG. 18C), a Tandem CAR with 20AA linker (4(G₄S); FIG. 18D), and a Parallel CAR (FIG. 18E).

FIG. 19 shows expression quantification of CAR constructs, as determined by flow cytometry. T cells were transduced with Hu08BBz CAR, 806BBz CAR, Hu08/806_(G₄S) bi-specific CAR, Hu08/806_2(G₄S) bi-specific CAR, Hu08/806_3(G₄S) bi-specific CAR, Hu08/806_4(G₄S) bi-specific CAR, and Hu08BBz_P2A_806BBz parallel CAR. CAR expression was detected with either biotin labelled protein L, and streptavidin conjugated PE, or streptavidin conjugated PE alone.

FIG. 20 illustrates the stimulation of T cells comprising Hu08BBz CAR and 806BBz CAR, the Hu08/806 bi-specific CARs, and Hu08BBz_P2A_806BBz parallel CAR. Each CAR T cell population was cocultured with the target-overexpressing 5077 glioma stem cell line. CAR1 (Hu08BBz) and CAR2 (806BBz) were single CAR constructs, 5AA, 10AA, 15AA, and 20AA were varying length tandem bi-specific CAR constructs (Hu08/806_(G₄S), Hu08/806_2(G₄S), Hu08/806_3(G₄S), Hu08/806_4(G₄S)), and 2A was a parallel bi-specific CAR construct (Hu08BBz_P2A_806BBz). The stimulation of T cells was illustrated by APC-conjugated anti-CD69 antibody staining, the median fluorescence intensity (MFI) was quantified on CD4⁺ (FIG. 20, top) and CD8⁺ (FIG. 20, bottom) CAR-positive T cells after 24hrs co-culture, controlled with un-transduced T cells. Statistically significant differences were calculated by one-way ANOVA with post hoc Tukey test. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$. Data are presented as means $\pm$ SEM.

FIGs. 21A-21F illustrate flow-based intracellular cytokine [IFN γ (FIGs. 21A and 21D), IL2 (FIGs. 21B and 21E), and TNF α (FIGs. 21C and 21F)] staining of each tandem bi-specific and parallel CAR T cell of FIGs 18-20, co-cultured with target-overexpressing 5077 glioma stem cell line. Percentage of cytokine positive T cells was demonstrated in CD4⁺ (FIGs. 21A-21C) and CD8⁺ (FIGs. 21D-21F) T cell subgroups. One-way ANOVA post hoc Tukey test. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Data are presented as means $\pm$ SEM.

FIGs. 22A-22D illustrate the bioluminescence-based cytotoxicity assay performed to test the killing ability of 806/Hu08 tandem bi-specific CAR T cells, when cocultured with target 5077 cell line not expressing EGFRvIII and IL13R α 2 (5077_R α 2- ν III-), or overexpressing IL13R α 2 alone (5077_R α 2+ ν III-), EGFRvIII alone (5077_R α 2- ν III+), or EGFRvIII and IL13R α 2 (5077_R α 2+ ν III+), and controlled with un-transduced T cells

(UTD). FIG. 2A illustrates the bioluminescence-based cytotoxicity assay of the Hu08/806_(G4S) bi-specific CAR. The linker between two scFv is GGGGS (SEQ ID NO:157). Data are presented as means $\pm$ SEM. FIG. 2B illustrates the bioluminescence-based cytotoxicity assay of the Hu08/806_2(G4S) bi-specific CAR. The linker between two scFv is GGGGSx2 (SEQ ID NO:181). Data are presented as means $\pm$ SEM. FIG. 2C illustrates the bioluminescence-based cytotoxicity assay of the Hu08/806_3(G4S) bi-specific CAR. The linker between two scFv is GGGGSx3 (SEQ ID NO:158). Data are presented as means $\pm$ SEM. FIG. 2D illustrates the bioluminescence-based cytotoxicity assay of the Hu08/806_4(G4S) bi-specific CAR. The linker between two scFv is GGGGSx4 (SEQ ID NO:160). Data are presented as means $\pm$ SEM.

FIGs. 23A-23D illustrate the *in vitro* killing of the parallel bi-specific CAR construct (Hu08BBz_P2A_806BBz). A bioluminescence-based cytotoxicity assay was performed to test the killing ability of 806BBz/Hu08BBz (Hu08BBz_P2A_806BBz) parallel bi-specific CAR T cells, when cocultured with the target- 5077 cell line overexpressing IL13R α 2 alone (5077_R α 2+_vIII-), EGFRvIII alone (5077_R α 2-_vIII+), or EGFRvIII and IL13R α 2 (5077_R α 2+_vIII+) and D270 glioma cell line overexpressing EGFRvIII and IL13R α 2 (D270_R α 2+_vIII+), and controlled with un-transduced T cells (UTD). Data are presented as means $\pm$ SEM.

FIG. 24 illustrates that 806BBz/Hu08BBz (Hu08BBz_P2A_806BBz) parallel bi-specific CAR T cells reduced tumor growth and enhanced animal survival. 806BBz/Hu08BBz bi-specific CAR T cells or the same number of un-transduced T cells (UTD) were i.v. infused in D270 subcutaneously implanted NSG mice (n=8 per group). Tumor volume measurements (FIG. 24, top) were performed to evaluate the tumor growth. Linear regression was used to test for significant differences between the experimental groups. Endpoint was predefined by the mouse hunch, inability to ambulate, or tumor reaching 2 cm in any direction, as predetermined IACUC-approved morbidity endpoint. Survival based on time to endpoint was plotted using a Kaplan-Meier curve (FIG. 24, bottom, Prism software). Statistically significant differences were determined using log-rank test. ****p < 0.0001. Data are presented as means $\pm$ SEM.

FIGs. 25A-25D illustrate T cell activation induced by an anti-IL13R α 2/CD3 bispecific T cell engager (Hu07BiTE) in IL13R α 2-positive cells. Fresh media (FIG. 25A), and conditioned media from un-transduced (UTD) T cells (FIG. 25B), Hu08BBz CAR Transduced T cells (FIG. 25C), and Hu07BiTE transduced T cells (FIG. 25D) were collected

and used in the co-culture with 5077 cell line (Top, IL13R α 2-) or 4892 cell line (Bottom, IL13R α 2+). CD69 was stained to demonstrate T cell activation. Human CD8 was stained to distinguish the CD4-positive and CD8-positive subgroups of T cells along the x axis.

FIG. 26 illustrates the binding of an anti-IL13R α 2/CD3 (Hu08OKT3) bispecific T cell engager to the IL13R α 2 *in vitro*. 293T cells were transfected with plasmid pTRPE CFP (a fluorescent gene) or pTRPE Hu08BiTE. Supernatant was collected 2 days later. Direct ELISA was performed to detect Hu08OKT3 BiTE binding with recombinant protein IL13R α 2.

FIG. 27 illustrates the binding of two anti-EGFR/CD3 (C225BiTE and 806BiTE) bispecific T cell engagers to the EGFR *in vitro*. T cells were transduced with pTRPE Hu08BBz, pTRPE C225BiTE, or pTRPE 806BiTE, controlled with un-transduced T cells (UTD) and Hu8BBz CAR. Supernatant was collected 7 days later. Direct ELISA was performed to detect BiTE's binding with recombinant protein EGFR wild type or EGFRvIII.

FIGs. 28A-28B illustrate the differential effect of the two anti-EGFR/CD3 (C225BiTE and 806BiTE) bispecific T cell engagers on wild type 5077 cells. Moreover, glioma stem cell line 5077 expresses low-level EGFR, but does not express the IL13R α 2. 806BiTE and C225BiTE transduced T cells were cocultured with 5077 cells expressing wild type or 5077 cells overexpressing EGFRvIII, and a killing assay (FIG. 28A) and cytokine secretion quantification assay (FIG. 28B) were performed. FIG. 28A illustrates that 806BiTE transduced T cells only killed EGFRvIII overexpressed 5077 cells, while C225BiTE transduced T cells killed 5077 wild type and EGFRvIII overexpressed 5077 cells. FIG. 28B illustrates that 806BiTE induced INF γ , IL-2, and TNF secretion only when 806BiTE transduced T cells were cocultured with EGFRvIII overexpressed 5077 cells, while C225BiTE transduced T cells stimulated INF γ , IL-2, and TNF secretion in the absence and presence of the EGFRvIII variant. No cytokine production was observed in the absence of target cells.

FIG. 29 illustrates T cell activation induced by an anti-IL13R α 2/CD3 (Hu08/KT3-T2A-mCherry) and anti-EGFRvIII/CD3 (80/KT3-T2A-mCherry) bispecific T cell engagers in IL13R α 2-positive cells and EGFRvIII-positive cells. Supernatant of un-transduced T cells (UTD), 806BBz CAR T cells, 806BiTE T cells, Hu08BBz CAR T cells and Hu08BiTE T cells was collected and used in the co-culture of untransduced T cells with target overexpressing 5077 GSC line and D270 glioma cell line. CD69 was stained to demonstrate

T cell activation. Human CD8 was stained to distinguish the CD4-positive and CD8-positive subgroups of T cells along the x axis.

FIGs. 30A-30D illustrate schematics of bispecific constructs used in BiTE/CAR experimentation. FIG. 30A shows a schematic of a parallel bispecific polynucleotide sequence comprising a first nucleotide sequence encoding a Hu08BBz CAR, a second nucleotide sequence encoding a 806BBz CAR, and a third nucleotide encoding a fluorescent marker (an 806BBz/Hu08BBz bispecific construct). FIG. 30B shows a schematic polynucleotide sequence comprising a first nucleotide sequence encoding an anti-EGFRvIII/CD3 bispecific T cell engager, a second nucleotide encoding Hu08CAR, and a third nucleotide encoding a fluorescent marker (an 806BiTE/Hu08CAR bispecific construct). FIG. 30C shows a schematic polynucleotide sequence comprising a first nucleotide sequence encoding an anti-IL13R α 2/CD3 bispecific T cell engager, a second nucleotide encoding 806CAR, and a third nucleotide encoding a fluorescent marker (an Hu08BiTE/806CAR bispecific construct). FIG. 30D shows a schematic polynucleotide sequence comprising a first nucleotide sequence encoding an anti-EGFRvIII/CD3 bispecific T cell engager, a second nucleotide encoding anti-IL13R α 2/CD3 bispecific T cell engager, and a third nucleotide encoding a fluorescent marker (an 806BiTE/Hu08BiTE bispecific construct). Self-cleaving sequences (P2A and/or T2A) link the CAR, the bispecific T cell engager, and the fluorescent marker in the same open reading frame.

FIGs. 31A-31D show the amino acid and nucleic acid sequences for 806BBz/Hu08BBz set forth as SEQ ID Nos: 173-174 (FIG. 31A), 806BiTE/Hu08BBz set forth as SEQ ID Nos: 175-176 (FIG. 31B), Hu08BiTE/806BBz set forth as SEQ ID Nos: 177-178 (FIG. 31C), and 806BiTE/Hu08BiTE set forth as SEQ ID Nos: 179-180 (FIG. 31D).

FIGs. 32A-32D illustrate the bioluminescence-based cytotoxicity assay performed to test the killing ability of 806BiTE/Hu08BBz bi-specific T cells, when cocultured with target overexpressed (EGFRvIII/IL13R α 2) cell lines, controlled with un-transduced T cells (UTD). Data are presented as means $\pm$ SEM. FIG. 32A shows the cytotoxic effect in the 5077 cell line overexpressing EGFRvIII alone (5077_R α 2-_vIII+). FIG. 32B shows the cytotoxic effect in the 5077 cell line overexpressing IL13R α 2 alone (5077_R α 2+_vIII-). FIG. 32C shows the cytotoxic effect in the 5077 cell line overexpressing IL13R α 2 and EGFRvIII (5077_R α 2+_vIII+). FIG. 32D shows the cytotoxic effect in the D270 cell line overexpressing IL13R α 2 and EGFRvIII (D270_R α 2+_vIII+).

FIGs. 33A-33B show that the 806BiTE/Hu08BBz bi-specific T cells reduced tumor growth and enhanced animal survival. 806BiTE/Hu08BBz bi-specific T cells or the same number of un-transduced T cells (UTD) were i.v. infused in D270 subcutaneously implanted NSG mice (n=8 per group). FIG. 33A shows reduced tumor size in animals treated with 806BiTE/Hu08BBz bi-specific T cells. Tumor volume measurements were performed to evaluate the tumor growth. Linear regression was used to test for significant differences between the experimental groups. Endpoint was predefined by the mouse hunch, inability to ambulate, or tumor reaching 2 cm in any direction, as predetermined IACUC-approved morbidity endpoint. FIG. 33B shows enhanced survival in animals treated with 806BiTE/Hu08BBz bi-specific T cells. Survival based on time to endpoint was plotted using a Kaplan-Meier curve (Prism software). Statistically significant differences were determined using log rank test. *** $p < 0.001$, **** $p < 0.0001$. Data are presented as means $\pm$ SEM.

FIGs. 34A-34D illustrate the bioluminescence-based cytotoxicity assay performed to test the killing ability of Hu08BiTE/806BBz bi-specific T cells, when cocultured with target overexpressed (EGFRvIII/IL13R α 2) 5077 cell line and D270 glioma cell line, controlled with un-transduced T cells (UTD). Data are presented as means $\pm$ SEM. FIG. 34A shows the cytotoxic effect in the 5077 cell line overexpressing EGFRvIII alone (5077_R α 2- vIII+). FIG. 34B shows the cytotoxic effect in the 5077 cell line overexpressing IL13R α 2 alone (5077_R α 2+ vIII-). FIG. 34C shows the cytotoxic effect in the 5077 cell line overexpressing IL13R α 2 and EGFRvIII (5077_R α 2+ vIII+). FIG. 34D shows the the cytotoxic effect in D270 cell line overexpressing IL13R α 2 and EGFRvIII (D270_R α 2+ vIII+).

FIGs. 35A-35B show that Hu08BiTE/806BBz bi-specific T cells reduced tumor growth and enhanced animal survival. Hu08BiTE/806BBz bi-specific T cells or the same number of un-transduced T cells (UTD) were i.v. infused in D270 subcutaneously implanted NSG mice (n=8 per group). FIG. 35A shows reduced tumor size in animals treated with Hu08BiTE/806BBz bi-specific T cells. Tumor volume measurements were performed to evaluate the tumor growth. Linear regression was used to test for significant differences between the experimental groups. Endpoint was predefined by the mouse hunch, inability to ambulate, or tumor reaching 2 cm in any direction, as predetermined IACUC-approved morbidity endpoint. FIG. 35B shows enhanced survival in animals treated with Hu08BiTE/806BBz bi-specific T cells. Survival based on time to endpoint was plotted using a Kaplan-Meier curve (Prism software). Statistically significant differences were determined using log-rank test. ** $p < 0.01$, **** $p < 0.0001$. Data are presented as means $\pm$ SEM.

FIGs. 36A-36D illustrate the bioluminescence-based cytotoxicity assay performed to test the killing ability of 806BiTE/Hu08BiTE bi-specific T cells, when cocultured with target overexpressed (EGFRvIII/IL13R α 2) 5077 cell line and D270 glioma cell line, controlled with un-transduced T cells (UTD). Data are presented as means $\pm$ SEM. FIG. 36A shows the cytotoxic effect in the 5077 cell line overexpressing EGFRvIII alone (5077_R α 2-_{vIII}+). FIG. 36B shows the cytotoxic effect in the 5077 cell line overexpressing IL13R α 2 alone (5077_R α 2+_{vIII}-). FIG. 36C shows the cytotoxic effect in the 5077 cell line overexpressing IL13R α 2 and EGFRvIII (5077_R α 2+_{vIII}+). FIG. 36D shows the the cytotoxic effect in D270 cell line overexpressing IL13R α 2 and EGFRvIII (D270_R α 2+_{vIII}+).

FIGs. 37A-37B show that 806BiTE/Hu08BiTE bi-specific T cells reduced tumor growth and enhanced animal survival. 806BiTE/Hu08BiTE bi-specific T cells or the same number of un-transduced T cells (UTD) were i.v. infused in D270 subcutaneously implanted NSG mice (n=8 per group). FIG. 37A shows reduced tumor size in animals treated with 806BiTE/Hu08BiTE bi-specific T cells. Tumor volume measurements were performed to evaluate the tumor growth. Linear regression was used to test for significant differences between the experimental groups. Endpoint was predefined by the mouse hunch, inability to ambulate, or tumor reaching 2 cm in any direction, as predetermined IACUC-approved morbidity endpoint. FIG. 37B shows enhanced survival in animals treated with 806BiTE/Hu08BiTE bi-specific T cells. Survival based on time to endpoint was plotted using a Kaplan-Meier curve (Prism software). Statistically significant differences were determined using log-rank test. ***p < 0.001, ****p < 0.0001. Data are presented as means $\pm$ SEM.

FIG. 38 illustrates the spread of therapeutic to a contralateral ventricle following intraventricular injections to the other ventricle to show the feasibility of intratumoral injection. 5 μ L of Trypan Blue were injected into the right ventricle, 1-2 mm to the right and 0.3 mm anterior to the bregma, to a depth of 3.0 mm. Animals were euthanized within 15 minutes of injection and brains examined for spread of Trypan Blue to the contralateral ventricle. Blue stain seen in both ventricles indicates the ability to both inject therapeutics into the right ventricle and obtain spread of the therapeutics to the left, contralateral ventricle.

DETAILED DESCRIPTION

The present invention provides compositions and methods for modified immune cells or precursors thereof (*e.g.*, modified T cells) comprising a chimeric antigen receptor (CAR)

capable of binding human IL13R α 2. In some embodiments, the invention provides compositions and methods for modified immune cells or precursors thereof comprising a first CAR capable of binding IL13R α 2, and a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. The provided compositions and methods are useful for treating cancer (*e.g.* glioma, high-grade astrocytoma, and glioblastoma).

It is to be understood that the methods described in this disclosure are not limited to particular methods and experimental conditions disclosed herein as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Furthermore, the experiments described herein, unless otherwise indicated, use conventional molecular and cellular biological and immunological techniques within the skill of the art. Such techniques are well known to the skilled worker, and are explained fully in the literature. See, *e.g.*, Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, N.Y. (1987-2008), including all supplements, Molecular Cloning: A Laboratory Manual (Fourth Edition) by MR Green and J. Sambrook and Harlow et al., Antibodies: A Laboratory Manual, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (2013, 2nd edition).

A. Definitions

Unless otherwise defined, scientific and technical terms used herein have the meanings that are commonly understood by those of ordinary skill in the art. In the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The use of “or” means “and/or” unless stated otherwise. The use of the term “including,” as well as other forms, such as “includes” and “included,” is not limiting.

Generally, nomenclature used in connection with cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein is well-known and commonly used in the art. The methods and techniques provided herein are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's

specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

That the disclosure may be more readily understood, select terms are defined below.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

“About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

“Activation,” as used herein, refers to the state of a T cell that has been sufficiently stimulated to induce detectable cellular proliferation. Activation can also be associated with induced cytokine production, and detectable effector functions. The term “activated T cells” refers to, among other things, T cells that are undergoing cell division.

As used herein, to “alleviate” a disease means reducing the severity of one or more symptoms of the disease.

The term “antigen” as used herein is defined as a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen.

Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an “antigen” as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, 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.

Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid.

As used herein, the term “autologous” is meant to refer to any material derived from the same individual to which it is later to be re-introduced into the individual.

5 A “co-stimulatory molecule” refers to the cognate binding partner on a T cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the T cell, such as, but not limited to, proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and a Toll ligand receptor.

10 A “co-stimulatory signal”, as used herein, refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to T cell proliferation and/or upregulation or downregulation of key molecules.

A “disease” is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal’s health continues to deteriorate. In contrast, a “disorder” in an animal is a state of health in which the animal is
15 able to maintain homeostasis, but in which the animal’s state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal’s state of health.

The term “downregulation” as used herein refers to the decrease or elimination of gene expression of one or more genes.

20 “Effective amount” or “therapeutically effective amount” are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result or provides a therapeutic or prophylactic benefit. Such results may include, but are not limited to an amount that when administered to a mammal, causes a detectable level of immune suppression or tolerance
25 compared to the immune response detected in the absence of the composition of the invention. The immune response can be readily assessed by a plethora of art-recognized methods. The skilled artisan would understand that the amount of the composition administered herein varies and can be readily determined based on a number of factors such as the disease or condition being treated, the age and health and physical condition of the
30 mammal being treated, the severity of the disease, the particular compound being administered, and the like.

“Encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence

of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

As used herein “endogenous” refers to any material from or produced inside an organism, cell, tissue or system.

The term “epitope” as used herein is defined as a small chemical molecule on an antigen that can elicit an immune response, inducing B and/or T cell responses. An antigen can have one or more epitopes. Most antigens have many epitopes; i.e., they are multivalent. In general, an epitope is roughly about 10 amino acids and/or sugars in size. Preferably, the epitope is about 4-18 amino acids, more preferably about 5-16 amino acids, and even more most preferably 6-14 amino acids, more preferably about 7-12, and most preferably about 8-10 amino acids. One skilled in the art understands that generally the overall three-dimensional structure, rather than the specific linear sequence of the molecule, is the main criterion of antigenic specificity and therefore distinguishes one epitope from another. Based on the present disclosure, a peptide used in the present invention can be an epitope.

As used herein, the term “exogenous” refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term “expand” as used herein refers to increasing in number, as in an increase in the number of T cells. In one embodiment, the T cells that are expanded ex vivo increase in number relative to the number originally present in the culture. In another embodiment, the T cells that are expanded ex vivo increase in number relative to other cell types in the culture. The term “ex vivo,” as used herein, refers to cells that have been removed from a living organism, (e.g., a human) and propagated outside the organism (e.g., in a culture dish, test tube, or bioreactor).

The term “expression” as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter.

“Expression vector” refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression

system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., Sendai viruses, lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

5 “Identity” as used herein refers to the subunit sequence identity between two polymeric molecules particularly between two amino acid molecules, such as, between two polypeptide molecules. When two amino acid sequences have the same residues at the same positions; e.g., if a position in each of two polypeptide molecules is occupied by an arginine, then they are identical at that position. The identity or extent to which two amino acid
10 sequences have the same residues at the same positions in an alignment is often expressed as a percentage. The identity between two amino acid sequences is a direct function of the number of matching or identical positions; e.g., if half (e.g., five positions in a polymer ten amino acids in length) of the positions in two sequences are identical, the two sequences are 50% identical; if 90% of the positions (e.g., 9 of 10), are matched or identical, the two amino
15 acids sequences are 90% identical.

 The term “immune response” as used herein is defined as a cellular response to an antigen that occurs when lymphocytes identify antigenic molecules as foreign and induce the formation of antibodies and/or activate lymphocytes to remove the antigen.

 The term “immunosuppressive” is used herein to refer to reducing overall immune
20 response.

 “Isolated” means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not “isolated,” but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is “isolated.” An isolated nucleic acid or protein can exist in substantially purified form,
25 or can exist in a non-native environment such as, for example, a host cell.

 A “lentivirus” as used herein refers to a genus of the Retroviridae family. Lentiviruses are unique among the retroviruses in being able to infect non-dividing cells; they can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of
30 lentiviruses. Vectors derived from lentiviruses offer the means to achieve significant levels of gene transfer *in vivo*.

 By the term “modified” as used herein, is meant a changed state or structure of a molecule or cell of the invention. Molecules may be modified in many ways, including

chemically, structurally, and functionally. Cells may be modified through the introduction of nucleic acids.

By the term “modulating,” as used herein, is meant mediating a detectable increase or decrease in the level of a response in a subject compared with the level of a response in the subject in the absence of a treatment or compound, and/or compared with the level of a response in an otherwise identical but untreated subject. The term encompasses perturbing and/or affecting a native signal or response thereby mediating a beneficial therapeutic response in a subject, preferably, a human.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. “A” refers to adenosine, “C” refers to cytosine, “G” refers to guanosine, “T” refers to thymidine, and “U” refers to uridine.

The term “oligonucleotide” typically refers to short polynucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, C, G), this also includes an RNA sequence (i.e., A, U, C, G) in which “U” replaces “T.”

Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

“Parenteral” administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, or infusion techniques.

The term “polynucleotide” as used herein is defined as a chain of nucleotides. Furthermore, nucleic acids are polymers of nucleotides. Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are polynucleotides, which can be hydrolyzed into the monomeric “nucleotides.” The monomeric nucleotides can be hydrolyzed into nucleosides. As used herein polynucleotides include, but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR, and the like, and by synthetic means.

As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked

by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

By the term "specifically binds," as used herein with respect to an antibody, is meant an antibody which recognizes a specific antigen, but does not substantially recognize or bind other molecules in a sample. For example, an antibody that specifically binds to an antigen from one species may also bind to that antigen from one or more species. But, such cross-species reactivity does not itself alter the classification of an antibody as specific. In another example, an antibody that specifically binds to an antigen may also bind to different allelic forms of the antigen. However, such cross reactivity does not itself alter the classification of an antibody as specific. In some instances, the terms "specific binding" or "specifically binding," can be used in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

By the term "stimulation," is meant a primary response induced by binding of a stimulatory molecule (e.g., a TCR/CD3 complex) with its cognate ligand thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex. Stimulation can mediate altered expression of certain molecules, such as downregulation of TGF-beta, and/or reorganization of cytoskeletal structures, and the like.

A "stimulatory molecule," as the term is used herein, means a molecule on a T cell that specifically binds with a cognate stimulatory ligand present on an antigen presenting cell.

A “stimulatory ligand,” as used herein, means a ligand that when present on an antigen presenting cell (e.g., an aAPC, a dendritic cell, a B-cell, and the like) can specifically bind with a cognate binding partner (referred to herein as a “stimulatory molecule”) on a T cell, thereby mediating a primary response by the T cell, including, but not limited to, activation, initiation of an immune response, proliferation, and the like. Stimulatory ligands are well-known in the art and encompass, inter alia, an MHC Class I molecule loaded with a peptide, an anti-CD3 antibody, a superagonist anti-CD28 antibody, and a superagonist anti-CD2 antibody.

The term “subject” is intended to include living organisms in which an immune response can be elicited (e.g., mammals). A “subject” or “patient,” as used therein, may be a human or non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. Preferably, the subject is human.

A “target site” or “target sequence” refers to a nucleic acid sequence that defines a portion of a nucleic acid to which a binding molecule may specifically bind under conditions sufficient for binding to occur. In some embodiments, a target sequence refers to a genomic nucleic acid sequence that defines a portion of a nucleic acid to which a binding molecule may specifically bind under conditions sufficient for binding to occur.

As used herein, the term “T cell receptor” or “TCR” refers to a complex of membrane proteins that participate in the activation of T cells in response to the presentation of antigen. The TCR is responsible for recognizing antigens bound to major histocompatibility complex molecules. TCR is composed of a heterodimer of an alpha (α) and beta (β) chain, although in some cells the TCR consists of gamma and delta (γ/δ) chains. TCRs may exist in alpha/beta and gamma/delta forms, which are structurally similar but have distinct anatomical locations and functions. Each chain is composed of two extracellular domains, a variable and constant domain. In some embodiments, the TCR may be modified on any cell comprising a TCR, including, for example, a helper T cell, a cytotoxic T cell, a memory T cell, regulatory T cell, natural killer T cell, and gamma delta T cell.

The term “therapeutic” as used herein means a treatment and/or prophylaxis. A therapeutic effect is obtained by suppression, remission, or eradication of a disease state.

“Transplant” refers to a biocompatible lattice or a donor tissue, organ or cell, to be transplanted. An example of a transplant may include but is not limited to skin cells or tissue, bone marrow, and solid organs such as heart, pancreas, kidney, lung and liver. A transplant

can also refer to any material that is to be administered to a host. For example, a transplant can refer to a nucleic acid or a protein.

The term “transfected” or “transformed” or “transduced” as used herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

To “treat” a disease as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject.

A “vector” is a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term “vector” includes an autonomously replicating plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, Sendai viral vectors, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

B. Chimeric Antigen Receptors

The present invention provides a chimeric antigen receptor (CAR) capable of binding IL13R α 2. In certain embodiments, the CAR comprises an antigen binding domain capable of binding IL13R α 2, a transmembrane domain, and an intracellular domain. Also provided are compositions and methods for modified immune cells or precursors thereof, e.g., modified T

cells, comprising the CAR. Thus, in some embodiments, the immune cell has been genetically modified to express the CAR. Also provided are nucleic acids encoding said CARs, vectors encoding said nucleic acids, and modified cells (*e.g.* modified T cells) comprising said CARs, vectors, or nucleic acids.

5 A subject CAR of the invention comprises an antigen binding domain capable of binding IL13R α 2, a transmembrane domain, and an intracellular domain. A subject CAR of the invention may optionally comprise a hinge domain. Accordingly, a subject CAR of the invention comprises an antigen binding domain capable of binding IL13R α 2, a hinge domain, a transmembrane domain, and an intracellular domain.

10 The antigen binding domain may be operably linked to another domain of the CAR, such as the transmembrane domain or the intracellular domain, both described elsewhere herein, for expression in the cell. In one embodiment, a first nucleic acid sequence encoding the antigen binding domain is operably linked to a second nucleic acid encoding a transmembrane domain, and further operably linked to a third a nucleic acid sequence
15 encoding an intracellular domain.

 The antigen binding domains described herein can be combined with any of the transmembrane domains described herein, any of the intracellular domains or cytoplasmic domains described herein, or any of the other domains described herein that may be included in a CAR of the present invention. A subject CAR of the present invention may also include a
20 hinge domain as described herein. A subject CAR of the present invention may also include a spacer domain as described herein. In some embodiments, each of the antigen binding domain, transmembrane domain, and intracellular domain is separated by a linker.

 In certain embodiments, the CAR is capable of binding human IL13R α 2. In certain embodiments, the CAR is capable of binding canine IL13R α 2. In certain embodiments, the CAR is capable of binding canine IL13R α 2 and human IL13R α 2.

 In one aspect, the invention includes an isolated antigen receptor (CAR) comprising an antigen-binding domain capable of binding human IL13R α 2, a transmembrane domain, and an intracellular domain. The antigen-binding domain comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence VKWAGGSTDYNSALMS (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID

NO: 5), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7).

In another aspect, the invention includes an isolated CAR comprising an antigen-binding domain capable of binding IL13R α 2, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises: a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence QGTTALATRFFD (SEQ ID NO: 14); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence KASQDVGTA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence QHHYSAPWT (SEQ ID NO: 18).

Tolerable variations of the CAR sequences will be known to those of skill in the art. For example, in some embodiments the CAR comprises an amino acid sequence that has at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any of the amino acid sequences set forth in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 12, 13, 14, 15, 16, 17, or 18.

In another aspect, the invention includes an isolated CAR capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises: a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.

In another aspect, the invention includes an isolated CAR capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises: a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20.

In another aspect, the invention includes an isolated CAR capable of binding IL13R α 2, comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or SEQ ID NO: 24 or SEQ ID NO: 55 or SEQ ID NO: 56.

In another aspect, the invention includes an isolated CAR capable of binding IL13R α 2, comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 92 or SEQ ID NO: 94 or SEQ ID NO: 111 or SEQ ID NO: 113.

In certain embodiments, the CAR is capable of binding a GBM stem cell.

Antigen-Binding Domain

The antigen-binding domain of a CAR is an extracellular region of the CAR for binding to a specific target antigen including proteins, carbohydrates, and glycolipids. In certain embodiments, the antigen-binding domain is capable of binding IL13R α 2. In certain embodiments, the antigen-binding domain is capable of binding human IL13R α 2. In certain embodiments, the antigen-binding domain is capable of binding canine IL13R α 2. In certain embodiments, the antigen-binding domain is capable of binding human IL13R α 2 and canine IL13R α 2.

In certain embodiments, the antigen-binding domain comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 8. In certain embodiments, the antigen-binding domain comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9. In certain embodiments, the antigen-binding domain comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antigen-binding domain comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 20.

In certain embodiments, the antigen-binding domain comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence of SEQ ID NO: 1, HCDR2 comprises the amino acid sequence of SEQ ID NO: 2, and HCDR3 comprises the amino acid sequence of SEQ ID NO: 4; and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence of SEQ ID NO: 5, LCDR2 comprises the amino acid sequence of SEQ ID NO: 6, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 7.

In certain embodiments, the antigen-binding domain comprises: a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence of SEQ ID NO: 1, HCDR2 comprises the amino acid sequence of SEQ ID NO: 3, and HCDR3 comprises the amino acid sequence of SEQ ID NO: 4; and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence of SEQ ID NO: 5, LCDR2 comprises the amino acid sequence of SEQ ID NO: 6, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 7.

In certain embodiments, the antigen-binding domain comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence of SEQ ID NO: 12, HCDR2 comprises the amino acid sequence of SEQ ID NO: 13, and HCDR3 comprises the amino acid sequence of SEQ ID NO: 14; and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18.

In certain embodiments, the antigen-binding domain comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence of SEQ ID NO: 12, HCDR2 comprises the amino acid sequence of SEQ ID NO: 13, and HCDR3 comprises the amino acid sequence of SEQ ID NO: 15; and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18.

In certain embodiments, the antigen-binding domain is selected from the group consisting of a full length antibody or antigen-binding fragment thereof, a Fab, a single-chain variable fragment (scFv), or a single-domain antibody. In certain embodiments, the antigen-binding domain comprises an scFv capable of binding IL13R α 2. In certain embodiments, the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 10. In certain embodiments, the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 11. In certain embodiments, the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 21. In certain embodiments, the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 22.

In certain embodiments, the antigen-binding domain is selected from the group consisting of a full length antibody or antigen-binding fragment thereof, a Fab, a single-chain variable fragment (scFv), or a single-domain antibody. In certain embodiments, the antigen-binding domain comprises an scFv capable of binding IL13R α 2. In certain embodiments, the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 125. In certain
5 embodiments, the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 127. In certain embodiments, the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 129. In certain embodiments, the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 131.

10 Tolerable variations of the antigen-binding domain sequences will be known to those of skill in the art. For example, in some embodiments the antigen-binding domain comprises an amino acid sequence that has at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least
15 98%, or at least 99% sequence identity to any of the amino acid sequences set forth in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11.

 Tolerable variations of the antigen-binding domain sequences will be known to those of skill in the art. For example, in some embodiments the antigen-binding domain comprises an amino acid sequence that has at least 80%, at least 81%, at least 82%, at least 83%, at least
20 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any of the amino acid sequences set forth in SEQ ID NO: 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22.

 Tolerable variations of the antigen-binding domain sequences will be known to those
25 of skill in the art. For example, in some embodiments the antigen-binding domain comprises an amino acid sequence that has at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any of the amino acid sequences set forth in SEQ
30 ID NO: 2, 77, 79, 82, 84, 86, 88, 90, 127, or 129.

 Tolerable variations of the antigen-binding domain sequences will be known to those of skill in the art. For example, in some embodiments the antigen-binding domain comprises an amino acid sequence that has at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least

91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any of the amino acid sequences set forth in SEQ ID NO: 96, 98, 99, 100, 103, 105, 107, 109, 129, or 131.

The antigen binding domain can include any domain that binds to the antigen and may include, but is not limited to, a monoclonal antibody, a polyclonal antibody, a synthetic antibody, a human antibody, a humanized antibody, a non-human antibody, and any fragment thereof. In some embodiments, the antigen binding domain portion comprises a mammalian antibody or a fragment thereof. The choice of antigen binding domain may depend upon the type and number of antigens that are present on the surface of a target cell.

In some embodiments, the antigen binding domain is selected from the group consisting of an antibody, an antigen binding fragment (Fab), and a single-chain variable fragment (scFv). In some embodiments, a IL13R α 2 binding domain of the present invention is selected from the group consisting of a IL13R α 2-specific antibody, a IL13R α 2-specific Fab, and a IL13R α 2-specific scFv. In one embodiment, a IL13R α 2 binding domain is a IL13R α 2-specific antibody. In one embodiment, a IL13R α 2 binding domain is a IL13R α 2-specific Fab. In one embodiment, a IL13R α 2 binding domain is a IL13R α 2-specific scFv.

As used herein, the term “single-chain variable fragment” or “scFv” is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of an immunoglobulin (*e.g.*, mouse or human) covalently linked to form a VH::VL heterodimer.

The heavy (VH) and light chains (VL) are either joined directly or joined by a peptide-encoding linker, which connects the N-terminus of the VH with the C-terminus of the VL, or the C-terminus of the VH with the N-terminus of the VL. In some embodiments, the antigen binding domain (*e.g.*, IL13R α 2 binding domain) comprises an scFv having the configuration from N-terminus to C-terminus, VH – linker – VL. In some embodiments, the antigen binding domain comprises an scFv having the configuration from N-terminus to C-terminus, VL – linker – VH. Those of skill in the art would be able to select the appropriate configuration for use in the present invention.

The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility. The linker can link the heavy chain variable region and the light chain variable region of the extracellular antigen-binding domain. Non-limiting examples of linkers are disclosed in Shen et al., *Anal. Chem.* 80(6):1910-1917 (2008) and WO 2014/087010, the contents of which are hereby incorporated by reference in their entireties. Various linker sequences are known in the art, including, without limitation, glycine serine (GS) linkers such as (GS)_n, (GSGGS)_n (SEQ ID NO:148), (GGGS)_n (SEQ ID NO:149), and (GGGGS)_n (SEQ

ID NO:150), where n represents an integer of at least 1. Exemplary linker sequences can comprise amino acid sequences including, without limitation, GGSG (SEQ ID NO:151), GGSGG (SEQ ID NO:152), GSGSG (SEQ ID NO:153), GSGGG (SEQ ID NO:154), GGGSG (SEQ ID NO:155), GSSSG (SEQ ID NO:156), GGGGS (SEQ ID NO:157),
 5 GGGGSGGGGSGGGGS (SEQ ID NO:158) and the like. Those of skill in the art would be able to select the appropriate linker sequence for use in the present invention. In one embodiment, an antigen binding domain of the present invention comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH and VL is separated by the linker sequence having the amino acid sequence GGGGSGGGGSGGGGS
 10 (SEQ ID NO:158), which may be encoded by the nucleic acid sequence GGTGGCGGTGGCTCGGGCGGTGGTGGGTGGGTGGCGGCGGATCT (SEQ ID NO:159).

Despite removal of the constant regions and the introduction of a linker, scFv proteins retain the specificity of the original immunoglobulin. Single chain Fv polypeptide antibodies
 15 can be expressed from a nucleic acid comprising VH- and VL-encoding sequences as described by Huston, et al. (Proc. Nat. Acad. Sci. USA, 85:5879-5883, 1988). See, also, U.S. Patent Nos. 5,091,513, 5,132,405 and 4,956,778; and U.S. Patent Publication Nos. 20050196754 and 20050196754. Antagonistic scFvs having inhibitory activity have been described (see, e.g., Zhao et al., Hyrbidoma (Larchmt) 2008 27(6):455-51; Peter et al., J
 20 Cachexia Sarcopenia Muscle 2012 August 12; Shieh et al., J Immunol 2009 183(4):2277-85; Giomarelli et al., Thromb Haemost 2007 97(6):955-63; Fife et al., J Clin Invest 2006 116(8):2252-61; Brocks et al., Immunotechnology 1997 3(3):173-84; Moosmayer et al., Ther Immunol 1995 2(10):31-40). Agonistic scFvs having stimulatory activity have been described (see, e.g., Peter et al., J Bio Chem 2003 25278(38):36740-7; Xie et al., Nat Biotech 1997
 25 15(8):768-71; Ledbetter et al., Crit Rev Immunol 1997 17(5-6):427-55; Ho et al., Biochim Biophys Acta 2003 1638(3):257-66).

As used herein, “Fab” refers to a fragment of an antibody structure that binds to an antigen but is monovalent and does not have a Fc portion, for example, an antibody digested by the enzyme papain yields two Fab fragments and an Fc fragment (e.g., a heavy (H) chain
 30 constant region; Fc region that does not bind to an antigen).

As used herein, “F(ab')₂” refers to an antibody fragment generated by pepsin digestion of whole IgG antibodies, wherein this fragment has two antigen binding (ab') (bivalent) regions, wherein each (ab') region comprises two separate amino acid chains, a part of a H chain and a light (L) chain linked by an S—S bond for binding an antigen and where

the remaining H chain portions are linked together. A “F(ab’)2” fragment can be split into two individual Fab’ fragments.

In some embodiments, the antigen binding domain may be derived from the same species in which the CAR will ultimately be used. For example, for use in humans, the antigen binding domain of the CAR may comprise a human antibody or a fragment thereof. In some embodiments, the antigen binding domain may be derived from a different species in which the CAR will ultimately be used. For example, for use in humans, the antigen binding domain of the CAR may comprise a murine antibody or a fragment thereof.

In some embodiments, a CAR of the present disclosure may have affinity for one or more target antigens on one or more target cells. In some embodiments, a CAR may have affinity for one or more target antigens on a target cell. In such embodiments, the CAR is a bispecific CAR, or a multispecific CAR. In some embodiments, the CAR comprises one or more target-specific binding domains that confer affinity for one or more target antigens. In some embodiments, the CAR comprises one or more target-specific binding domains that confer affinity for the same target antigen. For example, a CAR comprising one or more target-specific binding domains having affinity for the same target antigen could bind distinct epitopes of the target antigen. When a plurality of target-specific binding domains is present in a CAR, the binding domains may be arranged in tandem and may be separated by linker peptides. For example, in a CAR comprising two target-specific binding domains, the binding domains are connected to each other covalently on a single polypeptide chain, through an oligo- or polypeptide linker, an Fc hinge region, or a membrane hinge region.

Transmembrane Domain

CARs of the present invention may comprise a transmembrane domain that connects the antigen binding domain of the CAR to the intracellular domain of the CAR. The transmembrane domain of a subject CAR is a region that is capable of spanning the plasma membrane of a cell (*e.g.*, an immune cell or precursor thereof). The transmembrane domain is for insertion into a cell membrane, *e.g.*, a eukaryotic cell membrane. In some embodiments, the transmembrane domain is interposed between the antigen binding domain and the intracellular domain of a CAR.

In some embodiments, the transmembrane domain is naturally associated with one or more of the domains in the CAR. In some embodiments, the transmembrane domain can be selected or modified by one or more amino acid substitutions 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.

The transmembrane domain may be derived either from a natural or a synthetic source.

Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein, *e.g.*, a Type I transmembrane protein. Where the source is synthetic, the transmembrane domain may be any artificial sequence that facilitates insertion of the CAR into a cell membrane, *e.g.*, an artificial hydrophobic sequence. Examples of the transmembrane domain of particular use in this invention include, without limitation, transmembrane domains derived from (*i.e.* comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD7, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134 (OX-40), CD137 (4-1BB), CD154 (CD40L), Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, or a transmembrane domain derived from a killer immunoglobulin-like receptor (KIR). In one embodiment, the transmembrane domain comprises a transmembrane domain of CD8. In one embodiment, the transmembrane domain of CD8 is a transmembrane domain of CD8 alpha.

In some embodiments, the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. Preferably a
5 triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain.

The transmembrane domains described herein can be combined with any of the antigen binding domains described herein, any of the intracellular domains described herein, or any of the other domains described herein that may be included in a subject CAR.

10 In some embodiments, the transmembrane domain further comprises a hinge region. A subject CAR of the present invention may also include a hinge region. The hinge region of the CAR is a hydrophilic region which is located between the antigen binding domain and the transmembrane domain. In some embodiments, this domain facilitates proper protein folding for the CAR. The hinge region is an optional component for the CAR. The hinge region may
15 include a domain selected from Fc fragments of antibodies, hinge regions of antibodies, CH2 regions of antibodies, CH3 regions of antibodies, artificial hinge sequences or combinations thereof. Examples of hinge regions include, without limitation, a CD8a hinge, artificial hinges made of polypeptides which may be as small as, three glycines (Gly), as well as CH1 and CH3 domains of IgGs (such as human IgG4).

In some embodiments, a subject CAR of the present disclosure includes a hinge region that connects the antigen binding domain with the transmembrane domain, which, in turn, connects to the intracellular domain. The hinge region is preferably capable of supporting the antigen binding domain to recognize and bind to the target antigen on the target cells (see, *e.g.*, Hudecek et al., *Cancer Immunol. Res.* (2015) 3(2): 125-135). In some embodiments, the hinge region is a flexible domain, thus allowing the antigen binding domain to have a structure to optimally recognize the specific structure and density of the target antigens on a cell such as tumor cell (Hudecek et al., *supra*). The flexibility of the hinge region permits the hinge region to adopt many different conformations.

In some embodiments, the hinge region is an immunoglobulin heavy chain hinge region. In some embodiments, the hinge region is a hinge region polypeptide derived from a receptor (*e.g.*, a CD8-derived hinge region).

The hinge region can have a length of from about 4 amino acids to about 50 amino acids, *e.g.*, from about 4 aa to about 10 aa, from about 10 aa to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 40 aa, or from about 40 aa to about 50 aa. In some embodiments, the hinge region can have a length of greater than 5 aa, greater than 10 aa, greater than 15 aa, greater than 20 aa, greater than 25 aa, greater than 30 aa, greater than 35 aa, greater than 40 aa, greater than 45 aa, greater than 50 aa, greater than 55 aa, or more.

Suitable hinge regions can be readily selected and can be of any of a number of suitable lengths, such as from 1 amino acid (*e.g.*, Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and can be 1, 2, 3, 4, 5, 6, or 7 amino acids. Suitable hinge regions can have a length of greater than 20 amino acids (*e.g.*, 30, 40, 50, 60 or more amino acids).

For example, hinge regions include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n, (GSGGS)_n (SEQ ID NO:148) and (GGGS)_n (SEQ ID NO:149), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers can be used; both Gly and Ser are relatively unstructured, and therefore can serve as a neutral tether between components. Glycine polymers can be used; glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see, *e.g.*, Scheraga, *Rev. Computational. Chem.* (1992) 2: 73-142). Exemplary hinge regions can comprise amino acid sequences including, but not limited to, GGSG (SEQ

ID NO:151), GGSGG (SEQ ID NO:152), GSGSG (SEQ ID NO:153), GSGGG (SEQ ID NO:154), GGGSG (SEQ ID NO:155), GSSSG (SEQ ID NO:156), and the like.

In some embodiments, the hinge region is an immunoglobulin heavy chain hinge region. Immunoglobulin hinge region amino acid sequences are known in the art; see, e.g.,
 5 Tan et al., *Proc. Natl. Acad. Sci. USA* (1990) 87(1):162-166; and Huck et al., *Nucleic Acids Res.* (1986) 14(4): 1779-1789. As non-limiting examples, an immunoglobulin hinge region can include one of the following amino acid sequences: DKTHT (SEQ ID NO:182); CPPC (SEQ ID NO:183); CPEPKSCDTPPPCP (SEQ ID NO:184) (see, e.g., Glaser et al., *J. Biol. Chem.* (2005) 280:41494-41503); ELKTPLGDTTHT (SEQ ID NO:185); KSCDKTHTCP
 10 (SEQ ID NO:186); KCCVDCP (SEQ ID NO:187); KYGPPCP (SEQ ID NO:188); EPKSCDKTHTCPPCP (SEQ ID NO:189) (human IgG1 hinge); ERKCCVECPCPCP (SEQ ID NO:190) (human IgG2 hinge); ELKTPLGDTTHTCPRCP (SEQ ID NO:191) (human IgG3 hinge); SPNMVPHAHHAQ (SEQ ID NO:192) (human IgG4 hinge); and the like.

The hinge region can comprise an amino acid sequence of a human IgG1, IgG2, IgG3, or IgG4, hinge region. In one embodiment, the hinge region can include one or more amino acid substitutions and/or insertions and/or deletions compared to a wild-type (naturally-occurring) hinge region. For example, His229 of human IgG1 hinge can be substituted with Tyr, so that the hinge region comprises the sequence EPKSCDKTYTCPPCP (SEQ ID NO:193); see, e.g., Yan et al., *J. Biol. Chem.* (2012) 287: 5891-5897. In one embodiment, the hinge region can comprise an amino acid sequence derived from human CD8, or a variant thereof.

Intracellular Domain

15 A subject CAR of the present invention also includes an intracellular domain. In certain embodiments, the intracellular domain comprises a costimulatory signaling domain and an intracellular signaling domain. The intracellular domain of the CAR is responsible for activation of at least one of the effector functions of the cell in which the CAR is expressed (e.g., immune cell). The intracellular domain transduces the effector function signal and
 20 directs the cell (e.g., immune cell) to perform its specialized function, e.g., harming and/or destroying a target cell.

Examples of an intracellular domain for use in the invention include, but are not limited to, the cytoplasmic portion of a surface receptor, co-stimulatory molecule, and any molecule that acts in concert to initiate signal transduction in the T cell, as well as any

derivative or variant of these elements and any synthetic sequence that has the same functional capability.

Examples of the intracellular domain include, without limitation, the ζ chain of the T cell receptor complex or any of its homologs, e.g., η chain, Fc ϵ RI γ and β chains, MB 1 (Iga) chain, B29 (Ig) chain, etc., human CD3 zeta chain, CD3 polypeptides (Δ , δ and ϵ), syk family tyrosine kinases (Syk, ZAP 70, etc.), src family tyrosine kinases (Lck, Fyn, Lyn, etc.), and other molecules involved in T cell transduction, such as CD2, CD5 and CD28. In one embodiment, the intracellular signaling domain may be human CD3 zeta chain, Fc γ RIII, Fc ϵ RI, cytoplasmic tails of Fc receptors, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptors, and combinations thereof.

In certain embodiments, the intracellular domain of the CAR includes any portion of one or more co-stimulatory molecules, such as at least one signaling domain from CD2, CD3, CD8, CD27, CD28, ICOS, 4-1BB, PD-1, any derivative or variant thereof, any synthetic sequence thereof that has the same functional capability, and any combination thereof. the intracellular domain comprises a costimulatory domain of a protein selected from the group consisting of proteins in the TNFR superfamily, CD28, 4-1BB (CD137), OX40 (CD134), PD-1, CD7, LIGHT, CD83L, DAP10, DAP12, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40, ICOS, NKG2C, and B7-H3 (CD276), or a variant thereof, or an intracellular domain derived from a killer immunoglobulin-like receptor (KIR). In certain embodiments, the intracellular domain comprises a costimulatory domain of 4-1BB.

Other examples of the intracellular domain include a fragment or domain from one or more molecules or receptors including, but not limited to, TCR, CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, CD86, common FcR gamma, FcR beta (Fc Epsilon RIb), CD79a, CD79b, Fcgamma RIIa, DAP10, DAP12, T cell receptor (TCR), CD8, CD27, CD28, 4-1BB (CD137), OX9, OX40, CD30, CD40, PD-1, ICOS, a KIR family protein, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRP1), CD127, CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM

(SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, other co-stimulatory molecules described herein, any derivative, variant, or fragment thereof, any synthetic sequence of a co-

5 stimulatory molecule that has the same functional capability, and any combination thereof.

Additional examples of intracellular domains include, without limitation, intracellular signaling domains of several types of various other immune signaling receptors, including, but not limited to, first, second, and third generation T cell signaling proteins including CD3, B7 family costimulatory, and Tumor Necrosis Factor Receptor (TNFR) superfamily receptors
 10 (see, e.g., Park and Brentjens, J. Clin. Oncol. (2015) 33(6): 651-653). Additionally, intracellular signaling domains may include signaling domains used by NK and NKT cells (see, e.g., Hermanson and Kaufman, Front. Immunol. (2015) 6: 195) such as signaling domains of NKp30 (B7-H6) (see, e.g., Zhang et al., J. Immunol. (2012) 189(5): 2290-2299), and DAP 12 (see, e.g., Topfer et al., J. Immunol. (2015) 194(7): 3201-3212), NKG2D,
 15 NKp44, NKp46, DAP10, and CD3z.

In certain embodiments, the intracellular domain comprises an intracellular signaling domain selected from the group consisting of cytoplasmic signaling domains of a human CD3 zeta chain (CD3 ζ), Fc γ RIII, Fc ϵ RI, a cytoplasmic tail of an Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or a variant thereof. In certain embodiments, the intracellular domain comprises an intracellular domain of CD3 ζ .

Intracellular domains suitable for use in a subject CAR of the present invention include any desired signaling domain that provides a distinct and detectable signal (*e.g.*, increased production of one or more cytokines by the cell; change in transcription of a target gene; change in activity of a protein; change in cell behavior, *e.g.*, cell death; cellular
 20 proliferation; cellular differentiation; cell survival; modulation of cellular signaling responses; etc.) in response to activation of the CAR (*i.e.*, activated by antigen and dimerizing agent). In some embodiments, the intracellular domain includes at least one (*e.g.*, one, two, three, four, five, six, etc.) ITAM motif as described below. In some embodiments, the intracellular domain includes DAP10/CD28 type signaling chains. In some embodiments,
 25 the intracellular domain is not covalently attached to the membrane bound CAR, but is instead diffused in the cytoplasm.

Intracellular domains suitable for use in a subject CAR of the present invention include immunoreceptor tyrosine-based activation motif (ITAM)-containing intracellular signaling polypeptides. In some embodiments, an ITAM motif is repeated twice in an intracellular domain, where the first and second instances of the ITAM motif are separated from one another by 6 to 8 amino acids. In one embodiment, the intracellular domain of a subject CAR comprises 3 ITAM motifs.

In some embodiments, intracellular domains includes the signaling domains of human immunoglobulin receptors that contain immunoreceptor tyrosine based activation motifs (ITAMs) such as, but not limited to, FcγRI, FcγRIIA, FcγRIIC, FcγRIIIA, FcRL5 (see, *e.g.*, Gillis et al., *Front. Immunol.* (2014) 5:254).

A suitable intracellular domain can be an ITAM motif-containing portion that is derived from a polypeptide that contains an ITAM motif. For example, a suitable intracellular domain can be an ITAM motif-containing domain from any ITAM motif-containing protein. Thus, a suitable intracellular domain need not contain the entire sequence of the entire protein from which it is derived. Examples of suitable ITAM motif-containing polypeptides include, but are not limited to: DAP12, FCER1G (Fc epsilon receptor I gamma chain), CD3D (CD3 delta), CD3E (CD3 epsilon), CD3G (CD3 gamma), CD3Z (CD3 zeta), and CD79A (antigen receptor complex-associated protein alpha chain).

In one embodiment, the intracellular domain is derived from DAP12 (also known as TYROBP; TYRO protein tyrosine kinase binding protein; KARAP; PLOSL; DNAX-activation protein 12; KAR-associated protein; TYRO protein tyrosine kinase-binding protein; killer activating receptor associated protein; killer-activating receptor-associated protein; etc.). In one embodiment, the intracellular domain is derived from FCER1G (also known as FCRG; Fc epsilon receptor I gamma chain; Fc receptor gamma-chain; fc-epsilon RI-gamma; fcRgamma; fceRI gamma; high affinity immunoglobulin epsilon receptor subunit gamma; immunoglobulin E receptor, high affinity, gamma chain; etc.). In one embodiment, the intracellular domain is derived from T-cell surface glycoprotein CD3 delta chain (also known as CD3D; CD3-DELTA; T3D; CD3 antigen, delta subunit; CD3 delta; CD3d antigen, delta polypeptide (TiT3 complex); OKT3, delta chain; T-cell receptor T3 delta chain; T-cell surface glycoprotein CD3 delta chain; etc.). In one embodiment, the intracellular domain is derived from T-cell surface glycoprotein CD3 epsilon chain (also known as CD3e, T-cell surface antigen T3/Leu-4 epsilon chain, T-cell surface glycoprotein CD3 epsilon chain, AI504783, CD3, CD3epsilon, T3e, etc.). In one embodiment, the intracellular domain is derived from T-cell surface glycoprotein CD3 gamma chain (also known as CD3G, T-cell

receptor T3 gamma chain, CD3-GAMMA, T3G, gamma polypeptide (TiT3 complex), etc.). In one embodiment, the intracellular domain is derived from T-cell surface glycoprotein CD3 zeta chain (also known as CD3Z, T-cell receptor T3 zeta chain, CD247, CD3-ZETA, CD3H, CD3Q, T3Z, TCRZ, etc.). In one embodiment, the intracellular domain is derived from

5 CD79A (also known as B-cell antigen receptor complex-associated protein alpha chain; CD79a antigen (immunoglobulin-associated alpha); MB-1 membrane glycoprotein; ig-alpha; membrane-bound immunoglobulin-associated protein; surface IgM-associated protein; etc.). In one embodiment, an intracellular domain suitable for use in an FN3 CAR of the present disclosure includes a DAP10/CD28 type signaling chain. In one embodiment, an intracellular

10 domain suitable for use in an FN3 CAR of the present disclosure includes a ZAP70 polypeptide. In some embodiments, the intracellular domain includes a cytoplasmic signaling domain of TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, or CD66d. In one embodiment, the intracellular domain in the CAR includes a cytoplasmic signaling domain of human CD3 zeta.

15 While usually the entire intracellular domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular 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 intracellular domain includes any truncated portion of the intracellular domain sufficient to transduce the effector function signal.

20 The intracellular domains described herein can be combined with any of the antigen binding domains described herein, any of the transmembrane domains described herein, or any of the other domains described herein that may be included in the CAR.

Table 1: Sequences used in the invention

SEQ ID NO:	Name	Amino Acid/ Nucleotide Sequence
1	Hu07 HCDR1	TKYGVH
2	Mu07 HCDR2	VKWAGGSTDYNSALMS
3	Hu07 HCDR2	GVKWAGGSTDYNSALMS
4	Hu07 HCDR3	DHRDAMDY
5	Hu07 LCDR1	TASLSVSSTYLH
6	Hu07 LCDR2	STSNLAS
7	Hu07 LCDR3	HQYHRSPLT
8	Hu07 VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLTKYGVHWVRQAPGKGLEWVGVKWAGGSTDYNSALMSRFTISKDNAKNSLYLQMN SLRAEDTAVYYCARDHRDAMDYWGQGTLVTVSS
9	Hu07 VL	DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGSSPKLWIYSTSNLASGVPSRFSGSGSGTSYTLTISSLQPEDFATYYC

		HQYHRSPLTFGGGKVEIK
10	Hu07 scFv (VH > VL)	EVQLVESGGGLVQPGGSLRLSCAASGFSLTKEYGVHWVRQAPGKGLEWVGKWKAGGSTDYNSALMSRFTISKDNKNSLYLQMN SLRAEDTAVYYCARDHRDAMDYWGQGTLLTVSSGGGGSGGGGGSDIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGSSPKLWIYSTSNLASGVPSRFSGSGSGTSTLTISLQPEDFATYYCHQYHRSPLTFGGGKVEIK
11	Hu07 scFv (VL > VH)	DIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGSSPKLWIYSTSNLASGVPSRFSGSGSGTSTLTISLQPEDFATYYCHQYHRSPLTFGGGKVEIKGGGGSGGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSLTKEYGVHWVRQAPGKGLEWVGKWKAGGSTDYNSALMSRFTISKDNKNSLYLQMN SLRAEDTAVYYCARDHRDAMDYWGQGTLLTVSS
12	Hu08 HCDR1	SRNGMS
13	Hu08 HCDR2	TVSSGGSYIYYADSVK
14	Mu08 HCDR3	QGTTALATRFFD
15	Hu08 HCDR3	QGTTALATRFFDV
16	Hu08 LCDR1	KASQDVGTAVA
17	Hu08 LCDR2	SASYRST
18	Hu08 LCDR3	QHHYSAPWT
19	Hu08 VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQTPDKRLEWVATVSSGGSYIYYADSVKGRFTISRDNKNSLYLQMS SLRAEDTAVYYCARQGTTALATRFFDVWGQGTLLTVSS
20	Hu08 VL	DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQIPGKAPKLLIYSASYRSTGVPDRFSGSGSGTDFSFHISLQPEDFATYYCQHHYSAPWTFGGGKVEIK
21	Hu08 scFv (VH > VL)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQTPDKRLEWVATVSSGGSYIYYADSVKGRFTISRDNKNSLYLQMS SLRAEDTAVYYCARQGTTALATRFFDVWGQGTLLTVSSGGGGSGGGGGSDIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQIPGKAPKLLIYSASYRSTGVPDRFSGSGSGTDFSFHISLQPEDFATYYCQHHYSAPWTFGGGKVEIK
22	Hu08 scFv (VL > VH)	DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQIPGKAPKLLIYSASYRSTGVPDRFSGSGSGTDFSFHISLQPEDFATYYCQHHYSAPWTFGGGKVEIKGGGGSGGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQTPDKRLEWVATVSSGGSYIYYADSVKGRFTISRDNKNSLYLQMS SLRAEDTAVYYCARQGTTALATRFFDVWGQGTLLTVSS
23	Hu07 CAR (VH > VL)	MALPVTALLPLALLHAARPGSEVQLVESGGGLVQPGGSLRLSCAASGFSLTKEYGVHWVRQAPGKGLEWVGKWKAGGSTDYNSALMSRFTISKDNKNSLYLQMN SLRAEDTAVYYCARDHRDAMDYWGQGTLLTVSSGGGGSGGGGGSDIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWYQQKPGSSPKLWIYSTSNLASGVPSRFSGSGSGTSTLTISLQPEDFATYYCHQYHRSPLTFGGGKVEIKSGTTTPAPRPPTAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA PLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSDAPAYKQGGNQLYNELNLGRREEYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
24	Hu08 CAR (VH > VL)	MALPVTALLPLALLHAARPGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQTPDKRLEWVATVSSGGSYIYYADSVKGRFTISRDNKNSLYLQMS SLRAEDTAVYYCARQGTTALATRFFDVWGQGTLLTVSSGGGGSGGGGGSDIQMTQSP

		SSLSASVGDVRTITCKASQDVGTA VAWYQQIPGKAPKLLIYSA SYRSTGVPDRFSGSGSGTDFSFIISSLPEDFATYYCQHHYSAP WTFGGGTKVEIKSGTITPAPRPPTPAPTIASRPEACRPA AGGA VHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKRGR KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS RSADAPAYKQGGNQLYNELNLGRREEYDVLDRRGRDPEMG GKPRRNPNQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR
25	806 HCDR1	GYSITSDFAWN
26	806 HCDR2	GYISYSGNTRYNPSLK
27	806 HCDR3	VTAGRGFPYW
28	806 LCDR1	HSSQDINSNIG
29	806 LCDR2	HGTNLDD
143	806 LCDR2	HGINLDD
30	806 LCDR3	VQYAQFPWT
31	806 VH	DVQLQESGPSLVKPSQSLSTCTVTGYISITSDFAWNWI RQFPG NKLEWMGYISYSGNTRYNPSLKSRISITRDTSKNQFFLQLNSVT IEDTATYYCVTAGRGFPYWQGTLVTVA
139	806 VH nucleotide sequence	gatgtccagctgcaagagctgtgcccctagcctggtaagcctagccagagcctgagcctgacatgt accgtgaccggctacagcatcaccagcgacttcgctggaactggatcagacagttccccggcaa caagctggaatggatggctacatcagctacagcggaacacccgggtacaaccccagcctgaag tccccgatctccatcaccagagacaccagcaagaaccagttcttctgcagctgaacagcgtgacc atcgaggacacggccactactactgtgtgacagccggcagaggcttccctatlggggacaggg aacctgtgacacagttgtgtgt
194	806 VH nucleotide sequence	GACGTACA ACTGCAAGAATCCGGGCCGAGTTTGGTCA AGCCCTCTCAATCTCTTTCTCTCACITGACGGTCAACC GGATACTCCATAACCAGCGATTTTGCCTGGAATTGGAT TCGACAATTTCCAGGGAATAAATTGGAATGGATGGGA TATATCAGTTATTCTGGTAATACCAGATACAACCCGTC ATTGAAAAGTCGCATCTCTATAACACGAGACACTTCA AAGAATCAGTTCTTCTTCAGCTCAATTCTGTAACCAT CGAAGATACTGCTACTTATTACTGTGTAACGGCGGGTC GAGGATTCCCCTACTGGGGCCAGGGTACACTGGTTACT GTTTCCGCC
32	806 VL	DILMTQSPSSMSVSLGDTVSI TCHSSQDINSNIGWLQRRPGKSF KGLIYHGTLNLDDEVPSRFSGSGSGADYSLTISSLESEDFADYYC VQYAQFPWTFGGGTKLEIKR
140	806 VL nucleotide sequence	gatatcctgatgacacagagccccagcagcatgtctgttccttggcgataccgtgtccatcacct gtcacagcagccaggacatcaacagcaacatcggtggtgcagcagaggcctggcaagtctttt aagggcctgatctaccacggcaccacacctggatgatgaggtgccagcagatttccggctctgga agcggagccgactactcctgacaatcagcagcctggaagcagaggacttcgcgattactactg cgtgcagtacgcccagtttcttggaccttggaggcggcacaaagctggaaatcaagcgg
195	806 VL nucleotide sequence	GATATTCTGATGACTCAATCTCCGTCTTCTATGAGCGTGAGC TIGGGTGACACCGTCAGCATCACCTGTCAATCCAGCCAGGA TATAAACTCAAATATCGGCTGGCTCCAGCAACGCCAGGCA AGTCATTCAAGGGGCTTATTTATCATGGCACCAATCTTGAC GATGAAGTCCCATCACGCTTCAGCGGATCAGGCTCAGGTGC GGACTATTCTTGACTATAAGTTCCCTCGAATCTGAGGATTT CGCCGACTATTATTGCGTACAATACGCCCAGTTTCCCTGGA CCTTCGGAGGCGGCACCAAATTGGAGATAAAAAGG
33	806 scFv nucleotide sequence	GATGTCCAGCTGCAAGAGTCTGGCCCTAGCCTGGTCAAGCC TAGCCAGAGCCTGAGCCTGACATGTACCGTGACCGGCTACA GCATCACCAGCGACTTCGCCTGGA ACTGGATCAGACAGTTC

	(VH>VL)	CCCGGCAACAAGCTGGAATGGATGGGCTACATCAGCTACA GCGGCAACACCCGGTACAACCCAGCCTGAAGTCCCGGATC TCCATCACCAGAGACACCAGCAAGAACCAGTTCTTCTGCA GCTGAACAGCGTGACCATCGAGGACACCGCCACCTACTACT GTGTGACAGCCGGCAGAGGCTTCCCTTATTGGGGACAGGGA ACCCTGGTACAGTGTCTGCTGGTGGCGGAGGATCTGGCGG AGGCGGATCTTCTGGCGGTGGCTCTGATATCCTGATGACAC AGAGCCCCAGCAGCATGTCTGTGTCCCTGGGCGATACCGTG TCCATCACCTGTACAGCAGCCAGGACATCAACAGCAACAT CGGCTGGCTGCAGCAGAGGCCTGGCAAGTCTTTTAAGGGCC TGATCTACCACGGCACCAACCTGGATGATGAGGTGCCCAGC AGATTTTCCGGCTCTGGAAGCGGAGCCGACTACTCCCTGAC AATCAGCAGCCTGGAAAGCGAGGACTTCGCCGATTACTACT GCGTGCAGTACGCCAGTTTCCTTGGACCTTTGGAGGCGGC ACAAAGCTGGAAATCAAGCGG
34	806 scFv amino acid sequence (VH>VL)	DVQLQESGPSLVKPSQSLSLTCTVTGYSTITSDFAWNWIRQFPG NKLEWMGYISYSGNTRYNP SLKSRISITRDTSKNQFFLQLNSVT IEDTATYYCVTAGRGFPYWGQGLVTVSAGGGGSGGGGSSG GGSDILMTQSPSSMSVSLGDTVSI TCHSSQDINSNIGWLQQRPG KSFKGLIYHGTNLDDEVPSRFSGSGSGADYSLTISSESEDFAD YYCVQYAQFPWTFGGGKLEIKR
141	806 scFv nucleotide sequence (VL>VH)	GATATTCTGATGACTCAATCTCCGTCTTCTATGAGCGTGAGC TTGGGTGACACCGTCAGCATCACCTGTCATTCCAGCCAGGA TATAAACTCAAATATCGGCTGGCTCCAGCAACGCCCAGGCA AGTCATTCAAGGGGCTTATTTATCATGGCACCAATCTTGAC GATGAAGTCCCATCACGCTTCAGCGGATCAGGCTCAGGTGC GGACTATTCTTGACTATAAGTTCCCTCGAATCTGAGGATTT CGCCGACTATTATTGCGTACAATACGCCCAGTTTCCCTGGA CCTTCGGAGGCGGCACCAAATTGGAGATAAAAAGGGGTGG AGGAGGATCAGGCGGGGTGGAAGCGGCGGAGGAGGCAG CGACGTACAACCTGCAAGAATCCGGGCCGAGTTTGGTCAAGC CCTCTCAATCTCTTTCTCTCACTTGCACGGTCACCGGATACT CCATAACCAGCGATTTTTCGTGGAATTGGATTTCGACAATTT CCAGGGAATAAATTGGAATGGATGGGATATATCAGTTATTC TGGTAATACCAGATACAACCCGTCATTGAAAAGTCGCATCT CTATAACACGAGACACTTCAAAGAATCAGTTCTTCTTCAG CTCAATTCTGTAACCATCGAAGATACTGCTACTTATTACTGT GTAACGGCGGGTCGAGGATTCCCTACTGGGGCCAGGGTAC ACTGGTTACTGTTTCCGCC
142	806 scFv amino acid sequence (VL>VH)	DILMTQSPSSMSVSLGDTVSI TCHSSQDINSNIGWLQQRPGKSF KGLIYHGTNLDDEVPSRFSGSGSGADYSLTISSESEDFADYYC VQYAQFPWTFGGGKLEIKRGGGGSGGGGSGGGGSDVQLQE SGPSLVKPSQSLSLTCTVTGYSTITSDFAWNWIRQFPGNKLEWM GYISYSGNTRYNP SLKSRISITRDTSKNQFFLQLNSVTIEDTATY YCVTAGRGFPYWGQGLVTVSA
35	806-BBZ-CAR	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTG CTGCTCCACGCCCGCCAGGCCGGGATCCGATGTCAGCTGCA AGAGTCTGGCCCTAGCCTGGTCAAGCCTAGCCAGAGCCTGA GCCTGACATGTACCGTGACCGGCTACAGCATCACCAGCGAC TTCGCCTGGAACCTGGATCAGACAGTTCCCCGGCAACAAGCT GGAATGGATGGGCTACATCAGCTACAGCGGCAACACCCGG TACAACCCAGCCTGAAGTCCCGGATCTCCATCACCAGAGA CACCAGCAAGAACCAGTTCTTCTGCGAGCTGAACAGCGTGA CCATCGAGGACACCGCCACCTACTACTGTGTGACAGCCGGC

		AGAGGCTTCCCTTATTGGGGACAGGGAACCCTGGTCACAGT GTCTGCTGGTGGCGGAGGATCTGGCGGAGGCGGATCTTCTG GCGGTGGCTCTGATATCCTGATGACACAGAGCCCCAGCAGC ATGTCTGTGTCCCTGGGCGATACCGTGTCCATCACCTGTAC AGCAGCCAGGACATCAACAGCAACATCGGCTGGCTGCAGC AGAGGCCTGGCAAGTCTTTTAAGGGCCTGATCTACCACGGC ACCAACCTGGATGATGAGGTGCCAGCAGATTTTCCGGCTC TGGAAGCGGAGCCGACTACTCCCTGACAATCAGCAGCCTGG AAAGCGAGGACTTCGCCGATTACTACTGCGTGCAGTACGCC CAGTTTCCTTGGACCTTTGGAGGCGGCACAAAGCTGGAAAT CAAGCGGGCTAGCACCCTACCCAGCACCAGGGCCACCC ACCCCGGCTCCTACCATCGCCTCCAGCCTCTGTCCCTGCGT CCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATA CCCGGGGTCTTGA CTTCGCCTGCGATATCTACATTTGGGCCC CTCTGGCTGGTACTTGGGGGTCCTGCTGCTTTCACTCGTGA TCACTCTTTACTGTAAGCGCGGTGCGAAGAAGCTGCTGTAC ATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCA AGAGGAGGACGGCTGTTTCATGCCGGTTCAGAGGAGGAG GAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCG CAGATGCTCCAGCCTACAAGCAGGGGCAGAACCCAGCTCTA CAACGAACTCAATCTTGGTCCGAGAGAGGAGTACGACGTG CTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGA AGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGA GCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATT GGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGAC GGACTGTACCAGGGACTCAGCACCCGCCACCAAGGACACCT ATGACGCTCTTCACATGCAGGCCCTGCCGCTCGGTGA
36	806-BBZ-CAR	MALPVTALLLPLALLLHAARPGSDVQLQESGPSLVKPSQSLSL TCTVTGYSITSDFAWNWIROFPGNKLEWMGYISYSGNTRYNP SLKSRISITRDTSKNQFFLQLNSVTIEDTATYYCVTAGRGFPYW GQGTLVTVSAGGGGSGGGGSSGGSDILMTQSPSSMSVSLGD TVSITCHSSQDINSNIGWLQQRPGKSFGLIYHGTNLDDEVPSR FSGSGSGADYSLTISSLEDFADYYCVQYAQFPWTFGGGTKL EIKRASTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFK QPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY KQGQNQLYNELNLGRREEYDVLDRRGRDPGEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHGDLGYQLST ATKDTYDALHMQALPPR
196	806-BBZ-CAR	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTG CTGCTGCATGCCGCTAGACCCGGATCCGATATTCTGATGAC TCAATCTCCGTCTTCTATGAGCGTGAGCTTGGGTGACACCG TCAGCATCACCTGTCATTCCAGCCAGGATATAAACTCAAAT ATCGGCTGGCTCCAGCAACGCCAGGCAAGTCATTCAAGGG GCTTATTTATCATGGCACCAATCTTGACGATGAAGTCCCAT CACGCTTCAGCGGATCAGGCTCAGGTGCGGACTATTCTTG ACTATAAGTTCCTCGAATCTGAGGATTTGCGCGACTATTAT TGCGTACAATACGCCAGTTTCCCTGGACCTTCGGAGGCGG CACCAAATTGGAGATAAAAAGGGGTGGAGGAGGATCAGGC GGGGGTGGAAGCGGCGGAGGAGGCAGCGACGTACAACCTGC AAGAATCCGGGGCCGAGTTTGGTCAAGCCCTCTCAATCTCTT TCTCTCACTTGACCGGTACCGGATACTCCATAACCAGCGA TTTTGCGTGGAATTGGATTCGACAATTTCCAGGGAATAAAT TGGAATGGATGGGATATATCAGTTATTCTGGTAATACCAGA TACAACCCGTCATTGAAAAGTCGCATCTCTATAACACGAGA

		CACTTCAAAGAATCAGTTCTTCCTTCAGCTCAATTCTGTAAC CATCGAAGATACTGCTACTTATTACTGTGTAACGGCGGGTC GAGGATTCCCCTACTGGGGCCAGGGTACACTGGTTACTGTT TCCGCCTCCGGAACCACGACGCCAGCGCCGCGACCAACCAAC ACCGGCGCCCAACATCGCGTCGCAGCCCCTGTCCCTGCGCC CAGAGGCGTGC CGGCCAGCGGCGGGGGGCGCAGTGCACAC GAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCGC CCTTGGCCGGGACTTGTGGGGTCTTCTCCTGTCACTGGTTA TCACCCTTTACTGCAAACGGGGCAGAAAGAACTCCTGTAT ATATTCAAACAACCATTTATGAGACCAGTACAACTACTCA AGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAA GAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGGAGCG CAGACGCCCCCGGTACAAGCAGGGCCAGAACCAGCTCTA TAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTT TTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAA AGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGA ACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATT GGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGAT GGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTA CGACGCCCTTACATGCAGGCCCTGCCCCCTCGC
197	806-BBZ-CAR	MALPVTALLLPLALLHAARPGSDILMTQSPSSMSVSLGDTVSI TCHSSQDINSNIGWLQORPGKSFKGLIYHGTNLDDEVPSRFSGS GSGADYSLTISSESEDFADYYCVQYAQFPWTFGGGTKLEIKR GGGGSGGGSGGGGSDVQLQESGPSLVKPSQSLTCTVTGY SITSDFAWNWIQFPGNKLEWMGYISYSGNTRYNP SLKSRISIT RDTSKNQFFLQLNSVTIEDTATYYCVTAGRGFPYWGQGLVT VSASGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYWAPLAGTCGVLLLSLVITLYCKRGRKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQ GQNQLYNELNLRREEYDVLDRRRGRDPEMGGKPRRKNPQE GLYNELQKDKMAEAYSEIGMKGERRRGKHGHDGLYQGLSTAT KDTYDALHMQALPPR
37	806-KIR-CAR	ATGGGGGGACTTGAACCCTGCAGCAGGTTCTGCTCCTGCC TCTCCTGCTGGCTGTAAAGTGGTCTCCGTCCTGTCCAGGTCCA GGCCCAGAGCGATTGCAGTTGCTCTACGGTGAGCCCGGGCG TGCTGGCAGGGATCGTGATGGGAGACCTGGTGCTGACAGTG CTCATTGCCCTGGCCGTGTA CTTCCTGGGCCGGCTGGTCCCT CGGGGGCGAGGGGCTGCGGAGGCAGCGACCCGGAACAGC GTATCACTGAGACCGAGTCGCCTTATCAGGAGCTCCAGGGT CAGAGGTCGGATGTCTACAGCGACCTCAACACACAGAGGC CGTATTACAAAGTCGAGGGCGGCGGAGAGGGCAGAGGAAG TCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTA GGATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCC TTGCTGCTCCACGCCGCCAGGCCGGGATCCGATGTCCAGCT GCAAGAGTCTGGCCCTAGCCTGGTCAAGCCTAGCCAGAGCC TGAGCCTGACATGTACCGTGACCGGCTACAGCATCACCAGC GACTTCGCCTGGA ACTGGATCAGACAGTTCCCCGGCAACAA GCTGGAATGGATGGGCTACATCAGCTACAGCGGCAACACC CGGTACAACCCAGCCTGAAGTCCCGGATCTCCATCACCAG AGACACCAGCAAGAACCAGTTCTTCCTGCAGCTGAACAGCG TGACCATCGAGGACACCGCCACCTACTACTGTGTGACAGCC GGCAGAGGCTTCCCTTATTGGGGACAGGGAACCTGGTCAC AGTGTCTGCTGGTGGCGGAGGATCTGGCGGAGGCGGATCTT CTGGCGGTGGCTCTGATATCCTGATGACACAGAGCCCCAGC AGCATGTCTGTGTCCCTGGGCGATACCGTGTCCATCACCTG

		TCACAGCAGCCAGGACATCAACAGCAACATCGGCTGGCTG CAGCAGAGGCCTGGCAAGTCTTTTAAGGGCCTGATCTACCA CGGCACCAACCTGGATGATGAGGTGCCCAGCAGATTTTCCG GCTCTGGAAGCGGAGCCGACTACTCCCTGACAATCAGCAGC CTGGAAAGCGAGGACTTCGCCGATTACTACTGCGTGCAGTA CGCCCAGTTTCCTTGGACCTTTGGAGGCGGCACAAAGCTGG AAATCAAGCGGGCTAGCGGTGGCGGAGGTTCTGGAGGTGG GGGTTCCTCACCCACTGAACCAAGCTCCAAAACCGGTAACC CCAGACACCTGCATGTTCTGATTGGGACCTCAGTGGTCAAA ATCCCTTTTACCATCCTCCTCTTCTTTCTCCTTCATCGCTGGT GCTCCAACAAAAAAATGCTGCTGTAATGGACCAAGAGCC TGCAGGGAACAGAACAGTGAACAGCGAGGATTCTGATGAA CAAGACCATCAGGAGGTGTCATACGCATAA
38	806-KIR-CAR	MGGLEPCSRFLLPLLLAVSGLRPVQVQAQSDCSCSTVSPGVL AGIVMGDLVLTVLIALAVYFLGRLVPRGRGAAEAATRKQRITE TESPYQELQGQRSDVYSDLNTQRPYYKVEGGGEGRGSLLTCG DVEENPGPRMALPVTALLPLALLLHAARPGSDVQLQESGPSL VKPSQSLSLTCTVTGYSTDFAWNWRQFPGNKLEWMGYISY SGNTRYNPSLKSRSITRDTSKNQFFLQLNSVTIEDTATYYCVT AGRGFPYWQGTLVTVSAGGGGSGGGGSSGGGSDILMTQSPS SMSVSLGDTVSTCHSSQDINSNIGWLQQRPGKSFGLIYHGTN LDDEVPSRFSGSGSGADYSLTISSLESEDFADYYCVQYAQFPW TFGGGKLEIKRASGGGGSGGGGSSPTEPSSKTGNPRHLHLVIG TSVVKIPFTILLFLLHRWC SNKKNAAVMDQEPAGNRTVNSD SDEQDHQEVSYA
39	ABT-806 (humanized 806) VH	CAGGTTTCAGCTGCAAGAGTCTGGCCCTGGCCTGGTCAAGCC TAGCCAAACACTGAGCCTGACCTGTACCGTGTCCGGCTACA GCATCAGCAGCGACTTCGCCTGGAAGTGGATCAGACAGCCT CCTGGCAAAGGACTGGAATGGATGGGCTACATCAGCTACA GCGGCAACACCAGATACCAGCCTAGCCTGAAGTCCCGGATC ACCATCAGCAGAGACACCAGCAAGAACCAGTTCTTCCTGAA GCTGAACAGCGTGACAGCCGCCGATACCGCCACCTACTATT GTGTGACAGCTGGCAGAGGCTTCCCCTATTGGGGACAGGGA ACACTGGTCACCGTTAGCTCT
40	ABT-806 (humanized 806) VL	GATATCCAGATGACACAGAGCCCCAGCAGCATGTCCGTGTC CGTGGGAGACAGAGTGACCATCACCTGTACAGCAGCCAG GACATCAACAGCAACATCGGCTGGCTGCAGCAGAAGCCCG GCAAGTCTTTTAAGGGCCTGATCTACCACGGCACCAACCTG GATGATGGCGTGCCAGCAGATTTTCTGGCAGCGGCTCTGG CACCGACTACACCCTGACCATATCTAGCCTGCAGCCTGAGG ACTTCGCCACCTATTACTGCGTGACGTACGCCAGTTTCTT GGACCTTTGGAGGCGGCACAAAGCTGGAAATCAAGCGG
41	ABT-806 (humanized 806) scFv	CAGGTTTCAGCTGCAAGAGTCTGGCCCTGGCCTGGTCAAGCC TAGCCAAACACTGAGCCTGACCTGTACCGTGTCCGGCTACA GCATCAGCAGCGACTTCGCCTGGAAGTGGATCAGACAGCCT CCTGGCAAAGGACTGGAATGGATGGGCTACATCAGCTACA GCGGCAACACCAGATACCAGCCTAGCCTGAAGTCCCGGATC ACCATCAGCAGAGACACCAGCAAGAACCAGTTCTTCCTGAA GCTGAACAGCGTGACAGCCGCCGATACCGCCACCTACTATT GTGTGACAGCTGGCAGAGGCTTCCCCTATTGGGGACAGGGA ACACTGGTCACCGTTAGCTCTGATATCCAGATGACACAGAG CCCCAGCAGCATGTCCGTGTCCGTGGGAGACAGAGTGACCA TCACCTGTACAGCAGCCAGGACATCAACAGCAACATCGGC TGGCTGCAGCAGAAGCCCGGCAAGTCTTTTAAGGGCCTGAT

		CTACCACGGCACCAACCTGGATGATGGCGTGCCAGCAGAT TTTCTGGCAGCGGCTCTGGCACCAGCTACACCCTGACCATA TCTAGCCTGCAGCCTGAGGACTTCGCCACCTATTACTGCGT GCAGTACGCCCCAGTTTCCTTGGACCTTTGGAGGCGGCACAA AGCTGGAAATCAAGCGG
42	ABT-806 (humanized 806) VH	QVQ LQE SGP GLV KPS QTL SLT CTV SGY SIS SDF AWN WIR QPP GKG LEW MGY ISY SGN TRY QPS LKS RIT ISR DTS KNQ FFL KLN SVT AAD TAT YYC VTA GRG FPY WGQ GTL VTV SS
43	ABT-806 (humanized 806) VL	DIQ MTQ SPSS MSVS VGDR VTIT CHSS QDIN SNIG WLQQ KPGK SFGKLIYHG TNLD DGVP SRFS GSGS GTDY TLTI SSLQ PEDF ATYY CVQY AQFP WTFG GGTK LEIKR
44	ABT-806 (humanized 806) scFv	QVQ LQE SGP GLV KPS QTL SLT CTV SGY SIS SDF AWN WIR QPP GKG LEW MGY ISY SGN TRY QPS LKS RIT ISR DTS KNQ FFL KLN SVT AAD TAT YYC VTA GRG FPY WGQ GTL VTV SSDIQ MTQ SPSS MSVS VGDR VTIT CHSS QDIN SNIG WLQQ KPGK SFGKLIYHG TNLD DGVP SRFS GSGS GTDY TLTI SSLQ PEDF ATYY CVQY AQFP WTFG GGTK LEIKR
45	CD8 hinge	ACCACTACCCAGCACCGAGGCCACCCACCCCGGCTCCTAC CATCGCCTCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTA GACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGAC TTCGCCTGCGAT
46	CD8 trans- membrane domain	ATCTACATTTGGGCCCTCTGGCTGGTACTTGCGGGGTCCTG CTGCTTTCACCTCGTGATCACTCTTTACTGT
47	4-1BB intra- cellular domain	AAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACC CTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCT GTTTCATGCCGGTTCAGAGGAGGAGGAAGGCGGCTGCCGA ACTG
48	CD3-zeta	CGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAA GCAGGGGCGAGAACCAGCTCTACAACGAACCTCAATCTTGGTC GGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACG GGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCC CAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGG CAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAG AAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGC ACCGCCACCAAGGACACCTATGACGCTCTTCACATGCAGGC CCTGCCGCCTCGG
49	CD8 signal recognition peptide	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTG CTGCTCCACGCCCGCCAGGCCG
50	DAPI2	ATGGGGGGGACTTGAACCCTGCAGCAGGTTCTGCTCCTGCC TCTCCTGCTGGCTGTAAGTGGTCTCCGTCTGTCAGGTCCA GGCCCAGAGCGATTGCAGTTGCTCTACGGTGAGCCCGGGCG TGCTGGCAGGGATCGTGATGGGAGACCTGGTGCTGACAGTG CTCATTGCCCTGGCCGTGTACTTCCTGGGCCGGCTGGTCCCT CGGGGGCGAGGGGCTGCGGAGGCAGCGACCCGGAAACAGC GTATCACTGAGACCGAGTCGCCTTATCAGGAGCTCCAGGGT CAGAGGTCGGATGTCTACAGCGACCTCAACACACAGAGGC CGTATTACAAA
51	T2A	GTCGAGGGCGGCGGAGAGGGCAGAGGAAGTCTTCTAACAT GCGGTGACGTGGAGGAGAATCCCGGCCCTAGG
52	Linker + KIRS2	GGTGGCGGAGGTTCTGGAGGTGGGGGTTCTTCACCCACTGA ACCAAGCTCCAAAACCGGTAACCCAGACACCTGCATGTTT

		TGATTGGGACCTCAGTGGTCAAAATCCCTTTCACCATCCTCC TCTTCTTCTCCTTCATCGCTGGTGCTCCAACAAAAAATG CTGCTGTAATGGACCAAGAGCCTGCAGGGAACAGAACAGT GAACAGCGAGGATTCTGATGAACAAGACCATCAGGAGGTG TCATACGCATAA
53	(pASP79) C225 BiTE	METDTLLLWVLLLWVPGSTGDILLTQSPVILSVSPGERVSFSCR ASQSIGTNIHWYQQRNNGSPRLLIKYASESISGIPSRFSGSGSGT DFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKGGGGS GGGSGGGGSQVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYG VHWVRQSPGKGLEWLGVIWSSGNTDYNTPFTSRLSINKDNSK SQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLVT VSAGGGGSDIKLQQSGAELARPGASVKMSCKTSGYTFTRYTM HWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTDDKSS STAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTV SSVEGGSGGSGGSGGSGGVDDIQLTQSPAISASPGKVTMTCTC RASSVSVMNWYQKSGTSPKRWIYDTSKVASGVPYRFSGSG SGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELK
54	(pASP83) 806 BiTE	METDTLLLWVLLLWVPGSTGDILMTQSPSSMSVSLGDTVSTIC HSSQDINSNIGWLQQRPGKSFKGLIYHGTNLDDEVPSRFSGSGS GADYSLTISSLESEDFADYYCVQYAQFPWTFGGGTKLEIKRGG GGSGGGSGGGGSDVQLQESGPSLVKPSQSLSTCTVTGYST SDFAWNWRQFPNGKLEWMGYISYSGNTRYNPSLKSRSITRD TSKNQFFLQLNSVTIEDTATYYCVTAGRGFPYWGQGLVTVS AGGGGSDIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMH WVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTDDKSSS TAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVS SVEGGSGGSGGSGGSGGVDDIQLTQSPAISASPGKVTMTCTC ASSVSVMNWYQKSGTSPKRWIYDTSKVASGVPYRFSGSGS GTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELK
55	Hu07 CAR (VL>VH)	MALPVTALLPLALLHAARPGSDIQMTQSPSSLSASVGDRVTH TCTASLSVSSTYLHWYQKPGSSPKLWYSTSNLASGVPSRFS GSGSGTSYTLTISSLQPEDFATYYCHQYHRSPLTFGGGTKVEIK GGGSGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGF SLTKYGVHWVRQAPGKGLEWVGKWAAGGSDYNSALMSRF TISKDNAKNSLYLQMNSLRAEDTAVYYCARDHRDAMDYWG QGTLVTVSSSGTTTPAPRPPTPAPTASQPLSLRPEACRPAAGG AVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKRGRKRL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGK RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR
56	Hu08 CAR (VL>VH)	MALPVTALLPLALLHAARPGSDIQMTQSPSSLSASVGDRVTH TCKASQDVGTAVAWYQIPGKAPKLLIYSASVSTGVPRFS GSGSGTDFSFHSSLQPEDFATYYCQHHYSAPWTFGGGTKVEIK GGGSGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGF TFSRNGMSWVRQTPDKRLEWVATVSSGGSYIYYADSVKGRFT ISRDNKNSLYLQMNSLRAEDTAVYYCARQGTALATRFDDV WGQGTLLVTVSSSGTTTPAPRPPTPAPTASQPLSLRPEACRPA GGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRS ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR
57	Hu07 VH	GAAGTACAGCTGGTTGAGAGTGGCGGGGGTCTCGTACAGC

		CCGGCGGGTCTCTTAGGCTCTCCTGTGCTGCTTCTGGTTTCT CCTTGACTAAATACGGGGTACATTGGGTTCCGACGGCCCCCT GGCAAAGGTCTTGAATGGGTGGGCGTCAAGTGGGCTGGCG GAAGCACTGATTATAAATCCGCATTGATGTCCCGATTCACT ATTTCTAAGGATAATGCCAAGAACAGTCTCTATTTGCAAAT GAACTCCCTGAGAGCGGAGGATACTGCCGTTTACTACTGTG CACGGGATCACCGAGACGCTATGGATTACTGGGGTCAGGGT ACCCTGGTGACCGTAAGCTCC
58	Hu07 HCDR1	ACTAAATACGGGGTACAT
59	Hu07 HCDR2	GGCGTCAAGTGGGCTGGCGGAAGCACTGATTATAATTCGC ATTGATGTCC
60	Hu07 HCDR3	GATCACCGAGACGCTATGGATTAC
61	Hu07 VL	GACATACAAATGACACAGTCCCCCTCATCCTTGTCTGCTTCC GTAGGAGACCGGGTTACCATCACGTGCACCGCTTCTTTGTC CGTTTCAAGTACCTACCTCCACTGGTACCAGCAAAAACCCG GCAGCAGCCCCAAGTTGTGGATTTACTCAACTTCTAACTTG GCCTCAGGGGTACCGTCAAGATTTAGCGGATCTGGCAGTGG CACGAGTTATACTTTGACGATATCAAGCCTTCAACCGGAGG ATTTCCGCCACCTATTACTGTTCATCAGTATCATCGAAGCCCCCT TGACCTTTGGGGGAGGGACAAAAGTGGAATAAAAA
62	Hu07 LCDR1	ACCGCTTCTTTGTCCGTTTCAAGTACCTACCTCCAC
63	Hu07 LCDR2	TCAACTTCTAACTTGGCCTCA
64	Hu07 LCDR3	CATCAGTATCATCGAAGCCCCCTTGACC
65	Hu07 CAR (VH>VL)	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTG CTGCTGCATGCCGCTAGACCCGGATCCGAAGTACAGCTGGT TGAGAGTGGCGGGGGTCTCGTACAGCCCGGGCGGGTCTCTTA GGCTCTCCTGTGCTGCTTCTGGTTTCTCCTTGACTAAATACG GGGTACATIGGGTTCGCCAGGCCCCTGGAAGGTCTTGAA TGGGTGGGCGTCAAGTGGGCTGGCGGAAGCACTGATTATA ATTCCGCATTGATGTCCCGATTCACTATTTCTAAGGATAATG CCAAGAACAGTCTCTATTTGCAAATGAACTCCCTGAGAGCG GAGGATACTGCCGTTTACTACTGTGCACGGGATCACCGAGA CGCTATGGATTACTGGGGTCAGGGTACCCTGGTGACCGTAA GCTCCGGGGGAGGTGGAAGTGGTGGCGGTGGATCTGGTGG CGGCGGGTCAGACATACAAATGACACAGTCCCCCTCATCCT TGTCTGCTTCCGTAGGAGACCGGGTTACCATCACGTGCACC GCTTCTTTGTCCGTTTCAAGTACCTACCTCCACTGGTACCAG CAAAAACCCGGCAGCAGCCCCAAGTTGTGGATTTACTCAAC TTCTAACTTGGCCTCAGGGGTACCGTCAAGATTTAGCGGAT CTGGCAGTGGCAGCAGTTATACTTTGACGATATCAAGCCTT CAACCGGAGGATTTCCGCCACCTATTACTGTTCATCAGTATCA TCGAAGCCCCCTTGACCTTTGGGGGAGGGACAAAAGTGGA ATAAAATCCGGAACCACGACGCCAGCGCCGCGACCAACAA CACCGGCGCCCAACATCGCGTGCAGCCCCCTGTCCCTGCGC CCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACA CGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCG CCCTTGGCCGGGACTTGTGGGGTCTTCTCCTGTCACTGGTT ATCACCCCTTACTGCAAACGGGGCAGAAAGAACTCCTGTA TATATTCAAACAACCATTTATGAGACCAGTACAACTACTC AAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGA AGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGGAGC GCAGACGCCCCCGCGTACAAGCAGGGCCAGAACCAGCTCT ATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGA

		AAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATG AACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGAT TGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGAT GGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTA CGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC
66	Hu07 CAR (VL>VH)	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTG CTGCTGCATGCCGCTAGACCCGGATCCGACATACAAATGAC ACAGTCCCCCTCATCCTTGTCTGCTTCCGTAGGAGACCGGG TTACCATCACGTGCACCGCTTCTTTGTCCGTTTCAAGTACCT ACCTCCACTGGTACCAGCAAAAACCCGGCAGCAGCCCCAA GTTGTGGATTACTCAACTTCTAACTTGGCCTCAGGGGTACC GTCAAGATTTAGCGGATCTGGCAGTGGCAGGATTATACTT TGACGATATCAAGCCTTCAACCGGAGGATTTCCGCCACCTAT TACTGTCATCAGTATCATCGAAGCCCCCTTGACCTTTGGGGG AGGGACAAAAGTGGAATAAAAGGGGGAGGTGGAAGTGG TGGCGGTGGATCTGGTGGCGGCGGGTCAGAAGTACAGCTG GTTGAGAGTGGCGGGGGTCTCGTACAGCCCGGCGGGTCTCT TAGGCTCTCCTGTGCTGCTTCTGGTTTCTCCTTGACTAAATA CGGGGTACATTGGGTTCGCCAGGCCCTGGCAAAGGTCTTG AATGGGTGGGCGTCAAGTGGGCTGGCGGAAGCACTGATTA TAATTCGCAATTGATGTCCCGATTCACTATTTCTAAGGATAA TGCCAAGAACAGTCTCTATTTGCAAATGAACTCCCTGAGAG CGGAGGATACTGCCGTTTACTACTGTGCACGGGATCACCGA GACGCTATGGATTACTGGGGTCAGGGTACCCTGGTGACCGT AAGCTCCTCCGGAACCACGACGCCAGCGCCGCGACCACCA ACACCGGCGCCACCATCGCGTCGCAGCCCCCTGTCCCTGCG CCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCAC ACGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGC GCCCTTGGCCGGGACTTGTGGGGTCTTCTCCTGTCACTGGT TATCACCCCTTACTGCAAACGGGGCAGAAAGAACTCCTGT ATATATTCAAACAACCATTTATGAGACCAGTACAACTACT CAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAG AAGAAGGAGGATGTGAAGTGAAGTGAAGTTTCAGCAGGAG CGCAGACGCCCCCGCGTACAAGCAGGGCCAGAACAGCTC TATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATG TTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGG AAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAAT GAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGA TTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGA TGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCT ACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC
67	Hu08 VH	GAGGTTCAAGTTGGTAGAGTCAGGCGGTGGTCTGGTGCAGCC AGGTGGGTCCCTGCGCCTCAGCTGTGCAGCTTCCGGCTTTA CTTTCTCAAGGAATGGTATGTCCTGGGTACGGCAAACGCCG GACAAACGCCTTGAGTGGGTAGCTACCGTATCCTCTGGGGG CTCTTACATATACTATGCAGACTCTGTGAAAGGAAGATTTA CAATTTACGCGACAATGCAAAAAATAGTTTGTACCTCCAA ATGTCTAGTCTTAGGGCCGAGGATACTGCCGTCTACTACTG TGCACGCCAGGGAACGACGGCTCTTGCTACCCGATTTTTCG ACGTTTGGGGCCAAGGAACGTTGGTGACAGTTAGCAGT
68	Hu08 HCDR1	TCAAGGAATGGTATGTCC
69	Hu08 HCDR2	ACCGTATCCTCTGGGGGCTCTTACATATACTATGCAGACTCT GTGAAAGGA
70	Hu08 HCDR3	CAGGGAACGACGGCTCTTGCTACCCGATTTTTTCGACGTT

71	Hu08 VL	GACATCCAAATGACTCAGAGCCCCTCTAGCCTCAGTGCAAG CGTCGGAGACCGGGTGACCATCACCTGTAAAGCGTCCCAGG ATGTTGGAACGGCAGTAGCTTGGTATCAACAAATCCCAGGG AAGGCTCCAAAGCTCCTTATATACTCTGCTAGTTACAGGTC CACCGGGGTGCCCCGACCGATTCTCTGGCTCCGGGAGCGGCA CTGACTTTTCATTCATCATTAGTAGTCTTCAACCTGAGGACT TTGCCACCTATTATTGCCAGCACCACTACTCTGCGCCGTGG ACTTTCGGAGGAGGCACGAAGGTTGAAATTTAAA
72	Hu08 LCDR1	AAAGCGTCCCAGGATGTTGGAACGGCAGTAGCT
73	Hu08 LCDR2	TCTGCTAGTTACAGGTCCACC
74	Hu08 LCDR3	CAGCACCACTACTCTGCGCCGTGGACT
75	Hu08 CAR (VH>VL)	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTG CTGCTGCATGCCGCTAGACCCGGATCCGAGGTTCAAGTTGGT AGAGTCAGGCGGTGGTCTGGTGCAGCCAGGTGGGTCCCTGC GCCTCAGCTGTGCAGCTTCCGGCTTTACTTTCTCAAGGAATG GTATGTCCTGGGTACGGCAAACGCCGGACAAACGCCTTGAG TGGGTAGCTACCGTATCCTCTGGGGGCTCTTACATATACTAT GCAGACTCTGTGAAAGGAAGATTTACAATTTACCGCGACAA TGCAAAAAATAGTTTGTACCTCCAAATGTCTAGTCTTAGGG CCGAGGATACTGCCGTCTACTACTGTGCACGCCAGGGAACG ACGGCTCTTGCTACCCGATTTTTTCGACGTTTGGGGCCAAGG AACGTTGGTGACAGTTAGCAGTGGTGGAGGTGGGTCTGGCG GAGGTGGAAGTGGTGGAGGCGGGTCCGACATCCAAATGAC TCAGAGCCCCTCTAGCCTCAGTGCAAGCGTCCGAGACCGGG TGACCATCACCTGTAAAGCGTCCCAGGATGTTGGAACGGCA GTAGCTTGGTATCAACAAATCCCAGGGAAGGCTCCAAAGCT CCTTATATACTCTGCTAGTTACAGGTCCACCGGGGTGCCCG ACCGATTCTCTGGCTCCGGGAGCGGCACTGACTTTTCATTC ATCATTAGTAGTCTTCAACCTGAGGACTTTGCCACCTATTAT TGCCAGCACCACTACTCTGCGCCGTGGACTTTTCGGAGGAGG CACGAAGGTTGAAATTTAAATCCGGAACCAACGACGCCAGCG CCGCGACCACCAACACCGGCGCCCAACATCGCGTCGCAGCC CCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGG GGCGCAGTGACACAGAGGGGGCTGGACTTCGCTGTGATAT CTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCTCTC TCCTGTCACTGGTTATCACCCCTTACTGCAAAACGGGGCAGA AAGAACTCCTGTATATATTCAAACAACCATTTATGAGACC AGTACAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGA TTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGA AGTTCAGCAGGAGCGCAGACGCCCGCGTACAAGCAGGG CCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGA GAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACC CTGAGATGGGGGGAAGCCGAGAAGGAAGAACCCTCAGGA AGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAG GCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGG GCAAGGGGACGATGGCCTTTACCAGGGTCTCAGTACAGCC ACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCC CCCTCGC
76	Hu08 CAR (VL>VH)	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTG CTGCTGCATGCCGCTAGACCCGGATCCGACATCCAAATGAC TCAGAGCCCCTCTAGCCTCAGTGCAAGCGTCCGAGACCGGG TGACCATCACCTGTAAAGCGTCCCAGGATGTTGGAACGGCA GTAGCTTGGTATCAACAAATCCCAGGGAAGGCTCCAAAGCT CCTTATATACTCTGCTAGTTACAGGTCCACCGGGGTGCCCG

		ACCGATTCTCTGGCTCCGGGAGCGGCACTGACTTTTCATTC ATCATTAGTAGTCTTCAACCTGAGGACTTTGCCACCTATTAT TGCCAGCACCACACTACTCTGCGCCGTGGACTTTTCGGAGGAGG CACGAAGGTTGAAATTAAGGTGGAGGTGGGTCTGGCGGA GGTGGAAAGTGGTGGAGGCGGGTCCGAGGTTCACTTGGTAG AGTCAGGCGGTGGTCTGGTGCAGCCAGGTGGGTCCCTGCGC CTCAGCTGTGCAGCTTCCGGCTTTACTTTCTCAAGGAATGGT ATGTCCTGGGTACGGCAAACGCCGGACAAACGCCTTGAGTG GGTAGCTACCGTATCCTCTGGGGGCTCTTACATATACTATG CAGACTCTGTGAAAGGAAGATTTACAATTTACGCGACAAT GCAAAAAATAGTTTGTACCTCCAAATGTCTAGTCTTAGGGC CGAGGATACTGCCGTCTACTACTGTGCACGCCAGGGAACGA CGGCTCTTGCTACCCGATTTTTTCGACGTTTGGGGCCAAGGA ACGTTGGTGACAGTTAGCAGTTCCGGAACCACGACGCCAGC GCCGCGACCACCAACACCGGCGCCCAACATCGCGTGCAGC CCCTGTCCCTGCGCCCAGAGGGCGTGCCGGCCAGCGGCGGGG GGCGCAGTGCACACGAGGGGGCTGGACTTCGCTGTGATAT CTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTC TCCTGTCACTGGTTATCACCCCTTACTGCAAACGGGGCAGA AAGAACTCCTGTATATATTCAAACAACCATTTATGAGACC AGTACAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGA TTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGA AGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGG CCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGA GAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACC CTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGA AGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCCGAG GCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGG GCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCC ACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCC CCCTCGC
77	Mu07 VH	QVQLKESGPGLVAPSQSLINCTVSGFSLTKYGVHWIRQSPGK GLEWLGVK WAGGSTDYNSALMSRLTISKDNKSKVFLKMNS LQSDDSAMYCARDHRDAMDYWGQGTSVTVSS
78	Mu07 VH	CAAGTGCAATTGAAGGAGAGCGGGCCAGGTTTGGTCGCCC CCTCCCAATCATTGTCCATTAACGTGACCGTCTCTGGTTTTA GTTTGACCAAATATGGAGTTCACTGGATCAGACAATCACCT GGCAAAGGACTCGAGTGGCTGGGGGTCAAGTGGGCAGGAG GCTCTACCGATTACAATTCTGCCCTGATGAGCCGACTTACT ATAAGCAAAGACAATAATAAGAGCCAAGTTTTCTGAAAAT GAACAGCCTGCAGAGCGATGACTCAGCCATGTACTACTGCG CCAGAGACCACCGCGACGCTATGGATTATTGGGGGCAGGG CACCAGTGTCACGGTATCAAGC
79	Mu07 HCDR1	TKYGVH
80	Mu07 HCDR1	ACCAAATATGGAGTTCAC
81	Mu07 HCDR2	GTCAAGTGGGCAGGAGGCTCTACCGATTACAATTCTGCCCT GATGAGC
82	Mu07 HCDR3	DHRDAMDY
83	Mu07 HCDR3	GACCACCGCGACGCTATGGATTAT
84	Mu07 VL	QVVLTSQSPAIMSASPGERVMTCTASLSVSSTYLHWYHQKPG SSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAAT YYCHQYHRSPLTFGSGTKLELK
85	Mu07 VL	CAGGTCGTGCTTACTCAGAGTCCCGCTATAATGAGTGCCAG TCCAGGTGAGCGGGTGACAATGACGTGTACGGCTAGTCTTT

		CTGTATCCAGTACTTATCTGCACTGGTATCATCAGAAACCG GGTAGCTCACC GAAGCTGTGGATCTACTCCACCTCCAATTT GGCATCTGGAGTTCAGCTAGGTTTCAGCGGTAGCGGCAGCG GGACATCCTACTCCCTGACAATTTCAAGCATGGAGGCGGAA GACGCGGCCACTTACTATTGTTCATCAATACCACCGGTCTCC ACTCACCTTTGGGAGTGGCACTAAACTTGAGCTTAAG
86	Mu07 LCDR1	TASLSVSSTYLH
87	Mu07 LCDR1	ACGGCTAGTCTTTCTGTATCCAGTACTTATCTGCAC
88	Mu07 LCDR2	STSNLAS
89	Mu07 LCDR2	TCCACCTCCAATTTGGCATCT
90	Mu07 LCDR3	HQYHRSPLT
91	Mu07 LCDR3	CATCAATACCACCGGTCTCCACTCACC
92	Mu07 CAR (VH>VL)	MALPVTALLLPLALLLHAARPGSQVQLKESGPGLVAPSQSLSI NCTVSGFSLTKYGVHWRQSPGKGLEWLGVKWAGGSTDYNS ALMSRLTISKDNKSKQVFLKMNSLQSDDSAMYYCARDHRDA MDYWGGQTSVTVSSGGGGSGGGSGGGGSQVVLTQSPAIMS ASPGERVMTCTASLSVSSTYLHWYHQPSSPKLWYYSTSNL ASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSPLT FGSGTKLELKS GTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAFLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYKQGQNLQLYNELNLGRREEYDVLDKRRGRDPGEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLY QGLSTATKDTYDALHMQALPPR
93	Mu07 CAR (VH>VL)	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTG CTGCTGCATGCCGCTAGACCCGGATCCCAAGTGCAATTGAA GGAGAGCGGGCCAGGTTTGGTGC GCCCCTCCCAATCATTGT CCATTAACTGTACCGTCTCTGGTITTTAGTTTGACCAATATG GAGTTCACCTGGATCAGACAATCACCTGGCAAAGGACTCGA GTGGCTGGGGGTCAAGTGGGCAGGAGGCTCTACCGATTAC AATTCTGCCCTGATGAGCCGACTTACTATAAGCAAAGACAA TAATAAGAGCCAAGTTTTCTGAAAATGAACAGCCTGCAGA GCGATGACTCAGCCATGTACTACTGCGCCAGAGACCACCGC GACGCTATGGATTATTGGGGGCAGGGCACCAGTGTCACGGT ATCAAGCGGTGGTGGGGGGT CAGGCGGAGGCGGTAGTGGA GGGGGAGGCAGTCAGGTCGTGCTTACTCAGAGTCCCGCTAT AATGAGTGCCAGTCCAGGTGAGCGGGTGACAATGACGTGT ACGGCTAGTCTTTCTGTATCCAGTACTTATCTGCACTGGTAT CATCAGAAACCGGGTAGCTCACC GAAGCTGTGGATCTACTC CACCTCCAATTTGGCATCTGGAGTTCCAGCTAGGTTTCAGCG GTAGCGGCAGCGGGACATCCTACTCCCTGACAATTTCAAGC ATGGAGGCGGAAGACGCGGCCACTTACTATGTTCATCAATA CCACCGGTCTCCACTCACCTTTGGGAGTGGCACTAAACTTG AGCTTAAGTCCGGAACCACGACGCCAGCGCCGCGACCACC AACACCGGCGCCACCATCGCGTCGCAGCCCTGTCCCTGC GCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCA CACGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGG CGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGG TTATCACCTTTACTGCAAACGGGGCAGAAAGAACTCCTG TATATATTC AAACAACCATTTATGAGACCAGTACAAACTAC TCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAA GAAGAAGGAGGATGTGAAGTGAAGTGAAGTTCAGCAGGA GCGCAGACGCCCCCGGTACAAGCAGGGGCCAGAACCAGCT CTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGAT

		GTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGG GAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAA TGAAGTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAG ATTGGGATGAAAGGCGAGCGCCGGAGGGCAAGGGGCACG ATGGCCTTTACCAGGTCTCAGTACAGCCACCAAGGACACC TACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC
94	Mu07 CAR (VL>VH)	MALPVTALLPLALLHAARPGSQVVLTSQSPAIMSASPGERV MTCTASLSVSSTYLHWYHQKPGSSPKLWYSTSNLASGVPARF SGSGSGTSYSLTISSMEAEDAATYYCHQYHRSPLTFGSGTKLE LKGGGSGGGGSGGGGSGVQLKESGPGLVAPSQSLINCTVS GFSLTKEYGVHWIRQSPGKGLEWLGVKWAGGSTDYNSALMSR LTISKDNNKSQVFLKMNSLQSDDSAMYYCARDHRDAMDYW GQGTSTVTVSSGTTTPAPRPPTPAPTASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYKQGQNLQYLNELNLGRREEYDVLDRRRGRDPEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLY QGLSTATKDTYDALHMQALPPR
95	Mu07 CAR (VL>VH)	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTG CTGCTGCATGCCGCTAGACCCGGATCCCAGGTCGTGCTTAC TCAGAGTCCCGCTATAATGAGTGCCAGTCCAGGTGAGCGGG TGACAATGACGTGTACGGCTAGTCTTTCTGTATCCAAGTACTT ATCTGCACTGGTATCATCAGAAACCGGGTAGCTCACCGAAG CTGTGGATCTACTCCACCTCCAATTTGGCATCTGGAGTICCA GCTAGGTTTACGCGGTAGCGGCAGCGGGACATCCTACTCCCT GACAATTTCAAGCATGGAGGCGGAAGACGCGGCCACTTAC TATTGTCATCAATACCACCGGTCTCCACTCACCTTTGGGAGT GGCACTAAACTTGAGCTTAAGGGTGGTGGGGGGTCAAGCG GAGGCGGTAGTGGAGGGGGAGGCAGTCAAGTGCAATTGAA GGAGAGCGGGCCAGGTTTGGTCGCCCCCTCCCAATCATTGT CCATTAACTGTACCGTCTCTGGTTTTAGTTTGACCAAAATATG GAGTTCACCTGGATCAGACAATCACCTGGCAAAGGACTCGA GTGGCTGGGGGTCAAGTGGGCAGGAGGCTCTACCGATTAC AATTCTGCCCTGATGAGCCGACTTACTATAAGCAAAGACAA TAATAAGAGCCAAGTTTTCTGAAAATGAACAGCCTGCAGA GCGATGACTCAGCCATGTACTACTGCGCCAGAGACCACCGC GACGCTATGGATTATTGGGGGCAGGGCACCAGTGTACGGT ATCATCCGGAACCACGACGCCAGCGCCGCGACCACCAACA CCGGCGCCACCATCGCGTCGCAGCCCCCTGTCCCTGCGCCC AGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACG AGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCGCC CTTGGCCGGGACTTGTGGGGTCTTCTCCTGTCACTGGTTAT CACCTTTACTGCAAACGGGGCAGAAAGAACTCCTGTATA TATTCAAACAACCATTATGAGACCAGTACAACTACTCAA GAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAG AAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGC AGACGCCCCCGCGTACAAGCAGGGCCAGAACCAGCTCTAT AACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTT TGGACAAGAGACGTGGCCGGGACCCTGAGATCGGGGGAAA GCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAA CTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTG GGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATG GCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTAC GACGCCCTTCACATGCAGGCCCTGCCCCCTCGC

96	Mu08 VH	EVQLVESGGDLVRPGGSLQLSCAASGFTFSRNGMSWVRQTPD RRLEWVATVSSGGSYIYYADSVKGRFTISRDNARNTLYLQMS SLKSEDTAMYYCARQGTTALATRFDDVWGAGTTVTVSS
97	Mu08 VH	GAGGTGCAACTCGTTGAATCAGGTGGGGACTTGGTGCGCCC AGGAGGTAGCCTGCAATTGAGCTGTGCTGCTAGCGGGTTCA CTTTTTACGGAACGGTATGTCTTGGGTACGGCAGACCCCT GACAGAAGACTGGAGTGGGTTGCAACTGTCACTTCTGGTGG CTCCTATATTTACTACGCAGACAGCGTAAAAGGGAGATTTA CCATAAGCCGGGATAATGCCCCGAAATACCTCTACCTCCAG ATGTCCTCCTTGAAAAGTGAGGACACGGCTATGTACTATTG CGCCAGACAAGGAACCACTGCACTTGCAACGAGATTTTTTG ACGTTTGGGGAGCCGGGACCACCGTAACTGTGAGTAGC
98	Mu08 HCDR1	SRNGMS
99	Mu08 HCDR1	TCACGGAACGGTATGTCT
100	Mu08 HCDR2	TVSSGGSYIYYADSVKG
101	Mu08 HCDR2	ACTGTCAGTTCTGGTGGCTCCTATATTTACTACGCAGACAG CGTAAAAGGG
102	Mu08 HCDR3	CAAGGAACCACTGCACTTGCAACGAGATTTTTTGAC
103	Mu08 VL	DIVMTQSHKFISTSVGDRVSITCKASQDVGTAVAWYQQIPGQS PKLLIYSASYRSTGIPDRFTGSGSGTDFSFHSSVQAEDLALYYC QHHYSAPWTFGGGTTLDIK
104	Mu08 VL	GACATTGTTATGACGCAGTCTCATAAGTTCATCTCTACATCC GTCGGGGACCGGGTGAGCATTACCTGTAAAGCCTCCAGGA TGTAGGTACAGCTGTTGCATGGTACCAGCAAATACCGGGTC AGTCTCCGAAACTCCTGATTACAGCGCCTCCTATCGAAGC ACCGGATACCTGATAGATTACTGGATCAGGTTACGGGAC AGACTTCAGTTTTATCATCAGCTCTGTGCAAGCAGGAGATC TCGCGCTTTACTACTGTCAGCATCATTACAGCGCTCCGTGG ACGTTTCGGCGGGCGGGACAACCTGGATATCAAA
105	Mu08 LCDR1	KASQDVGTAVA
106	Mu08 LCDR1	AAAGCCTCCAGGATGTAGGTACAGCTGTTGCA
107	Mu08 LCDR2	SASYRST
108	Mu08 LCDR2	AGCGCCTCCTATCGAAGCACC
109	Mu08 LCDR3	QHHYSAPWT
110	Mu08 LCDR3	CAGCATCATTACAGCGCTCCGTGGACG
111	Mu08 CAR (VH>VL)	MALPVTALLLPLALLHAARPGSEVQLVESGGDLVRPGGSLQ LSCAASGFTFSRNGMSWVRQTPDRRLEWVATVSSGGSYIYYA DSVKGRFTISRDNARNTLYLQMSSLKSEDTAMYYCARQGTTA LATRFDDVWGAGTTVTVSSGGGGSGGGGSGGGGSDIVMTQS HKFISTSVGDRVSITCKASQDVGTAVAWYQQIPGQSPKLLIYS ASYRSTGIPDRFTGSGSGTDFSFHSSVQAEDLALYYCQHHYSA PWTFGGGTTLDIKSGTTTPAPRPPTAPTASQPLSLRPEACRPA AGGAVHTRGLDFACDIYWAPLAGTCGVLLLLSLVITLYCKRGR KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS RSADAPAYKQGQNLQLYNELNLGRREEYDVLDKRRGRDPENMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR
112	Mu08 CAR (VH>VL)	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTG CTGCTGCATGCCGCTAGACCCGGATCCGAGGTGCAACTCGT TGAATCAGGTGGGGACTTGGTGCGCCCAGGAGGTAGCCTGC AATTGAGCTGTGCTGCTAGCGGGTTCACTTTTTACGGAAC GGTATGTCTTGGGTACGGCAGACCCCTGACAGAAGACTGGA GTGGGTTGCAACTGTCACTGTCTGTGGCTCCATATATTTACTA CGCAGACAGCGTAAAAGGGAGATTTACCATAAGCCGGGAT

		AATGCCCCGAAATACCCCTCTACCTCCAGATGTCCTCCTTGAA AAGTGAGGACACGGCTATGTACTATTGCGCCAGACAAGGA ACCACTGCACTTGCAACGAGATTTTTTGACGTTTGGGGAGC CGGGACCACCGTAACCTGTGAGTAGCGGGGGCGGTGGTAGC GGTGGAGGTGGGTACGGGGGTGGTGGTTCAGACATTGTTAT GACGCAGTCTCATAAGTTCATCTCTACATCCGTCGGGGACC GGGTGAGCATTACCTGTAAAGCCTCCCAGGATGTAGGTACA GCTGTTCATGGTACCAGCAAATACCGGGTCAGTCTCCGAA ACTCCTGATTTACAGCGCCTCCTATCGAAGCACCGGGATAC CTGATAGATTTACTGGATCAGGTTCAGGGACAGACTTCAGT TTTATCATCAGCTCTGTGCAAGCAGAGGATCTCGCGCTTTA CTACTGTCAGCATCATTACAGCGCTCCGTGGACGTTTCGGCG GCGGGACAACCTGGATATCAAATCCGGAACACGACGCC AGCGCCGCGACCACCAACACCGGCGCCCAACATCGCGTCGC AGCCCCGTGCCCTGCGCCAGAGGCGTGCCGGCCAGCGGCG GGGGGCGCAGTGACACAGAGGGGGCTGGACTTCGCTGTG ATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTC CTTCTCCTGTCACTGGTTATCACCTTTACTGCAAACGGGGC AGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAG ACCAGTACAACTACTCAAGAGGAAGATGGCTGTAGCTGC CGATTTCCAGAAGAAGAAGAAGGAGGATGTGAAGTGAAG TGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCA GGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGA AGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGG ACCTTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCA GGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCG GAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGA GGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTAC AGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCC TGCCCCCTCGC
113	Mu08 CAR (VL>VH)	MALPVTALLPLALLHAARPGSDIVMTQSHKFISTSVGDRVSI TCKASQDVGTAVAWYQQIPGQSPKLLIYSASYRSTGIPDRFTG SGSGTDFSFHSSVQAEDLALYYCQHHSAPWTFGGGTLDIKG GGGSGGGGSGGGGSEVQLVESGGDLVRPGSLQLSCAASGFT FSRNGMSWVRQTPDRRLEWVATVSSGGSYIYYADSVKGRFTI SRDNARNTLYLQMSSLKSEDTAMYYCARQGTALATRFDDV WGAGTTTVTVSSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSA DAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPGEMGGKP RRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGK GHDGLY QGLSTATKDTYDALHMQALPPR
114	Mu08 CAR (VL>VH)	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTG CTGCTGCATGCCGCTAGACCCGGATCCGACATTGTTATGAC GCAGTCTCATAAGTTCATCTCTACATCCGTCGGGGACCGGG TGAGCATTACCTGTAAAGCCTCCCAGGATGTAGGTACAGCT GTTGCATGGTACCAGCAAATACCGGGTCAGTCTCCGAAACT CCTGATTTACAGCGCCTCCTATCGAAGCACCGGGATACCTG ATAGATTTACTGGATCAGGTTCAGGGACAGACTTCAGTTTT ATCATCAGCTCTGTGCAAGCAGAGGATCTCGCGCTTTACTA CTGTCAGCATCATTACAGCGCTCCGTGGACGTTTCGGCGGCG GGACAACCTGGATATCAAAGGGGGGCGGTGGTAGCGGTGG AGGTGGGTACGGGGGTGGTGGTTCAGAGGTGCAACTCGTTG AATCAGGTGGGGACTTGGTGCGCCCAAGGAGGTAGCCTGCA ATTGAGCTGTGCTGCTAGCGGGTTCACCTTTTACCGGAACG

		GTATGTCTTGGGTACGGCAGACCCCTGACAGAAGACTGGAG TGGGTTGCAACTGTCAGTTCTGGTGGCTCCTATATTTACTAC GCAGACAGCGTAAAAGGGAGATTTACCATAAGCCGGGATA ATGCCCCGAAATACCCCTCTACCTCCAGATGTCCTCCTTGAAA AGTGAGGACACGGCTATGTACTATTGCGCCAGACAAGGAA CCACTGCACCTTGCAACGAGATTTTTTGACGTTTGGGGAGCC GGGACCACCGTAACGTGTGAGTTCCGGAACCACGACGCCAG CGCCGCGACCAACACACCGGCGCCACCATCGCGTCGCAG CCCCTGTCCCTGCGCCCAAGGGCGTGCCGGCCAGCGGCGGG GGGCGCAGTGACACAGAGGGGGCTGGACTTCGCCTGTGAT ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCTT TCTCCTGTCACTGGTTATCACCTTTACTGCAAACGGGGCA GAAAGAACTCCTGTATATATTCAAACAACCATTTATGAGA CCAGTACAACTACTCAAGAGGAAGATGGCTGTAGCTGCC GATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGT GAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAG GGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAA GAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGA CCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAG GAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGG AGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAG GGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACA GCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCT GCCCCCTCGC
115	Secreting signaling	METDTLLLWVLLLWVPGSTG
116	Secreting signaling	ATGGAAACAGATACATTGTTGTTGTGGGTACTCCTGCTGTG GGTCCCTGGGAGCACCGGT
117	C225 scFv	DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNGSPR LLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQN NNWPTTFGAGTKLELKGGGSGGGGSGGGGSGVQLKQSGPG LVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKLEWLGVWS GGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSDNTAIYYCA RALIYYDYEFAYWGQGILVTVSA
118	C225 scFv	GACATACTTCTACACAATCTCCCGTGATTCTCAGCGTATCA CCAGGTGAAAGGGTGAGCTTCTCTGTGCGCCAGCCAATC CATCGGGACTAATATCCACTGGTATCAGCAGCGAACGAATG GGAGCCCAACGGCTTCTTATTAAGTACGCCAGTGAGTCAATT TCAGGTATCCCGAGCCGATTCAAGTGAAGTGGGAGTGGGA CTGACTTCACTTTGAGCATCAATTCGTCGAGTCTGAGGAC ATAGCCGATTATTATTGCCAACAGAATAACAACCTGGCCGAC TACTTTTGGGGCGGGTACAAAACCTCGAACTCAAGGGTGGGG GTGGATCTGGCGGAGGTGGGTCCGGGGGGGGAGGCTCTCA AGTCCAGCTCAAACAAAGCGGACCGGGATTGGTGCAACCC TCTCAATCTCTCTCCATAACGTGTACGGTGTCCGGTTTTTCT CTCACCAACTACGGTGTCCATTGGGTACGGCAATCTCCAGG CAAGGGCCTGGAATGGCTTGGTGTATCTGGAGCGGCGGGA ATACTGACTATAATAACCCATTACGAGCAGGCTCAGCATT AACAAAGACAATTCAAAGTCACAAGTATTCTTCAAGATGAA CTCACTTCAGTCCAATGATACTGCAATATACTACTGCGCGA GAGCCCTTACATACTATGACTATGAGTTCGCTTACTGGGGT CAAGGTACGTTGGTCAACCGTCTCCGCC
119	806 BiTE scFv	DILMTQSPSSMSVSLGDTVSIHCHSSQDINSNIGWLQQRPGKSF KGLIYHGNTLDDDEVPSRFSGSGSGADYSLTISSESEDFADYYC

		VQYAQFPWTFGGGKLEIKRGGGSGGGGSGGGGSDVQLQE SGPSLVKPSQSLSLTCTVTGYSTSDFAWNWIRQFPGNKLEWM GYISYSGNTRYNPISLKSRSITRDTSKNQFFLQLNSVTIEDTATY YCVTAGRGFPYWGGTLVTVSA
120	806 BiTE scFv	GATATTCTGATGACTCAATCTCCGTCCTTCTATGAGCGTGAGC TTGGGTGACACCGTCAGCATCACCTGTCTATCCAGCCAGGA TATAAACTCAAATATCGGCTGGCTCCAGCAACGCCAGGCA AGTCATTCAAGGGGCTTATTTATCATGGCACCAATCTTGAC GATGAAGTCCCATCACGCTTCAGCGGATCAGGCTCAGGTGC GGACTATTCTTGACTATAAGTTCCCTCGAATCTGAGGATTT CGCCGACTATTATTGCGTACAATACGCCCAGTTTCCCTGGA CCTTCGGAGGCGGCACCAAATTGGAGATAAAAAGGGGTGG AGGAGGATCAGGCGGGGGTGGAAAGCGGCGGAGGAGGCAG CGACGTACAACCTGCAAGAATCCGGGCCGAGTTTGGTCAAGC CCTCTCAATCTCTTTCTCTCACTTGCACGGTCACCGGATACT CCATAACCAGCGATTTTTCGTGGAATTGGATTTCGACAATTT CCAGGGAATAAATTGGAATGGATGGGATATATCAGTTATTC TGGTAATACCAGATACAACCCGTCATTGAAAAGTCGCATCT CTATAACACGAGACACTTCAAAGAATCAGTTCTTCCCTTCAG CTCAATTCTGTAACCATCGAAGATACTGCTACTTATTACTGT GTAACGGCGGGTCGAGGATTCCTTACTGGGGCCAGGGTAC ACTGGTTACTGTTCCGCC
121	OKT3 scFv	DIKLQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRP GQGLEWIGYINPSRGYTNYNQKFKDKATLTIDKSSSTAYMQL SSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSG SGGSGGSGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSY MNWYQQKSGTSPKRWTYDTSKVASGVPYRFSGSGSGTSYSLTI SSMEAEDAATYYCQQWSSNPLTFGAGTKLELK
122	OKT3 scFv	GATATTAAGCTCCAGCAATCAGGGGCAGAATTGGCCCCGCC CGGTGCAAGCGTGAAAATGTCCTGCAAGACTAGCGGATAC ACTTTTACCAGATACACGATGCACTGGGTAAACAGCGACC GGGGCAAGGCTTGGAGTGGATCGGATATATTAACCCAAGTC GCGGCTACACGAATTACAACCAGAAATTCAAAGACAAGGC AACACTGACCACAGATAAATCATCATCTACCGGTATATGC AACTGAGTTCACCTACTAGCGAGGATTCTCGGATATATTAC TGTGCGCGGTACTACGACGACCATTACTGTCTGGACTATTG GGGTCAAGGCACCAACCCTTACTGTGAGTTCAGTAGAAGGAG GCAGTGGGGGCTCTGGAGGGAGCGGTGGCTCAGGAGGGGT AGACGACATCCAACCTGACGCAATCTCCGGCTATAATGTCAG CGTCTCCGGGGGAAAAAGTAACGATGACTTGTGCGCGCTCC AGCAGCGTCTCTTATATGAACTGGTATCAACAGAAGAGTGG GACGAGTCCTAAGCGATGGATATATGATACAAGCAAAGTT GCGAGCGGAGTCCCGTATCGCTTCTCTGGAAGTGGCAGCGG AACCTCTTACTCCCTCACGATCAGCAGCATGGAGGCGGAGG ACGCAGCCACCTACTACTGTCAGCAGTGGTCTTCCAACCT CTGACATTCCGAGCCGGTACAAAACCTTGAAGTGAAG
123	C225 BiTE	ATGGAAACAGATACATTGTTGTTGTTGGGTACTCCTGCTGTG GGTCCCTGGGAGCACCGGTGACATACTTCTCACACAATCTC CCGTGATTCTCAGCGTATCACCAGGTGAAAGGGTGAGCTTC TCTTGTCGCGCCAGCCAATCCATCGGGACTAATATCCACTG GTATCAGCAGCGAACGAATGGGAGCCCACGGCTTCTTATTA AGTACGCCAGTGAGTCAATTTAGGTATCCCGAGCCGATTTC AGTGGAAGTGGGAGTGGGACTGACTTCACTTTGAGCATCAA TTCCGTCGAGTCTGAGGACATAGCCGATTATTATTGCCAAC

		AGAATAACAACCTGGCCGACTACTTTTGGGGCGGGTACAAA ACTCGAACTCAAGGGTGGGGGTGGATCTGGCGGAGGTGGG TCCGGGGGGGGAGGCTCTCAAGTCCAGCTCAAACAAAGCG GACCGGGATTGGTGCAACCCCTCTCAATCTCTCCATAACG TGTACGGTGTCCGGTTTTTCTCTCACCAACTACGGTGTCCAT TGGGTACGGCAATCTCCAGGCAAGGGCCTGGAATGGCTTGG TGTTATCTGGAGCGGCGGGAATACTGACTATAATACCCCAT TCACGAGCAGGCTCAGCATTAACAAAGACAATTCAAAGTC ACAAGTATTCTTCAAGATGAACTCACTTCAGTCCAATGATA CTGCAATATACTACTGCGCGAGAGCCCTTACATACTATGAC TATGAGTTCGCTTACTGGGGTCAAGGTACGTTGGTCACCGT CTCCGCCGGCGGAGGAGGAAGTGATATTAAGCTCCAGCAA TCAGGGGCAGAATTGGCCCCGCCCGGTGCAAGCGTGAAAA TGTCTGCAAGACTAGCGGATACACTTTTACCAGATACACG ATGCACTGGGTAAACAGCGACCGGGGCAAGGCTTGGAGT GGATCGGATATATTAACCCAAGTCGCGGCTACACGAATTAC AACCAGAAATTCAAAGACAAGGCAACACTGACCACAGATA AATCATCATCTACCGCGTATATGCAACTGAGTTCACCTACT AGCGAGGATTCTGCGGTATATTACTGTGCGCGGTACTACGA CGACCATTACTGTCTGGACTATTGGGGTCAAGGCACCACCC TTAAGTGTGAGTTCAGTAGAAGGAGGCAGTGGGGGCTCTGGA GGGAGCGGTGGCTCAGGAGGGGTAGACGACATCCAAGTGA CGCAATCTCCGGCTATAATGTCAGCGTCTCCGGGGGAAAAA GTAACGATGACTTGTGCGCGGTCCAGCAGCGTCTCTTATAT GAACTGGTATCAACAGAAGAGTGGGACGAGTCTTAAGCGA TGGATATATGATACAAGCAAAGTTGCGAGCGGAGTCCCGTA TCGCTTCTCTGGAAGTGGCAGCGGAACCTCTTACTCCCTCA CGATCAGCAGCATGGAGGCGGAGGACGCAGCCACCTACTA CTGTCAGCAGTGGTCTTCCAACCCTCTGACATTTCGGAGCCG GTACAAAACCTTGAAGTGAAG
124	806 BiTE	ATGGAAACAGATACATTGTTGTTGTGGGTACTCCTGCTGTG GGTCCCTGGGAGCACCGGTGATATTCTGATGACTCAATCTC CGTCTTCTATGAGCGTGAGCTTGGGTGACACCGTCAGCATC ACCTGTCAATTCCAGCCAGGATATAAACTCAAATATCGGCTG GCTCCAGCAACGCCCAGGCAAGTCATTCAAGGGGCTTATTT ATCATGGCACCAATCTTGACGATGAAGTCCCATCACGCTTC AGCGGATCAGGCTCAGGTGCGGACTATTCTTGACTATAAG TTCCCTCGAATCTGAGGATTTTCGCCGACTATTATTGCGTACA ATACGCCCAGTTTCCCTGGACCTTCGGAGGCGGCACCAAAT TGGAGATAAAAAGGGGTGGAGGAGGATCAGGCGGGGGTGG AAGCGGCGGAGGAGGCAGCGACGTACAAGTGAAGAATCC GGGCCGAGTTTGGTCAAGCCCTCTCAATCTCTTCTCTCACT TGCACGGTCACCGGATACTCCATAACCAGCGATTTTGCGTG GAATTGGATTTCGACAATTTCCAGGGAATAAATTGGAATGGA TGGGATATATCAGTTATTCTGGTAATACCAGATACAACCCG TCATTGAAAAGTCGCATCTCTATAACACGAGACACTTCAA GAATCAGTTCTTCCCTCAGCTCAATTCTGTAACCATCGAAG ATACTGCTACTTATTACTGTGTAACGGCGGGTCGAGGATTC CCCTACTGGGGCCAGGGTACACTGGTTACTGTTTCCGCCGG AGGAGGAGGAAGTGATATTAAGCTCCAGCAATCAGGGGCA GAATTGGCCCCGCCCGGTGCAAGCGTGAAAATGTCCTGCAA GACTAGCGGATACACTTTTACCAGATACACGATGCACTGGG TTAAACAGCGACCGGGGCAAGGCTTGGAGTGGATCGGATA TATTAACCCAAGTCGCGGCTACACGAATTACAACCAGAAAT TCAAAGACAAGGCAACACTGACCACAGATAAATCATCATCT

		ACCGCGTATATGCAACTGAGTTCACTTACTAGCGAGGATTC TGCGGTATATTACTGTGCGCGGTACTACGACGACCATTACT GTCTGGACTATTGGGGTCAAGGCACCACCCTTACTGTGAGT TCAGTAGAAGGAGGCAGTGGGGGCTCTGGAGGGAGCGGTG GCTCAGGAGGGGTAGACGACATCCAAGTACGCAATCTCC GGCTATAATGTCAGCGTCTCCGGGGGAAAAAGTAACGATG ACTTGTGCGCGCTCCAGCAGCGTCTCTTATATGAACTGGTA TCAACAGAAGAGTGGGACGAGTCCTAAGCGATGGATATAT GATACAAGCAAAGTTGCGAGCGGAGTCCCGTATCGCTTCTC TGGAAGTGGCAGCGGAACCTCTTACTCCCTCAGCATCAGCA GCATGGAGGCGGAGGACGAGCCACCTACTACTGTGTCAGCA GTGGTCTTCCAACCCTCTGACATTCGGAGCCGGTACAAAAC TTGAACTGAAA
125	Mu07 scFv (VH > VL)	QVQLKESGPGLVAPSQSLINCTVSGFSLTKYGVHWIRQSPGK GLEWLGVKWAGGSTDYNALMSRLTISKDNNKSQVFLKMNS LQSDDSAMYYCARDHRDAMDYWGQGSVTVSSGGGGSGGG GSGGGGSQVVLTSQSPAIMSASPGERVMTCTASLSVSSTYLH WYHQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSM EAEDAATYYCHQYHRSPLTFGSGTKLELK
126	Mu07 scFv (VH > VL)	CAAGTGCAATTGAAGGAGAGCGGGCCAGGTTTGGTTCGCCC CCTCCCAATCATTGTCCATTAAGTGTACCGTCTCTGGTTTAA GTTTGACCAAATATGGAGTTCAGTGGATCAGACAATCACCT GGCAAAGGACTCGAGTGGCTGGGGGTCAAGTGGGCAGGAG GCTCTACCGATTACAATTCGCCCCTGATGAGCCGACTTACT ATAAGCAAAGACAATAATAAGAGCCAAGTTTTTCTGAAAAT GAACAGCCTGCAGAGCGATGACTCAGCCATGTACTACTGCG CCAGAGACCACCGCGACGCTATGGATTATTGGGGGCAGGG CACCAGTGTACGGTATCAAGCGGTGGTGGGGGGTTCAGGC GGAGGCGGTAGTGGAGGGGGAGGCAGTCAGGTCGTGCTTA CTCAGAGTCCCGCTATAATGAGTGCCAGTCCAGGTGAGCGG GTGACAATGACGTGTACGGCTAGTCTTTCTGTATCCAGTAC TTATCTGCACTGGTATCATCAGAAACCGGGTAGCTCACCAG AGCTGTGGATCTACTCCACCTCCAATTTGGCATCTGGAGTTC CAGCTAGGTTTCAGCGGTAGCGGCAGCGGGACATCCTACTCC CTGACAATTTCAAGCATGGAGGCGGAAGACGCGGCCACTT ACTATTGTCAATCAATACCACCGGTCTCCACTCACCTTTGGGA GTGGCACTAAACTTGAGCTTAAG
127	Mu07 scFv (VL > VH)	QVVLTSQSPAIMSASPGERVMTCTASLSVSSTYLHWYHQKPG SSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAAT YYCHQYHRSPLTFGSGTKLELKGGGGSGGGSGGGGSQVQL KESGPGLVAPSQSLINCTVSGFSLTKYGVHWIRQSPGKLEW LGVKWAGGSTDYNALMSRLTISKDNNKSQVFLKMNSLQSD DSAMYYCARDHRDAMDYWGQGSVTVSS
128	Mu07 scFv (VL > VH)	CAGGTCGTGCTTACTCAGAGTCCCGCTATAATGAGTGCCAG TCCAGGTGAGCGGGTGACAATGACGTGTACGGCTAGTCTTT CTGTATCCAGTACTTATCTGCACTGGTATCATCAGAAACCG GGTAGCTCACCAGAGCTGTGGATCTACTCCACCTCCAATTT GGCATCTGGAGTTCAGCTAGGTTTCAGCGGTAGCGGCAGCG GGACATCCTACTCCCTGACAATTTCAAGCATGGAGGCGGAA GACGCGGCCACTTACTATTGTCAATACCACCGGTCTCC ACTCACCTTTGGGAGTGGCACTAAACTTGAGCTTAAGGGTG GTGGGGGGTCAAGGCGGAGGCGGTAGTGGAGGGGGAGGCAG TCAAGTGCAATTGAAGGAGAGCGGGCCAGGTTTGGTTCGCCC CCTCCCAATCATTGTCCATTAAGTGTACCGTCTCTGGTTTAA

		GTTTGACCAAATATGGAGTTCACTGGATCAGACAATCACCT GGCAAAGGACTCGAGTGGCTGGGGGTCAAGTGGGCAGGAG GCTCTACCGATTACAATTCTGCCCTGATGAGCCGACTTACT ATAAGCAAAGACAATAAAGAGCCAAGTTTTCTGAAAAT GAACAGCCTGCAGAGCGATGACTCAGCCATGTACTACTGCG CCAGAGACCACCGCGACGCTATGGATTATTGGGGGCAGGG CACCAGTGTCACGGTATCAAGC
129	Mu08 scFv (VH > VL)	EVQLVESGGDLVRPGGSLQLSCAASGFTFSRNGMSWVRQTPD RRLEWVATVSSGGSYIYYADSVKGRFTISRDNARNTLYLQMS SLKSEDTAMYCARQGTTALATRFDDVWGAGTTVTVSSGGG GSGGGSGGGSDIVMTQSHKFISTSVGDRVSITCKASQDVG TAVAWYQQIPGQSPKLLIYSASYRSTGIPDRFTGSGSGTDFSFHIS SVQAEDLALYYCQHHYSAPWTFGGGTTLTDIK
130	Mu08 scFv (VH > VL)	GAGGTGCAACTCGTTGAATCAGGTGGGGACTTGGTGCGCCC AGGAGGTAGCCTGCAATTGAGCTGTGCTGCTAGCGGGTTCA CTTTTTCACGGAACGGTATGTCTTGGGTACGGCAGACCCCT GACAGAAGACTGGAGTGGGTTGCAACTGTCAGTTCTGGTGG CTCCTATATTTACTACGCAGACAGCGTAAAAGGGAGATTTA CCATAAGCCGGGATAATGCCCCGAAATACCCTCTACCTCCAG ATGTCCTCCTTGAAAAGTGAGGACACGGCTATGTACTATTG CGCCAGACAAGGAACCACTGCACTTGCAACGAGATTTTTTG ACGTTTGGGGAGCCGGGACCAACCGTAACTGTGAGTAGCGG GGGCGGTGGTAGCGGTGGAGGTGGGTCAGGGGGTGGTGGT TCAGACATIGTTATGACGCAGTCTCATAAGTTTCATCTCTACA TCCGTCGGGGACCGGGTGAGCATTACCTGTAAAGCCTCCCA GGATGTAGGTACAGCTGTTGCATGGTACCAGCAAATACCGG GTCAGTCTCCGAAACTCCTGATTTACAGCGCCTCCTATCGA AGCACCGGGATACCTGATAGATTTACTGGATCAGGTTTCAGG GACAGACTTCAGTTTTATCATCAGCTCTGTGCAAGCAGAGG ATCTCGCGCTTTACTACTGTCAGCATCATTACAGCGCTCCGT GGACGTTCCGGCGGCGGGACAACCCTGGATATCAAA
131	Mu08 scFv (VL > VH)	DIVMTQSHKFISTSVGDRVSITCKASQDVGTA VAWYQQIPGQS PKLLIYSASYRSTGIPDRFTGSGSGTDFSFHSSVQAEDLALYYC QHHYSAPWTFGGGTTLTDIKGGGGSGGGSGGGSEVQLVESG GDLVRPGGSLQLSCAASGFTFSRNGMSWVRQTPDRRLEWVAT VSSGGSYIYYADSVKGRFTISRDNARNTLYLQMSLSEDTAM YYCARQGTTALATRFDDVWGAGTTVTVSS
132	Mu08 scFv (VL > VH)	GACATTGTTATGACGCAGTCTCATAAGTTTCATCTCTACATCC GTCGGGGACCGGGTGAGCATTACCTGTAAAGCCTCCCAGGA TGTAGGTACAGCTGTTGCATGGTACCAGCAAATACCGGGTC AGTCTCCGAAACTCCTGATTTACAGCGCCTCCTATCGAAGC ACCGGGATACCTGATAGATTTACTGGATCAGGTTTCAGGGAC AGACTTCAGTTTTATCATCAGCTCTGTGCAAGCAGAGGATC TCGCGCTTTACTACTGTCAGCATCATTACAGCGCTCCGTGG ACGTTCCGGCGGCGGGACAACCCTGGATATCAAAGGGGGCG GTGGTAGCGGTGGAGGTGGGTCAGGGGGTGGTGGTTTCAGA GGTGCAACTCGTTGAATCAGGTGGGGACTTGGTGCGCCCAG GAGGTAGCCTGCAATTGAGCTGTGCTGCTAGCGGGTTCACT TTTTACGGAACGGTATGTCTTGGGTACGGCAGACCCCTGA CAGAAGACTGGAGTGGGTTGCAACTGTCAGTTCTGGTGGCT CCTATATTTACTACGCAGACAGCGTAAAAGGGAGATTTACC ATAAGCCGGGATAATGCCCCGAAATACCCTCTACCTCCAGAT GTCTCCTTGAAAAGTGAGGACACGGCTATGTACTATTGCG CCAGACAAGGAACCACTGCACTTGCAACGAGATTTTTTGAC

133	Hu07 scFv (VL > VH)	GTTTGGGGAGCCGGGACCACCGTAACTGTGAGTAGC GACATACAAATGACACAGTCCCCCTCATCCTTGTCTGCTTCC GTAGGAGACCGGGTTACCATCACGTGCACCGCTTCTTTGTC CGTTTCAAGTACCTACCTCCACTGGTACCAGCAAAAACCCG GCAGCAGCCCCAAGTTGTGGATTACTCAACTTCTAACTTG GCCTCAGGGGTACCGTCAAGATTTAGCGGATCTGGCAGTGG CACGAGTTATACTTTGACGATATCAAGCCTTCAACCGGAGG ATTTCCGCCACCTATTACTGTATCATCAGTATCATCGAAGCCCCT TGACCTTTGGGGGAGGGACAAAAGTGGAAATAAAAGGGGG AGGTGGAAGTGGTGGCGGTGGATCTGGTGGCGGCGGGTCA GAAGTACAGCTGGTTGAGAGTGGCGGGGGTCTCGTACAGC CCGGCGGGTCTCTTAGGCTCTCCTGTGCTGCTTCTGGTTTCT CCTTGACTAAATACGGGGTACATTGGGTTCCGACGGCCCCCT GGCAAAGGTCTTGAATGGGTGGGCGTCAAGTGGGCTGGCG GAAGCACTGATTATAATTCCGCATTGATGTCCCGATTCACT ATTTCTAAGGATAATGCCAAGAACAGTCTCTATTTGCAAT GAACTCCCTGAGAGCGGAGGATACTGCCGTTTACTACTGTG CACGGGATCACCGAGACGCTATGGATTACTGGGGTCAGGGT ACCCTGGTGACCGTAAGCTCC
134	Hu08 scFv (VH > VL)	GAGGTTTCAGTTGGTAGAGTCAGGCGGTGGTCTGGTGCAGCC AGGTGGGTCCCTGCGCCTCAGCTGTGCAGCTTCCGGCTTTA CTTTCTCAAGGAATGGTATGTCCTGGGTACGGCAAACGCCG GACAAACGCCTTGAGTGGGTAGCTACCGTATCCTCTGGGGG CTCTTACATATACTATGCAGACTCTGTGAAAGGAAGATTTA CAATTTACGCGACAATGCAAAAAATAGTTTGTACCTCCAA ATGTCTAGTCTTAGGGCCGAGGATACTGCCGTCTACTACTG TGCACGCCAGGGAACGACGGCTCTTGCTACCCGATTTTTCG ACGTTTGGGGCCAAGGAACGTTGGTGACAGTTAGCAGTGGT GGAGGTGGGTCTGGCGGAGGTGGAAGTGGTGGAGGCGGGT CCGACATCCAAATGACTCAGAGCCCCCTCTAGCCTCAGTGCA AGCGTCGGAGACCGGGTGACCATCACCTGTAAAGCGTCCCA GGATGTTGGAACGGCAGTAGCTTGGTATCAACAAATCCCAG GGAAGGCTCCAAAGCTCCTTATATACTCTGCTAGTTACAGG TCCACCGGGGTGCCCGACCGATTCTCTGGCTCCGGGAGCGG CACTGACTTTTCATTATCATCATTAGTAGTCTTCAACCTGAGGA CTTTGCCACCTATTATTGCCAGCACCACTACTCTGCGCCGTG GACTTTCGGAGGAGGCACGAAGGTTGAAATTAA
135	Hu08 scFv (VL > VH)	GACATCCAAATGACTCAGAGCCCCCTCTAGCCTCAGTGCAAG CGTCGGAGACCGGGTGACCATCACCTGTAAAGCGTCCCAGG ATGTTGGAACGGCAGTAGCTTGGTATCAACAAATCCCAGGG AAGGCTCCAAAGCTCCTTATATACTCTGCTAGTTACAGGTC CACCGGGGTGCCCGACCGATTCTCTGGCTCCGGGAGCGGCA CTGACTTTTCATTATCATCATTAGTAGTCTTCAACCTGAGGACT TTGCCACCTATTATTGCCAGCACCACTACTCTGCGCCGTGG ACTTTCCGAGGAGGCACGAAGGTTGAAATTAAAGGTGGAG GTGGGTCTGGCGGAGGTGGAAGTGGTGGAGGCGGGTCCGA GGTTCAGTTGGTAGAGTCAGGCGGTGGTCTGGTGCAGCCAG GTGGGTCCCTGCGCCTCAGCTGTGCAGCTTCCGGCTTTACTT TCTCAAGGAATGGTATGTCCTGGGTACGGCAAACGCCGGAC AAACGCCTTGAGTGGGTAGCTACCGTATCCTCTGGGGGCTC TTACATATACTATGCAGACTCTGTGAAAGGAAGATTTACAA TTTCACGCGACAATGCAAAAAATAGTTTGTACCTCCAAATG TCTAGTCTTAGGGCCGAGGATACTGCCGTCTACTACTGTGC ACGCCAGGGAACGACGGCTCTTGCTACCCGATTTTTTCGACG

		TTTGGGGCCAAGGAACGTTGGTGACAGTTAGCAGT
136	Linker	GGCGGAGGAGGAAGT
137	Linker	GGAGGAGGAGGAAGT
138	Hu07 scFv (VH> VL)	GAAGTACAGCTGGTTGAGAGTGGCGGGGGTCTCGTACAGC CCGGCGGGTCTCTTAGGCTCTCCTGTGCTGCTTCTGGTTTCT CCTTGACTAAATACGGGGTACATTGGGTTCCGCCAGGCCCT GGCAAAGGTCTTGAATGGGTGGGCGTCAAGTGGGCTGGCG GAAGCACTGATTATAATTCCGCATTGATGTCCCGATTCACT ATTTCTAAGGATAATGCCAAGAACAGTCTCTATTGCAAAT GAACTCCCTGAGAGCGGAGGATACTGCCGTTTACTACTGTG CACGGGATCACCGAGACGCTATGGATTACTGGGGTCAGGGT ACCCTGGTGACCGTAAGCTCCGGGGGAGGTGGAAGTGGTG GCGGTGGATCTGGTGGCGGCGGGTCAGACATACAAATGAC ACAGTCCCCCTCATCCTTGTCTGCTTCCGTAGGAGACCGGG TTACCATCACGTGCACCGCTTCTTTGTCCGTTTCAAGTACCT ACCTCCACTGGTACCAGCAAAAACCCGGCAGCAGCCCCAA GTTGTGGATTACTCAACTTCTAACTTGGCCTCAGGGGTACC GTCAAGATTTAGCGGATCTGGCAGTGGCACGAGTTATACTT TGACGATATCAAGCCTTCAACCGGAGGATTTCCGCCACCTAT TACTGTCATCAGTATCATCGAAGCCCCTTGACCTTTGGGGG AGGGACAAAAGTGGAAATAAAA
144	806 Human VH	QVQLQESGPGLVKPSQTLSTCTVSGYSISSDFAWNWRQPPG KGLEWMGYISYSGNTRYQPSLKSRTISRDTSKNQFFLKLNSVT AADTATYYCVTAGRGFPYWGQGLVTVSS
145	806 Mature Human VH	EVQLQESGPGLVKPSQTLSTCTVSGYSISRDFAWNWRQPPG KGLEWMGYISYNGNTRYQPSLKSRTISRDTSKNQFFLKLNSV TAADTATYYCVTASRGFPYWGQGLVTVSS
146	806 Human VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQOKPGKSF KGLIYHGTLNDDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYC VQYAQFPWTFGGGTGLEIKR
147	806 Mature Human VL	DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQOKPGKSF KGLIYHGTLNDDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYC VQYAQFPWTFGGGTGLEIKR

C. Tandem and Parallel Bi-Specific CARs

Also provided herein is a tandem CAR, a cell (*e.g.* T cell) comprising a tandem CAR,
 5 an amino acid sequence comprising a tandem CAR, and a nucleic acid encoding a tandem
 CAR. A tandem CAR comprises two antigen binding domains that are separated by a linker,
 which are linked to a transmembrane domain and an intracellular domain (*e.g.* 4-1BB and/or
 CD3 ζ) (FIG. 17). In one aspect, the tandem CAR comprises a first antigen binding domain
 (*e.g.* a first scFv) separated by a linker from a second antigen binding domain (*e.g.* a second
 10 scFv), followed by a transmembrane domain and an intracellular domain (*e.g.* 4-1BB and/or
 CD3 ζ) (FIG. 17). The first and second antigen binding domains can bind two different
 antigens. For example, an exemplary tandem CAR comprises a first antigen binding domain

comprising an scFv capable of binding IL13R α 2 and the second antigen binding domain comprises an scFv capable of binding EGFR.

The linker in the tandem CAR that links the first and second antigen binding domains can be various sizes, *e.g.* any number of amino acids in length (FIGs. 18A-18D). For example, the linker can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids in length. In certain embodiments, the tandem CAR comprises a linker that is 5 amino acids in length. In certain embodiments, the tandem CAR comprises the amino acid sequence of SEQ ID NO: 163 and may be encoded by the nucleotide sequence of SEQ ID NO: 164. In certain embodiments, the tandem CAR comprises a linker that is 10 amino acids in length. In certain embodiments, the tandem CAR comprises the amino acid sequence of SEQ ID NO: 165 and may be encoded by the nucleotide sequence of SEQ ID NO: 166. In certain embodiments, the tandem CAR comprises a linker that is 15 amino acids in length. In certain embodiments, the tandem CAR comprises the amino acid sequence of SEQ ID NO: 167 and may be encoded by the nucleotide sequence of SEQ ID NO: 168.

Also provided herein is a parallel CAR, a cell (*e.g.* T cell) comprising a parallel CAR, an amino acid sequence comprising a parallel CAR, and a nucleic acid encoding a parallel CAR. A parallel CAR comprises two separate CARs linked by a cleavable linker (*e.g.* 2A linker). For example, an exemplary parallel CAR comprises a first antigen binding domain (*e.g.* scFv) linked to a first transmembrane domain and a first intracellular domain, a cleavable linker (*e.g.* 2A linker), and a second antigen binding domain (*e.g.* scFv) linked to a second transmembrane domain and a second intracellular domain. When the nucleic acid is expressed in the cell, the linker (*e.g.* 2A linker) is cleaved and two separate CARs are expressed on the surface of the cell. In certain embodiments, the parallel CAR comprises a first CAR capable of binding IL13R α 2 and a second CAR capable of binding EGFR. In certain embodiments, the parallel CAR comprises the amino acid sequence of SEQ ID NO: 171 and may be encoded by the nucleotide sequence of SEQ ID NO: 172.

D. BiTEs, BiTE/BiTEs, and BiTE /CAR combinations

Provided herein are Bispecific T Cell Engagers (BiTEs) and BiTE/ CAR combinations. BiTEs comprise a first antigen binding domain (*e.g.* first scFv) and a second antigen binding domain (*e.g.* second scFv) wherein the first scFv is capable of binding an antigen on a target cell (*e.g.* tumor cell) and the second scFv is capable of binding an antigen on an activating T cell (*e.g.* CD3, CD4, CD8, or TCR).

In one aspect, the invention includes a BiTE capable of binding IL13R α 2. In one aspect, the invention includes a BiTE capable of binding CD3 and IL13R α 2. In certain embodiments, the BiTE comprises any of the antigen binding domains disclosed herein that are capable of binding IL13R α 2. In certain embodiments, the BiTE comprises an antigen binding domain comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 1-22.

In one aspect, the invention includes a BiTE capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof (*e.g.* wild type EGFR (wtEGFR) or EGFR variant III (EGFRvIII)). In one aspect, the invention includes a BiTE capable of binding CD3 and EGFR or an isoform thereof. In certain embodiments, the BiTE comprises any of the antigen binding domains disclosed herein that are capable of binding EGFR or an isoform thereof. In certain embodiments, the BiTE comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 53 or 54.

In certain embodiments, the BiTE is inducible (*e.g.* comprises/ is driven by an inducible promoter).

Also provided herein are bispecific constructs comprising a first CAR and a second CAR (CAR/CAR, see *e.g.* FIG. 30A), a BiTE and a CAR (BiTE/CAR, see *e.g.* FIGs. 30B-30C), or a first BiTE and a second BiTE (BiTE/BiTE, see *e.g.* FIG. 30D).

The CAR/CAR can comprise any combination of any of the CARs disclosed herein. In certain embodiments the CAR/CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 173, which may be encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 174.

The BiTE/CAR can comprise any of the BiTEs disclosed herein, any of the CARs disclosed herein, and any combination thereof. In certain embodiments, the BiTE/CAR comprises a BiTE that is capable of binding EGFR or an isoform thereof, and a CAR that is capable of binding IL13R α 2. In certain embodiments the BiTE/CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 175, which may be encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 176. In certain embodiments, the BiTE/CAR comprises a BiTE that is capable of binding IL13R α 2, and a CAR that is capable of binding EGFR or an isoform thereof. In certain embodiments the BiTE/CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or

100% identical to SEQ ID NO: 177, which may be encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 178.

The BiTE/BiTE can comprise any of the BiTEs disclosed herein in any combination thereof. In certain embodiments, the BiTE/BiTE comprises a first BiTE that is capable of binding EGFR or an isoform thereof, and a second BiTE that is capable of binding IL13R α 2. In certain embodiments the BiTE/BiTE comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 179, which may be encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 180.

E. Nucleic Acids and Expression Vectors

The present disclosure provides a nucleic acid encoding a CAR. The nucleic acid of the present disclosure may comprises a polynucleotide sequence encoding any one of the
5 CARs, BiTEs, BiTE/CARs, or BiTE/BiTEs disclosed herein.

In one embodiment, a nucleic acid of the present disclosure comprises a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises: a heavy chain variable
10 region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence G VKWAGGSTDYNSALMS (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and a light chain variable region that comprises three light chain complementarity determining regions
15 (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7).

In one embodiment, the nucleic acid encodes a CAR comprising an antigen-binding domain comprising a heavy chain variable region encoded by a polynucleotide sequence at
20 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57.

In one embodiment, the nucleic acid encodes a CAR comprising an antigen-binding domain comprising a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

In one embodiment, the nucleic acid encodes a CAR comprising an antigen-binding
25 domain comprising a heavy chain variable region encoded by a polynucleotide sequence at

least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57 and/or a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

In one embodiment, the nucleic acid encodes a CAR wherein the antigen-binding
5 domain is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 133 or 138.

Also provided is a nucleic acid comprising a polynucleotide sequence encoding a
chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-
10 binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises: a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid
15 sequence QGTTALATRFDDV (SEQ ID NO: 14); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence QHHYSAPWT (SEQ ID NO: 18).

20 In one embodiment, the nucleic acid encodes a CAR comprising an antigen-binding domain comprising a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67.

In one embodiment, the nucleic acid encodes a CAR comprising an antigen-binding domain comprising a light chain variable region encoded by a polynucleotide sequence at
25 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

In one embodiment, the nucleic acid encodes a CAR comprising an antigen-binding domain comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%,
30 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

In one embodiment, the nucleic acid encodes a CAR comprising wherein the antigen-binding domain is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 134 or 135.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises: a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises: a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

In another aspect, the invention provides a nucleic acid comprising a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or SEQ ID NO: 66 or SEQ ID NO: 75 or SEQ ID NO: 76.

Also provided is a nucleic acid comprising a first polynucleotide sequence encoding a first CAR capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first and second CAR each comprise an antigen-binding domain, a transmembrane domain, and an intracellular domain.

In certain embodiments, the antigen-binding domain of the first CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence VKWAGGSTDYNSALMS (SEQ ID NO: 2) or GVKWAGGSTDYNSALMS (SEQ ID NO: 3), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7).

In certain embodiments, the antigen-binding domain of the first CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence TVSSGGSYTYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence QGTTALATRFFD (SEQ ID NO: 14); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence KASQDVGTA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence QHHYSAPWT (SEQ ID NO: 18).

In certain embodiments, the antigen-binding domain of the first CAR comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57; and/or a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

In certain embodiments, the antigen-binding domain of the first CAR comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67; and/or a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

In certain embodiments, the antigen-binding domain of the first CAR is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 138 or SEQ ID NO: 133 or SEQ ID NO: 134 or SEQ ID NO: 135.

In certain embodiments, the first polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or SEQ ID NO: 66 or SEQ ID NO: 75 or SEQ ID NO: 76.

In certain embodiments, the antigen-binding domain of the second CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFYPY (SEQ ID NO: 27); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid

sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGINLDD (SEQ ID NO: 143) or HGTNLDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

In certain embodiments, the antigen-binding domain of the second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31 and/or a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32. In certain embodiments, the antigen-binding domain of the second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 144 and/or a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 146. In certain embodiments, the antigen-binding domain of the second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 145 and/or a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 147.

In certain embodiments, the antigen-binding domain of the second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 42 and/or a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 43.

In certain embodiments, the antigen-binding domain of the second CAR is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or SEQ ID NO: 141 or SEQ ID NO: 41. In certain embodiments, the antigen-binding domain of the second CAR is a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34 or SEQ ID NO: 142 or SEQ ID NO: 44.

In certain embodiments, the second polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35 or SEQ ID NO: 37 or SEQ ID NO: 196. In certain embodiments, the second polynucleotide sequence encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 36 or SEQ ID NO: 38 or SEQ ID NO: 197.

Also provided is a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFFDV (SEQ ID NO: 15); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18); and the second CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFPYW (SEQ ID NO: 27); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGINLDD (SEQ ID NO: 143) or HGTNLDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

Also provided is a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first CAR comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57, or 67; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61, or 71; and the second CAR comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 139, or 194 and a light chain

variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 140, or 195.

Also provided is a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first CAR comprises a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 138, 133, 134, or 135; and the second CAR comprises a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 141.

Also provided is a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or 66 or 75 or 76; and the second polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35 or 196.

The invention also includes a nucleic acid comprising a first polynucleotide sequence encoding a first CAR capable of binding IL13R α 2, and a second polynucleotide sequence encoding an inhibitor of an immune checkpoint. In certain embodiments, the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3. In certain embodiments, the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody. In certain embodiments, the inhibitor of the immune checkpoint is an anti-CTLA-4 antibody.

Also provided is a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) capable of binding IL13R α 2, and a second polynucleotide sequence encoding an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. In certain embodiments, the second polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence that encodes SEQ ID NO: 53 or 54. In certain embodiments, the BiTE is capable of binding wild type EGFR (wtEGFR). In certain embodiments, the BiTE is capable of binding EGFR variant III (EGFRvIII).

In some embodiments, a nucleic acid of the present disclosure is provided for the production of a CAR as described herein, *e.g.*, in a mammalian cell. In some embodiments, a nucleic acid of the present disclosure provides for amplification of the CAR-encoding nucleic acid.

In some embodiments, a nucleic acid of the present disclosure comprises a first polynucleotide sequence and a second polynucleotide sequence. The first and second polynucleotide sequence may be separated by a linker. A linker for use in the present disclosure allows for multiple proteins to be encoded by the same nucleic acid sequence (*e.g.*, a multicistronic or bicistronic sequence), which are translated as a polyprotein that is dissociated into separate protein components. For example, a linker for use in a nucleic acid of the present disclosure comprising an IL13R α 2 CAR coding sequence and an EGFR CAR coding sequence, allows for the IL13R α 2CAR and EGFR CAR to be translated as a polyprotein that is dissociated into separate CARs. In certain embodiments, the nucleic acid comprises from 5' to 3' the first polynucleotide sequence, the linker, and the second polynucleotide sequence. In certain embodiments, the nucleic acid comprises from 5' to 3' the second polynucleotide sequence, the linker, and the first polynucleotide sequence.

5 In some embodiments, the linker comprises a nucleic acid sequence that encodes for an internal ribosome entry site (IRES). As used herein, "an internal ribosome entry site" or "IRES" refers to an element that promotes direct internal ribosome entry to the initiation codon, such as ATG, of a protein coding region, thereby leading to cap-independent translation of the gene. Various internal ribosome entry sites are known to those of skill in the art, including, without limitation, IRES obtainable from viral or cellular mRNA sources, *e.g.*, immunoglobulin heavy-chain binding protein (BiP); vascular endothelial growth factor (VEGF); fibroblast growth factor 2; insulin-like growth factor; translational initiation factor eIF4G; yeast transcription factors TFIID and HAP4; and IRES obtainable from, *e.g.*,
10 cardiovascular, rhinovirus, aphthovirus, HCV, Friend murine leukemia virus (FrMLV), and
15 Moloney murine leukemia virus (MoMLV). Those of skill in the art would be able to select the appropriate IRES for use in the present invention.

In some embodiments, the linker comprises a nucleic acid sequence that encodes for a self-cleaving peptide. As used herein, a "self-cleaving peptide" or "2A peptide" refers to an oligopeptide that allow multiple proteins to be encoded as polyproteins, which dissociate into
20 component proteins upon translation. Use of the term "self-cleaving" is not intended to imply a proteolytic cleavage reaction. Various self-cleaving or 2A peptides are known to those of skill in the art, including, without limitation, those found in members of the Picornaviridae

virus family, *e.g.*, foot-and-mouth disease virus (FMDV), equine rhinitis A virus (ERAV), Thosea asigna virus (TaV), and porcine tescho virus-1 (PTV-1); and carioviruses such as Theilovirus and encephalomyocarditis viruses. 2A peptides derived from FMDV, ERAV, PTV-1, and TaV are referred to herein as “F2A,” “E2A,” “P2A,” and “T2A,” respectively.

5 Those of skill in the art would be able to select the appropriate self-cleaving peptide for use in the present invention.

In some embodiments, a linker further comprises a nucleic acid sequence that encodes a furin cleavage site. Furin is a ubiquitously expressed protease that resides in the trans-golgi and processes protein precursors before their secretion. Furin cleaves at the COOH- terminus
10 of its consensus recognition sequence. Various furin consensus recognition sequences (or “furin cleavage sites”) are known to those of skill in the art, including, without limitation, Arg-X1-Lys-Arg (SEQ ID NO:117) or Arg-X1-Arg-Arg (SEQ ID NO:118), X2-Arg-X1-X3-Arg (SEQ ID NO:119) and Arg-X1-X1-Arg (SEQ ID NO:120), such as an Arg-Gln-Lys-Arg (SEQ ID NO:121), where X1 is any naturally occurring amino acid, X2 is Lys or Arg, and X3
15 is Lys or Arg. Those of skill in the art would be able to select the appropriate Furin cleavage site for use in the present invention.

In some embodiments, the linker comprises a nucleic acid sequence encoding a combination of a Furin cleavage site and a 2A peptide. Examples include, without limitation, a linker comprising a nucleic acid sequence encoding Furin and F2A, a linker comprising a
20 nucleic acid sequence encoding Furin and E2A, a linker comprising a nucleic acid sequence encoding Furin and P2A, a linker comprising a nucleic acid sequence encoding Furin and T2A. Those of skill in the art would be able to select the appropriate combination for use in the present invention. In such embodiments, the linker may further comprise a spacer sequence between the Furin and 2A peptide. Various spacer sequences are known in the art,
25 including, without limitation, glycine serine (GS) spacers such as (GS)*n*, (GSGGS)*n* (SEQ ID NO:148) and (GGGS)*n* (SEQ ID NO:149), where *n* represents an integer of at least 1. Exemplary spacer sequences can comprise amino acid sequences including, without limitation, GGSG (SEQ ID NO:151), GSGGG (SEQ ID NO:152), GSGSG (SEQ ID NO:153), GSGGG (SEQ ID NO:154), GGGSG (SEQ ID NO:155), GSSSG (SEQ ID
30 NO:156), and the like. Those of skill in the art would be able to select the appropriate spacer sequence for use in the present invention.

In some embodiments, a nucleic acid of the present disclosure may be operably linked to a transcriptional control element, *e.g.*, a promoter, and enhancer, etc. Suitable promoter and enhancer elements are known to those of skill in the art.

In certain embodiments, the nucleic acid encoding an exogenous CAR is in operable linkage with a promoter. In certain embodiments, the promoter is a phosphoglycerate kinase-1 (PGK) promoter.

For expression in a bacterial cell, suitable promoters include, but are not limited to, lacI, lacZ, T3, T7, gpt, lambda P and trc. For expression in a eukaryotic cell, suitable promoters include, but are not limited to, light and/or heavy chain immunoglobulin gene promoter and enhancer elements; cytomegalovirus immediate early promoter; herpes simplex virus thymidine kinase promoter; early and late SV40 promoters; promoter present in long terminal repeats from a retrovirus; mouse metallothionein-I promoter; and various art-known tissue specific promoters. Suitable reversible promoters, including reversible inducible promoters are known in the art. Such reversible promoters may be isolated and derived from many organisms, *e.g.*, eukaryotes and prokaryotes. Modification of reversible promoters derived from a first organism for use in a second organism, *e.g.*, a first prokaryote and a second a eukaryote, a first eukaryote and a second a prokaryote, etc., is well known in the art. Such reversible promoters, and systems based on such reversible promoters but also comprising additional control proteins, include, but are not limited to, alcohol regulated promoters (*e.g.*, alcohol dehydrogenase I (alcA) gene promoter, promoters responsive to alcohol transactivator proteins (A1cR), etc.), tetracycline regulated promoters, (*e.g.*, promoter systems including TetActivators, TetON, TetOFF, etc.), steroid regulated promoters (*e.g.*, rat glucocorticoid receptor promoter systems, human estrogen receptor promoter systems, retinoid promoter systems, thyroid promoter systems, ecdysone promoter systems, mifepristone promoter systems, etc.), metal regulated promoters (*e.g.*, metallothionein promoter systems, etc.), pathogenesis-related regulated promoters (*e.g.*, salicylic acid regulated promoters, ethylene regulated promoters, benzothiadiazole regulated promoters, etc.), temperature regulated promoters (*e.g.*, heat shock inducible promoters (*e.g.*, HSP-70, HSP-90, soybean heat shock promoter, etc.), light regulated promoters, synthetic inducible promoters, and the like.

In some embodiments, the promoter is a CD8 cell-specific promoter, a CD4 cell-specific promoter, a neutrophil-specific promoter, or an NK-specific promoter. For example, a CD4 gene promoter can be used; see, *e.g.*, Salmon et al. Proc. Natl. Acad. Sci. USA (1993) 90:7739; and Marodon et al. (2003) Blood 101:3416. As another example, a CD8 gene promoter can be used. NK cell-specific expression can be achieved by use of an NcrI (p46) promoter; see, *e.g.*, Eckelhart et al. Blood (2011) 117:1565.

For expression in a yeast cell, a suitable promoter is a constitutive promoter such as an ADH1 promoter, a PGK1 promoter, an ENO promoter, a PYK1 promoter and the like; or a regulatable promoter such as a GAL1 promoter, a GAL10 promoter, an ADH2 promoter, a PHOS promoter, a CUP1 promoter, a GALT promoter, a MET25 promoter, a MET3 promoter, a CYC1 promoter, a HIS3 promoter, an ADH1 promoter, a PGK promoter, a GAPDH promoter, an ADC1 promoter, a TRP1 promoter, a URA3 promoter, a LEU2 promoter, an ENO promoter, a TP1 promoter, and AOX1 (*e.g.*, for use in *Pichia*). Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. Suitable promoters for use in prokaryotic host cells include, but are not limited to, a bacteriophage T7 RNA polymerase promoter; a *trp* promoter; a *lac* operon promoter; a hybrid promoter, *e.g.*, a *lac/tac* hybrid promoter, a *tac/trc* hybrid promoter, a *trp/lac* promoter, a T7/*lac* promoter; a *trc* promoter; a *tac* promoter, and the like; an *araBAD* promoter; *in vivo* regulated promoters, such as an *ssaG* promoter or a related promoter (see, *e.g.*, U.S. Patent Publication No. 20040131637), a *pagC* promoter (Pulkkinen and Miller, *J. Bacteriol.* (1991) 173(1): 86-93; Alpuche-Aranda *et al.*, *Proc. Natl. Acad. Sci. USA* (1992) 89(21): 10079-83), a *nirB* promoter (Harborne *et al.*, *Mol. Micro.* (1992) 6:2805-2813), and the like (see, *e.g.*, Dunstan *et al.*, *Infect. Immun.* (1999) 67:5133-5141; McKelvie *et al.*, *Vaccine* (2004) 22:3243-3255; and Chatfield *et al.*, *Biotechnol.* (1992) 10:888-892); a *sigma70* promoter, *e.g.*, a consensus *sigma70* promoter (see, *e.g.*, GenBank Accession Nos. AX798980, AX798961, and AX798183); a stationary phase promoter, *e.g.*, a *dps* promoter, an *spv* promoter, and the like; a promoter derived from the pathogenicity island SPI-2 (see, *e.g.*, WO96/17951); an *actA* promoter (see, *e.g.*, Shetron-Rama *et al.*, *Infect. Immun.* (2002) 70:1087-1096); an *rpsM* promoter (see, *e.g.*, Valdivia and Falkow *Mol. Microbiol.* (1996) 22:367); a *tet* promoter (see, *e.g.*, Hillen, W. and Wissmann, A. (1989) In Saenger, W. and Heinemann, U. (eds), *Topics in Molecular and Structural Biology, Protein--Nucleic Acid Interaction*. Macmillan, London, UK, Vol. 10, pp. 143-162); an SP6 promoter (see, *e.g.*, Melton *et al.*, *Nucl. Acids Res.* (1984) 12:7035); and the like. Suitable strong promoters for use in prokaryotes such as *Escherichia coli* include, but are not limited to *Trc*, *Tac*, *T5*, *T7*, and *PLambda*. Non-limiting examples of operators for use in bacterial host cells include a lactose promoter operator (*LacI* repressor protein changes conformation when contacted with lactose, thereby preventing the *Lac* repressor protein from binding to the operator), a tryptophan promoter operator (when complexed with tryptophan, *TrpR* repressor protein has a conformation that binds the operator; in the absence of tryptophan, the *TrpR* repressor

protein has a conformation that does not bind to the operator), and a tac promoter operator (see, *e.g.*, deBoer et al., Proc. Natl. Acad. Sci. U.S.A. (1983) 80:21-25).

Other examples of suitable promoters include the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter

sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Other constitutive promoter sequences may also be used,

including, but not limited to a simian virus 40 (SV40) early promoter, a mouse mammary tumor virus (MMTV) or human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, a MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus

immediate early promoter, a Rous sarcoma virus promoter, the EF-1 alpha promoter, as well

as human gene promoters such as, but not limited to, an actin promoter, a myosin promoter, a hemoglobin promoter, and a creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as

part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired.

Examples of inducible promoters include, but are not limited to a metallothioneine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter. In certain embodiments, the invention provides a polynucleotide sequence encoding a CAR (*e.g.*

bispecific CAR, BiTE, tandem CAR, parallel CAR, and the like) comprising an inducible promoter. In certain embodiments, the inducible promoter promotes expression of the

operatively linked sequence (*e.g.* CAR) after T-cell activation. T cells (*e.g.* CAR T cells) can be modified with this promoter to express designed RNA or amino acids. In certain

embodiments, the inducible promoter comprises a nucleotide sequence that is 85%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 161.

In certain embodiments, the inducible promoter comprises a nucleotide sequence that is 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:

198. In certain embodiments, the sequence comprising SEQ ID NO: 198 is repeated to enhance T-cell expression level. For example, in certain embodiments, the inducible

promoter can comprise a nucleotide sequence that is 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 162.

In some embodiments, the locus or construct or transgene containing the suitable promoter is irreversibly switched through the induction of an inducible system. Suitable systems for induction of an irreversible switch are well known in the art, *e.g.*, induction of an

irreversible switch may make use of a Cre-lox-mediated recombination (see, *e.g.*, Fuhrmann-Benzakein, et al., Proc. Natl. Acad. Sci. USA (2000) 28:e99, the disclosure of which is incorporated herein by reference). Any suitable combination of recombinase, endonuclease, ligase, recombination sites, etc. known to the art may be used in generating an irreversibly switchable promoter. Methods, mechanisms, and requirements for performing site-specific recombination, described elsewhere herein, find use in generating irreversibly switched promoters and are well known in the art, see, *e.g.*, Grindley *et al.* Annual Review of Biochemistry (2006) 567-605; and Tropp, Molecular Biology (2012) (Jones & Bartlett Publishers, Sudbury, Mass.), the disclosures of which are incorporated herein by reference.

In some embodiments, a nucleic acid of the present disclosure further comprises a nucleic acid sequence encoding a CAR inducible expression cassette. In one embodiment, the CAR inducible expression cassette is for the production of a transgenic polypeptide product that is released upon CAR signaling. See, *e.g.*, Chmielewski and Abken, Expert Opin. Biol. Ther. (2015) 15(8): 1145-1154; and Abken, Immunotherapy (2015) 7(5): 535-544. In some embodiments, a nucleic acid of the present disclosure further comprises a nucleic acid sequence encoding a cytokine operably linked to a T-cell activation responsive promoter. In some embodiments, the cytokine operably linked to a T-cell activation responsive promoter is present on a separate nucleic acid sequence. In one embodiment, the cytokine is IL-12.

A nucleic acid of the present disclosure may be present within an expression vector and/or a cloning vector. An expression vector can include a selectable marker, an origin of replication, and other features that provide for replication and/or maintenance of the vector. Suitable expression vectors include, *e.g.*, plasmids, viral vectors, and the like. Large numbers of suitable vectors and promoters are known to those of skill in the art; many are commercially available for generating a subject recombinant construct. The following vectors are provided by way of example, and should not be construed in anyway as limiting: Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXR1, pSG (Stratagene) pSVK3, pBPV, pMSG and pSVL (Pharmacia).

Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding heterologous proteins. A selectable marker operative in the expression host may be present. Suitable expression vectors include, but are not limited to, viral vectors (*e.g.* viral vectors

based on vaccinia virus; poliovirus; adenovirus (see, *e.g.*, Li et al., Invest. Ophthalmol. Vis. Sci. (1994) 35: 2543-2549; Borrás et al., Gene Ther. (1999) 6: 515-524; Li and Davidson, Proc. Natl. Acad. Sci. USA (1995) 92: 7700-7704; Sakamoto et al., H. Gene Ther. (1999) 5: 1088-1097; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and
 5 WO 95/00655); adeno-associated virus (see, *e.g.*, Ali et al., Hum. Gene Ther. (1998) 9: 81-86, Flannery et al., Proc. Natl. Acad. Sci. USA (1997) 94: 6916-6921; Bennett et al., Invest. Ophthalmol. Vis. Sci. (1997) 38: 2857-2863; Jomary et al., Gene Ther. (1997) 4:683 690, Rolling et al., Hum. Gene Ther. (1999) 10: 641-648; Ali et al., Hum. Mol. Genet. (1996) 5: 591-594; Srivastava in WO 93/09239, Samulski et al., J. Vir. (1989) 63: 3822-3828;
 10 Mendelson et al., Virol. (1988) 166: 154-165; and Flotte et al., Proc. Natl. Acad. Sci. USA (1993) 90: 10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, *e.g.*, Miyoshi et al., Proc. Natl. Acad. Sci. USA (1997) 94: 10319-23; Takahashi et al., J. Virol. (1999) 73: 7812-7816); a retroviral vector (*e.g.*, Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey
 15 Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

Additional expression vectors suitable for use are, *e.g.*, without limitation, a lentivirus vector, a gamma retrovirus vector, a foamy virus vector, an adeno-associated virus vector, an adenovirus vector, a pox virus vector, a herpes virus vector, an engineered hybrid virus
 20 vector, a transposon mediated vector, and the like. Viral vector technology is well known in the art and is described, for example, in Sambrook et al., 2012, Molecular Cloning: A Laboratory Manual, volumes 1-4, Cold Spring Harbor Press, NY), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses.

25 In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (*e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

In some embodiments, an expression vector (*e.g.*, a lentiviral vector) may be used to introduce the CAR into an immune cell or precursor thereof (*e.g.*, a T cell). Accordingly, an
 30 expression vector (*e.g.*, a lentiviral vector) of the present invention may comprise a nucleic acid encoding for a CAR. In some embodiments, the expression vector (*e.g.*, lentiviral vector) will comprise additional elements that will aid in the functional expression of the CAR encoded therein. In some embodiments, an expression vector comprising a nucleic acid encoding for a CAR further comprises a mammalian promoter. In one embodiment, the

vector further comprises an elongation-factor-1-alpha promoter (EF-1 α promoter). Use of an EF-1 α promoter may increase the efficiency in expression of downstream transgenes (*e.g.*, a CAR encoding nucleic acid sequence). Physiologic promoters (*e.g.*, an EF-1 α promoter) may be less likely to induce integration mediated genotoxicity, and may abrogate the ability of the retroviral vector to transform stem cells. Other physiological promoters suitable for use in a vector (*e.g.*, lentiviral vector) are known to those of skill in the art and may be incorporated into a vector of the present invention. In some embodiments, the vector (*e.g.*, lentiviral vector) further comprises a non-requisite cis acting sequence that may improve titers and gene expression. One non-limiting example of a non-requisite cis acting sequence is the central polypurine tract and central termination sequence (cPPT/CTS) which is important for efficient reverse transcription and nuclear import. Other non-requisite cis acting sequences are known to those of skill in the art and may be incorporated into a vector (*e.g.*, lentiviral vector) of the present invention. In some embodiments, the vector further comprises a posttranscriptional regulatory element. Posttranscriptional regulatory elements may improve RNA translation, improve transgene expression and stabilize RNA transcripts. One example of a posttranscriptional regulatory element is the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE). Accordingly, in some embodiments a vector for the present invention further comprises a WPRE sequence. Various posttranscriptional regulator elements are known to those of skill in the art and may be incorporated into a vector (*e.g.*, lentiviral vector) of the present invention. A vector of the present invention may further comprise additional elements such as a rev response element (RRE) for RNA transport, packaging sequences, and 5' and 3' long terminal repeats (LTRs). The term "long terminal repeat" or "LTR" refers to domains of base pairs located at the ends of retroviral DNAs which comprise U3, R and U5 regions. LTRs generally provide functions required for the expression of retroviral genes (*e.g.*, promotion, initiation and polyadenylation of gene transcripts) and to viral replication. In one embodiment, a vector (*e.g.*, lentiviral vector) of the present invention includes a 3' U3 deleted LTR. Accordingly, a vector (*e.g.*, lentiviral vector) of the present invention may comprise any combination of the elements described herein to enhance the efficiency of functional expression of transgenes. For example, a vector (*e.g.*, lentiviral vector) of the present invention may comprise a WPRE sequence, cPPT sequence, RRE sequence, 5'LTR, 3' U3 deleted LTR' in addition to a nucleic acid encoding for a CAR.

Vectors of the present invention may be self-inactivating vectors. As used herein, the term "self-inactivating vector" refers to vectors in which the 3' LTR enhancer promoter region (U3 region) has been modified (*e.g.*, by deletion or substitution). A self-inactivating

vector may prevent viral transcription beyond the first round of viral replication. Consequently, a self-inactivating vector may be capable of infecting and then integrating into a host genome (*e.g.*, a mammalian genome) only once, and cannot be passed further. Accordingly, self-inactivating vectors may greatly reduce the risk of creating a replication-competent virus.

In some embodiments, a nucleic acid of the present invention may be RNA, *e.g.*, *in vitro* synthesized RNA. Methods for *in vitro* synthesis of RNA are known to those of skill in the art; any known method can be used to synthesize RNA comprising a sequence encoding a CAR of the present disclosure. Methods for introducing RNA into a host cell are known in the art. See, *e.g.*, Zhao et al. Cancer Res. (2010) 15: 9053. Introducing RNA comprising a nucleotide sequence encoding a CAR of the present disclosure into a host cell can be carried out *in vitro*, *ex vivo* or *in vivo*. For example, a host cell (*e.g.*, an NK cell, a cytotoxic T lymphocyte, etc.) can be electroporated *in vitro* or *ex vivo* with RNA comprising a nucleotide sequence encoding a CAR of the present disclosure.

In order to assess the expression of a polypeptide or portions thereof, the expression vector to be introduced into a cell may also contain either a selectable marker gene or a reporter gene, or both, to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In some embodiments, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, without limitation, antibiotic-resistance genes.

Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assessed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include, without limitation, genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tei et al., 2000 FEBS Letters 479: 79-82).

F. Modified Immune Cells

The present invention provides modified immune cells or precursors thereof (e.g., a T cell) comprising comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2 (e.g. human IL13R α 2 or canine IL13R α 2). Also provided are modified immune cells or precursors thereof comprising BiTEs, a BiTE/BiTEs, or BiTE /CARs. The invention also includes modified immune cells or precursors thereof comprising any of the nucleic acids disclosed herein or any of the vectors disclosed herein.

One aspect of the invention provides a modified immune cell or precursor cell thereof, comprising a CAR capable of binding IL13R α 2, wherein the CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs). HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYTYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFDDV (SEQ ID NO: 15). The CAR also comprises a light chain variable region that comprises three light chain complementarity determining regions (LCDRs). LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18).

Another aspect of the invention includes a modified immune cell or precursor cell thereof, comprising a CAR capable of binding IL13R α 2, wherein the CAR comprises: a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20.

Also provided is a modified immune cell or precursor cell thereof, comprising a CAR capable of binding IL13R α 2, wherein the CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or 11.

In another aspect, the invention provides a modified immune cell or precursor cell thereof, comprising a CAR capable of binding IL13R α 2, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 21 or 22.

Another aspect of the invention includes a modified immune cell or precursor cell thereof, comprising a first chimeric antigen receptor (CAR) comprising a first antigen-binding domain capable of binding IL13R α 2; and a second chimeric antigen receptor (CAR) comprising a second antigen-binding domain capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof.

Yet another aspect of the invention includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, and a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs). HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFDDV (SEQ ID NO: 15). The first CAR also comprises a light chain variable region that comprises three light chain complementarity determining regions (LCDRs). LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTA VA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18). The second CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs). HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFPYW (SEQ ID NO: 27). The second CAR also comprises a light chain variable region that comprises three light chain complementarity determining regions (LCDRs). LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGINLDD (SEQ ID NO: 143) or HGTNLDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

Still another aspect of the invention includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, and a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19 and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%,

95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20. The second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31 or SEQ ID NO: 42 or SEQ ID NO: 144 or SEQ ID NO: 145 and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32 or SEQ ID NO: 43 or SEQ ID NO: 146 or SEQ ID NO: 147.

Also provided is a modified immune cell or precursor cell thereof, comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 21 or SEQ ID NO: 22; and the second CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34 or SEQ ID NO: 44.

In certain embodiments, the second CAR is capable of binding an EGFR isoform selected from the group consisting of wild type EGFR (wtEGFR), mutated EGFR, EGFR^{A289V}, EGFR^{A289D}, EGFR^{A289T}, EGFR^{A289T}, EGFR^{R108K}, EGFR^{R108G}, EGFR^{G598V}, EGFR^{D126Y}, EGFR^{C628F}, EGFR^{R108K/A289V}, EGFR^{R108K/D126Y}, EGFR^{A289V/G598V}, EGFR^{A289V/C628F}, and EGFR variant II, or any combination thereof.

The modified cell can further comprise an inhibitor of an immune checkpoint, wherein the modified cell secretes the inhibitor of the immune checkpoint. Immune checkpoints include but are not limited to CTLA-4, PD-1, and TIM-3. Inhibitors of the immune checkpoint include but are not limited to an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody.

The modified cell can further comprise an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. The modified cell secretes the BiTE. In certain embodiments, the inducible BiTE comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 53 or 54. In certain embodiments, the BiTE is capable of binding wild type EGFR (wtEGFR). In certain embodiments, the BiTE is capable of binding EGFR variant III (EGFRvIII).

G. Sources of Immune Cells

In certain embodiments, a source of immune cells (*e.g.* T cells) is obtained from a subject for *ex vivo* manipulation. Sources of immune cells for *ex vivo* manipulation may also include, *e.g.*, autologous or heterologous donor blood, cord blood, or bone marrow. For example the source of immune cells may be from the subject to be treated with the modified immune cells of the invention, *e.g.*, the subject's blood, the subject's cord blood, or the subject's bone marrow. Non-limiting examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof. Preferably, the subject is a human.

Immune cells can be obtained from a number of sources, including blood, peripheral blood mononuclear cells, bone marrow, lymph node tissue, spleen tissue, umbilical cord, lymph, or lymphoid organs. Immune cells are cells of the immune system, such as cells of the innate or adaptive immunity, *e.g.*, myeloid or lymphoid cells, including lymphocytes, typically T cells and/or NK cells. Other exemplary cells include stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs). In some aspects, the cells are human cells. With reference to the subject to be treated, the cells may be allogeneic and/or autologous. The cells typically are primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen.

In certain embodiments, the immune cell is a T cell, *e.g.*, a CD8⁺ T cell (*e.g.*, a CD8⁺ naive T cell, central memory T cell, or effector memory T cell), a CD4⁺ T cell, a natural killer T cell (NKT cells), a regulatory T cell (Treg), a stem cell memory T cell, a lymphoid progenitor cell a hematopoietic stem cell, a natural killer cell (NK cell) or a dendritic cell. In some embodiments, the cells are monocytes or granulocytes, *e.g.*, myeloid cells, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, and/or basophils. In an embodiment, the target cell is an induced pluripotent stem (iPS) cell or a cell derived from an iPS cell, *e.g.*, an iPS cell generated from a subject, manipulated to alter (*e.g.*, induce a mutation in) or manipulate the expression of one or more target genes, and differentiated into, *e.g.*, a T cell, *e.g.*, a CD8⁺ T cell (*e.g.*, a CD8⁺ naive T cell, central memory T cell, or effector memory T cell), a CD4⁺ T cell, a stem cell memory T cell, a lymphoid progenitor cell or a hematopoietic stem cell.

In some embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, CD4⁺ cells, CD8⁺ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen- specificity, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine

secretion profile, and/or degree of differentiation. Among the sub-types and subpopulations of T cells and/or of CD4+ and/or of CD8+ T cells are naive T (TN) cells, effector T cells (TEFF), memory T cells and sub-types thereof, such as stem cell memory T (TSCM), central memory T (TCM), effector memory T (TEM), or terminally differentiated effector memory T cells, tumor-infiltrating lymphocytes (TIL), immature T cells, mature T cells, helper T cells, cytotoxic T cells, mucosa-associated invariant T (MAIT) cells, naturally occurring and adaptive regulatory T (Treg) cells, helper T cells, such as TH1 cells, TH2 cells, TH3 cells, TH17 cells, TH9 cells, TH22 cells, follicular helper T cells, alpha/beta T cells, and delta/gamma T cells. In certain embodiments, any number of T cell lines available in the art, may be used.

In some embodiments, the methods include isolating immune cells from the subject, preparing, processing, culturing, and/or engineering them. In some embodiments, preparation of the engineered cells includes one or more culture and/or preparation steps. The cells for engineering as described may be isolated from a sample, such as a biological sample, *e.g.*, one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered. Accordingly, the cells in some embodiments are primary cells, *e.g.*, primary human cells. The samples include tissue, fluid, and other samples taken directly from the subject, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (*e.g.* transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is or is derived from an apheresis or leukapheresis product.

Exemplary samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Samples include, in the

context of cell therapy, *e.g.*, adoptive cell therapy, samples from autologous and allogeneic sources.

In some embodiments, the cells are derived from cell lines, *e.g.*, T cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, and pig. In some embodiments, isolation of the cells includes one or more preparation and/or non-affinity based cell separation steps. In some examples, cells are washed, centrifuged, and/or incubated in the presence of one or more reagents, for example, to remove unwanted components, enrich for desired components, lyse or remove cells sensitive to particular reagents. In some examples, cells are separated based on one or more property, such as density, adherent properties, size, sensitivity and/or resistance to particular components.

In some examples, cells from the circulating blood of a subject are obtained, *e.g.*, by apheresis or leukapheresis. The samples, in some aspects, contain lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and/or platelets, and in some aspects contains cells other than red blood cells and platelets. In some embodiments, the blood cells collected from the subject are washed, *e.g.*, to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some aspects, a washing step is accomplished by tangential flow filtration (TFF) according to the manufacturer's instructions. In some embodiments, the cells are resuspended in a variety of biocompatible buffers after washing. In certain embodiments, components of a blood cell sample are removed and the cells directly resuspended in culture media. In some embodiments, the methods include density-based cell separation methods, such as the preparation of white blood cells from peripheral blood by lysing the red blood cells and centrifugation through a Percoll or Ficoll gradient.

In one embodiment, immune are obtained cells from the circulating blood of an individual are obtained by apheresis or leukapheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. The cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media, such as phosphate buffered saline (PBS) or wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations, for subsequent processing steps. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as,

for example, Ca-free, Mg-free PBS. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

In some embodiments, the isolation methods include the separation of different cell types based on the expression or presence in the cell of one or more specific molecules, such as surface markers, *e.g.*, surface proteins, intracellular markers, or nucleic acid. In some
5 embodiments, any known method for separation based on such markers may be used. In some
embodiments, the separation is affinity- or immunoaffinity-based separation. For example, the isolation in some aspects includes separation of cells and cell populations based on the cells' expression or expression level of one or more markers, typically cell surface markers,
10 for example, by incubation with an antibody or binding partner that specifically binds to such
markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner.

Such separation steps can be based on positive selection, in which the cells having
15 bound the reagents are retained for further use, and/or negative selection, in which the cells
having not bound to the antibody or binding partner are retained. In some examples, both fractions are retained for further use. In some aspects, negative selection can be particularly useful where no antibody is available that specifically identifies a cell type in a heterogeneous population, such that separation is best carried out based on markers expressed by cells other
20 than the desired population. The separation need not result in 100% enrichment or removal of
a particular cell population or cells expressing a particular marker. For example, positive selection of or enrichment for cells of a particular type, such as those expressing a marker, refers to increasing the number or percentage of such cells, but need not result in a complete absence of cells not expressing the marker. Likewise, negative selection, removal, or
25 depletion of cells of a particular type, such as those expressing a marker, refers to decreasing
the number or percentage of such cells, but need not result in a complete removal of all such cells.

In some examples, multiple rounds of separation steps are carried out, where the positively or negatively selected fraction from one step is subjected to another separation
30 step, such as a subsequent positive or negative selection. In some examples, a single
separation step can deplete cells expressing multiple markers simultaneously, such as by incubating cells with a plurality of antibodies or binding partners, each specific for a marker targeted for negative selection. Likewise, multiple cell types can simultaneously be positively

selected by incubating cells with a plurality of antibodies or binding partners expressed on the various cell types.

In some embodiments, one or more of the T cell populations is enriched for or depleted of cells that are positive for (marker⁺) or express high levels (marker^{high}) of one or more particular markers, such as surface markers, or that are negative for (marker⁻) or express relatively low levels (marker^{low}) of one or more markers. For example, in some aspects, specific subpopulations of T cells, such as cells positive or expressing high levels of one or more surface markers, e.g., CD28⁺, CD62L⁺, CCR7⁺, CD27⁺, CD127⁺, CD4⁺, CD8⁺, CD45RA⁺, and/or CD45RO⁺ T cells, are isolated by positive or negative selection techniques. In some cases, such markers are those that are absent or expressed at relatively low levels on certain populations of T cells (such as non-memory cells) but are present or expressed at relatively higher levels on certain other populations of T cells (such as memory cells). In one embodiment, the cells (such as the CD8⁺ cells or the T cells, e.g., CD3⁺ cells) are enriched for (*i.e.*, positively selected for) cells that are positive or expressing high surface levels of CD45RO, CCR7, CD28, CD27, CD44, CD 127, and/or CD62L and/or depleted of (*e.g.*, negatively selected for) cells that are positive for or express high surface levels of CD45RA. In some embodiments, cells are enriched for or depleted of cells positive or expressing high surface levels of CD 122, CD95, CD25, CD27, and/or IL7-Ra (CD 127). In some examples, CD8⁺ T cells are enriched for cells positive for CD45RO (or negative for CD45RA) and for CD62L. For example, CD3⁺, CD28⁺ T cells can be positively selected using CD3/CD28 conjugated magnetic beads (e.g., DYNABEADS® M-450 CD3/CD28 T Cell Expander).

In some embodiments, T cells are separated from a PBMC sample by negative selection of markers expressed on non-T cells, such as B cells, monocytes, or other white blood cells, such as CD 14. In some aspects, a CD4⁺ or CD8⁺ selection step is used to separate CD4⁺ helper and CD8⁺ cytotoxic T cells. Such CD4⁺ and CD8⁺ populations can be further sorted into sub-populations by positive or negative selection for markers expressed or expressed to a relatively higher degree on one or more naive, memory, and/or effector T cell subpopulations. In some embodiments, CD8⁺ cells are further enriched for or depleted of naive, central memory, effector memory, and/or central memory stem cells, such as by positive or negative selection based on surface antigens associated with the respective subpopulation. In some embodiments, enrichment for central memory T (TCM) cells is carried out to increase efficacy, such as to improve long-term survival, expansion, and/or engraftment following administration, which in some aspects is particularly robust in such

sub-populations. In some embodiments, combining TCM-enriched CD8⁺ T cells and CD4⁺ T cells further enhances efficacy.

In some embodiments, memory T cells are present in both CD62L⁺ and CD62L⁻ subsets of CD8⁺ peripheral blood lymphocytes. PBMC can be enriched for or depleted of CD62L⁻CD8⁺ and/or CD62L⁺CD8⁺ fractions, such as using anti-CD8 and anti-CD62L antibodies. In some embodiments, a CD4⁺ T cell population and a CD8⁺ T cell sub-population, *e.g.*, a sub-population enriched for central memory (TCM) cells. In some embodiments, the enrichment for central memory T (TCM) cells is based on positive or high surface expression of CD45RO, CD62L, CCR7, CD28, CD3, and/or CD 127; in some aspects, it is based on negative selection for cells expressing or highly expressing CD45RA and/or granzyme B. In some aspects, isolation of a CD8⁺ population enriched for TCM cells is carried out by depletion of cells expressing CD4, CD 14, CD45RA, and positive selection or enrichment for cells expressing CD62L. In one aspect, enrichment for central memory T (TCM) cells is carried out starting with a negative fraction of cells selected based on CD4 expression, which is subjected to a negative selection based on expression of CD 14 and CD45RA, and a positive selection based on CD62L. Such selections in some aspects are carried out simultaneously and in other aspects are carried out sequentially, in either order. In some aspects, the same CD4 expression-based selection step used in preparing the CD8⁺ cell population or subpopulation, also is used to generate the CD4⁺ cell population or subpopulation, such that both the positive and negative fractions from the CD4-based separation are retained and used in subsequent steps of the methods, optionally following one or more further positive or negative selection steps.

CD4⁺ T helper cells are sorted into naive, central memory, and effector cells by identifying cell populations that have cell surface antigens. CD4⁺ lymphocytes can be obtained by standard methods. In some embodiments, naive CD4⁺ T lymphocytes are CD45RO⁻, CD45RA⁺, CD62L⁺, CD4⁺ T cells. In some embodiments, central memory CD4⁺ cells are CD62L⁺ and CD45RO⁺. In some embodiments, effector CD4⁺ cells are CD62L⁻ and CD45RO⁻. In one example, to enrich for CD4⁺ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In some embodiments, the antibody or binding partner is bound to a solid support or matrix, such as a magnetic bead or paramagnetic bead, to allow for separation of cells for positive and/or negative selection.

In some embodiments, the cells are incubated and/or cultured prior to or in connection with genetic engineering. The incubation steps can include culture, cultivation, stimulation,

activation, and/or propagation. In some embodiments, the compositions or cells are incubated in the presence of stimulating conditions or a stimulatory agent. Such conditions include those designed to induce proliferation, expansion, activation, and/or survival of cells in the population, to mimic antigen exposure, and/or to prime the cells for genetic engineering, such as for the introduction of a recombinant antigen receptor. The conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, *e.g.*, nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells. In some embodiments, the stimulating conditions or agents include one or more agent, *e.g.*, ligand, which is capable of activating an intracellular signaling domain of a TCR complex. In some aspects, the agent turns on or initiates TCR/CD3 intracellular signaling cascade in a T cell. Such agents can include antibodies, such as those specific for a TCR component and/or costimulatory receptor, *e.g.*, anti-CD3, anti-CD28, for example, bound to solid support such as a bead, and/or one or more cytokines. Optionally, the expansion method may further comprise the step of adding anti-CD3 and/or anti CD28 antibody to the culture medium (*e.g.*, at a concentration of at least about 0.5 ng/ml). In some embodiments, the stimulating agents include IL-2 and/or IL-15, for example, an IL-2 concentration of at least about 10 units/mL.

In another embodiment, T cells are isolated from peripheral blood by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient. Alternatively, T cells can be isolated from an umbilical cord. In any event, a specific subpopulation of T cells can be further isolated by positive or negative selection techniques.

The cord blood mononuclear cells so isolated can be depleted of cells expressing certain antigens, including, but not limited to, CD34, CD8, CD14, CD19, and CD56. Depletion of these cells can be accomplished using an isolated antibody, a biological sample comprising an antibody, such as ascites, an antibody bound to a physical support, and a cell bound antibody.

Enrichment of a T cell population by negative selection can be accomplished using a combination of antibodies directed to surface markers unique to the negatively selected cells. A preferred method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4⁺ cells by

negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8.

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

15 T cells can also be frozen after the washing step, which does not require the monocyte-removal step. While not wishing to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and
20 parameters are known in the art and will be useful in this context, in a non-limiting example, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or other suitable cell freezing media. The cells are then frozen to -80°C at a rate of 1°C per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20°C or in
25 liquid nitrogen.

In one embodiment, the T cell is comprised within a population of cells such as peripheral blood mononuclear cells, cord blood cells, a purified population of T cells, and a T cell line. In another embodiment, peripheral blood mononuclear cells comprise the population of T cells. In yet another embodiment, purified T cells comprise the population of T cells.

30 In certain embodiments, T regulatory cells (Tregs) can be isolated from a sample. The sample can include, but is not limited to, umbilical cord blood or peripheral blood. In certain embodiments, the Tregs are isolated by flow-cytometry sorting. The sample can be enriched for Tregs prior to isolation by any means known in the art. The isolated Tregs can be cryopreserved, and/or expanded prior to use. Methods for isolating Tregs are described in

U.S. Patent Numbers: 7,754,482, 8,722,400, and 9,555,105, and U.S. Patent Application No. 13/639,927, contents of which are incorporated herein in their entirety.

H. Methods of Treatment

5 The modified immune cells (*e.g.*, T cells) described herein may be included in a composition for immunotherapy. The composition may include a pharmaceutical composition and further include a pharmaceutically acceptable carrier. A therapeutically effective amount of the pharmaceutical composition comprising the modified T cells may be administered.

10 In one aspect, the invention includes a method of treating a disease or condition in a subject comprising administering to a subject in need thereof an effective amount of a modified T cell of the present invention. In another aspect, the invention includes a method of treating a disease or condition in a subject comprising administering to a subject in need thereof a pharmaceutical composition comprising an effective amount of a modified T cell of
15 the present invention. In another aspect, the invention includes a method for adoptive cell transfer therapy comprising administering to a subject in need thereof an effective amount of a modified T cell of the present invention.

 Methods for administration of immune cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions. For example,
20 adoptive T cell therapy methods are described, *e.g.*, in US Patent Application Publication No. 2003/0170238 to Gruenberg et al; US Patent No. 4,690,915 to Rosenberg; Rosenberg (2011) Nat Rev Clin Oncol. 8(10):577-85). See, *e.g.*, Themeli et al. (2013) Nat Biotechnol. 31(10): 928-933; Tsukahara et al. (2013) Biochem Biophys Res Commun 438(1): 84-9; Davila et al. (2013) PLoS ONE 8(4): e61338. In some embodiments, the cell therapy, *e.g.*, adoptive T cell
25 therapy is carried out by autologous transfer, in which the cells are isolated and/or otherwise prepared from the subject who is to receive the cell therapy, or from a sample derived from such a subject. Thus, in some aspects, the cells are derived from a subject, *e.g.*, patient, in need of a treatment and the cells, following isolation and processing are administered to the same subject.

30 In some embodiments, the cell therapy, *e.g.*, adoptive T cell therapy, is carried out by allogeneic transfer, in which the cells are isolated and/or otherwise prepared from a subject other than a subject who is to receive or who ultimately receives the cell therapy, *e.g.*, a first subject. In such embodiments, the cells then are administered to a different subject, *e.g.*, a second subject, of the same species. In some embodiments, the first and second subjects are

genetically identical. In some embodiments, the first and second subjects are genetically similar. In some embodiments, the second subject expresses the same HLA class or supertype as the first subject.

5 In some embodiments, the subject has been treated with a therapeutic agent targeting the disease or condition, *e.g.* the tumor, prior to administration of the cells or composition containing the cells. In some aspects, the subject is refractory or non-responsive to the other therapeutic agent. In some embodiments, the subject has persistent or relapsed disease, *e.g.*, following treatment with another therapeutic intervention, including chemotherapy, radiation, and/or hematopoietic stem cell transplantation (HSCT), *e.g.*, allogenic HSCT. In some
10 embodiments, the administration effectively treats the subject despite the subject having become resistant to another therapy.

In some embodiments, the subject is responsive to the other therapeutic agent, and treatment with the therapeutic agent reduces disease burden. In some aspects, the subject is initially responsive to the therapeutic agent, but exhibits a relapse of the disease or condition
15 over time. In some embodiments, the subject has not relapsed. In some such embodiments, the subject is determined to be at risk for relapse, such as at a high risk of relapse, and thus the cells are administered prophylactically, *e.g.*, to reduce the likelihood of or prevent relapse. In some aspects, the subject has not received prior treatment with another therapeutic agent.

In some embodiments, the subject has persistent or relapsed disease, *e.g.*, following
20 treatment with another therapeutic intervention, including chemotherapy, radiation, and/or hematopoietic stem cell transplantation (HSCT), *e.g.*, allogenic HSCT. In some embodiments, the administration effectively treats the subject despite the subject having become resistant to another therapy.

The modified immune cells of the present invention can be administered to an animal,
25 preferably a mammal, even more preferably a human, to treat a cancer. In addition, the cells of the present invention can be used for the treatment of any condition related to a cancer, especially a cell-mediated immune response against a tumor cell(s), where it is desirable to treat or alleviate the disease. The types of cancers to be treated with the modified cells or pharmaceutical compositions of the invention include, carcinoma, blastoma, and sarcoma,
30 and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies *e.g.*, sarcomas, carcinomas, and melanomas. Other exemplary cancers include but are not limited breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer, thyroid cancer, and the like. The cancers may be non-solid tumors

(such as hematological tumors) or solid tumors. Adult tumors/cancers and pediatric tumors/cancers are also included. In one embodiment, the cancer is a solid tumor or a hematological tumor. In one embodiment, the cancer is a carcinoma. In one embodiment, the cancer is a sarcoma. In one embodiment, the cancer is a leukemia. In one embodiment the cancer is a solid tumor.

Solid tumors are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumors can be benign or malignant. Different types of solid tumors are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, melanoma, and CNS tumors (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma and brain metastases). In certain embodiments, the cancer is an astrocytoma. In certain embodiments, the cancer is a high-grade astrocytoma.

Carcinomas that can be amenable to therapy by a method disclosed herein include, but are not limited to, esophageal carcinoma, hepatocellular carcinoma, basal cell carcinoma (a form of skin cancer), squamous cell carcinoma (various tissues), bladder carcinoma, including transitional cell carcinoma (a malignant neoplasm of the bladder), bronchogenic carcinoma, colon carcinoma, colorectal carcinoma, gastric carcinoma, lung carcinoma, including small cell carcinoma and non-small cell carcinoma of the lung, adrenocortical carcinoma, thyroid carcinoma, pancreatic carcinoma, breast carcinoma, ovarian carcinoma, prostate carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, renal cell carcinoma, ductal carcinoma in situ or bile duct carcinoma, choriocarcinoma,

seminoma, embryonal carcinoma, Wilm's tumor, cervical carcinoma, uterine carcinoma, testicular carcinoma, osteogenic carcinoma, epithelial carcinoma, and nasopharyngeal carcinoma.

Sarcomas that can be amenable to therapy by a method disclosed herein include, but are not limited to, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, chordoma, osteogenic sarcoma, osteosarcoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's sarcoma, leiomyosarcoma, rhabdomyosarcoma, and other soft tissue sarcomas.

In certain exemplary embodiments, the modified immune cells of the invention are used to treat a myeloma, or a condition related to myeloma. Examples of myeloma or conditions related thereto include, without limitation, light chain myeloma, non-secretory myeloma, monoclonal gamopathy of undetermined significance (MGUS), plasmacytoma (e.g., solitary, multiple solitary, extramedullary plasmacytoma), amyloidosis, and multiple myeloma. In one embodiment, a method of the present disclosure is used to treat multiple myeloma. In one embodiment, a method of the present disclosure is used to treat refractory myeloma. In one embodiment, a method of the present disclosure is used to treat relapsed myeloma.

In certain exemplary embodiments, the modified immune cells of the invention are used to treat a melanoma, or a condition related to melanoma. Examples of melanoma or conditions related thereto include, without limitation, superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, acral lentiginous melanoma, amelanotic melanoma, or melanoma of the skin (e.g., cutaneous, eye, vulva, vagina, rectum melanoma). In one embodiment, a method of the present disclosure is used to treat cutaneous melanoma. In one embodiment, a method of the present disclosure is used to treat refractory melanoma. In one embodiment, a method of the present disclosure is used to treat relapsed melanoma.

In yet other exemplary embodiments, the modified immune cells of the invention are used to treat a sarcoma, or a condition related to sarcoma. Examples of sarcoma or conditions related thereto include, without limitation, angiosarcoma, chondrosarcoma, Ewing's sarcoma, fibrosarcoma, gastrointestinal stromal tumor, leiomyosarcoma, liposarcoma, malignant peripheral nerve sheath tumor, osteosarcoma, pleomorphic sarcoma, rhabdomyosarcoma, and synovial sarcoma. In one embodiment, a method of the present disclosure is used to treat synovial sarcoma. In one embodiment, a method of the present disclosure is used to treat liposarcoma such as myxoid/round cell liposarcoma, differentiated/dedifferentiated liposarcoma, and pleomorphic liposarcoma. In one

embodiment, a method of the present disclosure is used to treat myxoid/round cell liposarcoma. In one embodiment, a method of the present disclosure is used to treat a refractory sarcoma. In one embodiment, a method of the present disclosure is used to treat a relapsed sarcoma.

5 The cells of the invention to be administered may be autologous, with respect to the subject undergoing therapy.

 The administration of the cells of the invention may be carried out in any convenient manner known to those of skill in the art. The cells of the present invention may be administered to a subject by aerosol inhalation, injection, ingestion, transfusion, implantation
10 or transplantation. The compositions described herein may be administered to a patient transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In other instances, the cells of the invention are injected directly into a site of inflammation in the subject, a local disease site in the subject, a lymph node, an organ, a tumor, and the like.

15 In some embodiments, the cells are administered at a desired dosage, which in some aspects includes a desired dose or number of cells or cell type(s) and/or a desired ratio of cell types. Thus, the dosage of cells in some embodiments is based on a total number of cells (or number per kg body weight) and a desired ratio of the individual populations or sub-types, such as the CD4⁺ to CD8⁺ ratio. In some embodiments, the dosage of cells is based on a
20 desired total number (or number per kg of body weight) of cells in the individual populations or of individual cell types. In some embodiments, the dosage is based on a combination of such features, such as a desired number of total cells, desired ratio, and desired total number of cells in the individual populations.

 In some embodiments, the populations or sub-types of cells, such as CD8⁺ and CD4⁺
25 T cells, are administered at or within a tolerated difference of a desired dose of total cells, such as a desired dose of T cells. In some aspects, the desired dose is a desired number of cells or a desired number of cells per unit of body weight of the subject to whom the cells are administered, e.g., cells/kg. In some aspects, the desired dose is at or above a minimum number of cells or minimum number of cells per unit of body weight. In some aspects, among
30 the total cells, administered at the desired dose, the individual populations or sub-types are present at or near a desired output ratio (such as CD4⁺ to CD8⁺ ratio), e.g., within a certain tolerated difference or error of such a ratio.

 In some embodiments, the cells are administered at or within a tolerated difference of a desired dose of one or more of the individual populations or sub-types of cells, such as a

desired dose of CD4⁺ cells and/or a desired dose of CD8⁺ cells. In some aspects, the desired dose is a desired number of cells of the sub-type or population, or a desired number of such cells per unit of body weight of the subject to whom the cells are administered, e.g., cells/kg. In some aspects, the desired dose is at or above a minimum number of cells of the population or subtype, or minimum number of cells of the population or sub-type per unit of body weight. Thus, in some embodiments, the dosage is based on a desired fixed dose of total cells and a desired ratio, and/or based on a desired fixed dose of one or more, e.g., each, of the individual sub-types or sub-populations. Thus, in some embodiments, the dosage is based on a desired fixed or minimum dose of T cells and a desired ratio of CD4⁺ to CD8⁺ cells, and/or is based on a desired fixed or minimum dose of CD4⁺ and/or CD8⁺ cells.

In certain embodiments, the cells, or individual populations of sub-types of cells, are administered to the subject at a range of about one million to about 100 billion cells, such as, e.g., 1 million to about 50 billion cells (e.g., about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (e.g., about 20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases about 100 million cells to about 50 billion cells (e.g., about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells, about 30 billion cells, about 45 billion cells) or any value in between these ranges.

In some embodiments, the dose of total cells and/or dose of individual sub-populations of cells is within a range of between at or about 1×10^5 cells/kg to about 1×10^{11} cells/kg 10^4 and at or about 10^{11} cells/kilograms (kg) body weight, such as between 10^5 and 10^6 cells / kg body weight, for example, at or about 1×10^5 cells/kg, 1.5×10^5 cells/kg, 2×10^5 cells/kg, or 1×10^6 cells/kg body weight. For example, in some embodiments, the cells are administered at, or within a certain range of error of, between at or about 10^4 and at or about 10^9 T cells/kilograms (kg) body weight, such as between 10^5 and 10^6 T cells / kg body weight, for example, at or about 1×10^5 T cells/kg, 1.5×10^5 T cells/kg, 2×10^5 T cells/kg, or 1×10^6 T cells/kg body weight. In other exemplary embodiments, a suitable dosage range of modified cells for use in a method of the present disclosure includes, without limitation, from about 1×10^5 cells/kg to about 1×10^6 cells/kg, from about 1×10^6 cells/kg to about 1×10^7

cells/kg, from about 1×10^7 cells/kg about 1×10^8 cells/kg, from about 1×10^8 cells/kg about 1×10^9 cells/kg, from about 1×10^9 cells/kg about 1×10^{10} cells/kg, from about 1×10^{10} cells/kg about 1×10^{11} cells/kg. In an exemplary embodiment, a suitable dosage for use in a method of the present disclosure is about 1×10^8 cells/kg. In an exemplary embodiment, a suitable dosage for use in a method of the present disclosure is about 1×10^7 cells/kg. In other embodiments, a suitable dosage is from about 1×10^7 total cells to about 5×10^7 total cells. In some embodiments, a suitable dosage is from about 1×10^8 total cells to about 5×10^8 total cells. In some embodiments, a suitable dosage is from about 1.4×10^7 total cells to about 1.1×10^9 total cells. In an exemplary embodiment, a suitable dosage for use in a method of the present disclosure is about 7×10^9 total cells.

In some embodiments, the cells are administered at or within a certain range of error of between at or about 10^4 and at or about 10^9 CD4⁺ and/or CD8⁺ cells/kilograms (kg) body weight, such as between 10^5 and 10^6 CD4⁺ and/or CD8⁺ cells / kg body weight, for example, at or about 1×10^5 CD4⁺ and/or CD8⁺ cells/kg, 1.5×10^5 CD4⁺ and/or CD8⁺ cells/kg, 2×10^5 CD4⁺ and/or CD8⁺ cells/kg, or 1×10^6 CD4⁺ and/or CD8⁺ cells/kg body weight. In some embodiments, the cells are administered at or within a certain range of error of, greater than, and/or at least about 1×10^6 , about 2.5×10^6 , about 5×10^6 , about 7.5×10^6 , or about 9×10^6 CD4⁺ cells, and/or at least about 1×10^6 , about 2.5×10^6 , about 5×10^6 , about 7.5×10^6 , or about 9×10^6 CD8⁺ cells, and/or at least about 1×10^6 , about 2.5×10^6 , about 5×10^6 , about 7.5×10^6 , or about 9×10^6 T cells. In some embodiments, the cells are administered at or within a certain range of error of between about 10^8 and 10^{12} or between about 10^{10} and 10^{11} T cells, between about 10^8 and 10^{12} or between about 10^{10} and 10^{11} CD4⁺ cells, and/or between about 10^8 and 10^{12} or between about 10^{10} and 10^{11} CD8⁺ cells.

In some embodiments, the cells are administered at or within a tolerated range of a desired output ratio of multiple cell populations or sub-types, such as CD4⁺ and CD8⁺ cells or sub-types. In some aspects, the desired ratio can be a specific ratio or can be a range of ratios, for example, in some embodiments, the desired ratio (e.g., ratio of CD4⁺ to CD8⁺ cells) is between at or about 5: 1 and at or about 5: 1 (or greater than about 1:5 and less than about 5: 1), or between at or about 1:3 and at or about 3: 1 (or greater than about 1:3 and less than about 3: 1), such as between at or about 2: 1 and at or about 1:5 (or greater than about 1:5 and less than about 2: 1, such as at or about 5: 1, 4.5: 1, 4: 1, 3.5: 1, 3: 1, 2.5: 1, 2: 1, 1.9: 1, 1.8: 1, 1.7: 1, 1.6: 1, 1.5: 1, 1.4: 1, 1.3: 1, 1.2: 1, 1.1: 1, 1: 1, 1: 1.1, 1: 1.2, 1: 1.3, 1:1.4, 1: 1.5, 1: 1.6, 1: 1.7, 1: 1.8, 1: 1.9: 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, or 1:5. In some aspects, the tolerated difference is within about 1%, about 2%, about 3%, about 4% about 5%, about 10%,

about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50% of the desired ratio, including any value in between these ranges.

In some embodiments, a dose of modified cells is administered to a subject in need thereof, in a single dose or multiple doses. In some embodiments, a dose of modified cells is administered in multiple doses, e.g., once a week or every 7 days, once every 2 weeks or every 14 days, once every 3 weeks or every 21 days, once every 4 weeks or every 28 days. In an exemplary embodiment, a single dose of modified cells is administered to a subject in need thereof. In an exemplary embodiment, a single dose of modified cells is administered to a subject in need thereof by rapid intravenous infusion.

For the prevention or treatment of disease, the appropriate dosage may depend on the type of disease to be treated, the type of cells or recombinant receptors, the severity and course of the disease, whether the cells are administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the cells, and the discretion of the attending physician. The compositions and cells are in some embodiments suitably administered to the subject at one time or over a series of treatments.

In some embodiments, the cells are administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as an antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent. The cells in some embodiments are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. In some contexts, the cells are co-administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the cells are administered prior to the one or more additional therapeutic agents. In some embodiments, the cells are administered after the one or more additional therapeutic agents. In some embodiments, the one or more additional agents includes a cytokine, such as IL-2, for example, to enhance persistence. In some embodiments, the methods comprise administration of a chemotherapeutic agent.

In certain embodiments, the modified cells of the invention (e.g., a modified cell comprising a CAR) may be administered to a subject in combination with an inhibitor of an immune checkpoint. Examples of immune checkpoints include but are not limited to CTLA-4, PD-1, and TIM-3. Antibodies may be used to inhibit an immune checkpoint (e.g., an anti-PD1, anti-CTLA-4, or anti-TIM-3 antibody). For example, the modified cell may be administered in combination with an antibody or antibody fragment targeting, for example,

PD-1 (programmed death 1 protein). Examples of anti-PD-1 antibodies include, but are not limited to, pembrolizumab (KEYTRUDA®, formerly lambrolizumab, also known as MK-3475), and nivolumab (BMS-936558, MDX-1106, ONO-4538, OPDIVA®) or an antigen-binding fragment thereof. In certain embodiments, the modified cell may be administered in combination with an anti-PD-L1 antibody or antigen-binding fragment thereof. Examples of anti-PD-L1 antibodies include, but are not limited to, BMS-936559, MPDL3280A (TECENTRIQ®, Atezolizumab), and MEDI4736 (Durvalumab, Imfinzi). In certain embodiments, the modified cell may be administered in combination with an anti-CTLA-4 antibody or antigen-binding fragment thereof. An example of an anti-CTLA-4 antibody includes, but is not limited to, Ipilimumab (trade name Yervoy). Other types of immune checkpoint modulators may also be used including, but not limited to, small molecules, siRNA, miRNA, and CRISPR systems. Immune checkpoint modulators may be administered before, after, or concurrently with the modified cell comprising the CAR. In certain embodiments, combination treatment comprising an immune checkpoint modulator may increase the therapeutic efficacy of a therapy comprising a modified cell of the present invention.

Following administration of the cells, the biological activity of the engineered cell populations in some embodiments is measured, *e.g.*, by any of a number of known methods. Parameters to assess include specific binding of an engineered or natural T cell or other immune cell to antigen, *in vivo*, *e.g.*, by imaging, or *ex vivo*, *e.g.*, by ELISA or flow cytometry. In certain embodiments, the ability of the engineered cells to destroy target cells can be measured using any suitable method known in the art, such as cytotoxicity assays described in, for example, Kochenderfer et al., J. Immunotherapy, 32(7): 689-702 (2009), and Herman et al. J. Immunological Methods, 285(1): 25-40 (2004). In certain embodiments, the biological activity of the cells is measured by assaying expression and/or secretion of one or more cytokines, such as CD 107a, IFN γ , IL-2, and TNF. In some aspects the biological activity is measured by assessing clinical outcome, such as reduction in tumor burden or load.

In certain embodiments, the subject is provided a secondary treatment. Secondary treatments include but are not limited to chemotherapy, radiation, surgery, and medications.

In some embodiments, the subject can be administered a conditioning therapy prior to CAR T cell therapy. In some embodiments, the conditioning therapy comprises administering an effective amount of cyclophosphamide to the subject. In some embodiments, the conditioning therapy comprises administering an effective amount of fludarabine to the subject. In preferred embodiments, the conditioning therapy comprises administering an

effective amount of a combination of cyclophosphamide and fludarabine to the subject. Administration of a conditioning therapy prior to CAR T cell therapy may increase the efficacy of the CAR T cell therapy. Methods of conditioning patients for T cell therapy are described in U.S. Patent No. 9,855,298, which is incorporated herein by reference in its

entirety.

In some embodiments, a specific dosage regimen of the present disclosure includes a lymphodepletion step prior to the administration of the modified T cells. In an exemplary embodiment, the lymphodepletion step includes administration of cyclophosphamide and/or fludarabine.

In some embodiments, the lymphodepletion step includes administration of cyclophosphamide at a dose of between about 200 mg/m²/day and about 2000 mg/m²/day (*e.g.*, 200 mg/m²/day, 300 mg/m²/day, or 500 mg/m²/day). In an exemplary embodiment, the dose of cyclophosphamide is about 300 mg/m²/day. In some embodiments, the lymphodepletion step includes administration of fludarabine at a dose of between about 20 mg/m²/day and about 900 mg/m²/day (*e.g.*, 20 mg/m²/day, 25 mg/m²/day, 30 mg/m²/day, or 60 mg/m²/day). In an exemplary embodiment, the dose of fludarabine is about 30 mg/m²/day.

In some embodiment, the lymphodepletion step includes administration of cyclophosphamide at a dose of between about 200 mg/m²/day and about 2000 mg/m²/day (*e.g.*, 200 mg/m²/day, 300 mg/m²/day, or 500 mg/m²/day), and fludarabine at a dose of between about 20 mg/m²/day and about 900 mg/m²/day (*e.g.*, 20 mg/m²/day, 25 mg/m²/day, 30 mg/m²/day, or 60 mg/m²/day). In an exemplary embodiment, the lymphodepletion step includes administration of cyclophosphamide at a dose of about 300 mg/m²/day, and fludarabine at a dose of about 30 mg/m²/day.

In an exemplary embodiment, the dosing of cyclophosphamide is 300 mg/m²/day over three days, and the dosing of fludarabine is 30 mg/m²/day over three days.

Dosing of lymphodepletion chemotherapy may be scheduled on Days -6 to -4 (with a -1 day window, *i.e.*, dosing on Days -7 to -5) relative to T cell (*e.g.*, CAR-T, TCR-T, a modified T cell, *etc.*) infusion on Day 0.

In an exemplary embodiment, for a subject having cancer, the subject receives lymphodepleting chemotherapy including 300 mg/m² of cyclophosphamide by intravenous infusion 3 days prior to administration of the modified T cells. In an exemplary embodiment, for a subject having cancer, the subject receives lymphodepleting chemotherapy including

300 mg/m² of cyclophosphamide by intravenous infusion for 3 days prior to administration of the modified T cells.

In an exemplary embodiment, for a subject having cancer, the subject receives lymphodepleting chemotherapy including fludarabine at a dose of between about 20
5 mg/m²/day and about 900 mg/m²/day (*e.g.*, 20 mg/m²/day, 25 mg/m²/day, 30 mg/m²/day, or 60 mg/m²/day). In an exemplary embodiment, for a subject having cancer, the subject receives lymphodepleting chemotherapy including fludarabine at a dose of 30 mg/m² for 3 days.

In an exemplary embodiment, for a subject having cancer, the subject receives
10 lymphodepleting chemotherapy including cyclophosphamide at a dose of between about 200 mg/m²/day and about 2000 mg/m²/day (*e.g.*, 200 mg/m²/day, 300 mg/m²/day, or 500 mg/m²/day), and fludarabine at a dose of between about 20 mg/m²/day and about 900 mg/m²/day (*e.g.*, 20 mg/m²/day, 25 mg/m²/day, 30 mg/m²/day, or 60 mg/m²/day). In an exemplary embodiment, for a subject having cancer, the subject receives lymphodepleting
15 chemotherapy including cyclophosphamide at a dose of about 300 mg/m²/day, and fludarabine at a dose of 30 mg/m² for 3 days.

Cells of the invention can be administered in dosages and routes and at times to be determined in appropriate pre-clinical and clinical experimentation and trials. Cell compositions may be administered multiple times at dosages within these ranges.

20 Administration of the cells of the invention may be combined with other methods useful to treat the desired disease or condition as determined by those of skill in the art.

It is known in the art that one of the adverse effects following infusion of CAR T cells is the onset of immune activation, known as cytokine release syndrome (CRS). CRS is immune activation resulting in elevated inflammatory cytokines. CRS is a known on-target
25 toxicity, development of which likely correlates with efficacy. Clinical and laboratory measures range from mild CRS (constitutional symptoms and/or grade-2 organ toxicity) to severe CRS (sCRS; grade ≥ 3 organ toxicity, aggressive clinical intervention, and/or potentially life threatening). Clinical features include: high fever, malaise, fatigue, myalgia, nausea, anorexia, tachycardia/hypotension, capillary leak, cardiac dysfunction, renal
30 impairment, hepatic failure, and disseminated intravascular coagulation. Dramatic elevations of cytokines including interferon-gamma, granulocyte macrophage colony-stimulating factor, IL-10, and IL-6 have been shown following CAR T-cell infusion. One CRS signature is elevation of cytokines including IL-6 (severe elevation), IFN-gamma, TNF-alpha (moderate), and IL-2 (mild). Elevations in clinically available markers of inflammation including ferritin

and C-reactive protein (CRP) have also been observed to correlate with the CRS syndrome. The presence of CRS generally correlates with expansion and progressive immune activation of adoptively transferred cells. It has been demonstrated that the degree of CRS severity is dictated by disease burden at the time of infusion as patients with high tumor burden

5 experience a more sCRS.

Accordingly, the invention provides for, following the diagnosis of CRS, appropriate CRS management strategies to mitigate the physiological symptoms of uncontrolled inflammation without dampening the antitumor efficacy of the engineered cells (*e.g.*, CAR T cells). CRS management strategies are known in the art. For example, systemic
10 corticosteroids may be administered to rapidly reverse symptoms of sCRS (*e.g.*, grade 3 CRS) without compromising initial antitumor response.

In some embodiments, an anti-IL-6R antibody may be administered. An example of an anti-IL-6R antibody is the Food and Drug Administration-approved monoclonal antibody tocilizumab, also known as atilizumab (marketed as Actemra, or RoActemra). Tocilizumab is
15 a humanized monoclonal antibody against the interleukin-6 receptor (IL-6R). Administration of tocilizumab has demonstrated near-immediate reversal of CRS.

CRS is generally managed based on the severity of the observed syndrome and interventions are tailored as such. CRS management decisions may be based upon clinical signs and symptoms and response to interventions, not solely on laboratory values alone.

20 Mild to moderate cases generally are treated with symptom management with fluid therapy, non-steroidal anti-inflammatory drug (NSAID) and antihistamines as needed for adequate symptom relief. More severe cases include patients with any degree of hemodynamic instability; with any hemodynamic instability, the administration of tocilizumab is recommended. The first-line management of CRS may be tocilizumab, in
25 some embodiments, at the labeled dose of 8 mg/kg IV over 60 minutes (not to exceed 800 mg/dose); tocilizumab can be repeated Q8 hours. If suboptimal response to the first dose of tocilizumab, additional doses of tocilizumab may be considered. Tocilizumab can be administered alone or in combination with corticosteroid therapy. Patients with continued or progressive CRS symptoms, inadequate clinical improvement in 12-18 hours or poor
30 response to tocilizumab, may be treated with high-dose corticosteroid therapy, generally hydrocortisone 100 mg IV or methylprednisolone 1-2 mg/kg. In patients with more severe hemodynamic instability or more severe respiratory symptoms, patients may be administered high-dose corticosteroid therapy early in the course of the CRS. CRS management guidance may be based on published standards (Lee et al. (2019) *Biol Blood Marrow Transplant*,

doi.org/10.1016/j.bbmt.2018.12.758; Neelapu et al. (2018) *Nat Rev Clin Oncology*, 15:47; Teachey et al. (2016) *Cancer Discov*, 6(6):664-679).

Features consistent with Macrophage Activation Syndrome (MAS) or Hemophagocytic lymphohistiocytosis (HLH) have been observed in patients treated with CAR-T therapy (Henter, 2007), coincident with clinical manifestations of the CRS. MAS appears to be a reaction to immune activation that occurs from the CRS, and should therefore be considered a manifestation of CRS. MAS is similar to HLH (also a reaction to immune stimulation). The clinical syndrome of MAS is characterized by high grade non-remitting fever, cytopenias affecting at least two of three lineages, and hepatosplenomegaly. It is associated with high serum ferritin, soluble interleukin-2 receptor, and triglycerides, and a decrease of circulating natural killer (NK) activity.

The modified immune cells comprising CAR of the present invention may be used in a method of treatment as described herein. In one aspect, the invention includes a method of treating cancer in a subject in need thereof, comprising administering to the subject any one of the modified immune or precursor cells disclosed herein. Yet another aspect of the invention includes a method of treating cancer in a subject in need thereof, comprising administering to the subject a modified immune or precursor cell generated by any one of the methods disclosed herein.

One aspect of the invention provides a method of treating glioblastoma in a subject in need thereof. The method comprises administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2. The CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFDDV (SEQ ID NO: 15); and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18).

Another aspect of the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises: a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20.

Yet another aspect of the invention includes a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 21 or SEQ ID NO: 22.

Another aspect of the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or SEQ ID NO: 24 or SEQ ID NO: 55 or SEQ ID NO: 56.

Any of the methods disclosed herein can further comprise administering an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the modified cell secretes the BiTE. In certain embodiments, the inducible BiTE comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 53 or 54. In certain embodiments, the BiTE is capable of binding wild type EGFR (wtEGFR). In certain embodiments, the BiTE is capable of binding EGFR variant III (EGFRvIII). In certain embodiments, the BiTE is co-administered with the modified T cell. In certain embodiments, the method further comprises administering an inducible BiTE capable of binding EGFR or an isoform thereof, and an inhibitor of an immune checkpoint, wherein the modified cell secretes the BiTE and the inhibitor of the immune checkpoint. In certain embodiments, the BiTE and the inhibitor of the immune checkpoint is co-administered with the modified T cell.

Also provided is a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising

a first CAR comprising a first antigen-binding domain capable of binding IL13R α 2; and second CAR comprising a second antigen-binding domain capable of binding EGFR or an isoform thereof.

In another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first CAR capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. The

first CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs) and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs). HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNALMS (SEQ ID NO: 3) or TVSSGGSYTYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFDDV (SEQ ID NO: 15). LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVAL (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18). The second CAR comprises a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs) and a light chain variable region that comprises three light chain complementarity determining regions (LCDRs). HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFYVW (SEQ ID NO: 27). LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGINLDD (SEQ ID NO: 143) or HGTNLDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

In yet another aspect, the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof. The first CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,

99%, or 100% identical to SEQ ID NO: 8 or 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20. The second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32. In certain embodiments, the second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 144 or SEQ ID NO: 145; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 146 or SEQ ID NO: 147.

In still another aspect, the invention includes a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or 11 or 21 or 22; and the second CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34, 44, or 142.

Another aspect of the invention provides a method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 24 or 55 or 56; and the second CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 36 or 38 or 197.

I. Expansion of Immune Cells

Whether prior to or after modification of cells to express a CAR, the cells can be activated and expanded in number using methods as described, for example, in U.S. Patent

Nos. 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;
6,867,041; and U.S. Publication No. 20060121005. For example, the T cells of the invention
may be expanded by contact with a surface having attached thereto an agent that stimulates a
CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule
on the surface of the T cells. In particular, T cell populations may be stimulated by contact
with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody
immobilized on a surface, or by contact with a protein kinase C activator (*e.g.*, bryostatin) in
conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the
surface of the T cells, a ligand that binds the accessory molecule is used. For example, T
cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under
conditions appropriate for stimulating proliferation of the T cells. Examples of an anti-CD28
antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besancon, France) and these can be used in
the invention, as can other methods and reagents known in the art (see, *e.g.*, ten Berge et al.,
Transplant Proc. (1998) 30(8): 3975-3977; Haanen et al., J. Exp. Med. (1999) 190(9): 1319-
1328; and Garland et al., J. Immunol. Methods (1999) 227(1-2): 53-63).

Expanding T cells by the methods disclosed herein can be multiplied by about 10
fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold, 200 fold,
300 fold, 400 fold, 500 fold, 600 fold, 700 fold, 800 fold, 900 fold, 1000 fold, 2000 fold,
3000 fold, 4000 fold, 5000 fold, 6000 fold, 7000 fold, 8000 fold, 9000 fold, 10,000 fold,
100,000 fold, 1,000,000 fold, 10,000,000 fold, or greater, and any and all whole or partial
integers therebetween. In one embodiment, the T cells expand in the range of about 20 fold
to about 50 fold.

Following culturing, the T cells can be incubated in cell medium in a culture
apparatus for a period of time or until the cells reach confluency or high cell density for
optimal passage before passing the cells to another culture apparatus. The culturing
apparatus can be of any culture apparatus commonly used for culturing cells *in vitro*.
Preferably, the level of confluence is 70% or greater before passing the cells to another
culture apparatus. More preferably, the level of confluence is 90% or greater. A period of
time can be any time suitable for the culture of cells *in vitro*. The T cell medium may be
replaced during the culture of the T cells at any time. Preferably, the T cell medium is
replaced about every 2 to 3 days. The T cells are then harvested from the culture apparatus
whereupon the T cells can be used immediately or cryopreserved to be stored for use at a

later time. In one embodiment, the invention includes cryopreserving the expanded T cells. The cryopreserved T cells are thawed prior to introducing nucleic acids into the T cell.

In another embodiment, the method comprises isolating T cells and expanding the T cells. In another embodiment, the invention further comprises cryopreserving the T cells
5 prior to expansion. In yet another embodiment, the cryopreserved T cells are thawed for electroporation with the RNA encoding the chimeric membrane protein.

Another procedure for *ex vivo* expansion cells is described in U.S. Pat. No. 5,199,942 (incorporated herein by reference). Expansion, such as described in U.S. Pat. No. 5,199,942 can be an alternative or in addition to other methods of expansion described herein. Briefly,
10 *ex vivo* culture and expansion of T cells comprises the addition to the cellular growth factors, such as those described in U.S. Pat. No. 5,199,942, or other factors, such as flt3-L, IL-1, IL-3 and c-kit ligand. In one embodiment, expanding the T cells comprises culturing the T cells with a factor selected from the group consisting of flt3-L, IL-1, IL-3 and c-kit ligand.

The culturing step as described herein (contact with agents as described herein or after
15 electroporation) can be very short, for example less than 24 hours such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 hours. The culturing step as described further herein (contact with agents as described herein) can be longer, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more days.

Various terms are used to describe cells in culture. Cell culture refers generally to
20 cells taken from a living organism and grown under controlled condition. A primary cell culture is a culture of cells, tissues or organs taken directly from an organism and before the first subculture. Cells are expanded in culture when they are placed in a growth medium under conditions that facilitate cell growth and/or division, resulting in a larger population of the cells. When cells are expanded in culture, the rate of cell proliferation is typically
25 measured by the amount of time required for the cells to double in number, otherwise known as the doubling time.

Each round of subculturing is referred to as a passage. When cells are subcultured, they are referred to as having been passaged. A specific population of cells, or a cell line, is sometimes referred to or characterized by the number of times it has been passaged. For
30 example, a cultured cell population that has been passaged ten times may be referred to as a P10 culture. The primary culture, i.e., the first culture following the isolation of cells from tissue, is designated P0. Following the first subculture, the cells are described as a secondary culture (P1 or passage 1). After the second subculture, the cells become a tertiary culture (P2 or passage 2), and so on. It will be understood by those of skill in the art that there may be

many population doublings during the period of passaging; therefore the number of population doublings of a culture is greater than the passage number. The expansion of cells (i.e., the number of population doublings) during the period between passaging depends on many factors, including but is not limited to the seeding density, substrate, medium, and time between passaging.

In one embodiment, the cells may be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN- γ , IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGF- β , and TNF- α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α -MEM, F-12, 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 of T cells. 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% CO₂).

The medium used to culture the T cells may include an agent that can co-stimulate the T cells. For example, an agent that can stimulate CD3 is an antibody to CD3, and an agent that can stimulate CD28 is an antibody to CD28. A cell isolated by the methods disclosed herein can be expanded approximately 10 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold, 200 fold, 300 fold, 400 fold, 500 fold, 600 fold, 700 fold, 800 fold, 900 fold, 1000 fold, 2000 fold, 3000 fold, 4000 fold, 5000 fold, 6000 fold, 7000 fold, 8000 fold, 9000 fold, 10,000 fold, 100,000 fold, 1,000,000 fold, 10,000,000 fold, or greater. In one embodiment, the T cells expand in the range of about 20 fold to about 50 fold, or more. In one embodiment, human T regulatory cells are expanded via anti-CD3 antibody coated KT64.86 artificial antigen presenting cells (aAPCs). Methods for expanding and activating T cells can be found in U.S. Patent Numbers: 7,754,482, 8,722,400, and 9,555, 105, contents of which are incorporated herein in their entirety.

In one embodiment, the method of expanding the T cells can further comprise isolating the expanded T cells for further applications. In another embodiment, the method of expanding can further comprise a subsequent electroporation of the expanded T cells followed by culturing. The subsequent electroporation may include introducing a nucleic acid encoding an agent, such as a transducing the expanded T cells, transfecting the expanded T cells, or electroporating the expanded T cells with a nucleic acid, into the expanded population of T cells, wherein the agent further stimulates the T cell. The agent may stimulate the T cells, such as by stimulating further expansion, effector function, or another T cell function.

J. Methods of Producing Genetically Modified Immune Cells

The present disclosure provides methods for producing or generating a modified immune cell or precursor thereof (*e.g.*, a T cell) of the invention for tumor immunotherapy, *e.g.*, adoptive immunotherapy.

In some embodiments, the CAR is introduced into a cell by an expression vector. Expression vectors comprising a nucleic acid sequence encoding a CAR of the present invention are provided herein. Suitable expression vectors include lentivirus vectors, gamma retrovirus vectors, foamy virus vectors, adeno associated virus (AAV) vectors, adenovirus vectors, engineered hybrid viruses, naked DNA, including but not limited to transposon mediated vectors, such as Sleeping Beauty, Piggybak, and Integrases such as Phi31. Some other suitable expression vectors include Herpes simplex virus (HSV) and retrovirus expression vectors.

In certain embodiments, the nucleic acid encoding a CAR is introduced into the cell via viral transduction. In certain embodiments, the viral transduction comprises contacting the immune or precursor cell with a viral vector comprising the nucleic acid encoding a CAR. In certain embodiments, the viral vector is an adeno-associated viral (AAV) vector. In certain embodiments, the AAV vector comprises a 5' ITR and a 3' ITR derived from AAV6. In certain embodiments, the AAV vector comprises a Woodchuck Hepatitis Virus post-transcriptional regulatory element (WPRE). In certain embodiments, the AAV vector comprises a polyadenylation (polyA) sequence. In certain embodiments, the polyA sequence is a bovine growth hormone (BGH) polyA sequence.

Adenovirus expression vectors are based on adenoviruses, which have a low capacity for integration into genomic DNA but a high efficiency for transfecting host cells.

Adenovirus expression vectors contain adenovirus sequences sufficient to: (a) support

packaging of the expression vector and (b) to ultimately express the CAR in the host cell. In some embodiments, the adenovirus genome is a 36 kb, linear, double stranded DNA, where a foreign DNA sequence (e.g., a nucleic acid encoding a CAR) may be inserted to substitute large pieces of adenoviral DNA in order to make the expression vector of the present

invention (see, e.g., Danthinne and Imperiale, *Gene Therapy* (2000) 7(20): 1707-1714).

Another expression vector is based on an adeno associated virus (AAV), which takes advantage of the adenovirus coupled systems. This AAV expression vector has a high frequency of integration into the host genome. It can infect nondividing cells, thus making it useful for delivery of genes into mammalian cells, for example, in tissue cultures or *in vivo*.

The AAV vector has a broad host range for infectivity. Details concerning the generation and use of AAV vectors are described in U.S. Patent Nos. 5,139,941 and 4,797,368.

Retrovirus expression vectors are capable of integrating into the host genome, delivering a large amount of foreign genetic material, infecting a broad spectrum of species and cell types and being packaged in special cell lines. The retroviral vector is constructed by inserting a nucleic acid (e.g., a nucleic acid encoding a CAR) into the viral genome at certain locations to produce a virus that is replication defective. Though the retroviral vectors are able to infect a broad variety of cell types, integration and stable expression of the CAR requires the division of host cells.

Lentiviral vectors are derived from lentiviruses, which are complex retroviruses that, in addition to the common retroviral genes *gag*, *pol*, and *env*, contain other genes with regulatory or structural function (see, e.g., U.S. Patent Nos. 6,013,516 and 5,994,136). Some examples of lentiviruses include the Human Immunodeficiency Viruses (HIV-1, HIV-2) and the Simian Immunodeficiency Virus (SIV). Lentiviral vectors have been generated by multiply attenuating the HIV virulence genes, for example, the genes *env*, *vif*, *vpr*, *vpu* and *nef* are deleted making the vector biologically safe. Lentiviral vectors are capable of infecting non-dividing cells and can be used for both *in vivo* and *ex vivo* gene transfer and expression, e.g., of a nucleic acid encoding a CAR (see, e.g., U.S. Patent No. 5,994,136).

Expression vectors including a nucleic acid of the present disclosure can be introduced into a host cell by any means known to persons skilled in the art. The expression vectors may include viral sequences for transfection, if desired. Alternatively, the expression vectors may be introduced by fusion, electroporation, biolistics, transfection, lipofection, or the like. The host cell may be grown and expanded in culture before introduction of the expression vectors, followed by the appropriate treatment for introduction and integration of the vectors. The host cells are then expanded and may be screened by virtue of a marker

present in the vectors. Various markers that may be used are known in the art, and may include hprt, neomycin resistance, thymidine kinase, hygromycin resistance, etc. As used herein, the terms "cell," "cell line," and "cell culture" may be used interchangeably. In some embodiments, the host cell an immune cell or precursor thereof, e.g., a T cell, an NK cell, or an NKT cell.

The present invention also provides genetically engineered cells which include and stably express a CAR of the present disclosure. In some embodiments, the genetically engineered cells are genetically engineered T-lymphocytes (T cells), naive T cells (TN), memory T cells (for example, central memory T cells (TCM), effector memory cells (TEM)), natural killer cells (NK cells), and macrophages capable of giving rise to therapeutically relevant progeny. In certain embodiments, the genetically engineered cells are autologous cells. In certain embodiments, the modified cell is resistant to T cell exhaustion.

Modified cells (e.g., comprising a CAR) may be produced by stably transfecting host cells with an expression vector including a nucleic acid of the present disclosure. Additional methods for generating a modified cell of the present disclosure include, without limitation, chemical transformation methods (e.g., using calcium phosphate, dendrimers, liposomes and/or cationic polymers), non-chemical transformation methods (e.g., electroporation, optical transformation, gene electrotransfer and/or hydrodynamic delivery) and/or particle-based methods (e.g., impalefection, using a gene gun and/or magnetofection). Transfected cells expressing a CAR of the present disclosure may be expanded *ex vivo*.

Physical methods for introducing an expression vector into host cells include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells including vectors and/or exogenous nucleic acids are well-known in the art. See, e.g., Sambrook et al. (2001), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York. Chemical methods for introducing an expression vector into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

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

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

Compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the nucleic acids in the host cell, a variety of assays may be performed. Such assays include, for example, molecular biology assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; biochemistry assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

In one embodiment, the nucleic acids introduced into the host cell are RNA. In another embodiment, the RNA is mRNA that comprises *in vitro* transcribed RNA or synthetic RNA. The RNA may be produced by *in vitro* transcription using a polymerase chain reaction (PCR)-generated template. DNA of interest from any source can be directly converted by PCR into a template for *in vitro* mRNA synthesis using appropriate primers and RNA polymerase. The source of the DNA may be, for example, genomic DNA, plasmid DNA, phage DNA, cDNA, synthetic DNA sequence or any other appropriate source of DNA.

PCR may be used to generate a template for *in vitro* transcription of mRNA which is then introduced into cells. Methods for performing PCR are well known in the art. Primers for use in PCR are designed to have regions that are substantially complementary to regions of the DNA to be used as a template for the PCR. “Substantially complementary,” as used herein, refers to sequences of nucleotides where a majority or all of the bases in the primer sequence are complementary. Substantially complementary sequences are able to anneal or

hybridize with the intended DNA target under annealing conditions used for PCR. The primers can be designed to be substantially complementary to any portion of the DNA template. For example, the primers can be designed to amplify the portion of a gene that is normally transcribed in cells (the open reading frame), including 5' and 3' UTRs. The primers may also be designed to amplify a portion of a gene that encodes a particular domain of interest. In one embodiment, the primers are designed to amplify the coding region of a human cDNA, including all or portions of the 5' and 3' UTRs. Primers useful for PCR are generated by synthetic methods that are well known in the art. "Forward primers" are primers that contain a region of nucleotides that are substantially complementary to nucleotides on the DNA template that are upstream of the DNA sequence that is to be amplified. "Upstream" is used herein to refer to a location 5' to the DNA sequence to be amplified relative to the coding strand. "Reverse primers" are primers that contain a region of nucleotides that are substantially complementary to a double-stranded DNA template that are downstream of the DNA sequence that is to be amplified. "Downstream" is used herein to refer to a location 3' to the DNA sequence to be amplified relative to the coding strand.

Chemical structures that have the ability to promote stability and/or translation efficiency of the RNA may also be used. The RNA preferably has 5' and 3' UTRs. In one embodiment, the 5' UTR is between zero and 3000 nucleotides in length. The length of 5' and 3' UTR sequences to be added to the coding region can be altered by different methods, including, but not limited to, designing primers for PCR that anneal to different regions of the UTRs. Using this approach, one of ordinary skill in the art can modify the 5' and 3' UTR lengths required to achieve optimal translation efficiency following transfection of the transcribed RNA.

The 5' and 3' UTRs can be the naturally occurring, endogenous 5' and 3' UTRs for the gene of interest. Alternatively, UTR sequences that are not endogenous to the gene of interest can be added by incorporating the UTR sequences into the forward and reverse primers or by any other modifications of the template. The use of UTR sequences that are not endogenous to the gene of interest can be useful for modifying the stability and/or translation efficiency of the RNA. For example, it is known that AU-rich elements in 3' UTR sequences can decrease the stability of mRNA. Therefore, 3' UTRs can be selected or designed to increase the stability of the transcribed RNA based on properties of UTRs that are well known in the art.

In one embodiment, the 5' UTR can contain the Kozak sequence of the endogenous gene. Alternatively, when a 5' UTR that is not endogenous to the gene of interest is being

added by PCR as described above, a consensus Kozak sequence can be redesigned by adding the 5' UTR sequence. Kozak sequences can increase the efficiency of translation of some RNA transcripts, but does not appear to be required for all RNAs to enable efficient translation. The requirement for Kozak sequences for many mRNAs is known in the art. In other embodiments the 5' UTR can be derived from an RNA virus whose RNA genome is stable in cells. In other embodiments various nucleotide analogues can be used in the 3' or 5' UTR to impede exonuclease degradation of the mRNA.

To enable synthesis of RNA from a DNA template without the need for gene cloning, a promoter of transcription should be attached to the DNA template upstream of the sequence to be transcribed. When a sequence that functions as a promoter for an RNA polymerase is added to the 5' end of the forward primer, the RNA polymerase promoter becomes incorporated into the PCR product upstream of the open reading frame that is to be transcribed. In one embodiment, the promoter is a T7 polymerase promoter, as described elsewhere herein. Other useful promoters include, but are not limited to, T3 and SP6 RNA polymerase promoters. Consensus nucleotide sequences for T7, T3 and SP6 promoters are known in the art.

In one embodiment, the mRNA has both a cap on the 5' end and a 3' poly(A) tail which determine ribosome binding, initiation of translation and stability mRNA in the cell. On a circular DNA template, for instance, plasmid DNA, RNA polymerase produces a long concatameric product which is not suitable for expression in eukaryotic cells. The transcription of plasmid DNA linearized at the end of the 3' UTR results in normal sized mRNA which is not effective in eukaryotic transfection even if it is polyadenylated after transcription.

On a linear DNA template, phage T7 RNA polymerase can extend the 3' end of the transcript beyond the last base of the template (Schenborn and Mierendorf, Nuc Acids Res., 13:6223-36 (1985); Nacheva and Berzal-Herranz, Eur. J. Biochem., 270:1485-65 (2003).

The polyA/T segment of the transcriptional DNA template can be produced during PCR by using a reverse primer containing a polyT tail, such as 100T tail (size can be 50-5000 T), or after PCR by any other method, including, but not limited to, DNA ligation or *in vitro* recombination. Poly(A) tails also provide stability to RNAs and reduce their degradation. Generally, the length of a poly(A) tail positively correlates with the stability of the transcribed RNA. In one embodiment, the poly(A) tail is between 100 and 5000 adenosines.

Poly(A) tails of RNAs can be further extended following *in vitro* transcription with the use of a poly(A) polymerase, such as E. coli polyA polymerase (E-PAP). In one

embodiment, increasing the length of a poly(A) tail from 100 nucleotides to between 300 and 400 nucleotides results in about a two-fold increase in the translation efficiency of the RNA. Additionally, the attachment of different chemical groups to the 3' end can increase mRNA stability. Such attachment can contain modified/artificial nucleotides, aptamers and other compounds. For example, ATP analogs can be incorporated into the poly(A) tail using poly(A) polymerase. ATP analogs can further increase the stability of the RNA.

5' caps also provide stability to RNA molecules. In a preferred embodiment, RNAs produced by the methods disclosed herein include a 5' cap. The 5' cap is provided using techniques known in the art and described herein (Cougot, et al., Trends in Biochem. Sci., 29:436-444 (2001); Stepinski, et al., RNA, 7:1468-95 (2001); Elango, et al., Biochim. Biophys. Res. Commun., 330:958-966 (2005)).

In some embodiments, the RNA is electroporated into the cells, such as *in vitro* transcribed RNA. Any solutes suitable for cell electroporation, which can contain factors facilitating cellular permeability and viability such as sugars, peptides, lipids, proteins, antioxidants, and surfactants can be included.

In some embodiments, a nucleic acid encoding a CAR of the present disclosure will be RNA, e.g., *in vitro* synthesized RNA. Methods for *in vitro* synthesis of RNA are known in the art; any known method can be used to synthesize RNA comprising a sequence encoding a CAR. Methods for introducing RNA into a host cell are known in the art. See, e.g., Zhao et al. Cancer Res. (2010) 15: 9053. Introducing RNA comprising a nucleotide sequence encoding a CAR into a host cell can be carried out *in vitro*, *ex vivo* or *in vivo*. For example, a host cell (e.g., an NK cell, a cytotoxic T lymphocyte, etc.) can be electroporated *in vitro* or *ex vivo* with RNA comprising a nucleotide sequence encoding a CAR.

The disclosed methods can be applied to the modulation of T cell activity in basic research and therapy, in the fields of cancer, stem cells, acute and chronic infections, and autoimmune diseases, including the assessment of the ability of the genetically modified T cell to kill a target cancer cell.

The methods also provide the ability to control the level of expression over a wide range by changing, for example, the promoter or the amount of input RNA, making it possible to individually regulate the expression level. Furthermore, the PCR-based technique of mRNA production greatly facilitates the design of the mRNAs with different structures and combination of their domains.

One advantage of RNA transfection methods of the invention is that RNA transfection is essentially transient and a vector-free. An RNA transgene can be delivered to a

lymphocyte and expressed therein following a brief *in vitro* cell activation, as a minimal expressing cassette without the need for any additional viral sequences. Under these conditions, integration of the transgene into the host cell genome is unlikely. Cloning of cells is not necessary because of the efficiency of transfection of the RNA and its ability to uniformly modify the entire lymphocyte population.

Genetic modification of T cells with *in vitro*-transcribed RNA (IVT-RNA) makes use of two different strategies both of which have been successively tested in various animal models. Cells are transfected with *in vitro*-transcribed RNA by means of lipofection or electroporation. It is desirable to stabilize IVT-RNA using various modifications in order to achieve prolonged expression of transferred IVT-RNA.

Some IVT vectors are known in the literature which are utilized in a standardized manner as template for *in vitro* transcription and which have been genetically modified in such a way that stabilized RNA transcripts are produced. Currently protocols used in the art are based on a plasmid vector with the following structure: a 5' RNA polymerase promoter enabling RNA transcription, followed by a gene of interest which is flanked either 3' and/or 5' by untranslated regions (UTR), and a 3' polyadenyl cassette containing 50-70 A nucleotides. Prior to *in vitro* transcription, the circular plasmid is linearized downstream of the polyadenyl cassette by type II restriction enzymes (recognition sequence corresponds to cleavage site). The polyadenyl cassette thus corresponds to the later poly(A) sequence in the transcript. As a result of this procedure, some nucleotides remain as part of the enzyme cleavage site after linearization and extend or mask the poly(A) sequence at the 3' end. It is not clear, whether this nonphysiological overhang affects the amount of protein produced intracellularly from such a construct.

In another aspect, the RNA construct is delivered into the cells by electroporation. See, e.g., the formulations and methodology of electroporation of nucleic acid constructs into mammalian cells as taught in US 2004/0014645, US 2005/0052630A1, US 2005/0070841A1, US 2004/0059285A1, US 2004/0092907A1. The various parameters including electric field strength required for electroporation of any known cell type are generally known in the relevant research literature as well as numerous patents and applications in the field. See e.g., U.S. Pat. No. 6,678,556, U.S. Pat. No. 7,171,264, and U.S. Pat. No. 7,173,116. Apparatus for therapeutic application of electroporation are available commercially, e.g., the MedPulser™ DNA Electroporation Therapy System (Inovio/Genetronics, San Diego, Calif.), and are described in patents such as U.S. Pat. No. 6,567,694; U.S. Pat. No. 6,516,223, U.S. Pat. No. 5,993,434, U.S. Pat. No. 6,181,964, U.S. Pat. No. 6,241,701, and U.S. Pat. No. 6,233,482;

electroporation may also be used for transfection of cells in vitro as described e.g. in US20070128708A1. Electroporation may also be utilized to deliver nucleic acids into cells in vitro. Accordingly, electroporation-mediated administration into cells of nucleic acids including expression constructs utilizing any of the many available devices and
5 electroporation systems known to those of skill in the art presents an exciting new means for delivering an RNA of interest to a target cell.

K. Pharmaceutical compositions and Formulations

Also provided are populations of immune cells of the invention, compositions
10 containing such cells and/or enriched for such cells, such as in which cells expressing the CAR make up at least 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more of the total cells in the composition or cells of a certain type such as T cells or CD8+ or CD4+ cells. Among the compositions are pharmaceutical compositions and formulations for administration, such as for adoptive cell therapy. Also provided are
15 therapeutic methods for administering the cells and compositions to subjects, *e.g.*, patients.

Also provided are compositions including the cells for administration, including pharmaceutical compositions and formulations, such as unit dose form compositions including the number of cells for administration in a given dose or fraction thereof. The pharmaceutical compositions and formulations generally include one or more optional
20 pharmaceutically acceptable carrier or excipient. In some embodiments, the composition includes at least one additional therapeutic agent.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to
25 which the formulation would be administered. A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative. In some aspects, the choice of carrier is determined in part by the particular cell and/or by the method of administration. Accordingly,
30 there are a variety of suitable formulations. For example, the pharmaceutical composition can contain preservatives. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some aspects, a mixture of two or more preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition. Carriers are

described, e.g., by Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; 5 preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as 10 polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

15 Buffering agents in some aspects are included in the compositions. Suitable buffering agents include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. In some aspects, a mixture of two or more buffering agents is used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition. Methods for 20 preparing administrable pharmaceutical compositions are known. Exemplary methods are described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21st ed. (May 1, 2005).

The formulations can include aqueous solutions. The formulation or composition may also contain more than one active ingredient useful for the particular indication, disease, or 25 condition being treated with the cells, preferably those with activities complementary to the cells, where the respective activities do not adversely affect one another. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. Thus, in some embodiments, the pharmaceutical composition further includes other pharmaceutically active agents or drugs, such as chemotherapeutic agents, e.g., asparaginase, 30 busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, and/or vincristine. The pharmaceutical composition in some embodiments contains the cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments

is monitored by periodic assessment of treated subjects. The desired dosage can be delivered by a single bolus administration of the cells, by multiple bolus administrations of the cells, or by continuous infusion administration of the cells.

Formulations include those for oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. In some embodiments, the cell populations are administered parenterally. The term "parenteral," as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, and intraperitoneal administration. In some embodiments, the cells are administered to the subject using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection. Compositions in some embodiments are provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may in some aspects be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof.

Sterile injectable solutions can be prepared by incorporating the cells in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, and/or colors, depending upon the route of administration and the preparation desired. Standard texts may in some aspects be consulted to prepare suitable preparations.

Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, and sorbic acid. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

The contents of the articles, patents, and patent applications, and all other documents and electronically available information mentioned or cited herein, are hereby incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. Applicants reserve the right to physically incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other physical and electronic documents.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods described herein may be made using suitable equivalents without departing from the scope of the embodiments disclosed herein. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto. Having now described certain embodiments in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting.

EXPERIMENTAL EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only, and the invention is not limited to these Examples, but rather encompasses all variations that are evident as a result of the teachings provided herein.

Materials and Methods

Study design: The aim of this study was to design fully humanized IL13R α 2 specific targeting CAR T cells and test the possibility of combinational therapy with different checkpoint blockades by systematic and local delivery for potential use as a therapeutic agent in patients with IL13R α 2 expressing tumors, such as malignant glioma. Canine IL13R α 2 targeting CAR T cells were also generated to treat canine malignancies expressing IL13R α 2. In this study, two murine IL13R α 2 targeting scFvs and their humanized scFvs CAR T cells

were cloned and tested by co-culturing with tumor cell lines or human normal cell lines *in vitro*. The function of humanized IL13R α 2 targeting CAR T cells were also tested by intravenous infusion into subcutaneous or orthotopic xenograft glioma mouse models. Expression of checkpoint receptors was detected after *in vitro* T cells stimulation. Tumor sizes were measured through caliper and compared between groups of IL13R α 2 targeting (Hu08BBz) or EGFRvIII targeting (2173BBz) CAR T cells combined with checkpoint blockade (anti-PD-1, anti-CTLA-4 and anti-TIM-3) in the glioma mouse model. IL13R α 2 targeting (Hu08BBz) CAR T cells were modified to express these different checkpoint blockade minibodies to further explore the feasibility of combinational therapy by this strategy *in vitro* and *in vivo*. Canine IL13R α 2 targeting CAR T cells were sorted out by co-culturing with canine IL13R α 2 protein and confirmed by co-culturing with different canine tumor cell lines. Human and canine protein component based canine IL13RA2 targeting CAR T cells were established and tested in a canine glioma orthotopic xenograft mouse model. Each experiment was performed multiple times with T cells derived from various normal donors.

Cell lines and culture: The human tumor cell lines (Sup-T1, Jurkat clone E6-1, A549 and 293T cells) and canine tumor cell lines (CLBL-1 and GL-1) were maintained in RPMI-1640 plus GlutaMAX-1, HEPES, pyruvate and penicillin/streptomycin (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) (R10 media). U87 was purchased from the American Type Culture Collection (ATCC) and maintained in MEM (Richter's modification) with components mentioned above. Human glioma cell line, U251, was provided by Dr. Jay Dorsey (Department of Radiation Oncology, University of Pennsylvania). Canine glioma cell line, J3T, was provided by Michael Berens (Cancer and Cell Biology Division, Tgen). Canine tumor cell lines, Camac2, Cacal3, Cacal5, BW-KOSA, CS-KOSA, MC-KOSA and SK-KOSA, were all cultured in Dulbecco's Modified Eagle Medium (DMEM) with penicillin/streptomycin (Thermo Fisher Scientific) and 10% FBS. Human glioma stem cell lines (5077, 5430, 4860, 5377, 5560, 4806 and 4892) were isolated from patient excised tumor tissue (Department of Neurosurgery, Perelman School of Medicine) and maintained in DMEM/F12 with penicillin/streptomycin, GlutaMAX-1, B27, epidermal growth factor and basic fibroblast growth factor (Corning). D270 glioma cells were grown and passaged in the right flank of NSG mouse to keep their glioma characteristics *in vivo*. Except J3T cell line, canine tumor cell lines were provided by Nicola Mason (School of Veterinary Medicine, University of Pennsylvania) and lentivirally transduced to express the click beetle green luciferase and green fluorescent protein (GFP)

under control of the EF-1 α promoter for *in vivo* study. Canine glioma cell line, J3T, was modified with the same procedure in our lab and used in the orthotopic xenograft canine glioma mouse model. Human primary cells, CD34⁺ bone marrow cells, human pulmonary microvascular endothelial cells, human small airway epithelial cells, human renal epithelial cells, human keratinocytes, human neuronal progenitor cells, human aortic smooth muscle cells and human pulmonary artery smooth muscle cells were purchased from PromoCell GmbH and maintained in culture for 3 to 7 passages in medium indicated by the vendor.

Vector constructs: A second-generation CAR structure in pGEM vector was provided by Jesse Rodriguez (Perelman School of Medicine, University of Pennsylvania) with leader sequence, hinge and transmembrane sequence of human CD8 α and the sequence of stimulation domain of human 4-1BB and CD3 ζ . The amino acid sequences of murine IL13R α 2 targeting scFvs (07/08) and the humanized versions (WO2014/072888) were reverse translated into nucleic acid sequence with codon optimization and ligated into BamHI and BspEI sites between the leader and hinge domain. Humanized 07/08 BBz CAR sequences were digested with XbaI and SalI from pGEM vector and ligated into pTRPE vector with the same enzyme sites. Humanized EGFRvIII targeting scFv was ligated into the Hu08BBz CAR structure between BamHI and BspEI to replace the humanized IL13R α 2 targeting scFv to construct humanized EGFRvIII targeting CAR with the same structure of humanized IL13R α 2 CAR. Minibodies secreting CAR structures were established by ligating the nucleic acid sequences of minibodies (anti-PD-1/CTLA-4/TIM-3 scFvs, CH3 domain of IgG1 and Strep-tag) with P2A ribosomal skipping sequence (J. H. Kim, et al. *PLoS One* 6, e18556 (2011)) into pTRPE vector on the 5' of Hu08BBz CAR structure. Canine IL13R α 2 CAR construct was generated by ligating the humanized 08 (Hu08) scFv sequence into the BamHI and BspEI sites of pGEM CD20 canine BBz with canine CD8 α leader sequence, hinge and transmembrane sequence and the sequence of costimulation domain of canine 4-1BB and CD3 ζ provided by Nicola Mason (School of Veterinary Medicine, University of Pennsylvania).

Human T cell transduction and culture *in vitro*: Human T cell transduction and culture was performed as previously described (L. A. Johnson, et al. *Sci Transl Med* 7, 275ra222 (2015)). Briefly, isolated T cells were derived from leukapheresis products obtained from the Human Immunology Core at the University of Pennsylvania using de-identified healthy donors under an institutional review board approved protocol. T cells were stimulated with Dynabeads Human T-Activator CD3/CD28 (Life Technologies) as a bead to cell ratio of 3:1. After 24hrs stimulation, lentivirus was added into the culture media and

thoroughly mixed to produce stably transduced CAR T cells. The concentration of the expanding human T cells was calculated on a Coulter Multisizer (Beckman Coulter) and maintained at $1.0\text{--}2.0 \times 10^6$ per mL in R10 media supplemented with 30IU/mL recombinant human IL2 (rhIL2; Proleukin, Chiron). Stably-transduced human CAR T cells used in the *in vivo* study were normalized to 30% CAR+ before transplantation.

Canine T cell culture and expansion in vitro: Canine T cells were collected from leukapheresis products obtained from peripheral blood of healthy research dogs at the University of Pennsylvania, Veterinary School of Medicine with Institutional Animal Care and Use Committee (IACUC) approval. The cells were cultured and expanded with cell-based artificial APCs (aAPCs) as described before (M. K. Panjwani, et al. *Mol Ther* 24, 1602-1614 (2016)). In brief, the human erythroleukemic cell line K562 transduced with lentiviral vector to stably express human FcγRII (CD32) and canine CD86 was used as artificial APCs, which were provided by Nicola Mason (School of Veterinary Medicine, University of Pennsylvania). Before expanding canine T cells, aAPCs were irradiated with 10,000 Rads and washed with R10 media. Canine T cells were cultured with aAPCs at 2:1 ratio to a final concentration of 1×10^6 canine T cells and 5×10^5 aAPCs per mL with 0.5μg/mL mouse anti-canine CD3 (Bio-Rad) in R10 media with 30IU/mL rhIL2. The concentration of the expanding canine T cells was calculated on a Coulter Multisizer (Beckman Coulter) and maintained at $1.0\text{--}2.0 \times 10^6$ per mL R10 media with rhIL2.

mRNA in vitro transcription and electroporation: RNA was synthesized and electroporated as previously described (M. K. Panjwani, et al. *Mol Ther* 24, 1602-1614 (2016)). Briefly, pGEM plasmids were linearized by digestion with SpeI. mRNA *in vitro* transcription was performed using the T7 mScript Standard mRNA production system (CellScript) as per the manufacturer's instructions to obtain capped and tailed mRNA. Production was aliquoted and stored at -80°C until use. Expanded T cells were washed three times with Opti-MEM media (Gibco) and resuspended at 1×10^8 cells/mL. 10mg mRNA was mixed with 1×10^7 T cells and moved into cuvettes for electroporation. After electroporated with 500V for 700μs, T cells were recovered in the R10 media with rhIL2.

Flow cytometry: For CAR detection, cells were stained with biotinylated protein L (GenScript), goat anti-mouse IgG and rabbit anti-mouse/human IgG (Jackson ImmunoResearch), and secondary detection was carried out by the addition of streptavidin-coupled PE/FITC (BD Biosciences). Before and after each staining, cells were washed three times with PBS containing 2% fetal bovine serum (FACS buffer). APC conjugated anti-IL13Rα1 (R&D Systems), PE conjugated anti-IL13Rα2 (BioLegend) with their isotypes and

non-conjugated anti-EGFRvIII antibody (Novartis) with PE conjugated anti-Rabbit IgG (BioLegend) secondary stain were used for detecting these targets. Except cell proliferation assay, the co-culture experiments used in the flow cytometry were set up in 96 well plate at 1:1 effector/target (E:T) ratio with 12 days expanded T cells after 24 or 48 hrs co-culture.

5 CFSE staining (Thermo Fisher) was performed as per the manufacturer's instructions, target cells were irradiated with 10,000 Rads ahead of co-culture with T cells. For 8 days co-culture, 75% more irradiated target cells were added on day 2. Spleen was minced and single cell suspensions washed through a cell strainer (40µm, Falcon), red blood cells were lysed with Ammonium-Chloride-Potassium (ACK) Lysing Buffer (Lonza). The size and concentration of cells was measured on a Coulter Multisizer (Beckman Coulter) after washing with PBS. Human CD4⁺ and CD8⁺ T cells was distinguished with live/dead viability stain (Thermo Fisher Scientific), followed by human CD45, CD3 and CD8 (BioLegend) stain in the spleen and tumor co-culture experiment (Fig. 11A). FITC conjugated anti-human CD69 (BioLegend) was used to detect the T cell stimulation. BV711 conjugated anti-human PD-1, 15 PE conjugated anti-human CTLA-4, BV605 conjugated anti-human TIM-3, BV605/PE conjugated anti-human PD-L1, PE conjugated anti-human CD80, BV711/PE conjugated anti-human CD86, FITC/PE conjugated anti-human galectin 9 and isotypes (BioLegend) were used to detect the expression of checkpoints and their ligands. Fluorescence was assessed using a BD LSR II flow cytometer and data were analyzed with FlowJo software.

20 ***Intracellular cytokine analysis:*** CAR transduced or untransduced T cells (2×10^6 cells per mL) were co-cultured with target cells (tumors, cell lines or human primary cells) in a 1:1 ratio in 96-well round bottom tissue culture plates, 37°C, 5% CO₂ for 16hrs, in R10 media in the presence of Golgi inhibitors monensin and brefeldin A (BD Bioscience); when protein was used to stimulated the T cells, human IL13Rα2 (R&D System)/canine IL13Rα2 25 (SinoBiological Inc.) or bovine serum albumin (Sigma-Aldrich) were coated on 24-wells flat bottom tissue culture plate for 16hrs before the stimulation of T cells. Cells were washed, stained with live/dead viability stain, followed by surface staining for human CD3 and CD8 (BioLegend) or canine CD3 and CD4 (Bio-Rad), then fixed and permeabilized, and intracellularly stained for human IFNγ, IL2 and TNFα or canine IFNγ. Cells were analyzed 30 by flow cytometry (BD LSR II) and gated on live, single-cell lymphocytes and CD3-positive lymphocytes.

Chromium release assays: Cytotoxicity of the CAR-expressing T cells was tested in a 4-hour ⁵¹Cr release assay, as described in L. A. Johnson, et al. *Sci Transl Med* 7, 275ra222 (2015). 1×10^6 target cells were labeled with radioactive ⁵¹Cr (50 µCi) for 1 hour at 37°C.

After labeling, cells were washed with 10mL of non-phenol red RPMI medium plus 5% FBS twice and resuspended at 1×10^6 cells/mL. Five thousand (100 μ l) labeled target cells was plated in each well of a 96-well plate. Effector cells were added in a volume of 100 μ l at different E:T ratios (1:1, 3:1, 10:1 and 30:1). Effector and targets were incubated together for 4 hours at 37°C. Supernatant from each well was collected and transferred onto the filter of a LumaPlate. The filter was allowed to dry overnight. Radioactivity released in the culture medium was measured using a β -emission reading liquid scintillation counter. Percentage specific lysis was calculated as follows: (sample counts – spontaneous counts)/(maximum counts – spontaneous counts) $\times$ 100.

Mouse models: All mouse experiments were conducted according to Institutional Animal Care and Use Committee (IACUC)-approved protocols and described in L. A. Johnson, et al. *Sci Transl Med* 7, 275ra222 (2015). For orthotopic models, 2×10^4 D270 cells or J3T cells were implanted intracranially into 6- to 8- week-old female NSG mice (JAX). The surgical implants were done using a stereotactic surgical setup with tumor cells implanted 2mm right and 0.1mm posterior to the bregma and 3mm into the brain. Before surgery and for 3 days after surgery, mice were treated with an analgesic and monitored for adverse symptoms in accordance with the IACUC. In subcutaneous models, NSG mice were injected with 5×10^5 D270 tumors subcutaneously in 100 μ l of PBS on day 0. CAR T cells were injected in 100 μ l of PBS intravenously via the tail vein a week later. Tumor size was measured by calipers in two dimensions, L $\times$ W, for the duration of the experiment. Tumor progression was also evaluated by luminescence emission on a Xenogen IVIS spectrum after intraperitoneal D-luciferin injection according to the manufacturer's directions (GoldBio). Anti-PD-1, anti-CTLA-4 and anti-TIM-3 checkpoint blockades (BioLegend) were intraperitoneally injected 200 μ g per mouse every four days from day six after tumor implantation, based on the dosage applied in other studies (S. F. Ngiew, et al. *Cancer Res* 71, 3540-3551 (2011); K. Sakuishi, et al. *J Exp Med* 207, 2187-2194 (2010); E. K. Moon, et al. *Clin Cancer Res* 22, 436-447 (2016); K. D. Lute, et al. *Blood* 106, 3127-3133 (2005). Survival was followed over time until predetermined IACUC-approved endpoint was reached.

Reverse transcription-polymerase chain reaction (RT-PCR): cDNA of canine tumor cell lines was synthesized with reverse transcription kit from the extracted RNA. Phusion polymerase (NEB) was used to amplify DNA fragments. Reaction was set up as indicated in the PCR protocol for Phusion polymerase. Primer designed for the experiments are IL13R α 1 forward: 5'-CAAATTGTACCCTCCAGGTTTCCCTC-3', reverse: 5'-

GAGTCGGCTGTGACTGAGCTA CAATG-3'; IL13Rα2 forward: 5'-CTATGCCACCAGACTACCTTAGTC-3', reverse: 5'-GATCGTTTTTCAGTAAAGCCCTTTGC-3'; GAPDH forward: 5'-GCCATCAATGACCCCTTCA TTGATC-3', reverse: 5'-GATCCACAACCTGATACATTGGGGGT-3'. After 35 cycles of
 5 reaction, PCR products were run on a 1% agarose gel and visualized in a gel documentation system (GDS touch, ENDURO).

Enzyme-linked immunosorbent assay (ELISA): For detecting anti-PD-1 and anti-CTLA-4 minibodies, T cells were transduced and maintained as described above, between 1.0-2.0×10⁶ cells/mL. 70mL supernatant from the day 11 of T cells expansion *in vitro* was
 10 collected and concentrated with Centricon Plus-70 as per the manufacturer's instructions. A standard direct ELISA was performed with DuoSet Ancillary Reagent Kit 2 (R&D systems). After coating with recombinant human PD-1 and CTLA-4 protein (Abcam), 96-well plate was loaded with the concentrated supernatants followed by peroxidase goat anti-human IgG (Jackson ImmunoResearch) detection antibody. For detecting canine IFN_γ, supernatant was
 15 collected from canine T cells and target cells 16hrs co-culture at 1:1 ratio. The detection was performed with canine IFN-gamma DuoSet ELISA kit (R&D Systems) as the introduction indicated.

2 photon microscopy: Mice were anaesthetized and maintained at a core temperature of 37 °C. Thinned-skull surgery was performed as described previously (G. Yang, et al. *Nat*
 20 *Protoc* 5, 201-208 (2010)). For ex vivo imaging, as described before (C. Konradt, et al. *Nat Microbiol* 1, 16001 (2016)), Cell Trace Violet (Life Technologies) and TRITC (Thermo) labeled CAR T cells were intravenously transplanted, four hours later, the mice were euthanized, and the spleen was removed immediately and placed in a heated chamber where specimens were constantly perfused with warmed (37°C), oxygenated medium (phenol-red
 25 free RPMI 1640 supplemented with 10% FBS, Gibco). The temperature in the imaging chamber was maintained at 37 °C using heating elements, and was monitored using a temperature-control probe (Fine Science Tools). Imaging was performed with a Leica SP5 two-photon microscope system (Leica Microsystems) equipped with a picosecond or femtosecond laser (Coherent). Images were obtained using a ×20 water-dipping lens. The
 30 resulting movies were analyzed with Volocity software (PerkinElmer).

Statistical analysis: Data are presented as means ± SEM. Cytotoxicity assays, intracellular cytokine analysis and median fluorescence intensity results of flow cytometry were analyzed with one-way Analysis of Variance (ANOVA) with post hoc Tukey test to compare the differences in each group. Unpaired *t* tests were used in the ex vivo staining of

mouse spleen and ELISA of canine IFN γ secretion and minibody detection. For the *in vivo* tumor study, linear regression was used to test for significant differences in the tumor size calipering and bioluminescence imaging. Survival curves were analyzed with Kaplan-Meier (log-rank test). All statistical analyses were performed with Prism software version 7.0

(GraphPad).

Example 1: Humanized IL13R α 2 targeting CAR T cells

The Human Protein Atlas illustrates that IL13R α 1 is widely expressed in normal human tissues (Fig. 7A), while IL13R α 2 is restricted to expression in testes (Fig. 7B). In contrast, the cancer genome atlas demonstrates IL13R α 2 was expressed in multiple different tumor samples with different tissue origins. Very high expression of IL13R α 2 was found in GBM (Fig. 7C). To make IL13R α 2-targeting CAR T cells, a second-generation CAR construct with human CD8 α hinge and transmembrane domains linked with human 4-1BB and CD3 ζ intracellular signaling domains was used. Human IL13R α 2 targeting murine scFv sequences, Mu07 and Mu08 (WO2014/072888), were cloned into the CAR backbone in the pGEM vector (Fig. 8A). mRNA encoding the IL13R α 2 CAR was made *in vitro* with the pGEM template. After mRNA electroporation into human T cells, the two CAR structures, Mu07BBz and Mu08BBz, were detected on the T cell surface (Fig. 8B). Three glioma cell lines (U87, U251 and D270) and two T cell cancer lines, Sup-T1 and Jurkat, were chosen as target cells for testing the specificity and function of the murine scFv based IL13R α 2 CAR T cells *in vitro*. IL13R α 2 was detected on all three glioma cell lines, but not on the Sup-T1 and Jurkat T cell cancer lines, confirming their negative control status (Fig. 8C). To determine antigen-specific CAR T cell activation, electroporated murine scFv based IL13R α 2 CAR T cells were co-cultured with target tumor cells. IFN γ production was only detected within CAR T cells co-cultured with IL13R α 2 positive tumor cell lines (Fig. 8D), and not detected within CAR T cells co-cultured with the negative control cell lines. The production of IL2 and TNF α also demonstrated as the same pattern as IFN γ production (Fig. 10A).

To avoid HAMA responses and anaphylaxis, humanized 07 (Hu07) and 08 (Hu08) scFvs (WO2014/072888) were utilized to generate humanized IL13R α 2 targeting CAR T cells. Hu07 and Hu08 scFvs were prepared by CDR grafting with frame back mutations. DP-54 and DPK9 were utilized as the human acceptor framework. Based on the binding activity and thermal stability of the humanized scFvs described previously (WO2014/072888), Hu07 and Hu08 sequences were chosen to be cloned into the second-generation CAR construct in the pGEM vector (Fig. 1B). After human T cell mRNA electroporation, the Mu07/08 and

Hu07/08 scFvs were detected on the T cell surface with anti-murine or anti-human IgG antibodies (Fig. 1A). All four structures were detected by the anti-murine IgG, but only the humanized CARs were recognized by anti-human IgG (Fig. 1A). To stably express the IL13R α 2 CARs on the human T cell surface, Hu07BBz and Hu08BBz CAR constructs were cloned into the pTRPE vector, which is a transfer plasmid used in lentivirus production (Fig. 1B). CAR expression was detected on the cell surface of transduced T cells (Fig. 1C). To determine specificity of both IL13R α 2 CARs, transduced IL13R α 2 CAR T cells were co-cultured with target cell lines that expressed neither IL13R α 1 nor IL13R α 2 (supT1 and Jurkat cells); IL13R α 1 only (the lung cancer cell line A549), IL13R α 2 only (D270) or both IL13R α 1 and IL13R α 2 (U87 and U251) (Fig. 1D). Both humanized 07/08BBz CAR constructs produced IFN γ when co-cultured with IL13R α 2 positive target cells (Fig. 1E). Additionally, the humanized IL13R α 2 targeting CAR T cells did not cross-react with IL13R α 1, as evidenced by a lack of IFN γ production when co-cultured with A549. IL2 and TNF α production also corresponded with the production of IFN γ (Fig. 10B). These co-culture results are consistent with those of murine scFv based CARs (Fig. 8D). To determine the ability of the humanized IL13R α 2 targeting CAR T cells to mediate antigen specific cytotoxicity, chromium release assays were performed at different effector/target (E:T) ratios (1:1, 3:1, 10:1, 30:1) of humanized IL13R α 2 targeting CAR T cells to target tumor cells. The humanized CAR T cells specifically killed IL13R α 2 positive target cells (U87, U251, D270) during four hours of co-culture, even at the lower E:T ratios (Fig. 1F). No killing activity above background was detected in the negative control groups. Hu07 and Hu08BBz CAR T cells (Fig. 9A) were also co-cultured with normal human primary cells. Different levels of IL13R α 1 expression were detected on several types of human primary cell (Fig. 9B), specifically human small airway epithelial cells, human renal epithelial cells and human keratinocytes. No stimulation was found in the co-cultured humanized IL13R α 2 targeting CAR T cells with either of these targets by intracellular cytokine (IFN γ , IL2 and TNF α) staining (Fig. 9C, Fig. 10C). IL13R α 2 expression was also detected on the co-cultured human aortic smooth muscle cells and pulmonary artery smooth muscle cells with IL13R α 2 antibody (clone 47) (Fig. 9B), which also induced stimulation of both CAR T cells (Hu07BBz and Hu08BBz) illustrated as the type I cytokine production (IFN γ , IL2 and TNF α) (Fig. 9C, Fig. 10C). Taken together, IL13R α 2 represented a viable target in glioblastoma and Hu07 and Hu08BBz CAR T cells target this receptor with a high degree of specificity.

Example 2: IL13R α 2 CAR T cells control tumor growth in vivo

To further test the function of the two humanized IL13R α 2 CAR T cells *in vivo*, subcutaneous and orthotopic glioma xenograft models were developed in NSG mice. The D270 glioma cell line was chosen for the *in vivo* work, based on pathophysiologic characteristics that closely match human primary glioma invasive and aggressive growth patterns. The status of the orthotopic implanted D270 glioma cells was monitored in the NSG mouse model using two-photon microscope after skull thinning.

The D270 cell line not only expressed IL13R α 2 endogenously, but also EGFRvIII (Fig. 2A). The expression of both targets was detected on day 0, 1, 2, 3, 5, 7 of D270 culture *in vitro* (Fig. 10D). This allowed inclusion of the previously described 2173BBz CAR T cells in this experiment (L. A. Johnson, et al. *Sci Transl Med* 7, 275ra222 (2015); D. M. O'Rourke, et al. *Sci Transl Med* 9, (2017)). 2173BBz is a humanized, EGFRvIII targeting CAR with the same CAR backbone as Hu07/08BBz. EGFRvIII targeting (2173BBz) and IL13R α 2 targeting (Hu08BBz) CAR T cells were co-cultured with D270 glioma cells at 1:1 ratio and CAR T cell activation determined by evaluating the median fluorescence intensity (MFI) of CD69 staining by flow cytometry (Figs. 11A-11B). The MFI of 2173BBz and Hu08BBz CAR T cells was significantly higher than the un-transduced (UTD) T cells, demonstrating CAR-mediated activation in the presence of target cells (Fig. 2B). The CD69 MFI of Hu08BBz was also significantly higher than the CD69 MFI of 2173BBz on the CD4⁺ and CD8⁺ subgroups of CAR T cells after 24 hours ($P<0.0001$ and $P=0.0021$) and 48 hours of co-culture ($P=0.0008$ and $P=0.0038$) (Fig. 2B), suggesting that the Hu08BBz CAR T cells were more activated in response to target cells compared to the 2173BBz CAR T cells.

To determine antigen specific proliferation, CFSE labelled UTD T cells, 2173BBz CAR T cells and Hu08BBz CAR T cells were co-cultured with the D270 cell line, as well as the target negative cell line A549. The intensity of CFSE signaling on UTD T cells and CAR positive T cells (2173BBz and Hu08BBz) was determined by flow cytometry (Fig. 10E). The MFI of both CAR T populations was progressively lower than the UTD T cells during 3, 5 and 8 days co-culture with D270 cell line ($P<0.0001$), indicating increased proliferation compared with the UTD T cells. The spleen, as an important lymphoid organ, is a reservoir of large amounts of lymphocytes. The status of CAR T cells in the spleen has been demonstrated to correspond with their function *in vivo*. CellTrace Violet and TRITC labeled CAR T cells were transplanted intravenously into an orthotopically implanted glioma NSG mouse model where they were visualized in the mouse spleen with 2 photon microscopy. To determine CAR mediated T cell expansion *in vivo*, 2×10^6 human CAR T cells (2173BBz and Hu08BBz) or UTD T cells were also intravenously infused into mice, 7 days after D270

subcutaneous implantation in NSG mice. Eleven days after T cell transfer, human T cells were counted in the spleen of three mice per group. There were 7 times more human T cells in mice treated with 2173BBz ($n=7.3 \times 10^5$) than treated with UTD T cells ($n=1 \times 10^5$), while there were 30 times more human T cells in the Hu08BBz ($n=3 \times 10^6$) group than the UTD group (Fig. 2C), but no statistical differences were detected between each group with one way Analysis of Variance (ANOVA).

To determine whether CAR T cells could control tumor growth, D270 cells were implanted subcutaneously and 7 days later 5×10^6 CAR T cells (2173BBz/Hu07BBz/Hu08BBz) or the same number of UTD T cells were administered via the intravenous route. Compared with UTD cells, all three CAR T cells tested (2173BBz/Hu07BBz/Hu08BBz) significantly inhibited tumor growth, as determined by caliper measurement ($P < 0.0001$), and decreased bioluminescent signal ($P < 0.0001$) as detected by *in vivo* imaging system (IVIS) and representative tumor size (Fig. 2D). For mice treated with the humanized IL13R α 2 CAR T cells (Hu07BBz and Hu08BBz), no tumor was palpable 16 days after intravenous (i.v.) T cell implantation. Only background signal (2×10^3 p/s/cm²/sr) was captured in the humanized IL13R α 2 CAR T groups via IVIS. Furthermore, no tumor recurrence was observed in either of the two groups (Hu07BBz and Hu08BBz) over 43 days, based on repeated flank palpation and bioluminescence imaging (BLI), significantly better tumor eradication ($P < 0.0001$) and overall survival ($P = 0.0012$) than the EGFRvIII (2173BBz) CAR T cells group in this mouse model (Fig. 2D). Next the effects of the humanized IL13R α 2 CAR T cells (Hu08BBz) was evaluated in an orthotopic glioma model. D270 glioma cells were implanted intracranially and 8 days later 8×10^5 CAR T cells (Hu08BBz) or control UTD T cells were administered intravenously. All mice in the UTD group became hunched and symptomatic by day 17-20 after tumor implantation and were euthanized based on the predetermined IACUC approved endpoint. Although 25% of the mice in the Hu08BBz group were euthanized during the same period (day 20), bioluminescent signals from the D270 tumor cells were not detected in any other mice in that treatment group ($P < 0.0001$) and mice treated with Hu08BBz showed a clear survival advantage over mice treated with UTD T cells (control group) ($P = 0.0027$) (Fig. 2E). These results suggest Hu07 and Hu08BBz have potent anti-tumor activity *in vivo*.

Example 3: Immune Checkpoint blockade selectively enhances the function of CAR T cells

Immune checkpoint receptors are expressed on the surface of T cells. The effects of the three most frequently studied immune checkpoint receptors (PD-1, CTLA-4 and TIM-3)

on CAR T cell function were evaluated to determine whether immune checkpoint blockade could augment CAR T cell function.

First, the expression of PD-1, CTLA-4 and TIM-3 on human T cells was assessed during *in vitro* expansion on day 0, 3, 7 and 13 (Fig. 12A). T cell activation was illustrated by the expression of CD69, which peaked on day 3 of *in vitro* culture. The percentage of checkpoint receptor (PD-1, CTLA-4 and TIM-3)-positive T cells also increased during early stimulation, and then decreased in both of CD4 and CD8 T cells subgroups. The ligands of PD-1 (PD-L1), CTLA-4 (CD80 and CD86) and TIM-3 (galectin-9) were also detected on the surface of the T cells. The percentage of ligand-positive T cells similarly fluctuated with time after T cell stimulation (Fig. 12A). Investigation of the D270 glioma cell line revealed expression of PD-L1 and galectin-9 (Fig. 12B), making it an appropriate tumor target to study the effects of checkpoint blockade on CAR T cells.

To determine the effects of CAR target engagement on checkpoint molecule expression over time, after 12 days of T cell expansion, the humanized EGFRvIII targeting CAR T cells (2173BBz) and the humanized IL13R α 2 targeting CAR T cells (Hu08BBz) were co-cultured with either D270 tumor cells (EGFRvIII and IL13R α 2+) or A549 tumor cells (EGFRvIII and IL13R α 2-, IL13R α 1+; negative control), *in vitro* for 24hrs or 48hrs (Fig. 11A and 11B). Compared with A549 cells co-culture groups or the group of UTD T cells, the expression of PD-1, CTLA-4 and TIM-3 on D270 cells co-cultured 2173BBz and Hu08BBz CAR T cells was increased at both time points (Fig. 3A). Interestingly, the expression level of these checkpoint receptors differed between the 2173BBz and Hu08BBz CAR T cells (Fig. 3A). CTLA-4 expression was higher on the Hu08BBz CAR T cells than on the 2173BBz CAR T cells during 24hrs and 48hrs of co-culture, in both CD4 ($P=0.0003$ and $P=0.0010$) and CD8 ($P=0.0006$ and $P=0.0050$) T cell subsets, which corresponded to the level of CD69 expression when co-cultured with the D270 cell line (Fig. 2B). Although PD-1 expression was not statistically different between 2173BBz and Hu08BBz CAR T cells after 24hrs of co-culture, it was significantly higher on the CD4 and CD8 positive 2173BBz CAR T cells than on the Hu08BBz CAR T cells after 48hrs of co-culture ($P=0.0021$ and $P=0.0456$). Finally, the expression of TIM-3 was higher with 2173BBz than Hu08BBz CAR T cells, independent of the duration of co-culture or the CD4 and CD8 subsets ($P=0.0371$ and $P=0.0026$ for 24hrs co-culture; $P=0.0002$ and $P=0.0004$ for 48hrs co-culture).

To further study if blocking the immune checkpoint receptors enhanced the tumor killing activity of the CAR T cell, 2173BBz and Hu08BBz CAR T cell treatment was combined with intraperitoneal (i.p.) administration of checkpoint inhibitor (anti-PD-1, anti-

CTLA-4 and anti-TIM-3) in NSG mice with intracranially-implanted D270 tumors. For the majority of mice, only background signal was detectable at later time points, making it difficult to show any benefit of combined checkpoint blockade. Therefore, to determine whether checkpoint blockade enhanced the anti-tumor effects of CAR T cell therapy, the number of CAR T cells administered was decreased and the effects of combination CAR T cells and checkpoint blockade on mice with subcutaneously implanted D270 glioma cells was studied. In this mouse model, intraperitoneal delivery of checkpoint inhibitor (anti-PD-1, anti-CTLA-4 and anti-TIM-3) did not have any effect on reducing tumor size, because the tumor grew at the same rate when mice were injected with UTD T cells ($P=0.1600$, 0.1194 and 0.4565) (Fig. 3B). Significant inhibition of tumor growth was seen when either 2173BBz or Hu08BBz CAR T cells were combined with anti-PD-1 and anti-TIM-3 ($P<0.0001$ and $P<0.0001$ for 2173BBz groups; and $P=0.0325$, and $P=0.0032$ for Hu08BBz groups). In addition, Hu08BBz CAR T cells also showed enhanced anti-tumor effects when used in combination with anti-CTLA-4, whereas 2173BBz CAR T cells did not benefit from CTLA-4 checkpoint blockade ($P=0.5817$ for 2173BBz groups; and $P<0.0001$ for Hu08BBz groups) (Fig. 3C).

Next, the effects of the different checkpoint inhibitors on tumor growth in mice treated with either the 2173BBz CAR T cells or Hu08BBz CAR T cells were compared (Fig. 3D). Combination therapy with either anti-PD-1 or anti-TIM-3 produced greater anti-tumor effects with 2173BBz CAR T cells than anti-CTLA-4 ($P<0.0001$), with combination anti-PD-1 having the greatest effect ($P=0.0185$ compared with anti-TIM-3 group). For the Hu08BBz CAR T cells, CTLA-4 blockade presented the best combinational therapy ($P=0.0010$ and $P<0.0001$). These results corresponded with the expression levels of checkpoint receptors on 2173BBz and Hu08BBz CAR T cells during co-culture with D270 tumor cells *in vitro* (Fig. 3A). Only combination therapy with anti-PD-1 prolonged survival in the 2173BBz CAR T cell treatment group ($P=0.0135$), whereas anti-CTLA-4 prolonged survival in the Hu08BBz CAR T cell treatment group ($P=0.0135$). The number and activation status of human T cells in mouse spleens was also compared between the 2173BBz CAR T cell group and the 2173BBz CAR T cell plus anti-PD-1 group (Fig. 12C). PD-1 expression was efficiently blocked in the combination anti-PD-1 group ($P=0.0034$ and 0.0037 respectively) and there was a higher percentage of CD69⁺ human T cells ($P=0.0006$ and 0.0340) and a larger percentage of CD8⁺ human T cells in this group compared to those treated with 2173BBz CAR T cells alone ($P=0.0177$ and 0.0022). Thus, checkpoint blockades enhanced the function of CAR T cells with specific efficacy on different CARs.

Example 4: IL13R α 2 CAR T cells are selectively enhanced by *in situ* secreted anti-CTLA-4 checkpoint blockade

Given the finding that systemic checkpoint blockade enhanced the anti-tumor effect of both 2173BBz and Hu08BBz CAR T cells, the effects of *in situ* checkpoint blockade was evaluated by modifying CAR T cells to secrete checkpoint inhibitors. It was rationalized that local checkpoint inhibition would enhance CAR T cell activity and would reduce adverse effects induced by systemic checkpoint blockade. T cells were transduced with the pTRPE vector containing the Hu08BBz CAR construct linked to anti-PD-1, anti-CTLA-4 and anti-TIM-3 constructs via the ribosomal skipping sequence (P2A) which enables simultaneous expression of the Hu08BBz CAR and the checkpoint inhibitor molecules. To decrease T cell burden of molecules secretion, the size of the checkpoint inhibitors was reduced by directly linking their scFvs with the CH3 domain of the human IgG1 molecule to generate minibodies (Fig. 4A). These are referred to as minibody secreting T-cells (MiST).

Surface expression of the Hu08BBz CAR on the Hu08BBz CAR T cells and on the Hu08BBz CAR T cells secreting the minibodies was confirmed by flow cytometry (Fig. 4B). Conditioned media from the CAR T cells was collected, concentrated and used in a standard direct ELISA to confirm the secretion and binding of anti-PD-1 and anti-CTLA-4 minibodies from CAR T cells to recombinant hPD-1 and hCTLA-4 ($P=0.0017$ and 0.0075) (Fig. 4C). To evaluate the specificity of the anti-TIM-3 minibody secreted from Hu08BBz CAR T cells, Hu08BBz CAR T cells were co-cultured with or without minibodies with the D270 tumor cell line for 24hrs or 48hrs and a competitive inhibition experiment was performed with fluorochrome-conjugated anti-TIM-3 antibody. The MFI determined binding of fluorochrome-conjugated anti-TIM-3 antibody was significantly lower in the anti-TIM-3 minibody secreting CAR T cell group, suggesting effective secretion and blockade by the TIM-3 minibody (Fig. 4D). Furthermore, with the exception of CD4 positive anti-TIM-3 secreting CAR T cells at 48hrs there was no statistical difference between TIM-3 expression on anti-TIM-3 secreting CAR T cells and UTD T cells in these co-cultures (CD4: $P=0.6642$ and CD8: $P=0.8771$ for 24hrs co-culture; CD4: $P=0.0014$ and CD8: $P=0.4578$ for 48hrs co-culture). The MFI determined binding of fluorochrome-conjugated anti-PD-1 and anti-CTLA-4 antibodies was also lower on anti-PD-1/anti-CTLA-4 MiSTs than non-minibody secreting CAR T cells (Hu08BBz) when co-cultured with D270 cells, demonstrating competitive binding by the anti-PD-1/anti-CTLA-4 minibodies secreted by MiST (anti-PD-1 MiST and Hu08BBz CD4: $P=0.0678$ and CD8: $P=0.0140$ for 24hrs co-culture; CD4:

$P<0.0001$ and CD8: $P=0.0012$ for 48hrs co-culture; anti-CTLA-4 MiST and Hu08BBz CD4: $P<0.0001$ and CD8: $P<0.0001$ for 24hrs co-culture; CD4: $P=0.0002$ and CD8: $P=0.0006$ for 48hrs co-culture) (Fig. 13A). Taken together this data suggests that Hu08BBz MiST secreting anti-PD-1, anti-CTLA-4 and anti-TIM-3 minibodies were successfully generated.

Next, the effects of minibody secretion on Hu08BBz CAR T cell activation was evaluated by D270 target cells *in vitro* for 48hrs, using CD69 expression as a marker of activation. The stimulation of Hu08BBz CAR T cells without minibody secretion was higher than the minibody secreting cells in the 24hrs or 48hrs of co-culture, but no statistical difference was seen with anti-CTLA-4 minibody secreting Hu08BBz CAR T cells in the CD8 positive T cell subgroup ($P=0.0614$ and 0.4561) (Fig. 13B). Among the minibody secreting groups, the stimulation of anti-PD-1 Hu08BBz CAR T cells was significantly lower than the other two groups in the 24hrs co-culture ($P<0.0001$) and in the 48hrs co-culture ($P=0.0008$ and 0.0010) of CD4 positive T cells. For the anti-CTLA-4 and anti-TIM-3 secreting Hu08BBz CAR T cells, the stimulation of anti-CTLA-4 secretion was higher than anti-TIM-3 secretion in the CD4⁺ CAR T cells at 24 hours ($P<0.0001$), while no difference was seen in the other subgroups (Fig. 13B). The cytokine secretion (IFN γ , IL2 and TNF α) of these CAR T cells when co-cultured with the D270 tumor cell line was also compared. Compared with the other Hu08BBz CAR T cell groups, a significantly lower percentage of the anti-PD-1 minibody secreting group secreted cytokines in each subgroup (Fig. 13B). A greater percentage of anti-CTLA-4 and anti-TIM-3 secreting groups produced IFN γ and TNF α than the no minibody secreting group. The production of IL2 was 1.5 fold more frequent in anti-CTLA-4 secreting group than in the anti-TIM-3 secreting group, and significantly higher in the CD4⁺ subgroup ($P=0.0001$) (Fig. 13C).

To determine the effects of checkpoint blockade via minibody secretion from CAR T cells, a sub-therapeutic dose of Hu08BBz CAR T cells (8×10^5 cells per mouse), the same amount of MiSTs or UTD T cells, was intravenously administered into NSG mice eight days after subcutaneous implantation of D270 cells. Despite this low dose, Hu08BBz CAR T cells mediated transient tumor regression until day 22, but the tumors progressed after this time point (Fig. 4E). Among the minibody secreting CAR T cell groups, only the anti-CTLA-4 minibody secreting CAR T cells prolonged the Hu08BBz CAR T cell function further inhibited the tumor growth ($p=0.0195$) (Fig. 4E), which was consistent with *in vitro* results and *in vivo* results using systemic checkpoint blockade. These results not only demonstrated the feasibility of MiST, but also confirmed the specificity of benefits from checkpoint blockades on CAR T cells.

Example 5: IL13R α 2 CAR T cells show potent anti-tumor activity against canine IL13R α 2+ tumors

Besides generating D270 glioma cell line, glioma tissues were also harvested from surgical excision to generate glioma stem cell lines. IL13R α 2 expression was detected on many of these lines (Fig. 5A). The expression was heterogeneous as demonstrated by the percentage of target positive cells and target expression level. Further considering the potential on target off tumor toxicity and specific benefits from checkpoint blockades, prior to their use in human glioblastoma patients, IL13R α 2 CAR T cells should be evaluated in a clinically relevant, spontaneous, large animal model of human disease.

To this end, the domestic dog develops high grade glioblastoma that mimics the biology and clinical course of the human disease and has been suggested as a preclinical mode for glioblastoma. Therefore, it was determined whether Hu07BBz and Hu08BBz CAR T cells recognize epitopes on canine IL13R α 2. First, the amino acid sequence of human IL13R α 2 and canine IL13R α 2 were compared and found to share 71.6% sequence homology (Fig. 14A). Next, human T cells were electroporated with Hu07BBz and Hu08BBz mRNA and the expression of both CARs was confirmed on the T cell surface (Fig. 5B).

Electroporated Hu07BBz and Hu08BBz human CAR T cells were then co-cultured with human and canine IL13R α 2 protein *in vitro* and activation was evaluated by IFN γ production. Both Hu07BBz and Hu08BBz CAR T cells produced IFN γ in response to human IL13R α 2 protein. Surprisingly, only Hu08BBz CAR T cells were activated by canine IL13R α 2. Neither Hu07BBz nor Hu08BBz CAR T cells were activated by bovine serum albumin (BSA) (negative control) (Fig. 5B). The expression of canine IL13R α 1 and IL13R α 2 mRNA levels in a variety of canine tumor cell lines was investigated. All four of the canine osteosarcoma cell lines (BW-KOSA, CS-KOSA, MC-KOSA and SK-KOSA) expressed canine IL13R α 2 (Fig. 5C). Low levels of IL13R α 2 mRNA expression were also detected in the canine leukemia cell line (GL-1) and lung cancer cell lines (Cacal3 and Cacal5), but not in the canine mammary carcinoma cell line (Camac2) or lymphoma cell line (CLBL-1). The expression of canine IL13R α 1 was detected in all canine tumor cell lines tested except of GL-1 and potentially CLBL-1 (Fig. 5C).

To determine whether Hu08BBz CAR T cells could be activated by canine IL13R α 2 expressed on the surface of tumor cells, mRNA electroporated human Hu07BBz and Hu08BBz CAR T cells or UTD T cells were co-cultured with the canine cell lines and cytokine production (IFN γ , IL2 and TNF α) evaluated by CD4 and CD8 CAR T cell

subgroups using flow cytometry. Strikingly, both CD4 and CD8 Hu08BBz CAR T cells produced IFN γ , IL2 and TNF α when co-cultured with the osteosarcoma cell lines (BW-KOSA, CS-KOSA, MC-KOSA and SK-KOSA), while a lower percentage of CD4 and CD8 Hu08BBz CAR T cells produced these cytokines in response to GL-1, Cacal3 and Cacal5 tumor cell lines (Fig. 5C). Cytokine production corresponded with the level of canine IL13R α 2 expression in these tumor cell lines. Although Camac2 expressed canine IL13R α 1, none of the CAR T cells co-cultured with these cells produced cytokines, demonstrating a lack of cross-reactivity of the IL13R α 2 CAR T cells with canine IL13R α 1. Furthermore, no cytokine positive T cells were detected in the un-transduced T cell group and Hu07BBz CAR T cell group (Fig. 5C).

Prior to testing the anti-tumor activity of Hu08BBz CAR T cells against IL13R α 2+ tumors, a canine osteosarcoma model was established in NSG mice. Three different doses of canine osteosarcoma tumor cells were implanted subcutaneously into the right flank of NSG mice and bioluminescence imaging was used to evaluate the tumor growth. The average radiance of the canine osteosarcoma mouse model with the MC-KOSA tumor cell line reached 1×10^7 p/s/cm²/sr and increased with time. The average radiance in the other canine osteosarcoma cell lines was much lower and did not consistently increase (Fig. 14B). NSG mice implanted with 5×10^6 MC-KOSA tumor cells showed a significant difference in radiance compared with the other tumor cell groups ($P < 0.0001$). The highest tolerated dose (5×10^6) of MC-KOSA was chosen to establish IL13R α 2+ osteosarcoma tumors in the NSG mice and 7 days after implantation, 2×10^6 Hu08BBz CAR transduced human T cells were intravenously administered. Tumor growth was significantly inhibited in the CAR T cell treatment group compared to the UTD T cell treatment group (Fig. 5D). These results highlighted the potential in generating canine CAR T cells to target canine IL13R α 2 positive tumors.

Example 6: Canine IL13R α 2 CAR T cells control canine tumor growth

In the next step toward evaluating IL13R α 2 CAR T cells in dogs with spontaneous glioblastoma, canine IL13R α 2 CAR T cells were generated and their antigen-specific function evaluated *in vitro* and *in vivo*. Primary canine T cells were electroporated with Hu08BBz CAR mRNA and co-cultured with different canine tumor cell lines for testing their activation. Canine IFN γ was secreted by Hu08BBz canine CAR T cells but not by UTD canine T cells when co-cultured with IL13R α 2 expressing tumor cells (all osteosarcoma cell lines plus Cacal5). Canine IFN γ was not secreted in response to IL13R α 2 negative canine cell

lines (Camac2 and CLBL-1). Interestingly, the canine glioma cell line, J3T induced the greatest amount of IFN γ by canine Hu08BBz CAR T cells, reaching the maximum detectable limit in this assay (1.26×10^4 pg/mL) (Fig. 6A).

To mimic the physiological expression and stimulation status of canine T cells, a second generation canine IL13R α 2 CAR was established by switching the human CD8 α domain and human 4-1BB and CD3 ζ intracellular signaling domains with canine CD8 α and canine 4-1BB and CD3 ζ (Fig. 6B). Canine IFN γ secretion was compared between Hu08-human-BBz (Hu08HuBBz) and Hu08-canine-BBz (Hu08CaBBz) canine CAR T cells when they were co-cultured with canine tumor cell lines (CLBL-1 and J3T). Hu07-human-BBz (Hu07HuBBz) CAR T cells were included as a negative control. Canine T cells expressing either the Hu08HuBBz or Hu08CaBBz CAR produced IFN γ in response to the J3T tumor cell line but not the CLBL-1 cell line and no significant difference in IFN γ production was detected between the two CAR constructs ($P=0.2736$) (Fig. 6C).

Next, J3T glioma cells were orthotopically injected in mice to further evaluate the function of these CAR T cells *in vivo*. The J3T tumor cell line was transduced with the click beetle green luciferase gene for visualizing in the *in vivo* imaging system. After J3T implantation, 1.2×10^7 mRNA electroporated Hu08HuBBz, Hu08CaBBz canine CAR T cells or UTD canine T cells were injected intravenously by mouse tail vein on days 7, 10 and 13. Bioluminescence imaging was performed until day 41 after tumor implantation. Both Hu08HuBBz and Hu08CaBBz CAR T cells mediated prolonged inhibition of tumor growth when compared to UTD T cells ($p < 0.0001$; $p = 0.0015$) (Fig. 6D). Canine T cells used in the second implantation were analyzed *in vitro*. Hu08HuBBz and Hu08CaBBz CAR constructs were detected on the surface of canine T cells (Fig. 6E left panels) although the expression of the canine CAR construct was less. Canine IFN γ production was also detected in the J3T co-cultured canine CAR T cells (Fig. 6E right panels). Thus, canine IL13R α 2 targeting CAR T cells were successfully generated for translational studies.

Example 7: Inducible CAR constructs

An inducible promoter, which can promote expression after T-cell activation, was generated and tested herein (FIGs. 15A-15B). The underlined portion of the promoter sequence shown in FIG. 15B can be partially repeated to enhance T-cell expression level. T cells/CAR T cells are modified with this promoter to express designed RNA or amino acids. A construct using this promoter was generated (FIG. 16A) and included a TDTomato gene for fluorescent expression. When Jurkat cells (a T cell tumor line) were stimulated with

PMA/Ionomycin, TD-Tomato expression was detected with flow cytometry, demonstrating promoter activation (FIG 16B).

Example 8: Tandem and parallel bi-specific CARs

5 Tandem (FIG. 17 top) and parallel (FIG. 17 bottom) bi-specific CARs, comprised of 806 and Hu08, were generated and tested herein. The tandem CARs contained linkers that were either 5 Amino Acids (5AA), 10 amino acids (10AA), 15 amino acids (15AA), or 20 amino acids (20AA) in length. Amino acid and nucleic acid sequences are exemplified for a tandem CAR with 5AA linker (FIG. 18A), a tandem CAR with 10AA linker (FIG. 18B), a
10 tandem CAR with 15AA linker (FIG. 18C), a tandem CAR with 20AA linker (FIG. 18D), and a parallel CAR (FIG. 18E). The amino acid sequence of 5AA is GGGGS ((G4S); SEQ ID NO:157). The amino acid sequence of 10AA is GGGGSGGGGS (2(G4S); SEQ ID NO:181). The amino acid sequence of 15AA is GGGGSGGGGSGGGGS (3(G4S); SEQ ID NO:158). The amino acid sequence of 20AA is GGGGSGGGGSGGGGSGGGGS (4(G4S);
15 SEQ ID NO:160).

Expression of each CAR construct was quantified by flow cytometry (FIG. 19). T cells were transduced with Hu08BBz CAR, 806BBz CAR, Hu08/806_(G4S) bi-specific CAR, Hu08/806_2(G4S) bi-specific CAR, Hu08/806_3(G4S) bi-specific CAR, Hu08/806_4(G4S) bi-specific CAR, and Hu08BBz_P2A_806BBz parallel CAR. CAR
20 expression was detected with either biotin labelled protein L and streptavidin conjugated PE, or streptavidin conjugated PE alone.

CD69-based T cell stimulation data is shown in FIG. 20. Each CAR T cell population was cocultured with the target-overexpressing 5077 glioma stem cell line. CAR1 (Hu08BBz) and CAR2 (806BBz) were single CAR constructs, 5AA, 10AA, 15AA, and 20AA were
25 varying length linked bis-specific CAR constructs ((Hu08/806_(G4S), Hu08/806_2(G4S), Hu08/806_3(G4S), Hu08/806_4(G4S)), and 2A was a parallel bi-specific CAR construct (Hu08BBz_P2A_806BBz). The stimulation of T cells was illustrated by APC-conjugated anti-CD69 antibody staining, the median fluorescence intensity (MFI) was quantified on CD4+ (FIG. 20, top) and CD8+ (FIG. 20, bottom) CAR-positive T cells after 24 hours of co-
30 culture, controlled with un-transduced T cells. Statistically significant differences were calculated by one-way ANOVA with post hoc Tukey test. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$. Data are presented as means $\pm$ SEM.

Flow-based intracellular cytokine [IFN γ (FIGs. 21A and 21D), IL2 (FIGs. 21B and 21E), and TNF α (FIGs. 21C and 21F)] staining was measured for each tandem bi-specific and parallel CAR T cell co-cultured with the target-overexpressing 5077 glioma stem cell line. The percentage of cytokine positive T cells was demonstrated in CD4 $^{+}$ (FIGs. 21A-21C) and CD8 $^{+}$ (FIGs. 21D-21F) T cell subgroups. One-way ANOVA post hoc Tukey test was performed with **p < 0.01, ***p < 0.001, ****p < 0.0001. Data are presented as means $\pm$ SEM.

A bioluminescence-based cytotoxicity assay was performed to test the killing ability of 806/Hu08 tandem bispecific CAR T cells, when cocultured with target 5077 cell line not expressing EGFRvIII and IL13R α 2 (5077_R α 2-_vIII-), or overexpressing IL13R α 2 alone (5077_R α 2+_vIII-), EGFRvIII alone (5077_R α 2-_vIII+), or EGFRvIII and IL13R α 2 (5077_R α 2+_vIII+), and controlled with un-transduced T cells (UTD) (FIG. 22A-D). The linker between two scFv is GGGGS (SEQ ID NO:157). Data are presented as means $\pm$ SEM. A bioluminescence-based cytotoxicity assay was performed to test the killing ability of 806/Hu08 tandem bi-specific CAR T cells, when cocultured with target overexpressed (EGFRvIII/IL13R α 2) 5077 cell line, controlled with un-transduced T cells (UTD) (FIG. 22B). The linker between two scFv is GGGGSx2 (SEQ ID NO:181). Data are presented as means $\pm$ SEM. FIG. 22C: Bioluminescence-based cytotoxicity assay was performed to test the killing ability of 806&Hu08 tandem bi-specific CAR T cells, when cocultured with target overexpressed (EGFRvIII/IL13R α 2) 5077 cell line, controlled with un-transduced T cells (UTD). The linker between two scFv is GGGGSx3 (SEQ ID NO:158). Data are presented as means $\pm$ SEM. FIG. 22D Bioluminescence-based cytotoxicity assay was performed to test the killing ability of 806&Hu08 tandem bi-specific CAR T cells, when cocultured with target overexpressed (EGFRvIII/IL13R α 2) 5077 cell line, controlled with un-transduced T cells (UTD). The linker between two scFv is GGGGSx4 (SEQ ID NO:160). Data are presented as means $\pm$ SEM.

In vitro killing was demonstrated with the parallel bi-specific CAR construct (FIGs 23A-23D). A bioluminescence-based cytotoxicity assay was performed to test the killing ability of 806BBz/Hu08BBz (Hu08BBz_P2A_806BBz) parallel bi-specific CAR T cells, when cocultured with the target-overexpressed (EGFRvIII/IL13R α 2) 5077 cell line and D270 glioma cell line, controlled with un-transduced T cells (UTD). Data are presented as means $\pm$ SEM.

806BBz/Hu08BBz (Hu08BBz_P2A_806BBz) parallel bi-specific CAR T cells or the same number of un-transduced T cells (UTD) were i.v. infused into D270 subcutaneously implanted NSG mice (n=8 per group) (FIG. 24). Tumor volume measurements (FIG. 24, top) were performed to evaluate the tumor growth. Linear regression was used to test for significant differences between the experimental groups. Endpoint was predefined by the mouse hunch, inability to ambulate, or tumor reaching 2 cm in any direction, as predetermined IACUC-approved morbidity endpoint. Survival based on time to endpoint was plotted using a Kaplan-Meier curve (FIG. 24, bottom, Prism software). Statistically significant differences were determined using log-rank test. ****p < 0.0001. Data are presented as means ± SEM (FIG. 24).

Example 9: BiTE®s

BiTEs were designed to target CD3 and an EGFR isoform or IL13Rα2, and thus bind to naïve T cells as well as tumor cells. In particular, anti-EGFR/CD3, anti-IL13Rα2/CD3 bispecific T cell engagers were generated. This in turn functions to bring the CAR into close proximity to the tumor cell. Conditioned media from fresh (FIG. 25A), un-transduced (UTD) (FIG. 25B), Hu08BBz CAR (FIG. 25C), and Hu07BiTE (FIG. 25D) transduced T cells was collected and used in the co-culture of un-transduced T cells with the 5077 cell line (FIGs. 25A-25D Top, IL13Rα2-) or the 4892 cell line (FIGs. 25A-25D Bottom, IL13Rα2+), controlled with fresh media. CD69 was stained to demonstrate T cell activation. Human CD8 was stained to distinguish the CD4-positive and CD8-positive subgroups of T cells along the x axis (FIGs. 25A-25D).

293T cells were transfected with plasmid pTRPE CFP (a fluorescent gene) or pTRPE Hu08BiTE (FIG. 26). Supernatant was collected 2 days later. Direct ELISA was performed to detect Hu08OKT3 BiTE binding with recombinant protein IL13Rα2. T cells were transduced with pTRPE Hu08BBz, pTRPE C225BiTE, or pTRPE 806BiTE, controlled with un-transduced T cells (UTD) (FIG. 27). Supernatant was collected 7 days later. Direct ELISA was performed to detect BiTE's binding with recombinant protein EGFR wild type or EGFRvIII. Results demonstrated that the BiTEs bind specifically to their intended target (FIGs. 26-27).

The glioma stem cell line 5077 was demonstrated to expresses low-level EGFR (FIGs. 28A-28B). 806BiTE and C225BiTE transduced T cells were cocultured with 5077 wild type or target transduced cells, and a killing assay and cytokine secretion quantification

assay was performed. 806BiTE secreted T cells only responded to EGFRvIII overexpressed 5077. C225BiTE secreted T cells respond to 5077 wild type (FIGs. 28A-28B).

Supernatant of un-transduced T cells (UTD), 806BBz CAR T cells, 806BiTE T cells, Hu08BBz CAR T cells and Hu08BiTE T cells was collected and used in the co-culture of untransduced T cells with target overexpressing 5077 GSC line and D270 glioma cell line (FIG. 29). CD69 was stained to demonstrate T cell activation. Human CD8 was stained to distinguish the CD4-positive and CD8-positive subgroups of T cells along the x axis (FIG. 29).

10 Example 10: BiTE /CAR combinations

Bispecific constructs were generated and used in BiTE/CAR experimentation (FIGs. 30A-30D). FIG. 30A shows a CAR/CAR bispecific construct, FIG. 30B shows an 806BiTE/Hu08BBz bispecific construct, FIG. 30C shows an Hu08BiTE/806BBz bispecific construct, and FIG. 30D shows an 806BiTE/Hu08BiTE bispecific construct. Amino acid and nucleic acid sequences are shown for 806BBz/Hu08BBz (FIG. 31A), 806BiTE/Hu08BBz (FIG. 31B), Hu08BiTE/806BBz (FIG. 31C), and 806BiTE/Hu08BiTE (FIG. 31D).

A bioluminescence-based cytotoxicity assay was performed to test the killing ability of 806BiTE/Hu08BBz bi-specific T cells, when cocultured with target overexpressed (EGFRvIII/IL13R α 2) cell lines, controlled with un-transduced T cells (UTD) (FIGs. 32A-32D). Data are presented as means $\pm$ SEM. FIG. 32A shows EGFRvIII+ GSC 5077, FIG. 32B shows IL13R α 2+ GSC 5077, FIG. 32C shows the double-positive GSC 5077, and FIG. 32D shows the doublepositive D270. Results demonstrated the BiTEs were capable of *in vitro* killing.

806BiTE/Hu08BBz bi-specific T cells or the same number of un-transduced T cells (UTD) were i.v. infused in a D270 subcutaneously implanted NSG mice (n=8 per group) (FIGs. 33A-33B). Tumor volume measurements were performed to evaluate the tumor growth (FIG. 33A). Linear regression was used to test for significant differences between the experimental groups. Endpoint was predefined by the mouse hunch, inability to ambulate, or tumor reaching 2 cm in any direction, as predetermined IACUC-approved morbidity endpoint. Survival based on time to endpoint was plotted using a Kaplan-Meier curve (Prism software) (FIG. 33B). Statistically significant differences were determined using log rank test. ***p < 0.001, ****p < 0.0001. Data are presented as means $\pm$ SEM. Results demonstrated the BiTEs were capable of killing *in vivo*.

A bioluminescence-based cytotoxicity assay was performed to test the killing ability of Hu08BiTE/806BBz bi-specific T cells, when cocultured with target overexpressed (EGFRvIII/IL13R α 2) 5077 cell line and D270 glioma cell line, controlled with un-transduced T cells (UTD) (FIGs. 34A-34D). Data are presented as means $\pm$ SEM. FIG. 34A shows EGFRvIII+ GSC 5077, FIG. 34B shows IL13R α 2+ GSC 5077, FIG. 34C shows the double-positive GSC 5077, and FIG. 34D shows the double-positive D270.

Hu08BiTE/806BBz bi-specific T cells or the same number of un-transduced T cells (UTD) were i.v. infused in a D270 subcutaneously implanted NSG mouse model (n=8 per group) (FIGs. 35A-35B). Tumor volume measurements were performed to evaluate the tumor growth (FIG. 35A). Linear regression was used to test for significant differences between the experimental groups. Endpoint was predefined by the mouse hunch, inability to ambulate, or tumor reaching 2 cm in any direction, as predetermined IACUC-approved morbidity endpoint. Survival based on time to endpoint was plotted using a Kaplan-Meier curve (Prism software) (FIG. 35B). Statistically significant differences were determined using log-rank test. **p < 0.01, ****p < 0.0001. Data are presented as means $\pm$ SEM.

A bioluminescence-based cytotoxicity assay was performed to test the killing ability of 806BiTE/Hu08BiTE bi-specific T cells, when cocultured with target overexpressed (EGFRvIII/IL13R α 2) 5077 cell line and D270 glioma cell line, controlled with un-transduced T cells (UTD) (FIGs. 36A-36D). Data are presented as means $\pm$ SEM. FIG. 36A shows EGFRvIII+ GSC 5077, FIG. 36B shows IL13R α 2+ GSC 5077, FIG. 36C shows the double-positive GSC 5077, and FIG. 36D shows the double-positive D270.

806BiTE/Hu08BiTE bi-specific T cells or the same number of un-transduced T cells (UTD) were i.v. infused in D270 subcutaneously implanted NSG mice (n=8 per group) (FIGs. 37A-37B). Tumor volume measurements were performed to evaluate the tumor growth (FIG. 37A). Linear regression was used to test for significant differences between the experimental groups. Endpoint was predefined by the mouse hunch, inability to ambulate, or tumor reaching 2 cm in any direction, as predetermined IACUC-approved morbidity endpoint. Survival based on time to endpoint was plotted using a Kaplan-Meier curve (Prism software) (FIG. 37B). Statistically significant differences were determined using log-rank test. ***p < 0.001, ****p < 0.0001. Data are presented as means $\pm$ SEM.

Example 11: Intraventricular injections

Trypan Blue (5uL) was injected into the right ventricle of mice, 1-2 mm to the right and 0.3 mm anterior to the bregma, to a depth of 3.0 mm. Animals were euthanized within 15 minutes of injection and brains examined for spread of Trypan Blue to the contralateral ventricle. Blue stain seen in both ventricles demonstrated the ability to both inject
5 therapeutics into the right ventricle and obtain spread of the therapeutics to the left, contralateral ventricle.

Other Embodiments

The recitation of a listing of elements in any definition of a variable herein includes
10 definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

The disclosures of each and every patent, patent application, and publication cited
15 herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed:

1. A chimeric antigen receptor (CAR) comprising an antigen-binding domain capable of binding human IL13R α 2, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence VKWAGGSTDYNSALMS (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7).

2. The CAR of claim 1, wherein the antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8.

3. The CAR of claim 1 or 2, wherein the antigen-binding domain comprises a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.

4. The CAR of any preceding claim, wherein the antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.

5. The CAR of any preceding claim, wherein the antigen-binding domain is selected from the group consisting of a full length antibody or antigen-binding fragment thereof, a Fab, a single-chain variable fragment (scFv), or a single-domain antibody.

6. The CAR of any preceding claim, wherein the antigen-binding domain is a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or 11.

7. A chimeric antigen receptor (CAR) comprising an antigen-binding domain capable of binding IL13R α 2, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence QGTTALATRFFD (SEQ ID NO: 14); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence QHHYSAPWT (SEQ ID NO: 18).

8. The CAR of claim 7, wherein the antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19.

9. The CAR of claim 7 or 8, wherein the antigen-binding domain comprises a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20.

10. The CAR of any one of claims 7-9, wherein the antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20.

11. The CAR of any one of claims 7-10, wherein the antigen-binding domain is selected from the group consisting of a full length antibody or antigen-binding fragment thereof, a Fab, a single-chain variable fragment (scFv), or a single-domain antibody.
12. The CAR of any one of claims 7-11, wherein the antigen-binding domain is a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 21 or 22.
13. The CAR of any one of claims 1-12, wherein the CAR is capable of binding IL13R α 2.
14. The CAR of any one of claims 1-13, wherein the CAR is capable of binding human IL13R α 2.
15. The CAR of any one of claims 1-14, wherein the CAR is capable of binding canine IL13R α 2.
16. The CAR of any one of claims 1-15, wherein the CAR is capable of binding human and canine IL13R α 2.
17. The CAR of any one of claims 1-16, wherein the transmembrane domain is selected from the group consisting of an artificial hydrophobic sequence, and a transmembrane domain of a type I transmembrane protein, an alpha, beta, or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, OX40 (CD134), 4-1BB (CD137), and CD154, or a transmembrane domain derived from a killer immunoglobulin-like receptor (KIR).
18. The CAR of any one of claims 1-17, wherein the transmembrane domain comprises a transmembrane domain of CD8.
19. The CAR of claim 18, wherein the transmembrane domain of CD8 is a transmembrane domain of CD8 alpha.
20. The CAR of any one of claims 1-19, wherein the intracellular domain comprises a costimulatory signaling domain and an intracellular signaling domain.

21. The CAR of any one of claims 1-20, wherein the intracellular domain comprises a costimulatory domain of a protein selected from the group consisting of proteins in the TNFR superfamily, CD28, 4-1BB (CD137), OX40 (CD134), PD-1, CD7, LIGHT, CD83L, DAP10, DAP12, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40, ICOS, NKG2C, and B7-H3 (CD276), or a variant thereof, or an intracellular domain derived from a killer immunoglobulin-like receptor (KIR).

22. The CAR of claim 21, wherein the intracellular domain comprises a costimulatory domain of 4-1BB.

23. The CAR of any one of claims 20-22, wherein the intracellular signaling domain comprises an intracellular domain selected from the group consisting of cytoplasmic signaling domains of a human CD3 zeta chain (CD3 ζ), Fc γ RIII, Fc ϵ RI, a cytoplasmic tail of an Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or a variant thereof.

24. The CAR of any one of claims 20-23, wherein the intracellular signaling domain comprises an intracellular domain of CD3 ζ .

25. A chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises:

a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8; and

a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.

26. A chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises:

a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19; and

a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20.

27. A chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or SEQ ID NO: 24 or SEQ ID NO: 55 or SEQ ID NO: 56.

28. A nucleic acid comprising a polynucleotide sequence encoding a CAR of any one of claims 1-28.

29. A nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence VKWAGGSTDYNSALMS (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7).

30. The nucleic acid of claim 29, wherein the antigen-binding domain comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57.

31. The nucleic acid of claim 29 or 30, wherein the antigen-binding domain comprises a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

32. The nucleic acid of any one of claims 29-31, wherein the antigen-binding domain comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

33. The nucleic acid of any one of claims 29-32, wherein the antigen-binding domain is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 138 or 133.

34. A nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence QGTALATRFFD (SEQ ID NO: 14); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence QHHYSAPWT (SEQ ID NO: 18).

35. The nucleic acid of claim 34, wherein the antigen-binding domain comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67.

36. The nucleic acid of claim 34 or 35, wherein the antigen-binding domain comprises a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

37. The nucleic acid of any one of claims 34-36, wherein the antigen-binding domain comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

38. The nucleic acid of any one of claims 34-37, wherein the antigen-binding domain is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 134 or 135.

39. The nucleic acid of any one of claims 29-38, wherein the transmembrane domain comprises a transmembrane domain of CD8 alpha.

40. The nucleic acid of any one of claims 29-39, wherein the intracellular domain comprises a costimulatory signaling domain and an intracellular signaling domain.

41. The nucleic acid of claim 40, wherein the costimulatory signaling domain comprises a costimulatory domain of 4-1BB.

42. The nucleic acid of claim 40 or 41, wherein the intracellular signaling domain comprises an intracellular domain of CD3 ζ .

43. A nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises:

a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57; and

a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

44. A nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, comprising an antigen-binding domain, a

transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises:

a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67; and

a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

45. A nucleic acid comprising a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or SEQ ID NO: 66 or SEQ ID NO: 75 or SEQ ID NO: 76.

46. A nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the first and second CAR each comprise an antigen-binding domain, a transmembrane domain, and an intracellular domain.

47. The nucleic acid of claim 46, wherein the antigen-binding domain of the first CAR comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence VKWAGGSTDYNSALMS (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7).

48. The nucleic acid of claim 46 or 47, wherein the antigen-binding domain of the first CAR comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57; and a light

chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61.

49. The nucleic acid of any one of claims 46–48, wherein the antigen-binding domain of the first CAR is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 138 or 133.

50. The nucleic acid of claim 46, wherein the antigen-binding domain of the first CAR comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence QGTTALATRFFD (SEQ ID NO: 14); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence QHHYSAPWT (SEQ ID NO: 18).

51. The nucleic acid of claim 50, wherein the antigen-binding domain of the first CAR comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 67; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 71.

52. The nucleic acid of claim 50 or 51, wherein the antigen-binding domain of the first CAR is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 134 or 135.

53. The nucleic acid of any one of claims 46–53, wherein the first polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or SEQ ID NO: 66 or SEQ ID NO: 75 or SEQ ID NO: 76.

54. The nucleic acid of any one of claims 46-53, wherein the antigen-binding domain of the second CAR comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFYPYW (SEQ ID NO: 27); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGTNLDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

55. The nucleic acid of claim 54, wherein the antigen-binding domain of the second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31.

56. The nucleic acid of claim 54 or 55, wherein the antigen-binding domain of the second CAR comprises a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32.

57. The nucleic acid of any one of claims 54-56, wherein the antigen-binding domain of the second CAR comprises a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32.

58. The nucleic acid of any one of claims 54-57, wherein the antigen-binding domain of the second CAR is a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 141.

59. The nucleic acid of any one of claims 46-58, wherein the second polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35 or SEQ ID NO: 196.

60. The nucleic acid of any one of claims 46-59, wherein the transmembrane domain of the first and/or second CAR is selected from the group consisting of an artificial hydrophobic sequence, and a transmembrane domain of a type I transmembrane protein, an alpha, beta, or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, OX40 (CD134), 4-1BB (CD137), and CD154, or a transmembrane domain derived from a killer immunoglobulin-like receptor (KIR).

61. The nucleic acid of any one of claims 46-60, wherein the transmembrane domain of the first and/or second CAR comprises a transmembrane domain of CD8 alpha.

62. The nucleic acid of any one of claims 46-61, wherein the intracellular domain of the first and/or second CAR comprises a costimulatory signaling domain and an intracellular signaling domain.

63. The nucleic acid of any one of claims 46-62, wherein the intracellular domain of the first and/or second CAR comprises a costimulatory domain of a protein selected from the group consisting of proteins in the TNFR superfamily, CD28, 4-1BB (CD137), OX40 (CD134), PD-1, CD7, LIGHT, CD83L, DAP10, DAP12, CD27, CD2, CD5, ICAM-1, LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40, ICOS, NKG2C, and B7-H3 (CD276), or a variant thereof, or an intracellular domain derived from a killer immunoglobulin-like receptor (KIR).

64. The nucleic acid of any one of claims 46-63, wherein the intracellular domain of the first and/or second CAR comprises a costimulatory domain of 4-1BB.

65. The nucleic acid of any one of claims 46-64, wherein the intracellular signaling domain of the first and/or second CAR comprises an intracellular domain selected from the group consisting of cytoplasmic signaling domains of a human CD3 zeta chain (CD3ζ), FcγRIII, FcεRI, a cytoplasmic tail of an Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or a variant thereof.

66. The nucleic acid of any one of claims 46–65, wherein the intracellular signaling domain of the first and/or second CAR comprises an intracellular domain of CD3 ζ .

67. A nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first CAR comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNALMS (SEQ ID NO: 3) or TVSSGGSYTYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFFDV (SEQ ID NO: 15); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18); and

the second CAR comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFPYW (SEQ ID NO: 27); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGTLNDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

68. A nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first CAR comprises:

a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 57 or 67; and

a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 61 or 71; and

the second CAR comprises:

a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 139 or 194; and

a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 140 or 195.

69. A nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first CAR comprises a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 133, 134, 135, or 138; and

the second CAR comprises a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 141.

70. A nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, and a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or SEQ ID NO: 66 or SEQ ID NO: 75 or SEQ ID NO: 76; and

the second polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35 or SEQ ID NO: 196.

71. A nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) capable of binding IL13R α 2, and a second polynucleotide sequence encoding an inhibitor of an immune checkpoint.

72. The nucleic acid of claim 71, wherein the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3.

73. The nucleic acid of claim 71 or 72, wherein the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody.

74. The nucleic acid of any one of claims 71-73, wherein the inhibitor of the immune checkpoint is an anti-CTLA-4 antibody.

75. A nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) capable of binding IL13R α 2, and a second polynucleotide sequence encoding an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof.

76. The nucleic acid of claim 75, wherein the second polynucleotide sequence comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence encoding SEQ ID NO: 53 or 54.

77. The nucleic acid of claim 75 or 76, wherein the BiTE is capable of binding wild type EGFR (wtEGFR).

78. The nucleic acid of claim 75 or 76, wherein the BiTE is capable of binding EGFR variant III (EGFRvIII).

79. The nucleic acid of any one of claims 46-78, wherein the first polynucleotide sequence and the second polynucleotide sequence is separated by a linker.

80. The nucleic acid of claim 79, wherein the linker comprises a nucleotide sequence encoding an internal ribosome entry site (IRES) or a self-cleaving peptide.

81. The nucleic acid of claim 80, wherein the self-cleaving peptide is a 2A peptide.

82. The nucleic acid of claim 81, wherein the 2A peptide is selected from the group consisting of porcine teschovirus-1 2A (P2A), Thoseaasigna virus 2A (T2A), equine rhinitis A virus 2A (E2A), and foot-and-mouth disease virus 2A (F2A).

83. The nucleic acid of claim 81 or 82, wherein the 2A peptide is T2A.

84. The nucleic acid of any one of claims 79-83, wherein the linker further comprises a furin cleavage site.

85. The nucleic acid of any one of claims 79-84, wherein the nucleic acid comprises from 5' to 3' the first polynucleotide sequence, the linker, and the second polynucleotide sequence.

86. The nucleic acid of any one of claims 79-84, wherein the nucleic acid comprises from 5' to 3' the second polynucleotide sequence, the linker, and the first polynucleotide sequence.

87. A vector comprising the nucleic acid of any one of claims 29-86.

88. The vector of claim 87, wherein the vector is an expression vector.

89. The vector of claim 87 or 88, wherein the vector is selected from the group consisting of a DNA vector, an RNA vector, a plasmid, a lentiviral vector, an adenoviral vector, an adeno-associated viral vector, and a retroviral vector.

90. The vector of any one of claims 87-89, further comprising an EF-1 a promoter.

91. The vector of any one of claims 87-90, further comprising a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE).

92. The vector of any one of claims 87-91, further comprising a rev response element (RRE).
93. The vector of any one of claims 87-92, further comprising a cPPT sequence.
94. The vector of any one of claims 87-93, wherein the vector is a self-inactivating vector.
95. A modified immune cell or precursor cell thereof, comprising the CAR of any one of claims 1-27, the nucleic acid of any one of claims 29-86, or the vector of any one of claims 87-93.
96. A modified immune cell or precursor cell thereof, comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises:
- a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFFDV (SEQ ID NO: 15); and
 - a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18).
97. A modified immune cell or precursor cell thereof, comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises:
- a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19; and
 - a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20.
98. A modified immune cell or precursor cell thereof, comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises a single-chain variable

fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or 11.

99. A modified immune cell or precursor cell thereof, comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 21 or 22.

100. The modified cell of any one of claims 96-99, wherein the CAR is capable of binding IL13R α 2.

101. The modified cell of any one of claims 96-100, wherein the CAR is capable of binding human IL13R α 2.

102. The modified cell of any one of claims 96-101, further comprising an inhibitor of an immune checkpoint, wherein the modified cell secretes the inhibitor of the immune checkpoint.

103. The modified cell of claim 102, wherein the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3.

104. The modified cell of claim 102 or 103, wherein the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody.

105. The modified cell of any one of claims 96-104, further comprising an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the modified cell secretes the BiTE.

106. The modified cell of claim 105, wherein the inducible BiTE comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 53 or 54.

107. The modified cell of claim 105 or 106, wherein the BiTE is capable of binding wild type EGFR (wtEGFR).

108. The modified cell of claim 105 or 106, wherein the BiTE is capable of binding EGFR variant III (EGFRvIII).

109. A modified immune cell or precursor cell thereof, comprising:

 a first chimeric antigen receptor (CAR) comprising a first antigen-binding domain capable of binding IL13R α 2; and

 a second chimeric antigen receptor (CAR) comprising a second antigen-binding domain capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof.

110. A modified immune cell or precursor cell thereof, comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

 the first CAR comprises:

 a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFDDV (SEQ ID NO: 15); and

 a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18); and

 the second CAR comprises:

 a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFPYW (SEQ ID NO: 27); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGTNLDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

111. A modified immune cell or precursor cell thereof, comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first CAR comprises:

a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19; and

a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20; and

the second CAR comprises:

a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31; and

a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32.

112. A modified immune cell or precursor cell thereof, comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or 11; and

the second CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34.

113. A modified immune cell or precursor cell thereof, comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 24; and

the second CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 36 or 197.

114. The modified cell of any one of claims 109-113, further comprising an inhibitor of an immune checkpoint, wherein the modified cell secretes the inhibitor of the immune checkpoint.

115. The modified cell of claim 114, wherein the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3.

116. The modified cell of claim 114 or 115, wherein the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody.

115. The modified cell of any one of claims 96-116, wherein the CAR is capable of binding human IL13R α 2.

116. The modified cell of any one of claims 109-115, wherein the second CAR is capable of binding an EGFR isoform selected from the group consisting of wild type EGFR (wtEGFR), mutated EGFR, EGFR^{A289V}, EGFR^{A289D}, EGFR^{A289T}, EGFR^{A289T}, EGFR^{R108K}, EGFR^{R108G}, EGFR^{G598V}, EGFR^{D126Y}, EGFR^{C628F}, EGFR^{R108K/A289V}, EGFR^{R108K/D126Y}, EGFR^{A289V/G598V}, EGFR^{A289V/C628F}, and EGFR variant II, or any combination thereof.

117. The modified cell of any one of claims 96-116, wherein the modified cell is a modified immune cell.

118. The modified cell of any one of claims 96-117, wherein the modified cell is a modified T cell.

119. The modified cell of any one of claims 96-118, wherein the modified cell is an autologous cell.

120. The modified cell of any one of claims 96-119, wherein the modified cell is an autologous cell obtained from a human subject.

121. A pharmaceutical composition comprising a therapeutically effective amount of the modified cell of any one of claims 96-120.

122. A method of treating a disease in a subject in need thereof, comprising administering to the subject an effective amount of the modified cell of any one of claims 96-120, or the pharmaceutical composition of claim 121.

123. The method of claim 122, wherein the disease is a cancer.

124. The method of claim 123, wherein the cancer is a glioma.

125. The method of claim 123 or 124, wherein the cancer is an astrocytoma.

126. The method of any one of claims 123-125, wherein the cancer is a high-grade astrocytoma.

127. The method of any one of claims 123-126, wherein the cancer is glioblastoma.

128. A method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFFDV (SEQ ID NO: 15); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO:

17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18).

129. A method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises:

a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19; and

a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20.

130. A method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 21 or SEQ ID NO: 22.

131. A method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or SEQ ID NO: 24 or SEQ ID NO: 55 or SEQ ID NO: 56.

132. The method of any one of claims 128-131, further comprising administering an inhibitor of an immune checkpoint, wherein the modified cell secretes the inhibitor of the immune checkpoint.

133. The method of claim 132, wherein the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3.

134. The method of claim 132 or 133, wherein the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody.

135. The method of any one of claims 132-134, wherein the inhibitor of the immune checkpoint is co-administered with the modified T cell.

136. The method of any one of claims 128-135, further comprising administering an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein the modified cell secretes the BiTE.

137. The method of claim 136, wherein the inducible BiTE comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 53 or 54.

138. The method of claim 136 or 137, wherein the BiTE is capable of binding wild type EGFR (wtEGFR).

139. The method of claim 136 or 137, wherein the BiTE is capable of binding EGFR variant III (EGFRvIII).

140. The method of any one of claims 136-139, wherein the BiTE is co-administered with the modified T cell.

141. The method of any one of claims 128-131, further comprising administering an inducible bispecific T cell engager (BiTE) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and an inhibitor of an immune checkpoint, wherein the modified cell secretes the BiTE and the inhibitor of the immune checkpoint.

142. The method of claim 141, wherein the BiTE and the inhibitor of the immune checkpoint is co-administered with the modified T cell.

143. A method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising:

a first chimeric antigen receptor (CAR) comprising a first antigen-binding domain capable of binding IL13R α 2; and

a second chimeric antigen receptor (CAR) comprising a second antigen-binding domain capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof.

144. A method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first CAR comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1) or SRNGMS (SEQ ID NO: 12), HCDR2 comprises the amino acid sequence GVKWAGGSTDYNSALMS (SEQ ID NO: 3) or TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4) or QGTTALATRFDDV (SEQ ID NO: 15); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASLSVSSTYLH (SEQ ID NO: 5) or KASQDVGTAVA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO: 6) or SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7) or QHHYSAPWT (SEQ ID NO: 18); and

the second CAR comprises:

a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNPSLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFPYW (SEQ ID NO: 27); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence HSSQDINSNIG (SEQ ID NO: 28), LCDR2 comprises the amino acid sequence HGTNLDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

145. A method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first CAR comprises:

a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8 or 19; and

a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9 or 20; and

the second CAR comprises:

a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 31; and

a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32.

146. A method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10 or 11; and

the second CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34.

147. A method of treating glioblastoma in a subject in need thereof, comprising administering to the subject an effective amount of a modified T cell comprising a first chimeric antigen receptor capable of binding IL13R α 2, and a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, wherein:

the first CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 24; and

the second CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 36 or 197.

148. The method of any one of claims 143-147, further comprising administering an inhibitor of an immune checkpoint, wherein the modified cell secretes the inhibitor of the immune checkpoint.

149. The method of claim 148, wherein the immune checkpoint is selected from the group consisting of CTLA-4, PD-1, and TIM-3.

150. The method of claim 148 or 149, wherein the inhibitor of the immune checkpoint is selected from the group consisting of an anti-CTLA-4 antibody, an anti-PD-1 antibody, and an anti-TIM-3 antibody.

151. The method of any one of claims 148-150, wherein the inhibitor of the immune checkpoint is co-administered with the modified T cell.

152. The nucleic acid of any one of claims 28-86, further comprising an inducible promoter, wherein the inducible promoter comprises a nucleotide sequence that is 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs. 161, 162, or 198.

153. A nucleic acid comprising a polynucleotide sequence encoding a CAR comprising a first antigen binding domain, a second antigen binding domain, a transmembrane domain, and an intracellular domain, wherein the first and second antigen binding domain are separate by a linker.

154. The nucleic acid of claim 153, wherein the linker comprises 5, 10, 15, or 20 amino acids.

155. The nucleic acid of claim 153, wherein the first antigen binding domain is capable of binding IL13R α 2, and the second antigen binding domain is capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof.

156. The nucleic acid of claim 155, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 163, 165, 167, or 169 and/or is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 164, 166, 168, or 170.

157. A nucleic acid comprising a polynucleotide sequence encoding a parallel CAR, wherein the parallel CAR comprises a first CAR and a second CAR, each comprising an antigen binding domain, a transmembrane domain, and an intracellular domain, wherein the first CAR and the second CAR are separate by a cleavable linker.

158. The nucleic acid of claim 157, wherein the parallel CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 171 and/or is encoded by a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 172.

159. A nucleic acid comprising a polynucleotide sequence encoding a BiTE and a CAR.

160. The nucleic acid of claim 159, wherein the BiTE comprises an antigen binding domain capable of binding EGFR or an isoform thereof, and the CAR comprises an antigen binding domain capable of binding IL13R α 2.

161. The nucleic acid of claim 159, wherein the BiTE comprises an antigen binding domain capable of binding IL13R α 2, and the CAR comprises an antigen binding domain capable of binding EGFR or an isoform thereof.

162. The nucleic acid of claim 159, wherein the polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 176 or SEQ ID NO: 178.

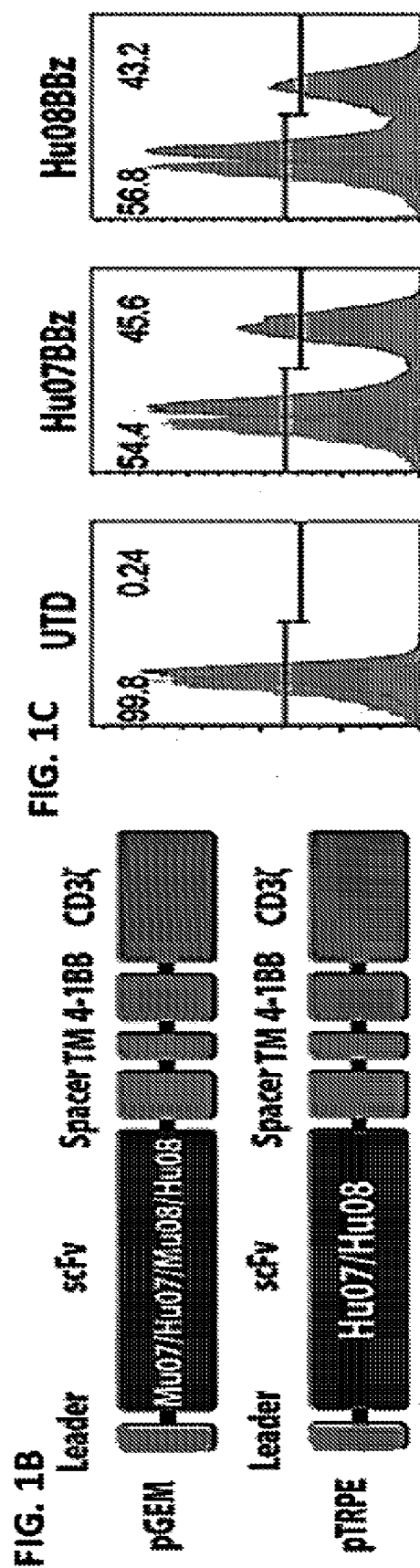
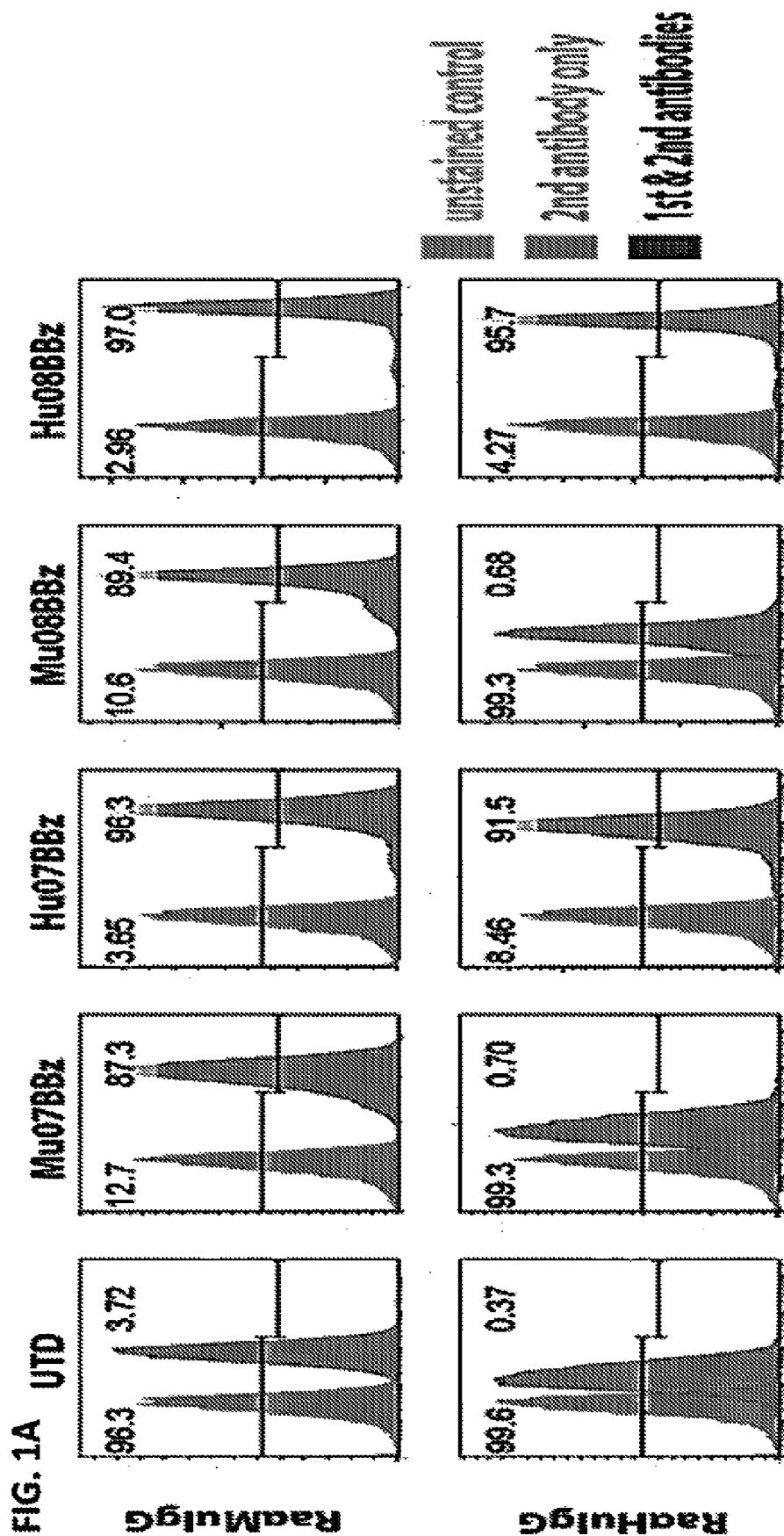
163. The nucleic acid of claim 159, wherein the polynucleotide sequence is encoded by an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 175 or SEQ ID NO: 177.

164. A nucleic acid comprising a polynucleotide sequence encoding a first BiTE and a second BiTE.

165. The nucleic acid of claim 164, wherein the first and/or second BiTE comprises an antigen binding domain capable of binding IL13R α 2, and/or an antigen binding domain capable of binding EGFR or an isoform thereof.

166. The nucleic acid of claim 164, wherein the polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 180.

167. The nucleic acid of claim 164, wherein the polynucleotide sequence encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 179.



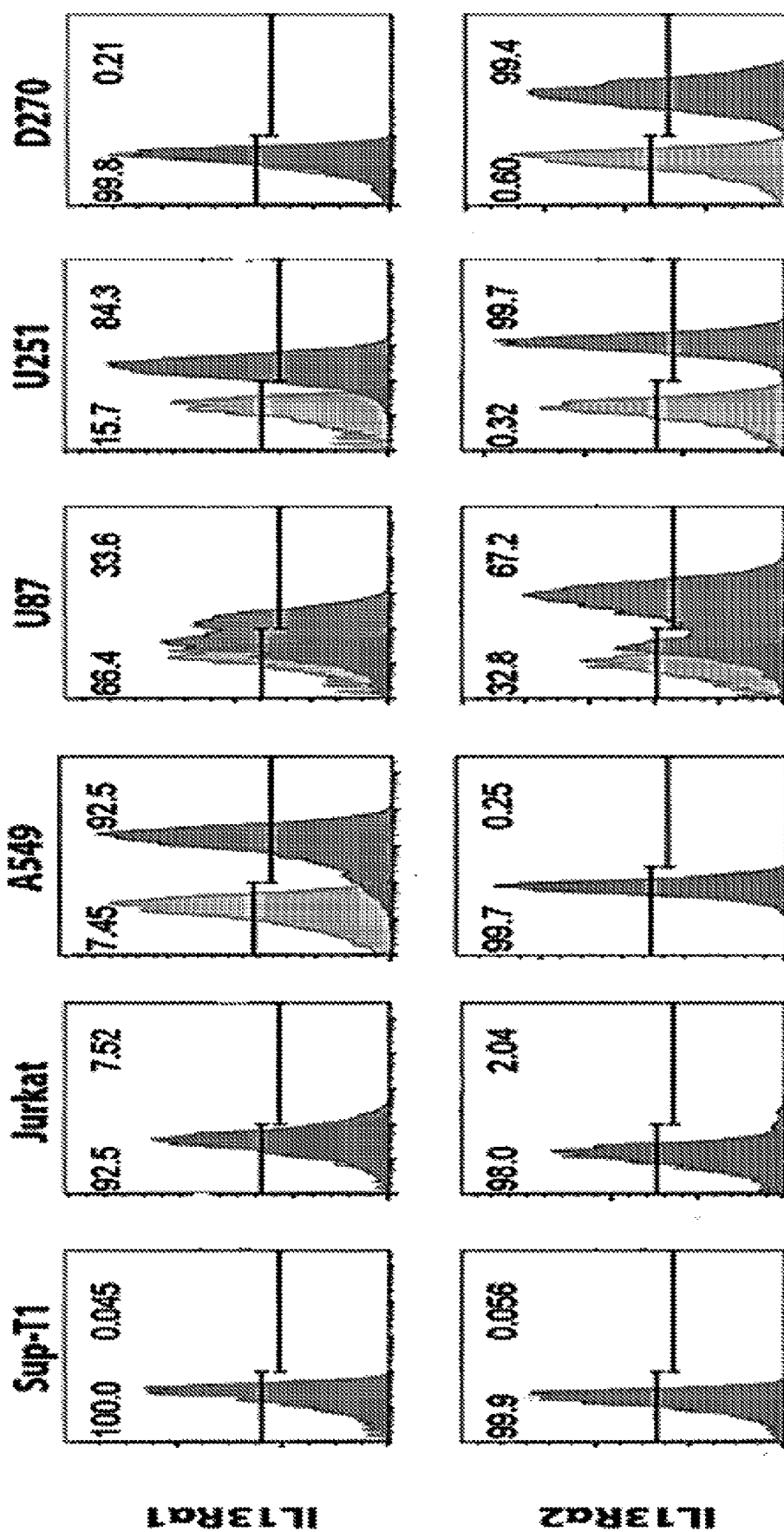
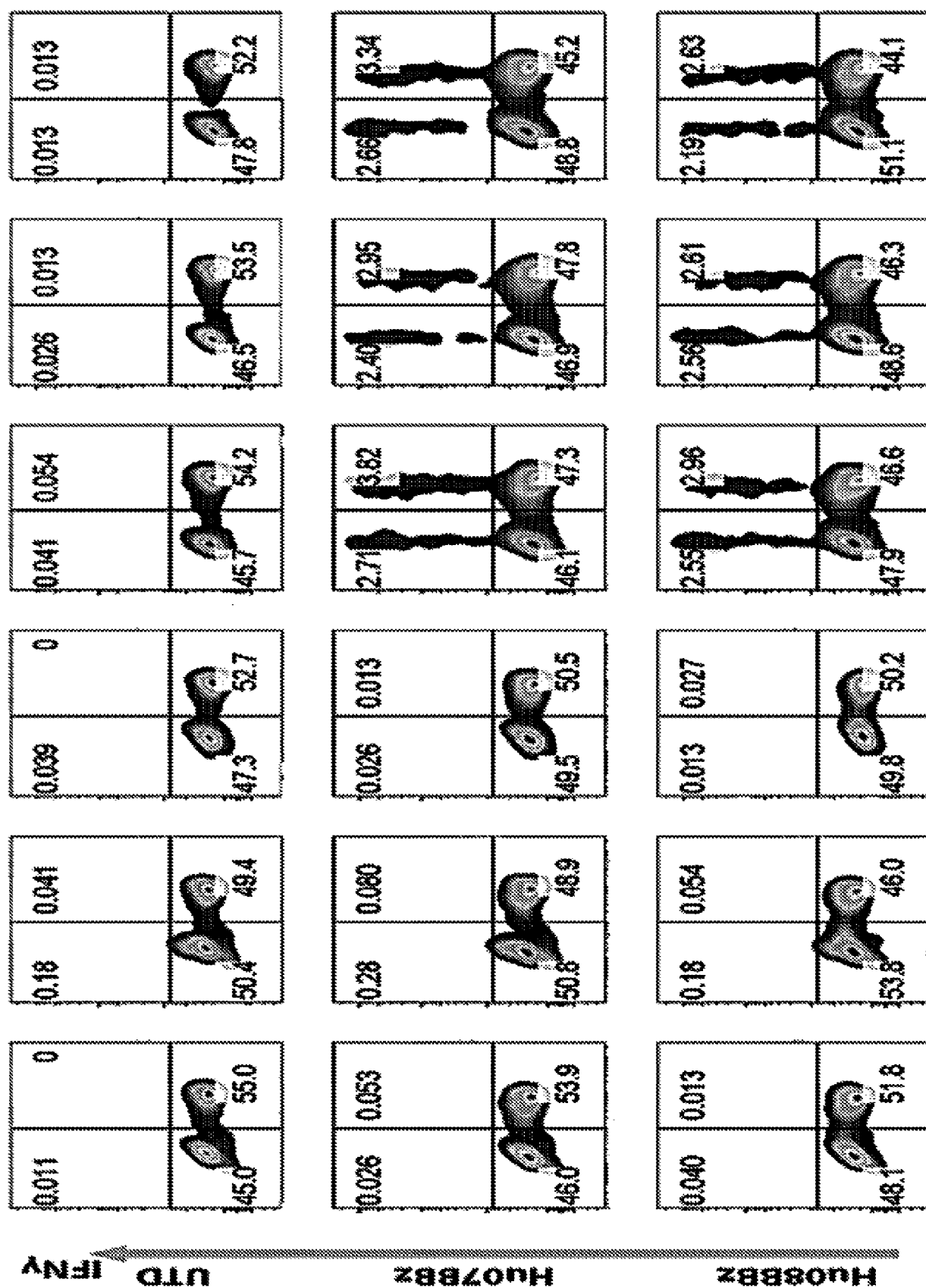


FIG. 1D



CD8

FIG. 1E

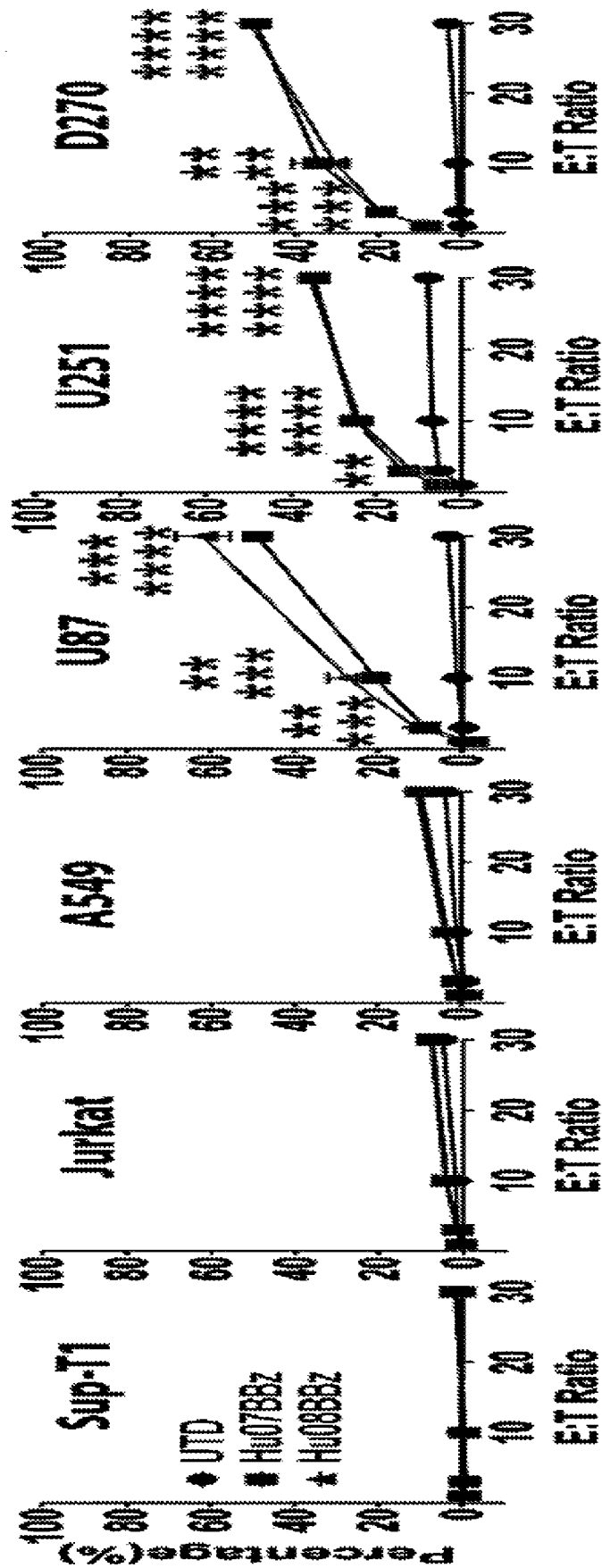


FIG. 1F

FIG. 2A

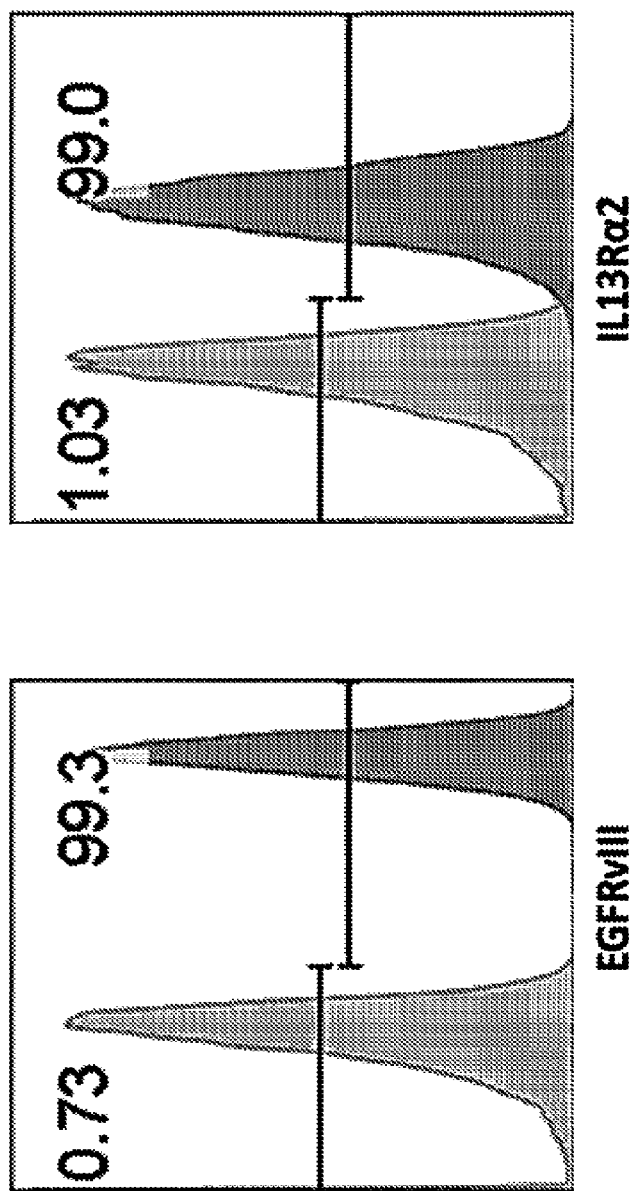


FIG. 2B

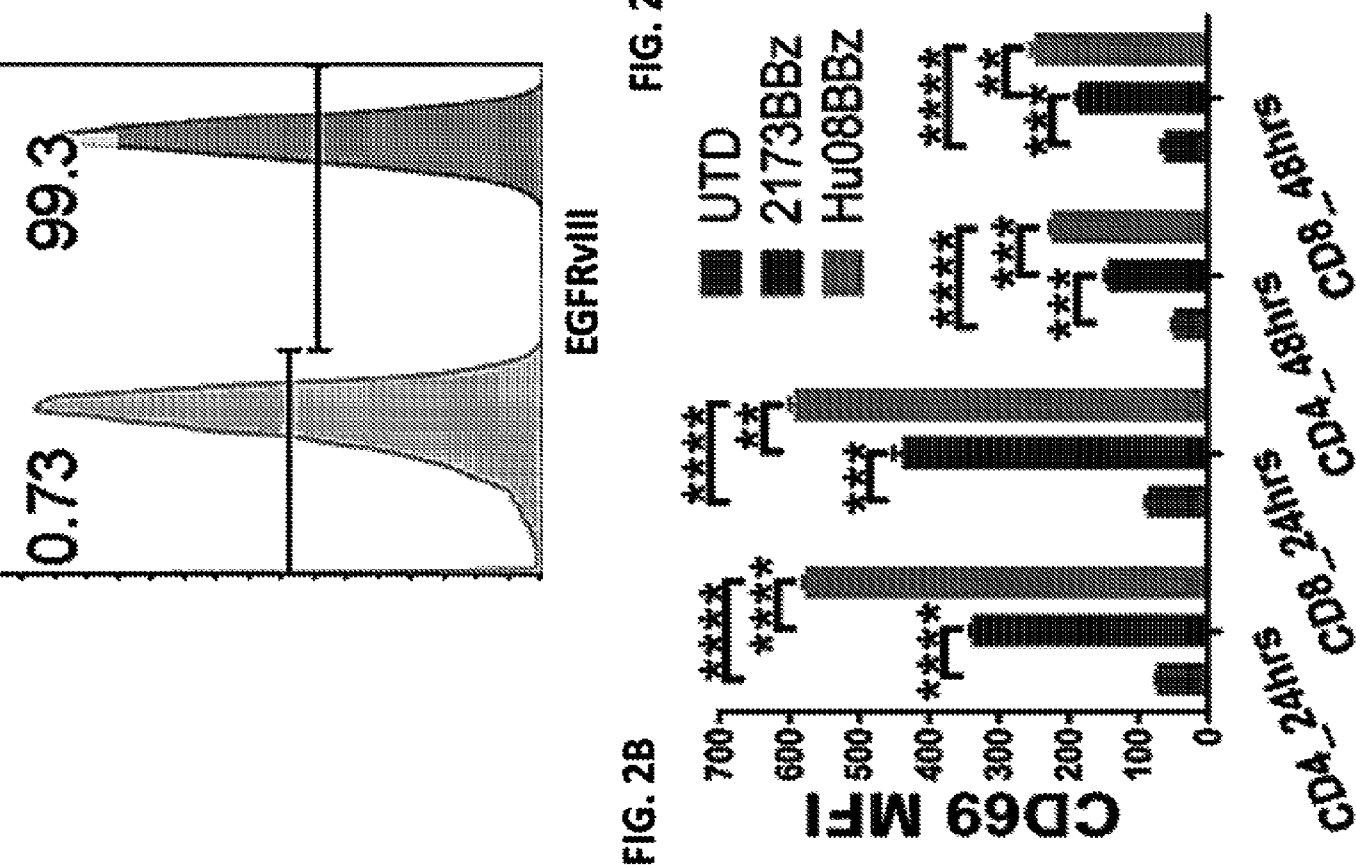
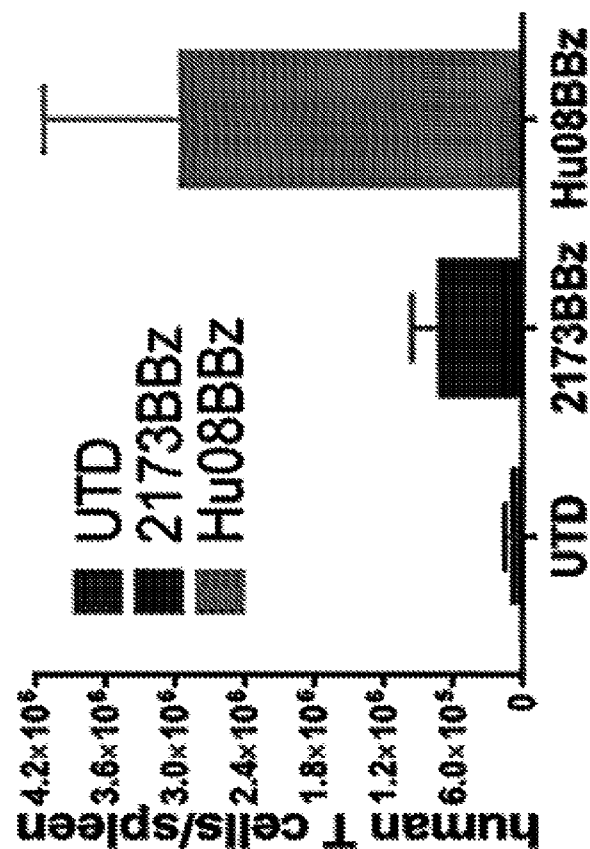


FIG. 2C



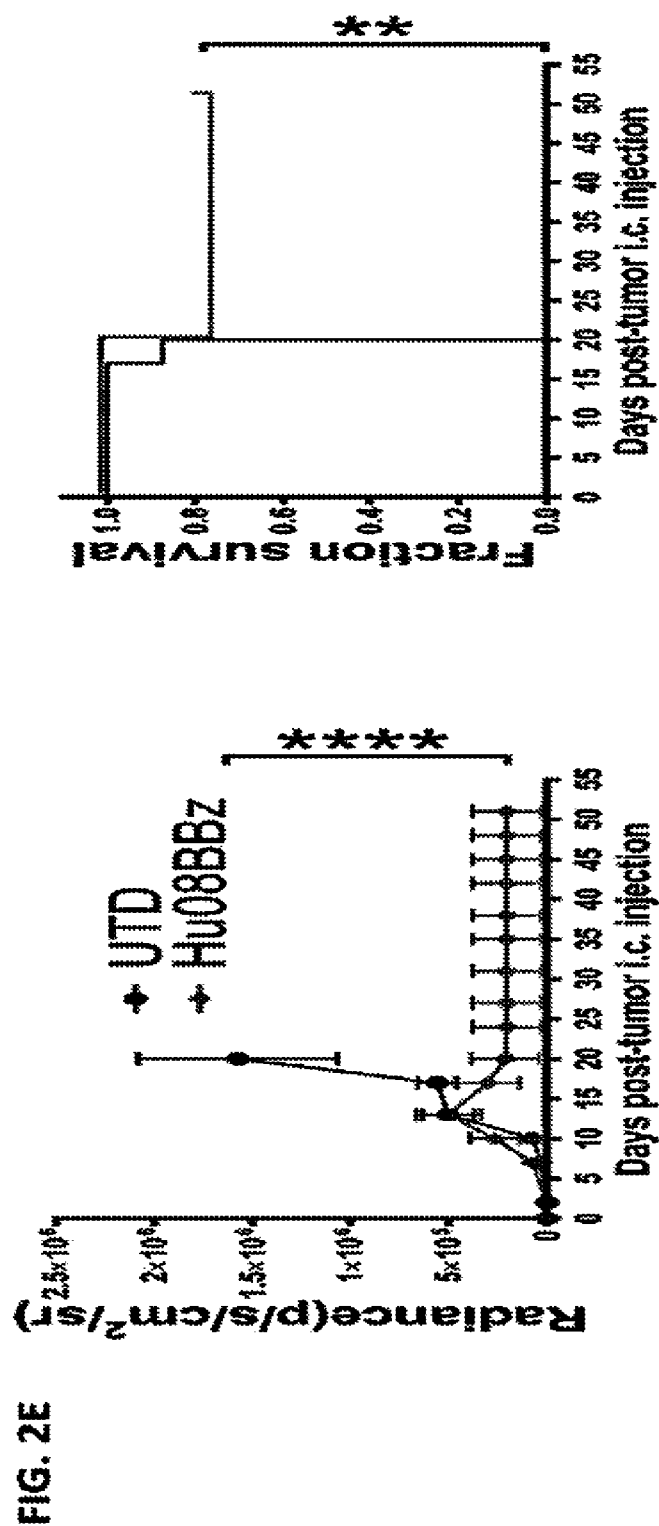
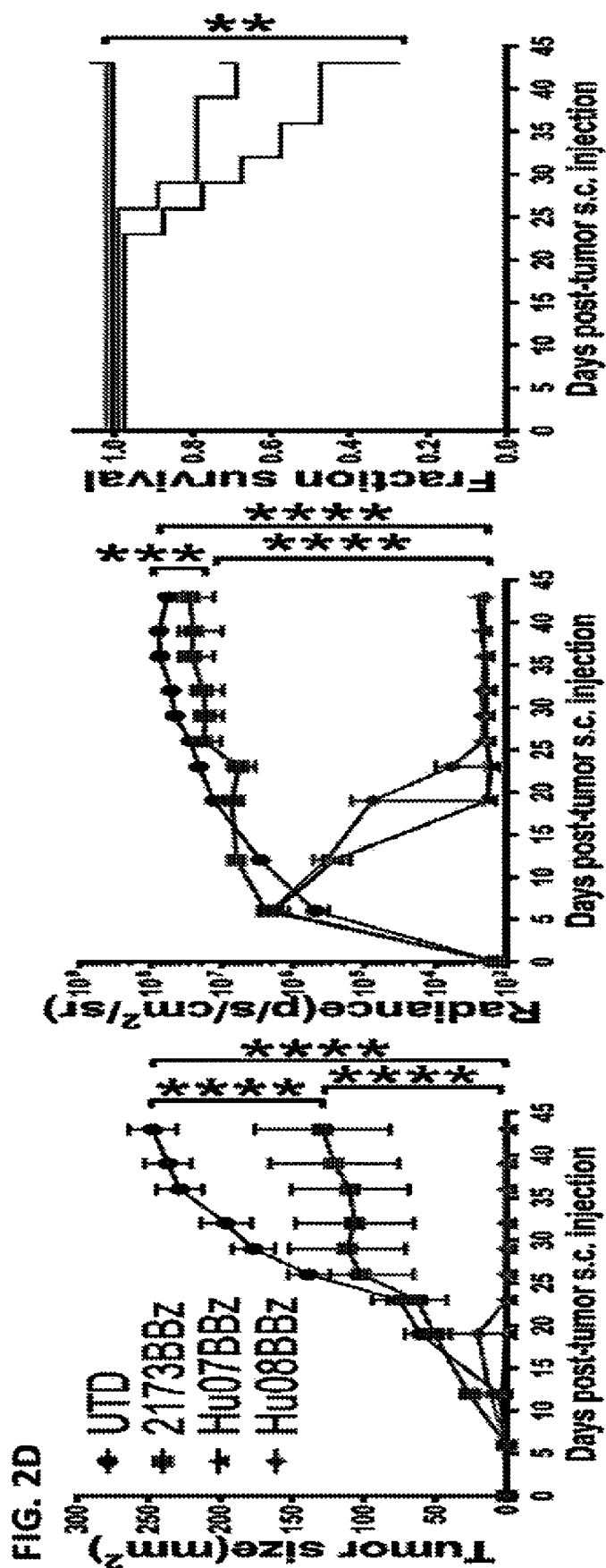


FIG. 3A

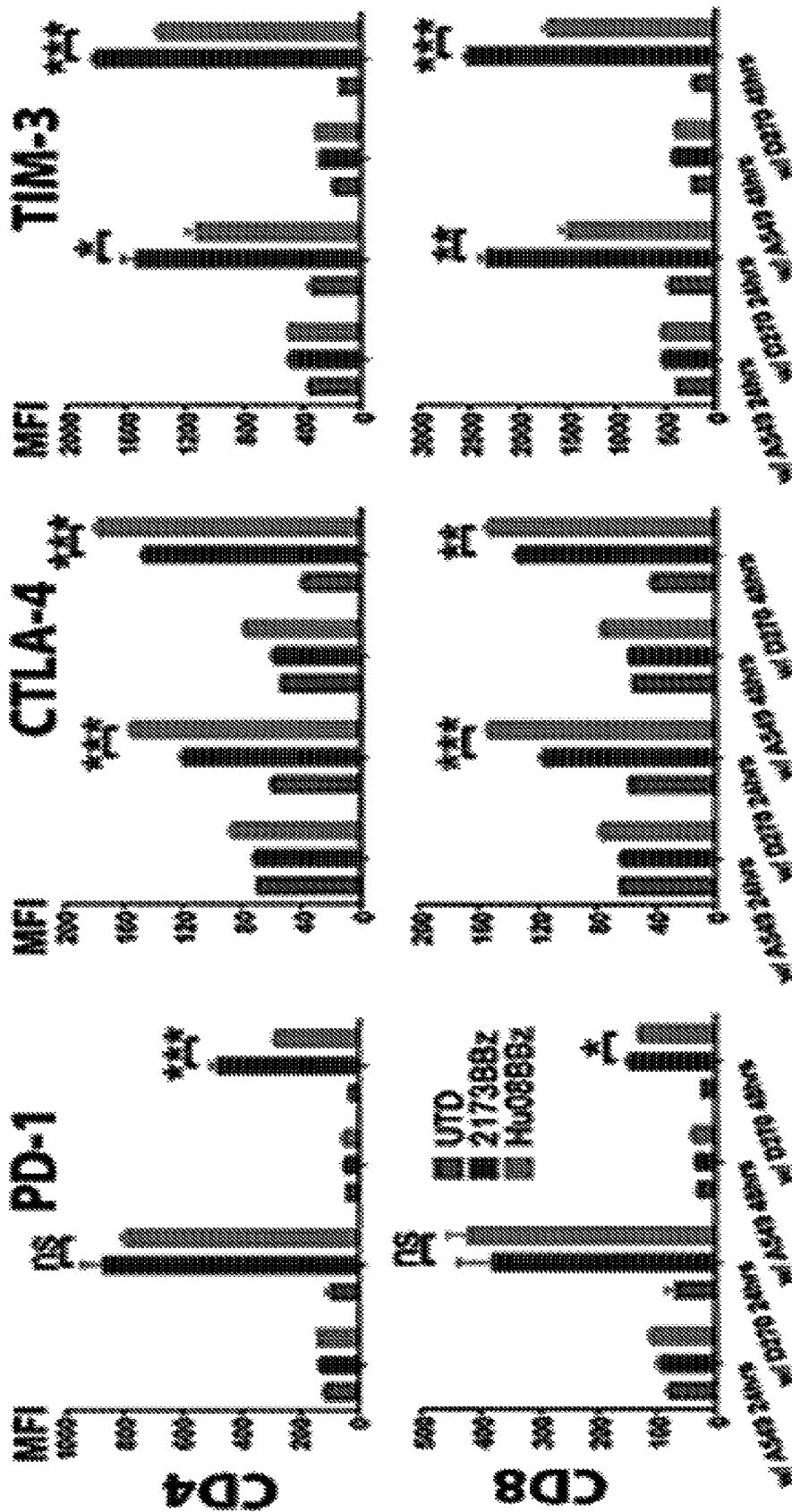
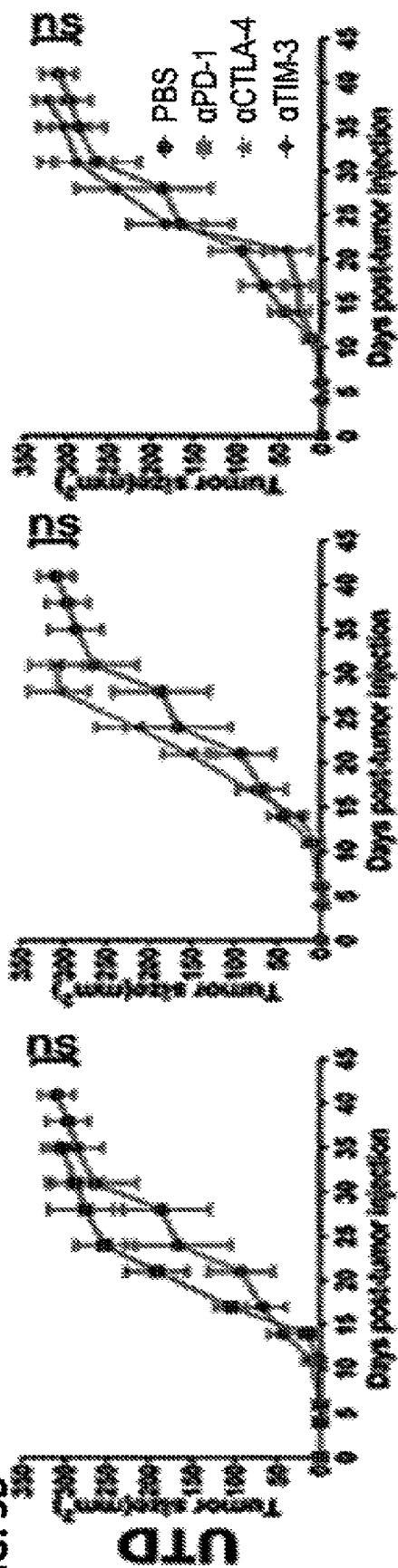


FIG. 3B



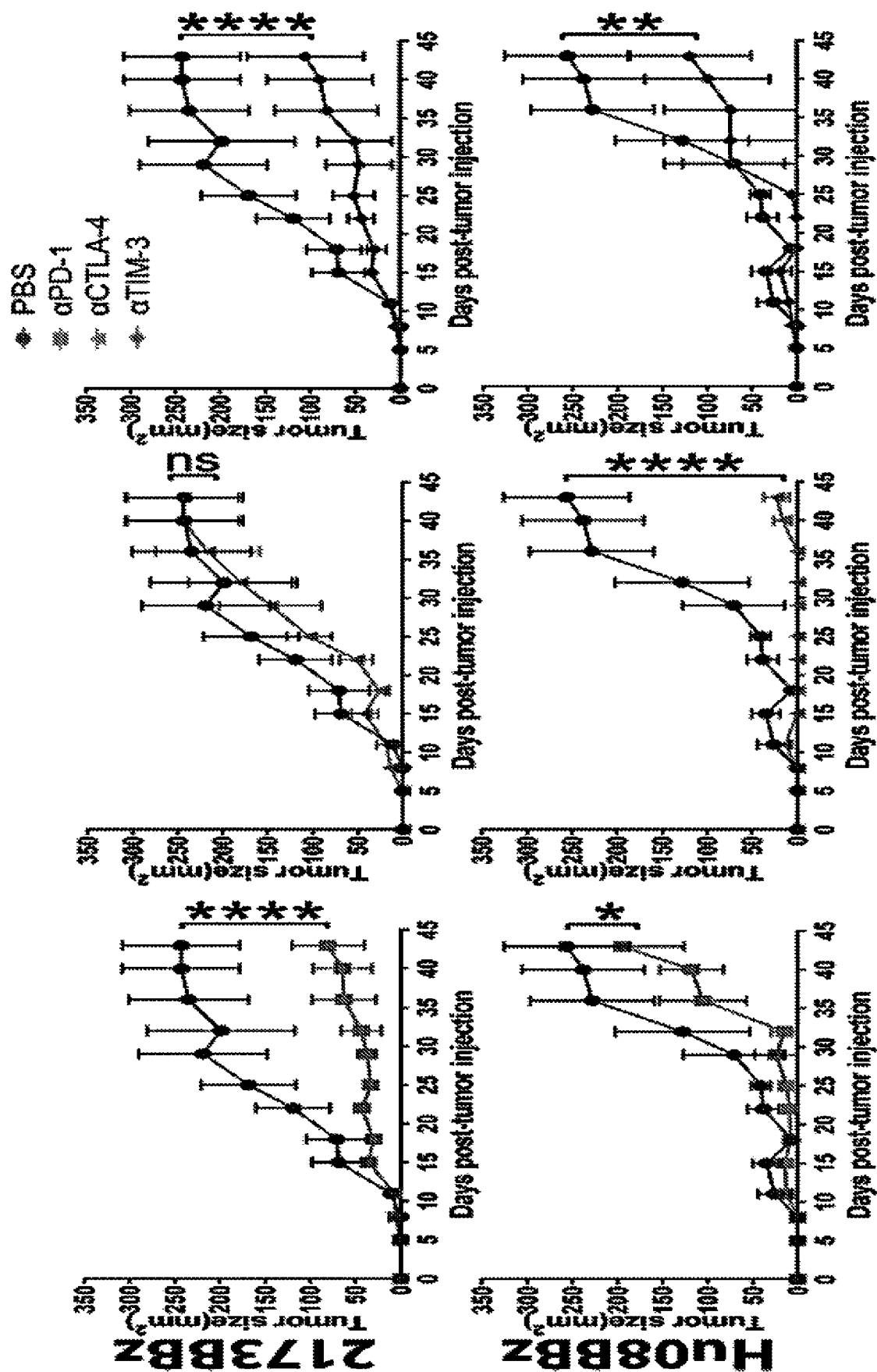


FIG. 3C

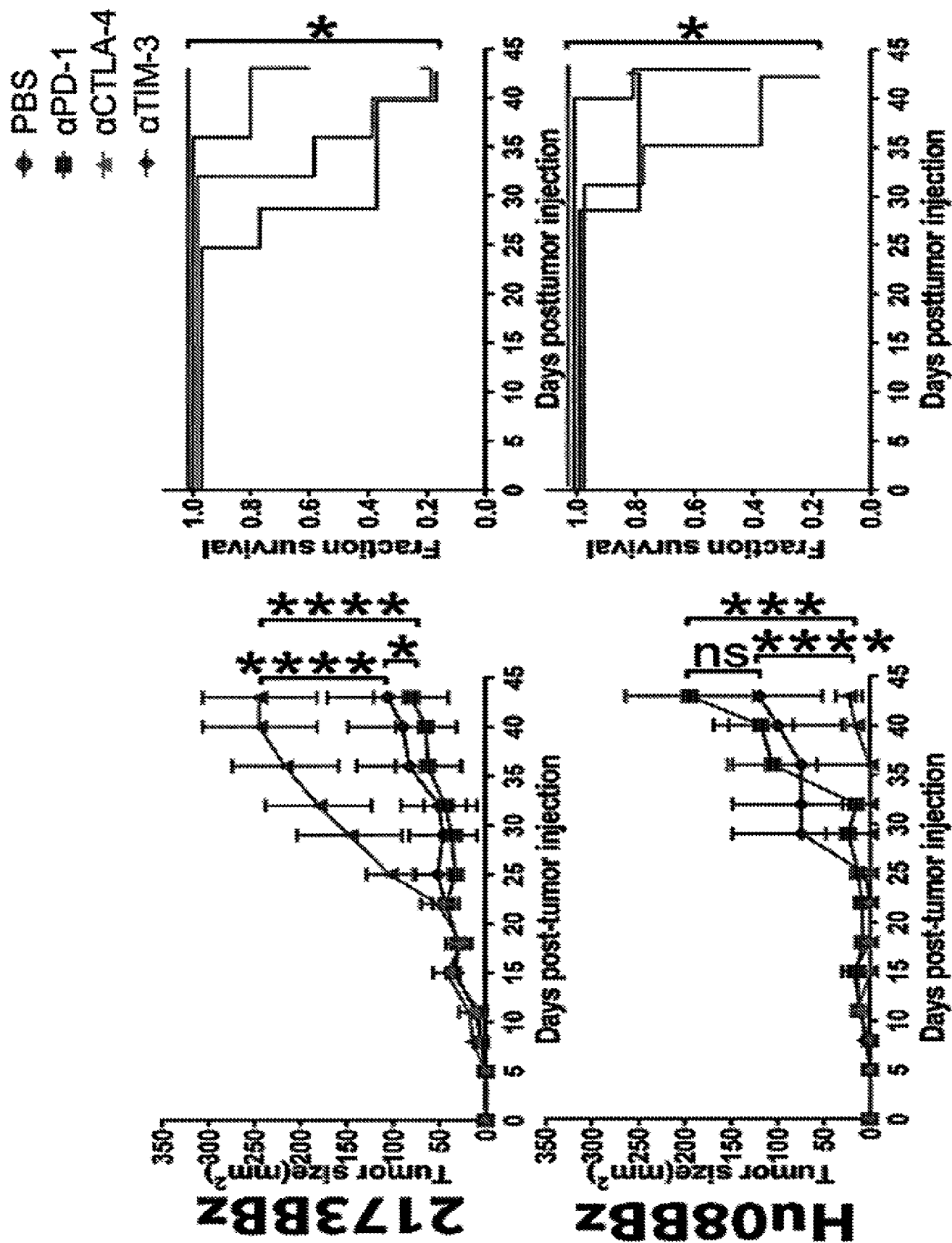


FIG. 3D

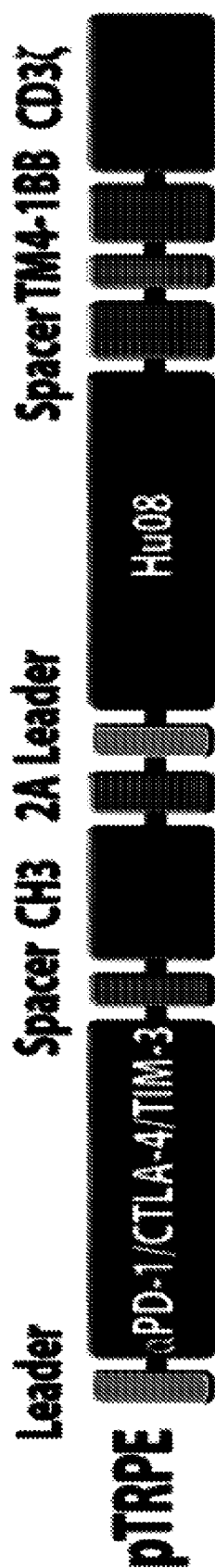


FIG. 4A

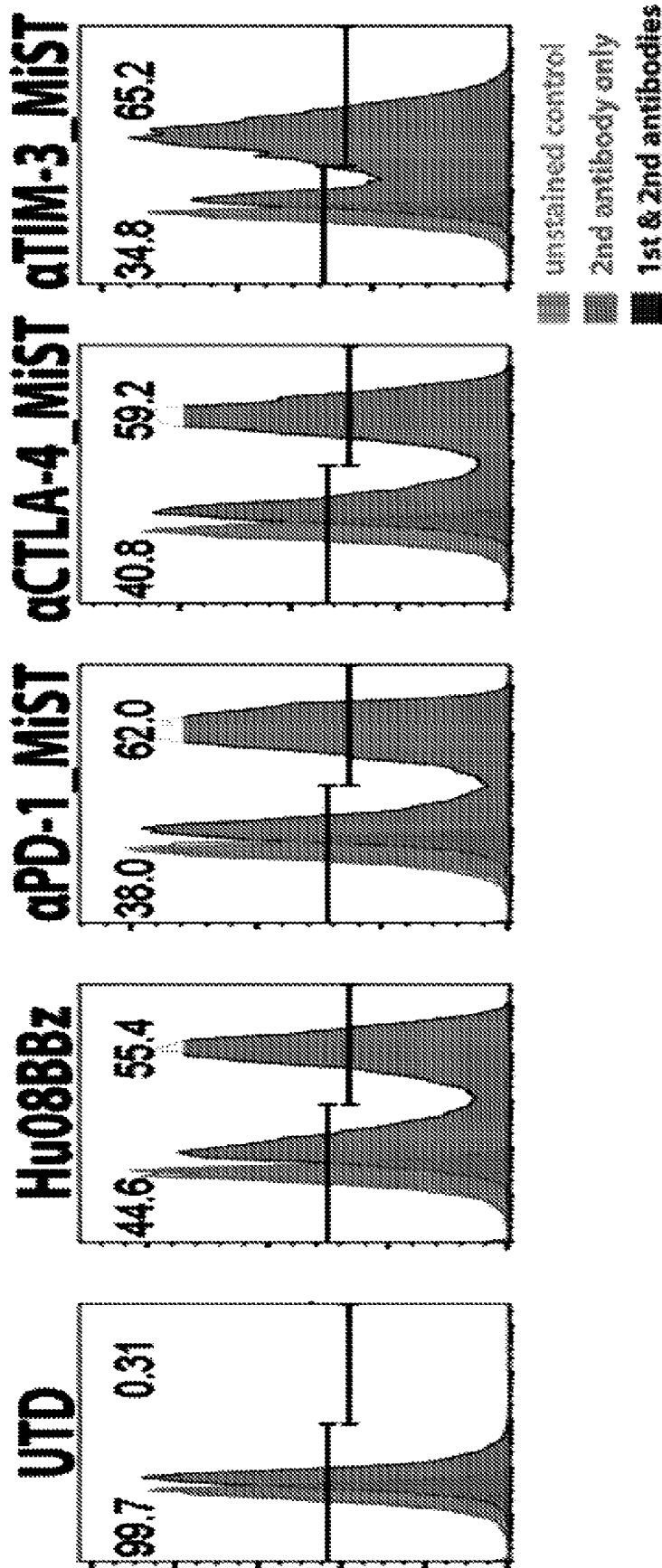


FIG. 4B

FIG. 4C

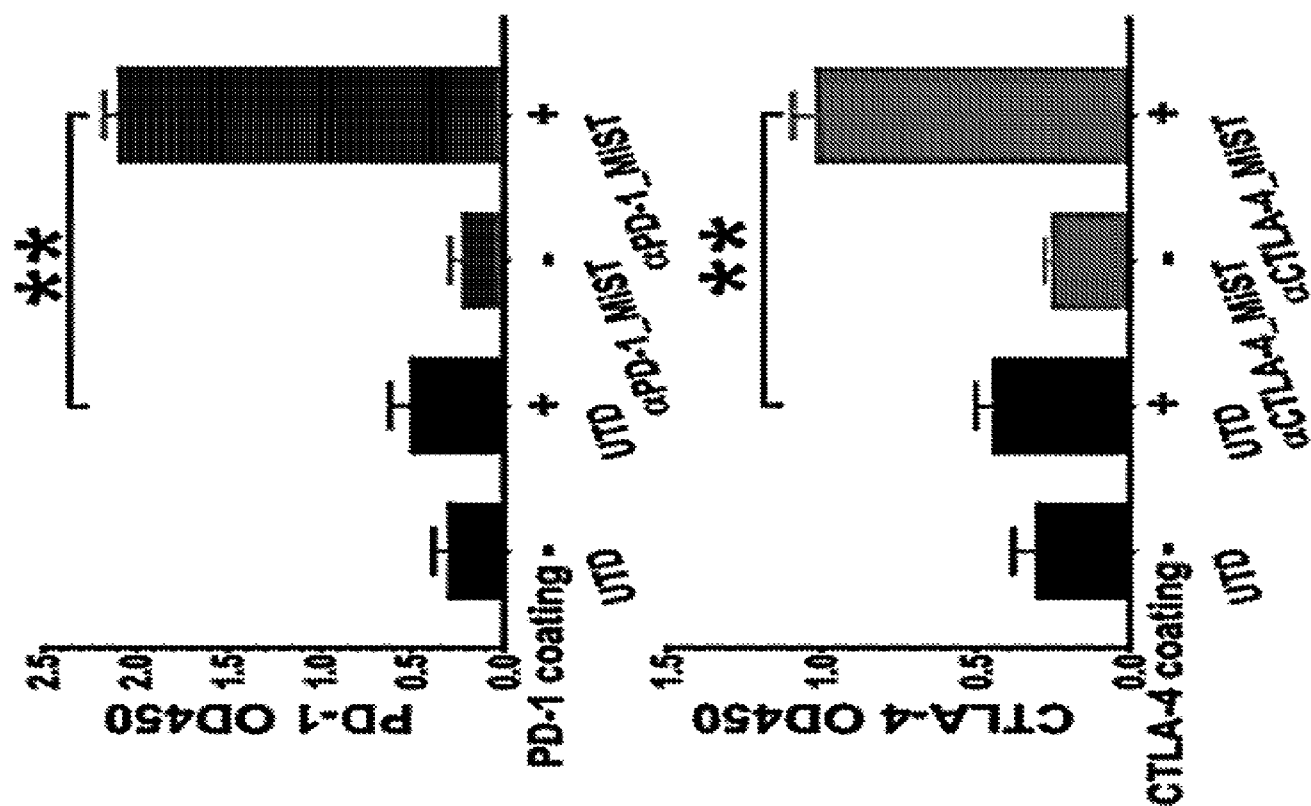
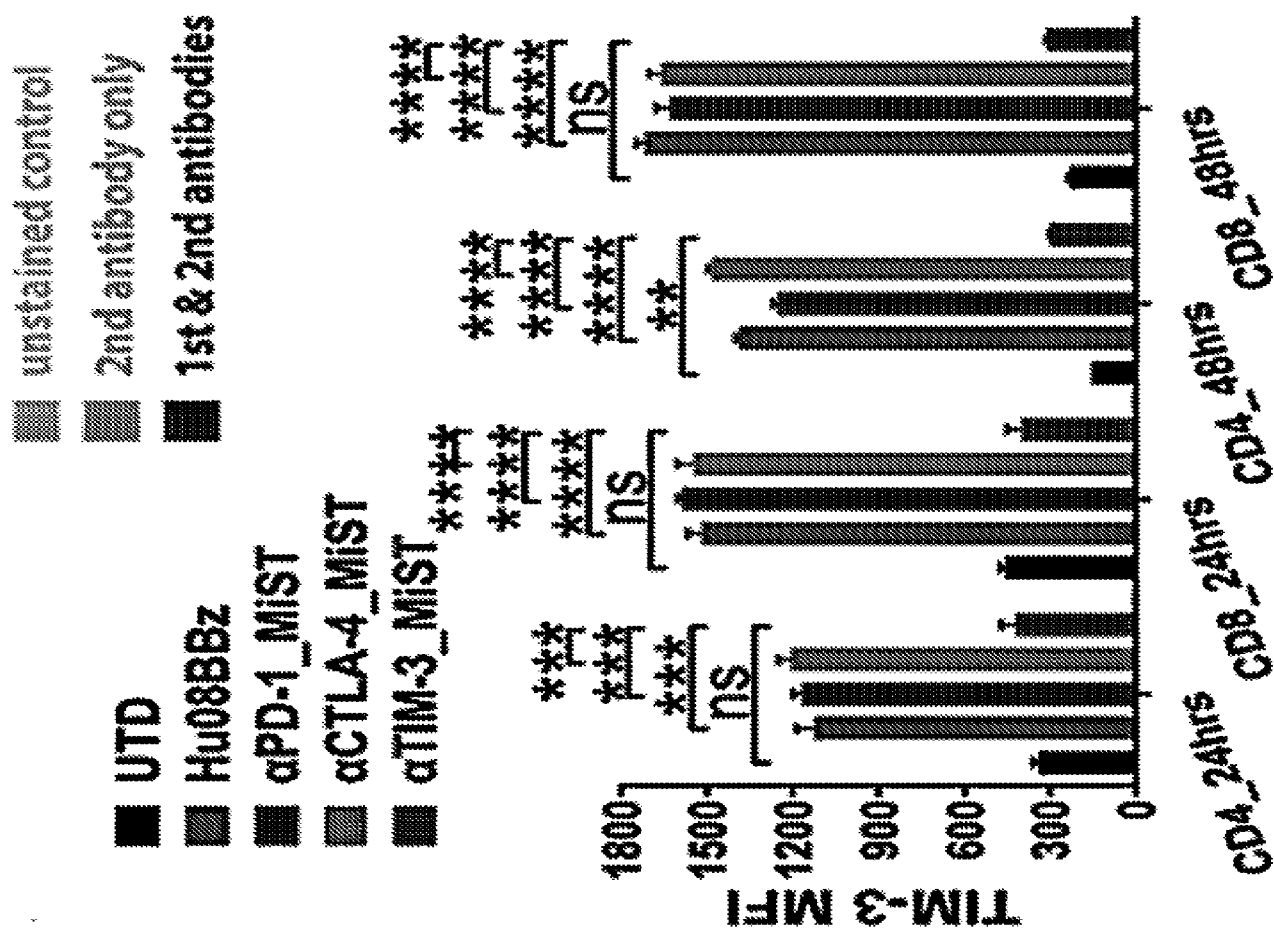


FIG. 4D



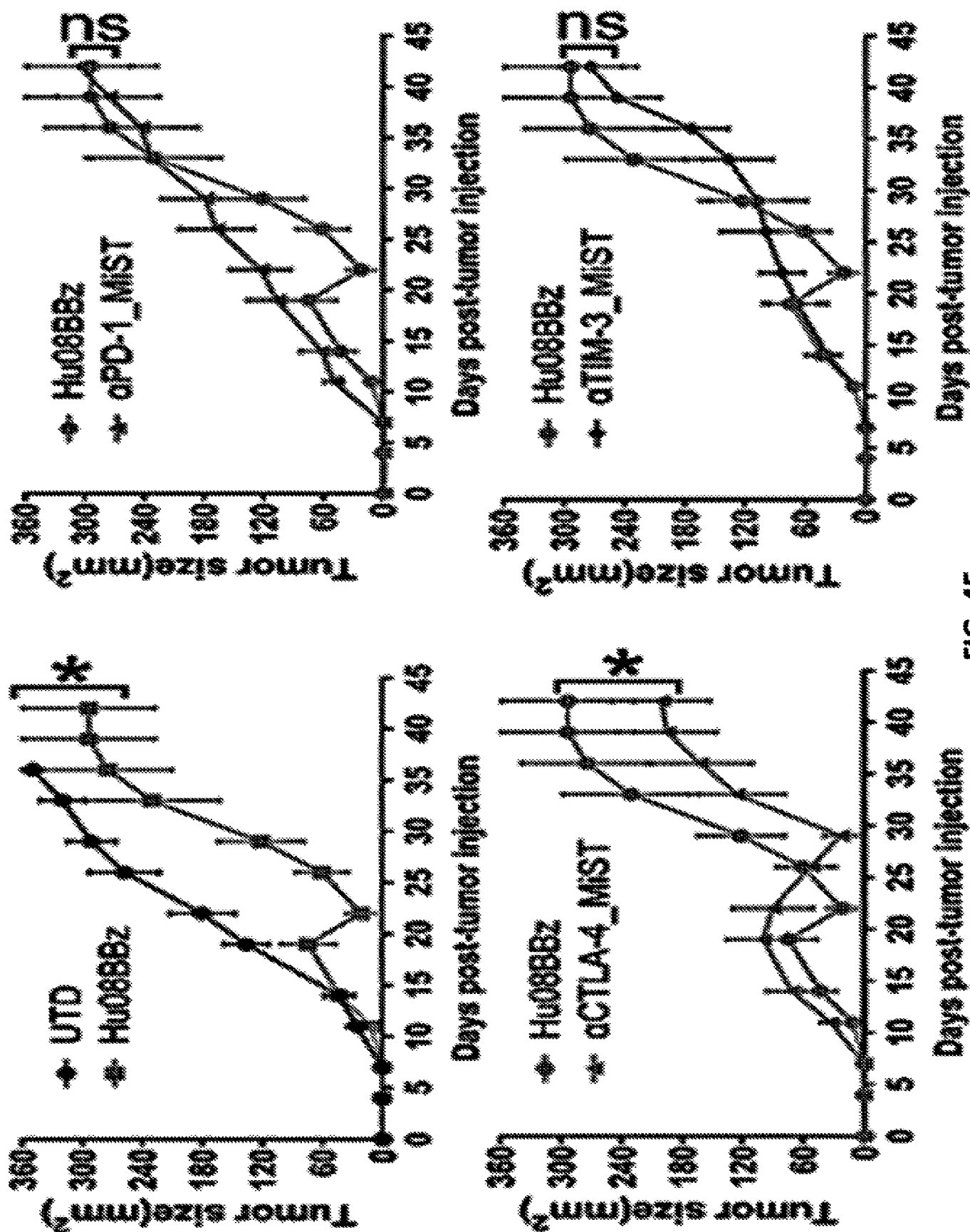


FIG. 4E

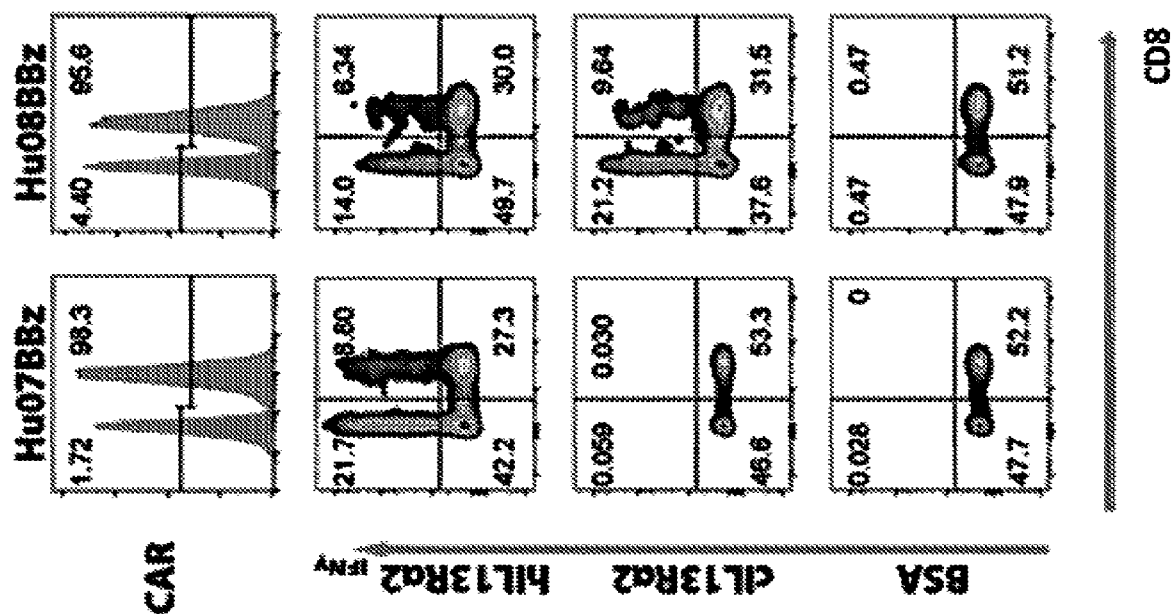


FIG. 5B

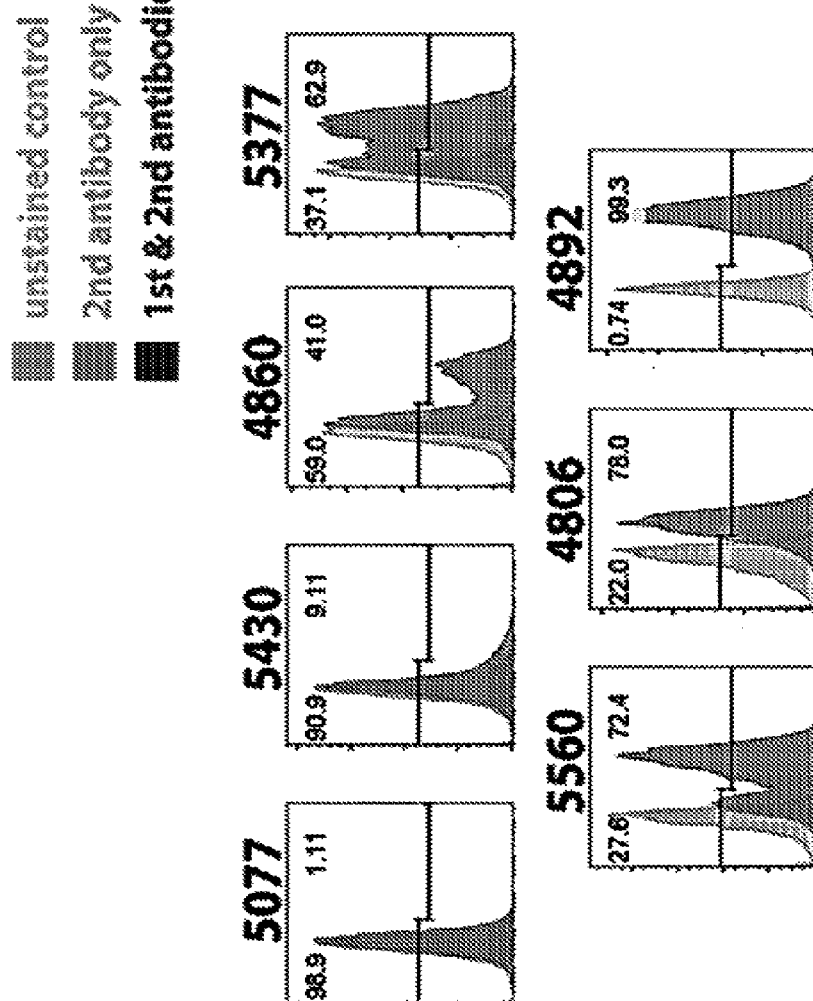


FIG. 5A

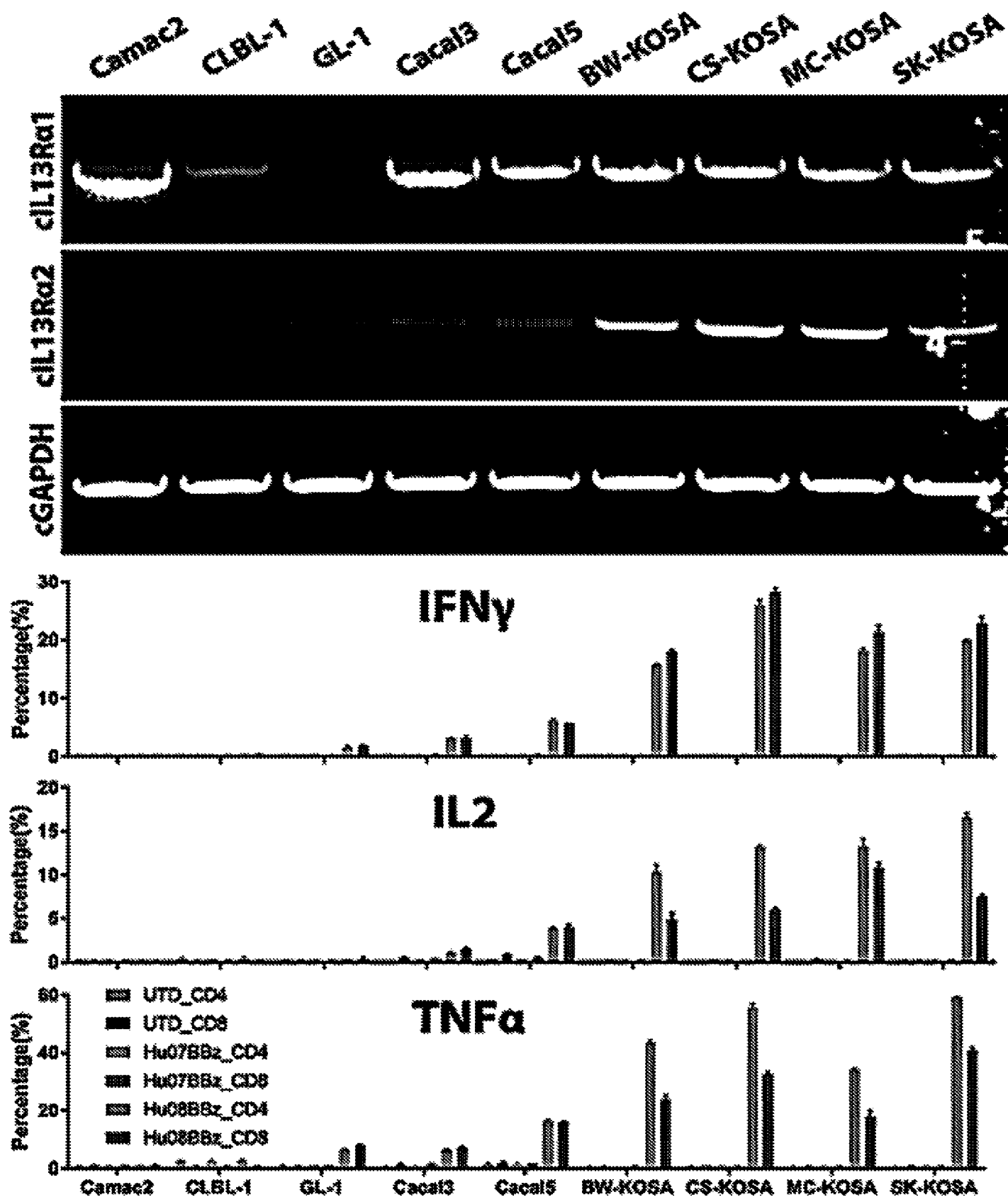


FIG. 5C

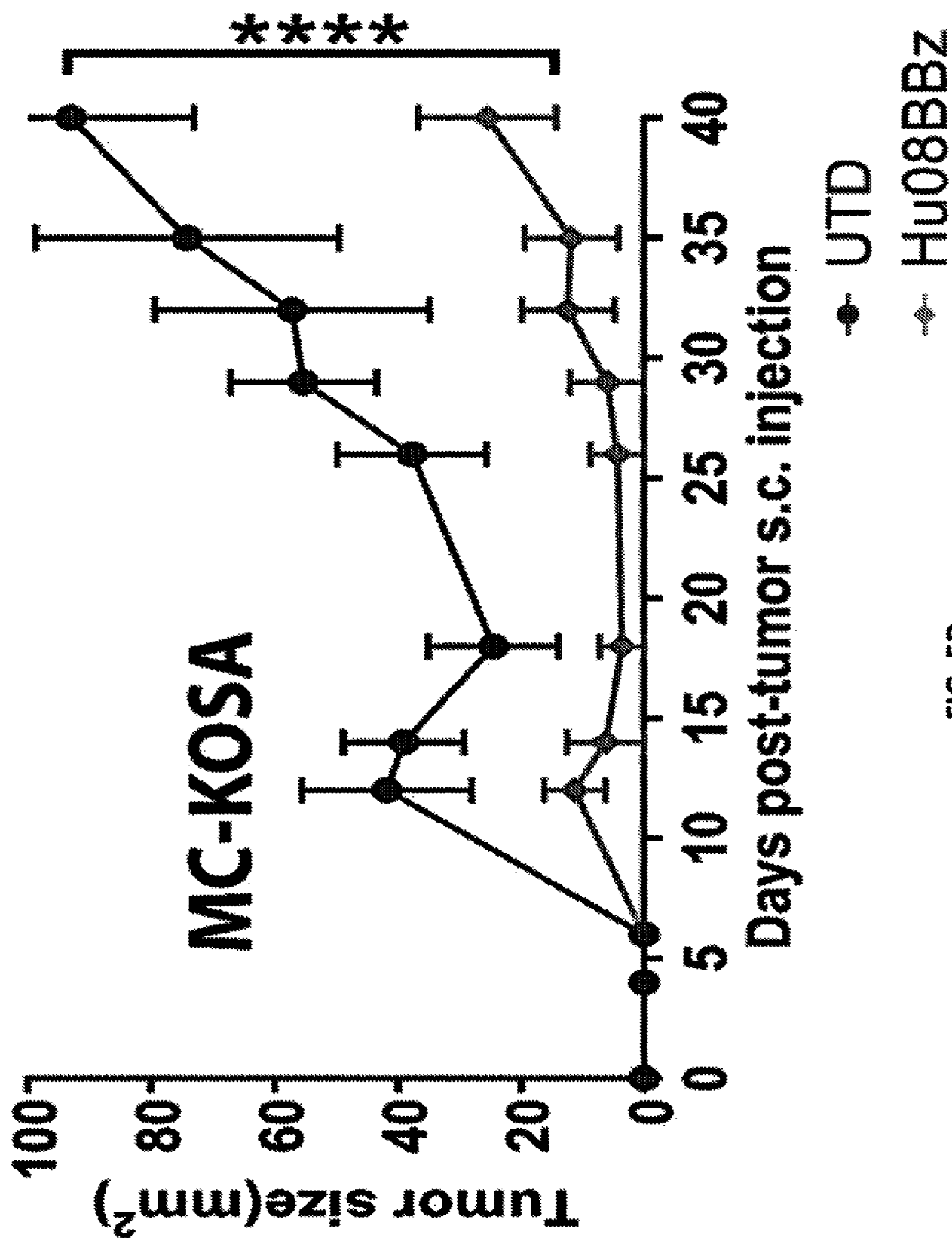


FIG. 5D



FIG. 6A

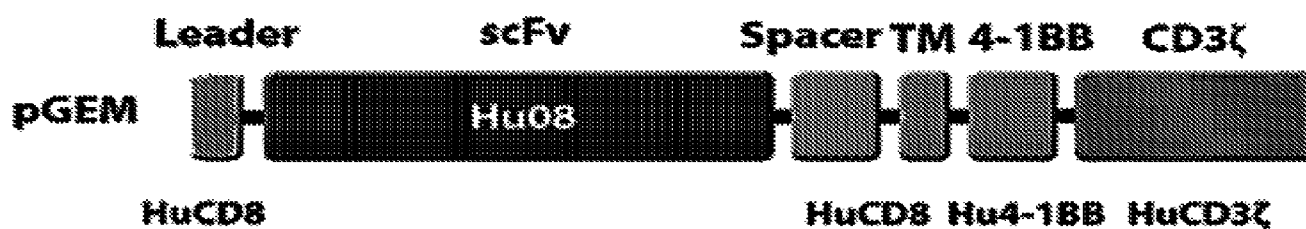


FIG. 6B

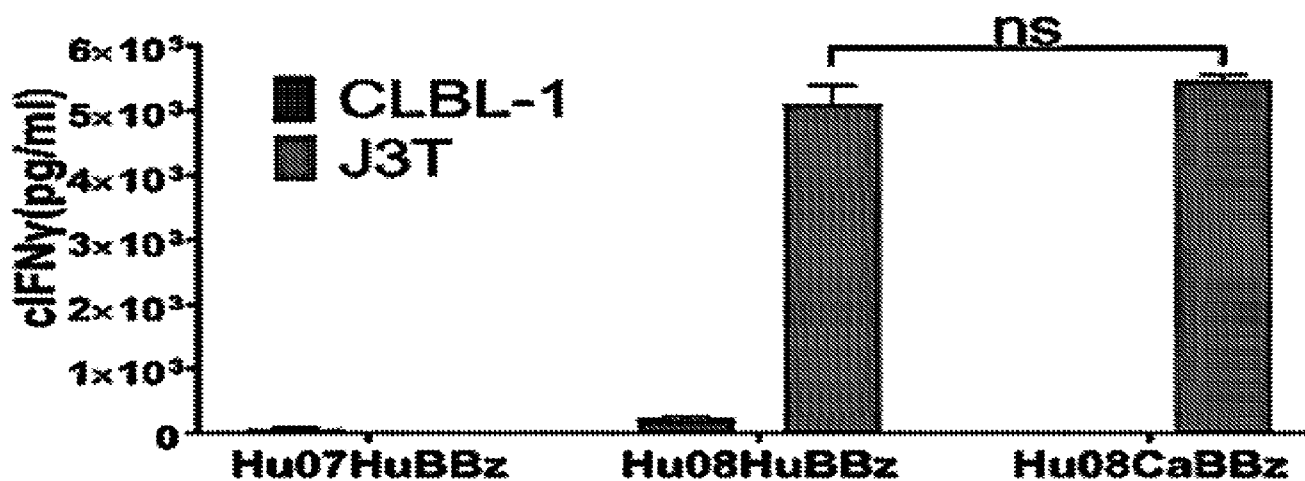


FIG. 6C

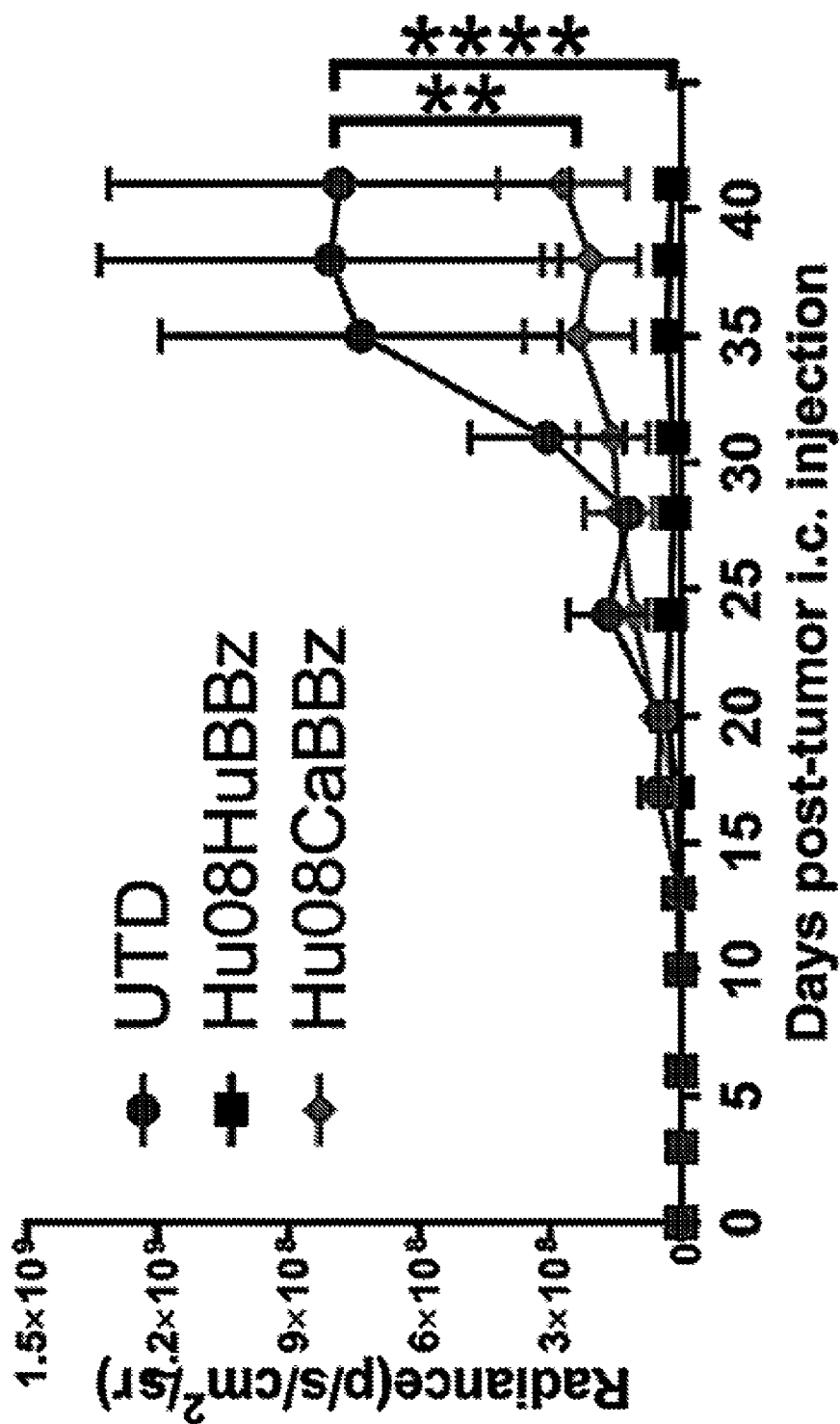


FIG. 6D

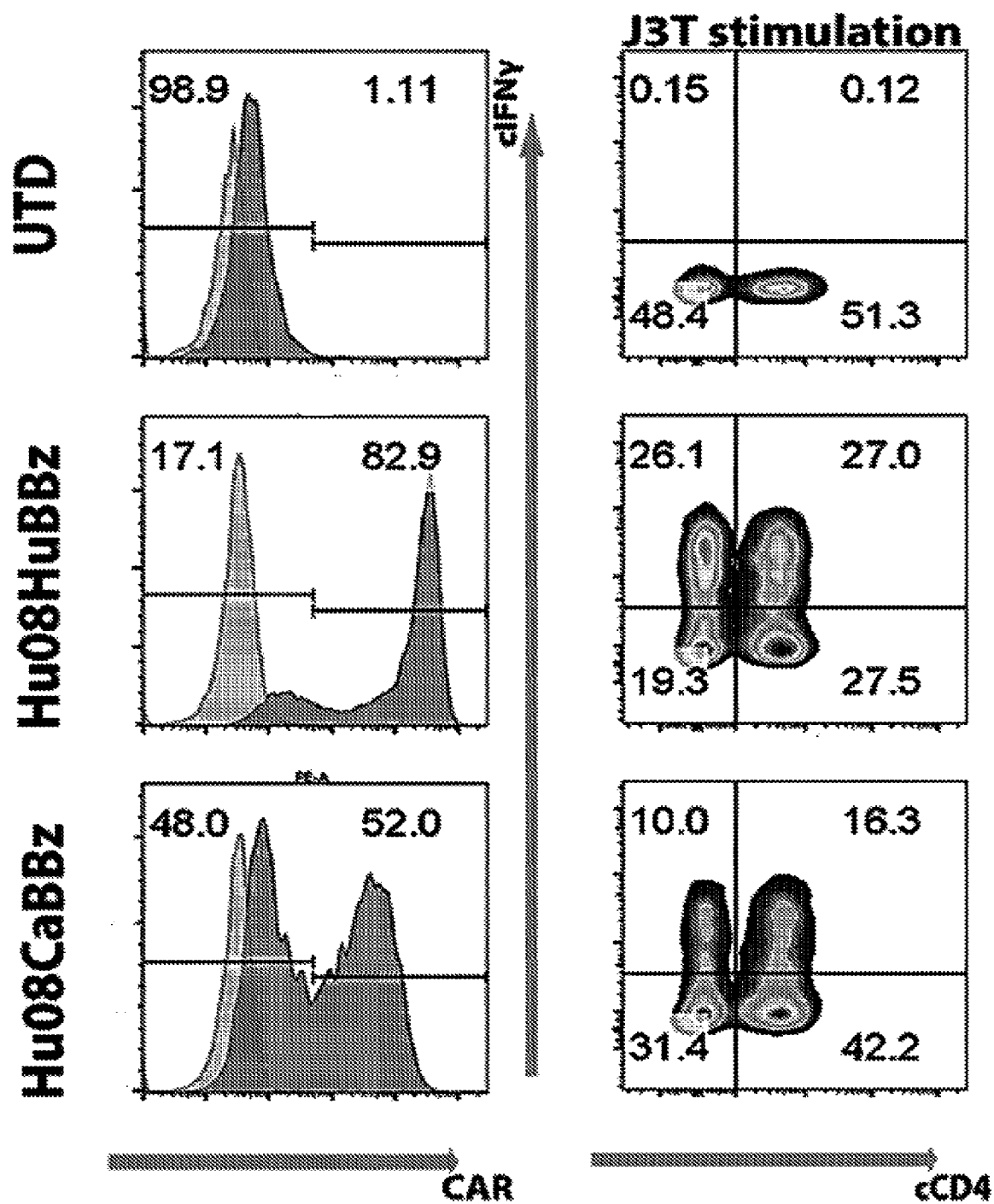


FIG. 6E

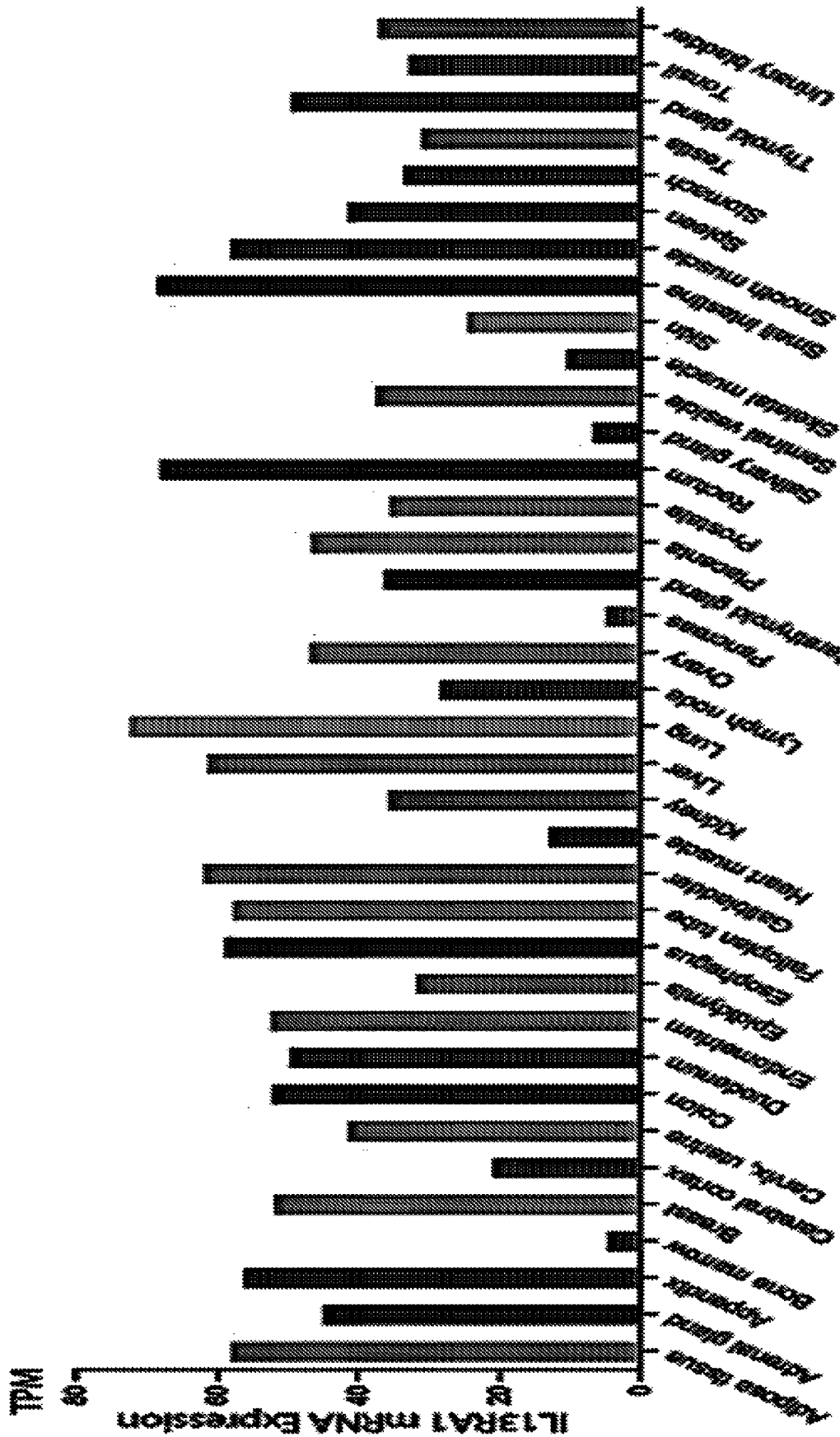


FIG. 7A

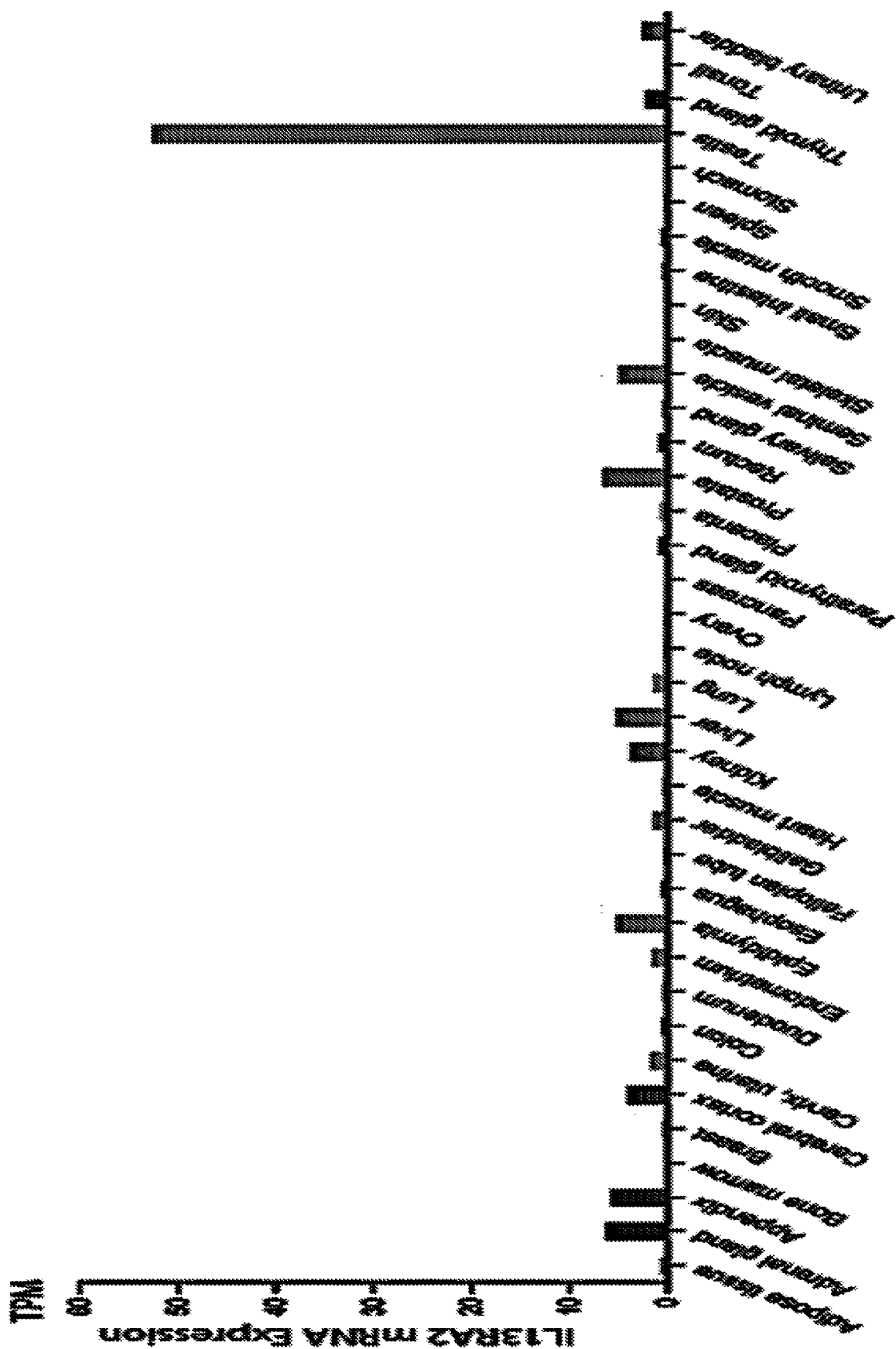


FIG. 7B

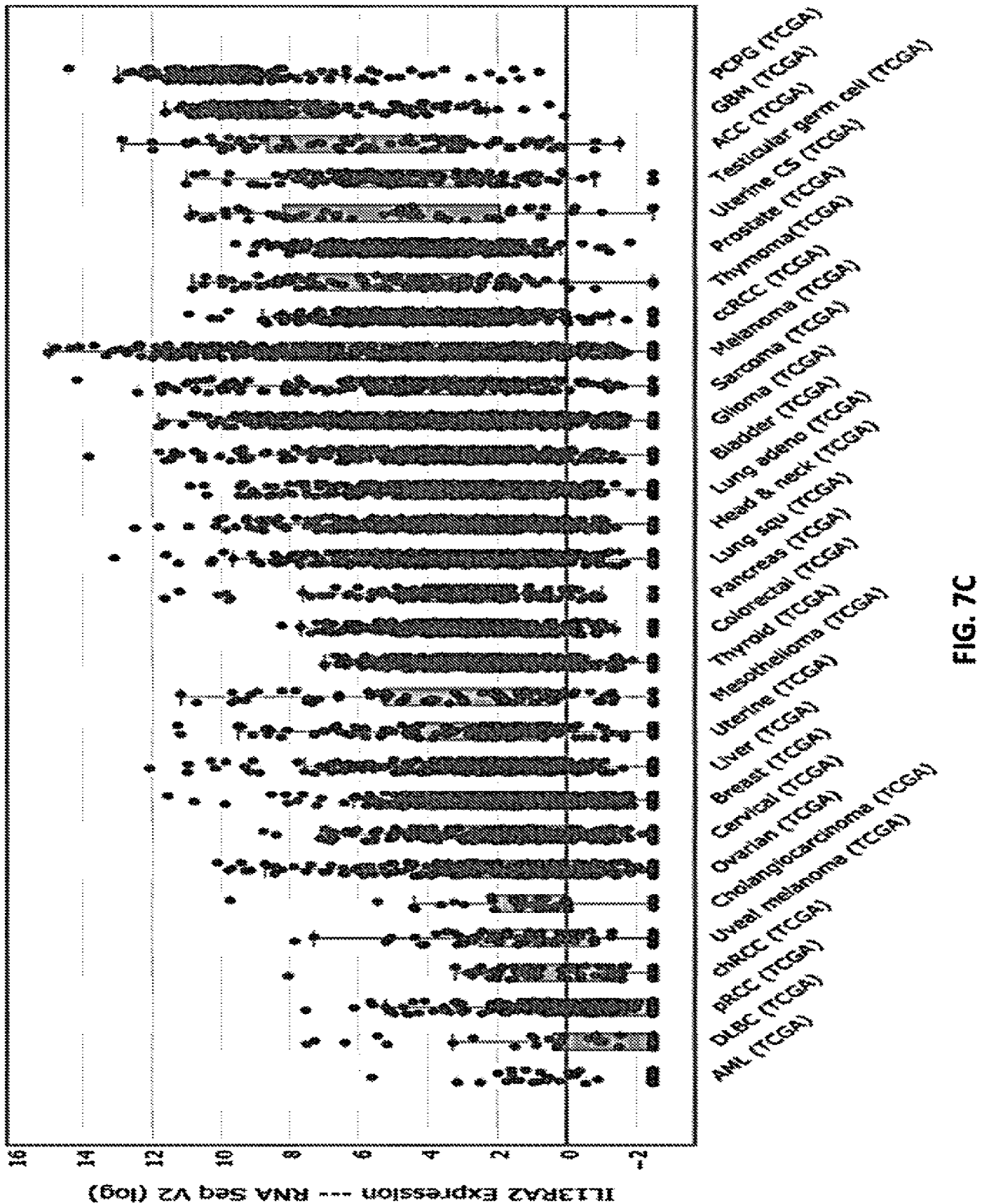


FIG. 7C

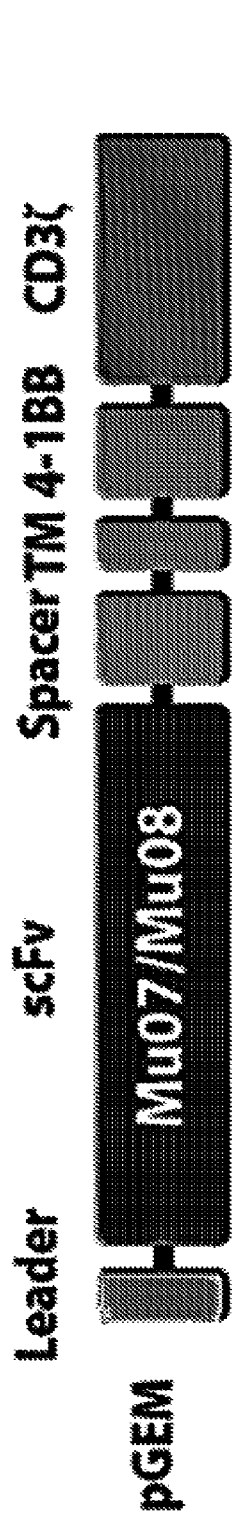


FIG. 8A

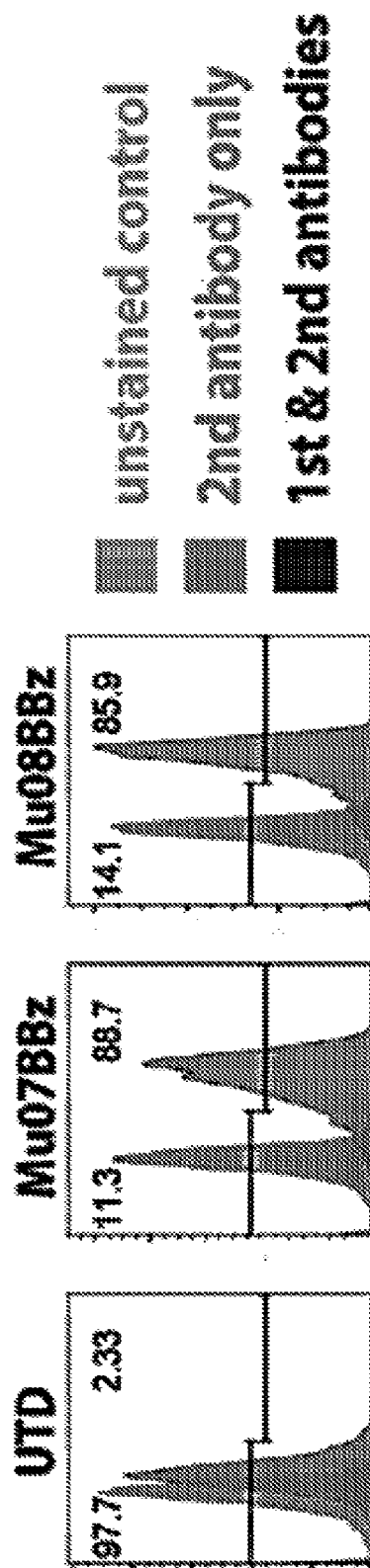


FIG. 8B

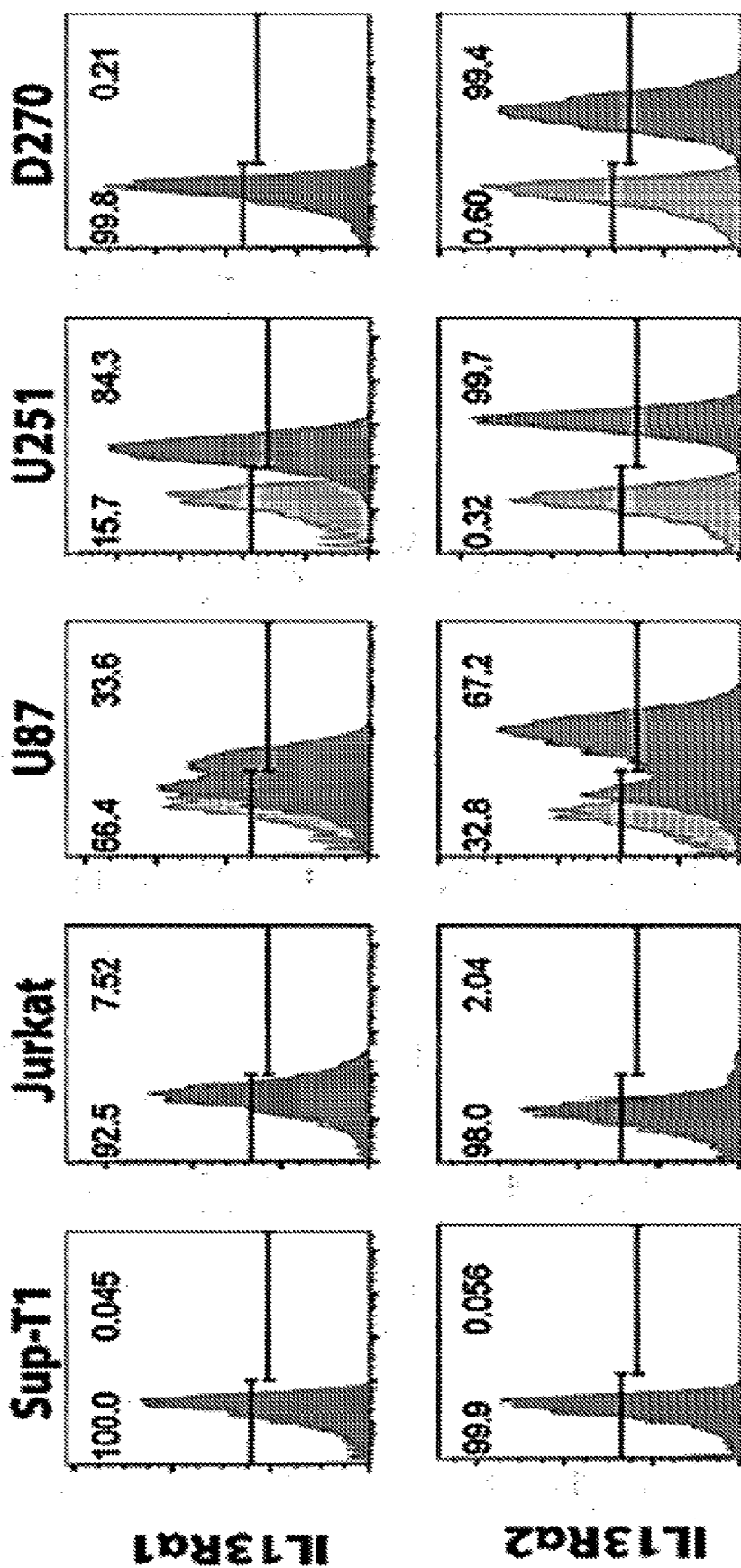


FIG. 8C

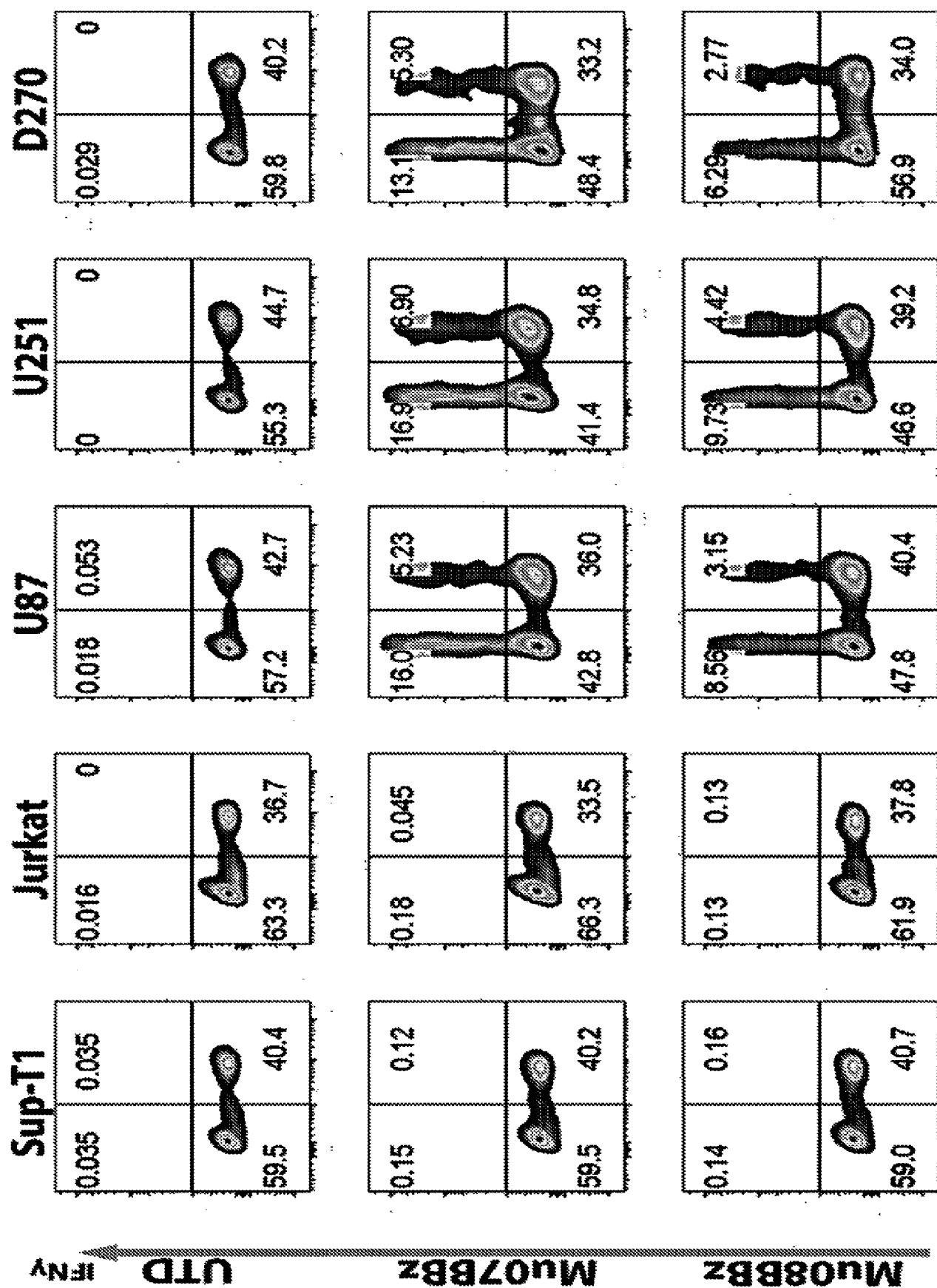


FIG. 8D

FIG. 9A

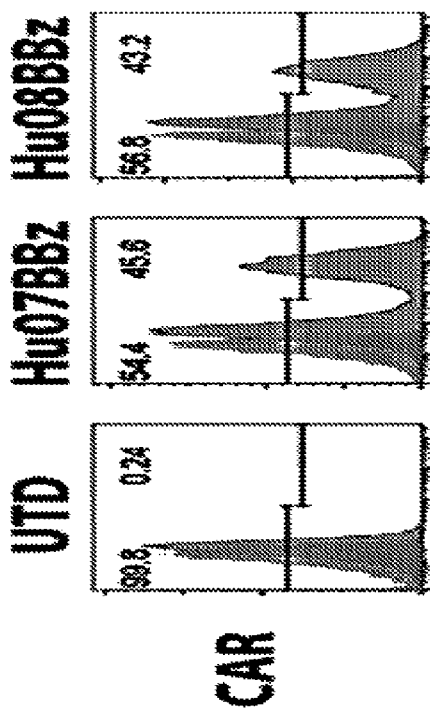
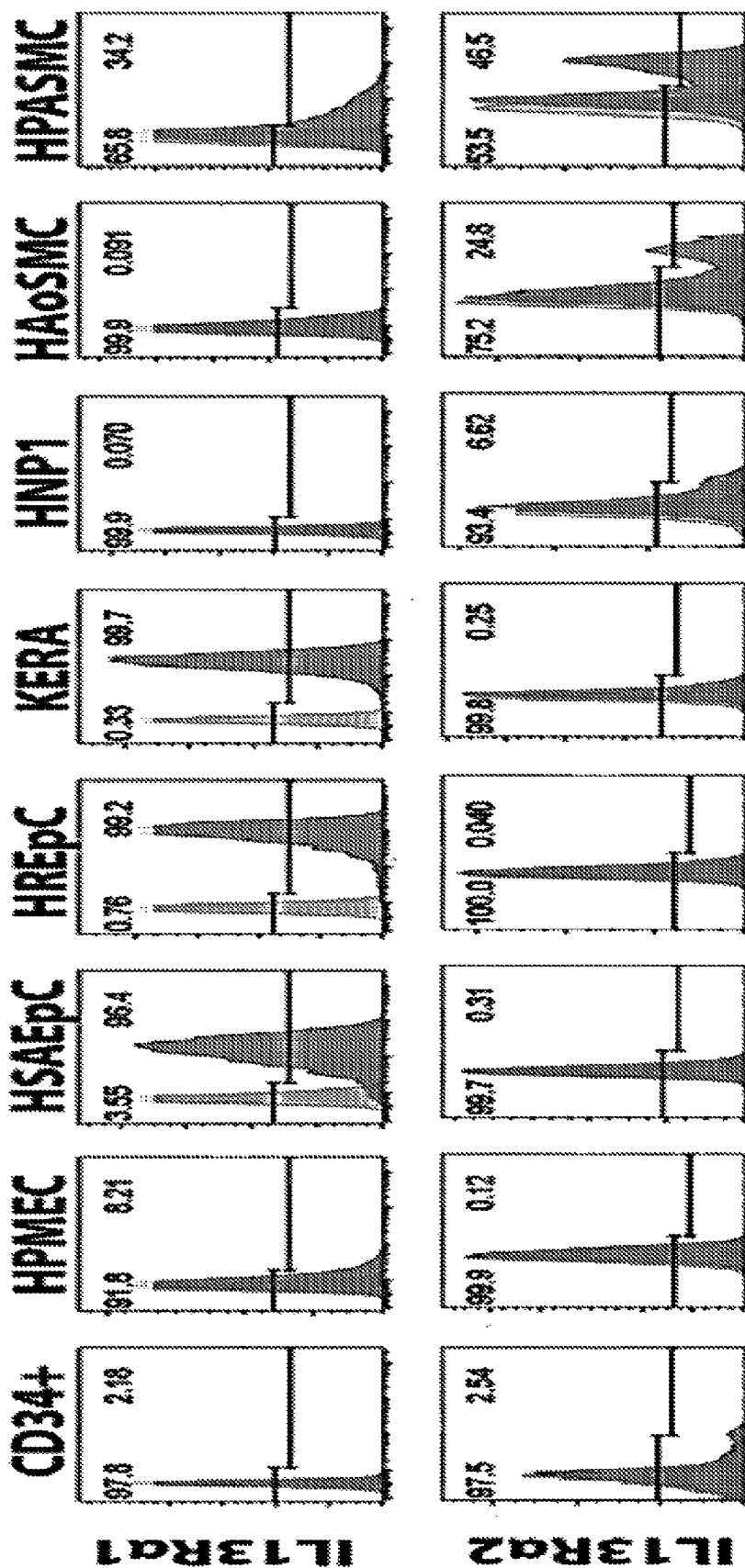


FIG. 9B



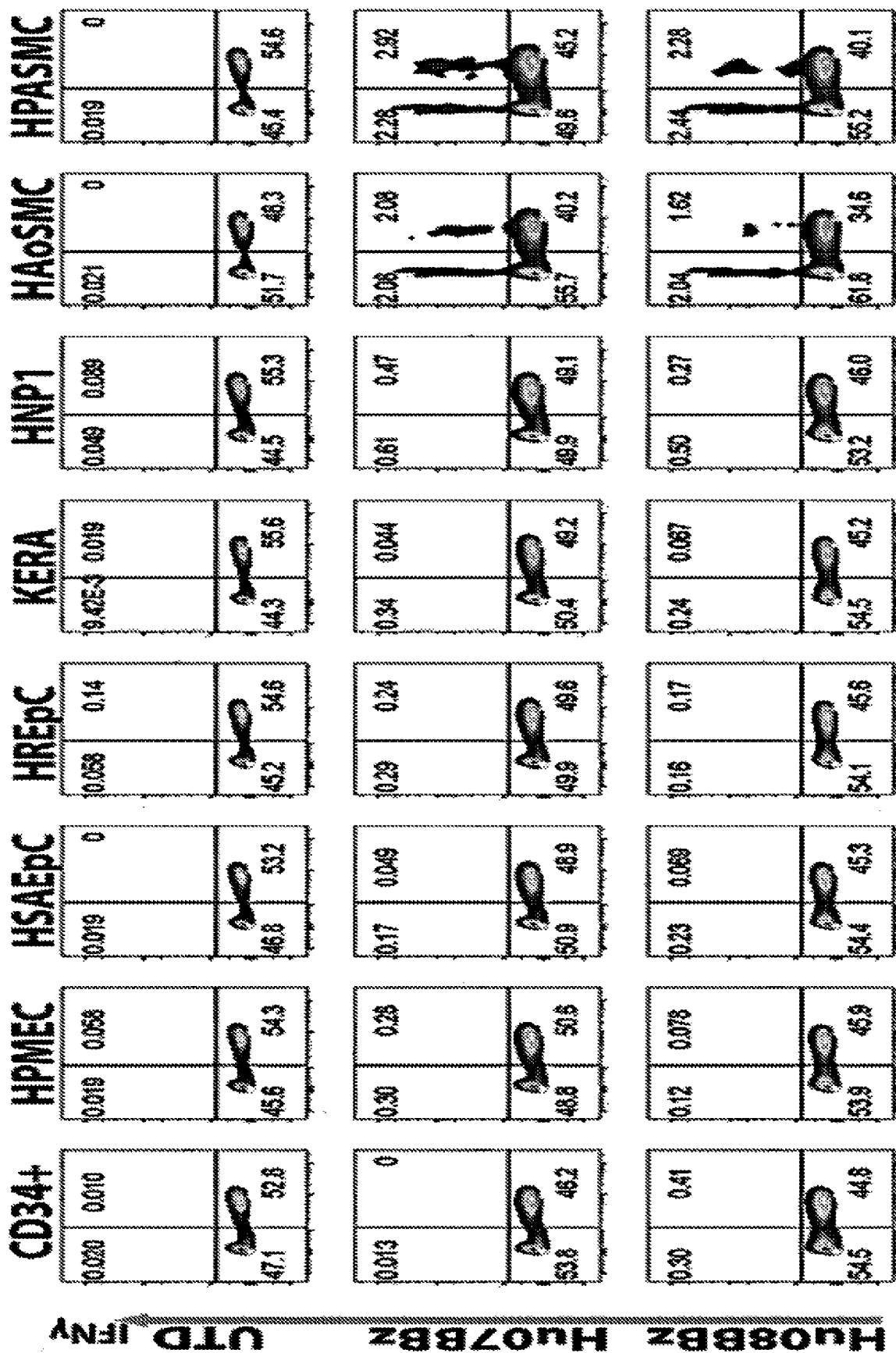
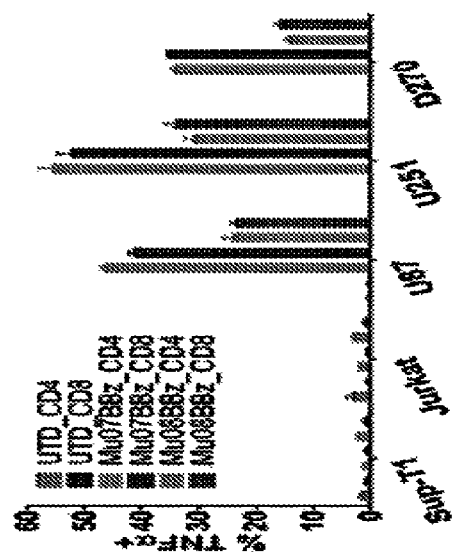
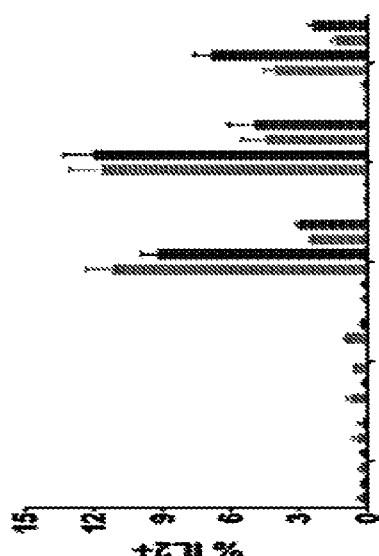
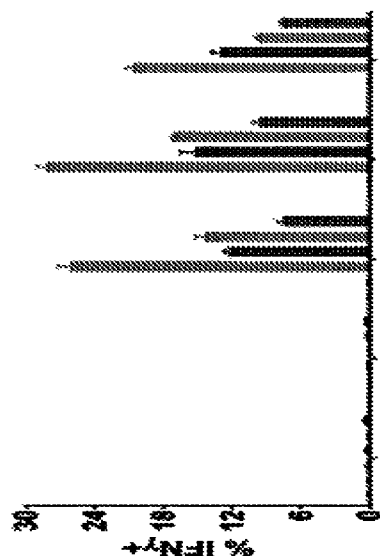


FIG. 9C

CD8

FIG. 10A



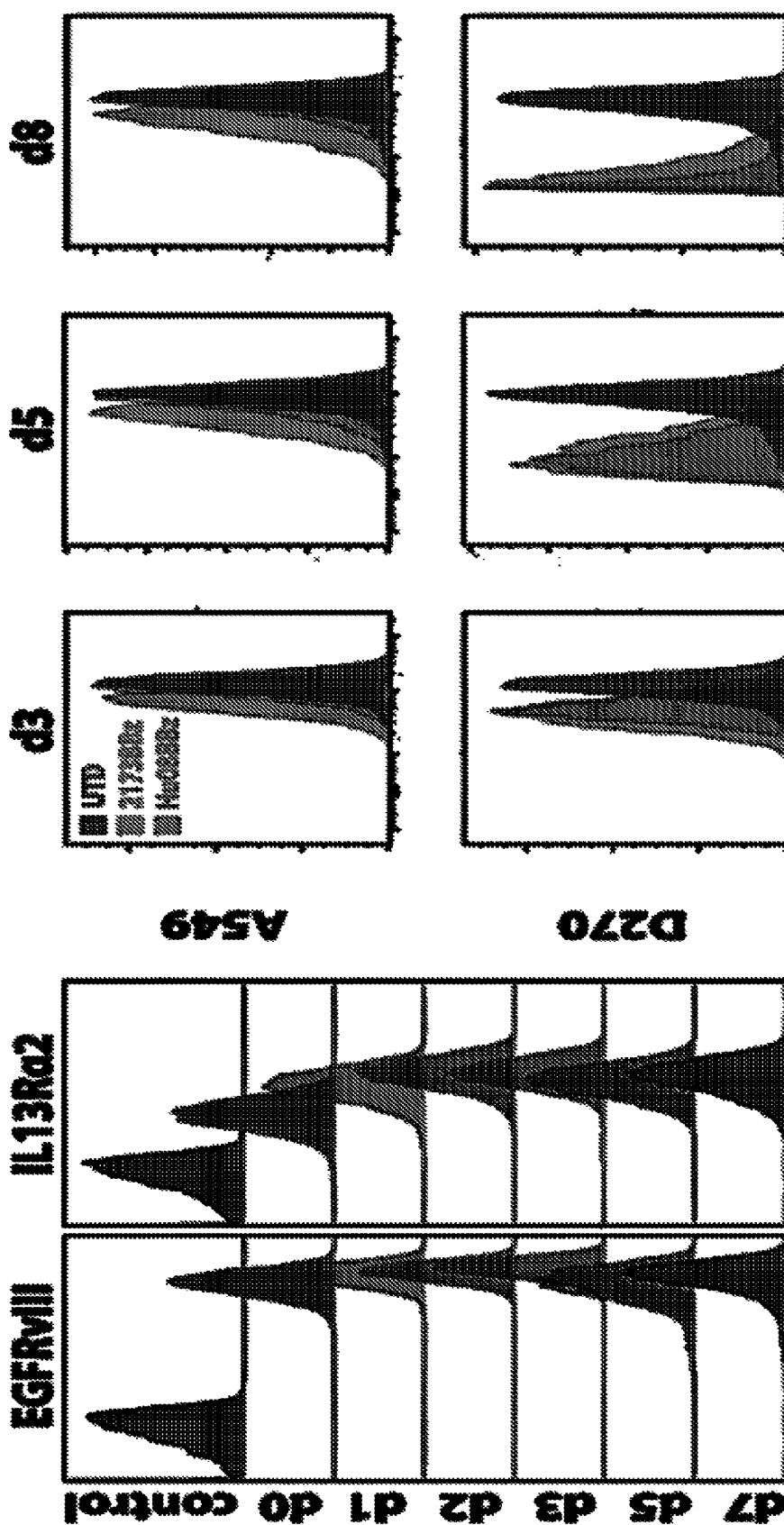


FIG. 10E

FIG. 10D

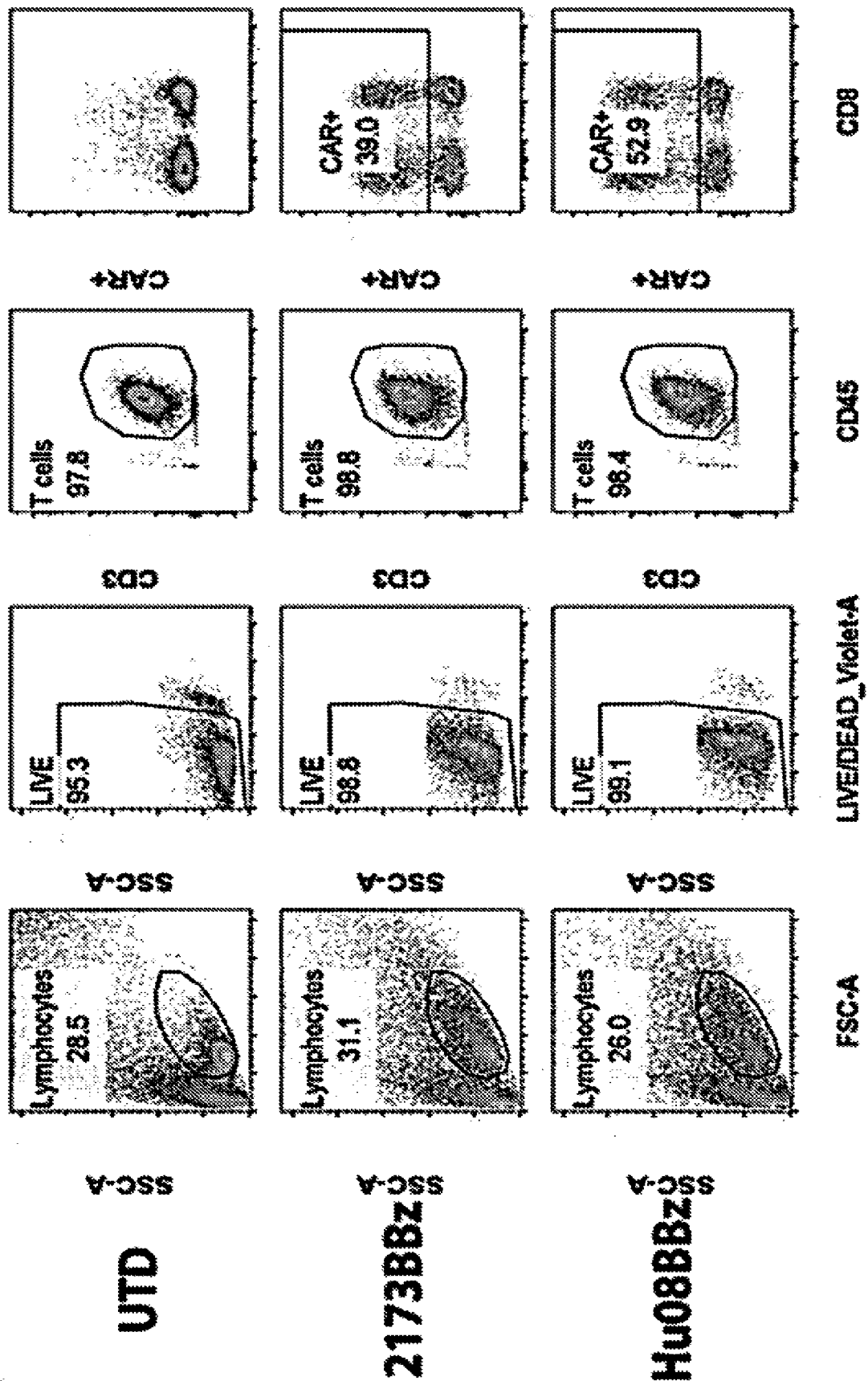
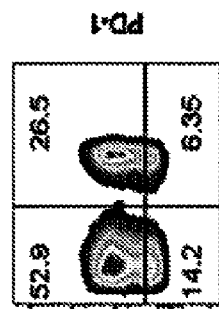
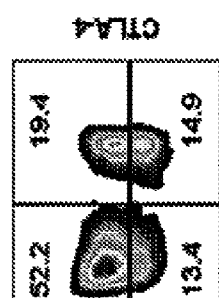
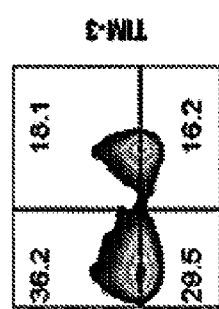
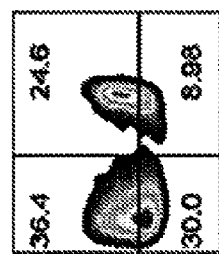
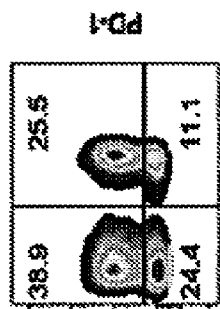
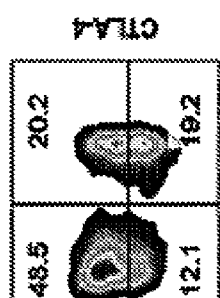
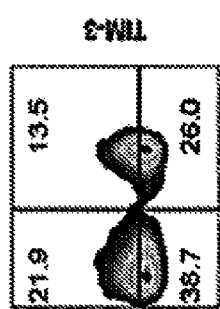
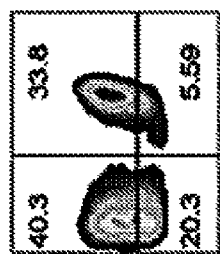
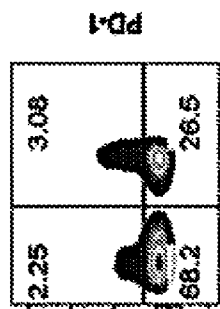
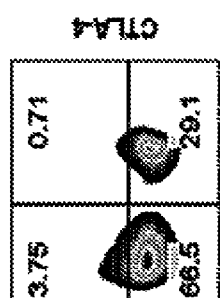
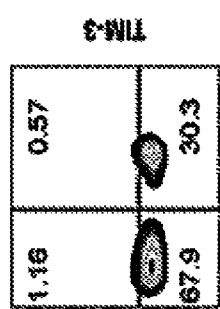
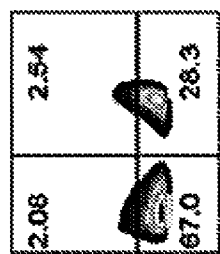


FIG. 11A

FIG. 11B 24hrs

CD69
UTD



48hrs

CD69
UTD

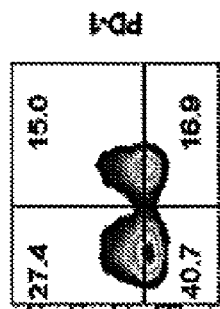
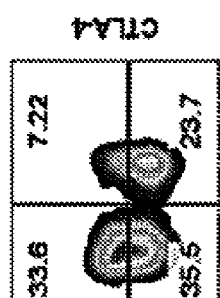
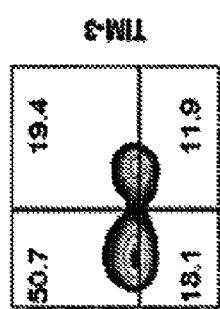
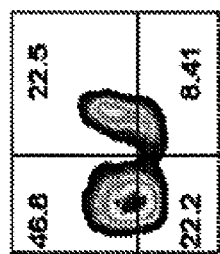
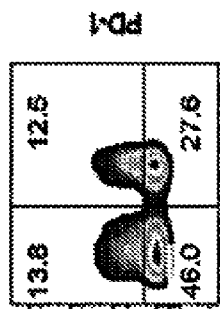
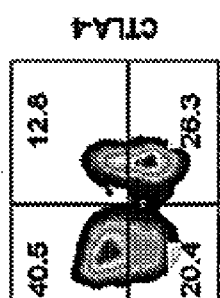
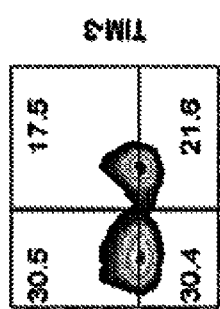
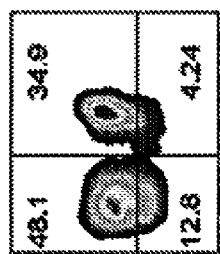
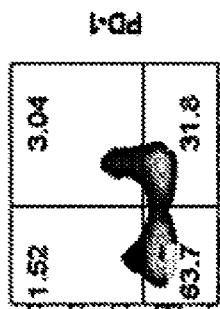
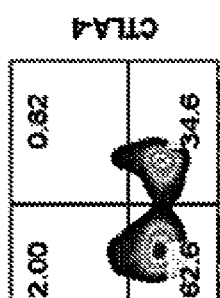
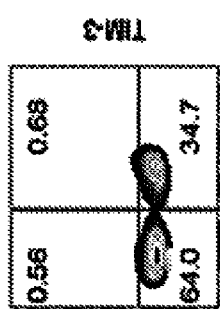
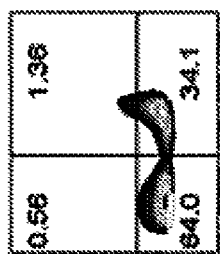


FIG. 12A

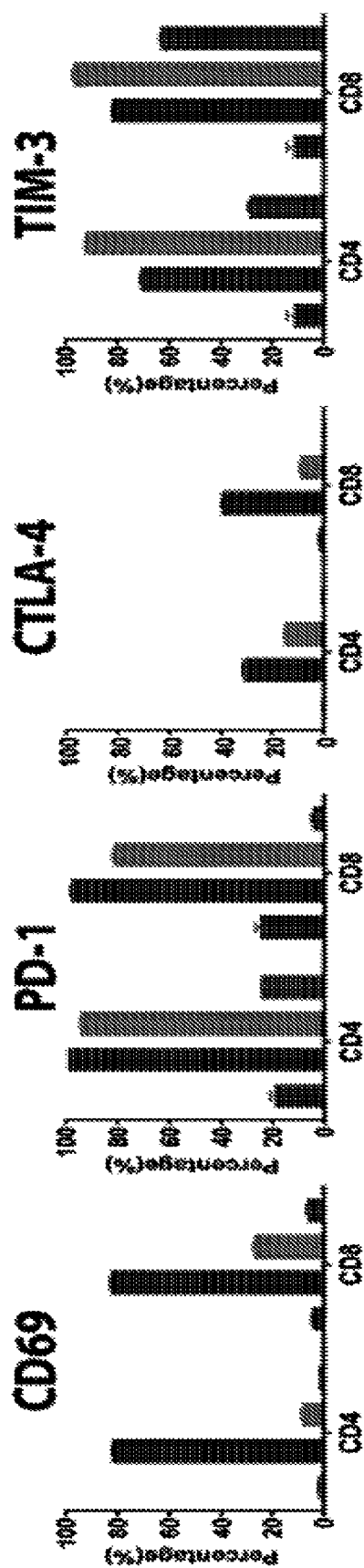
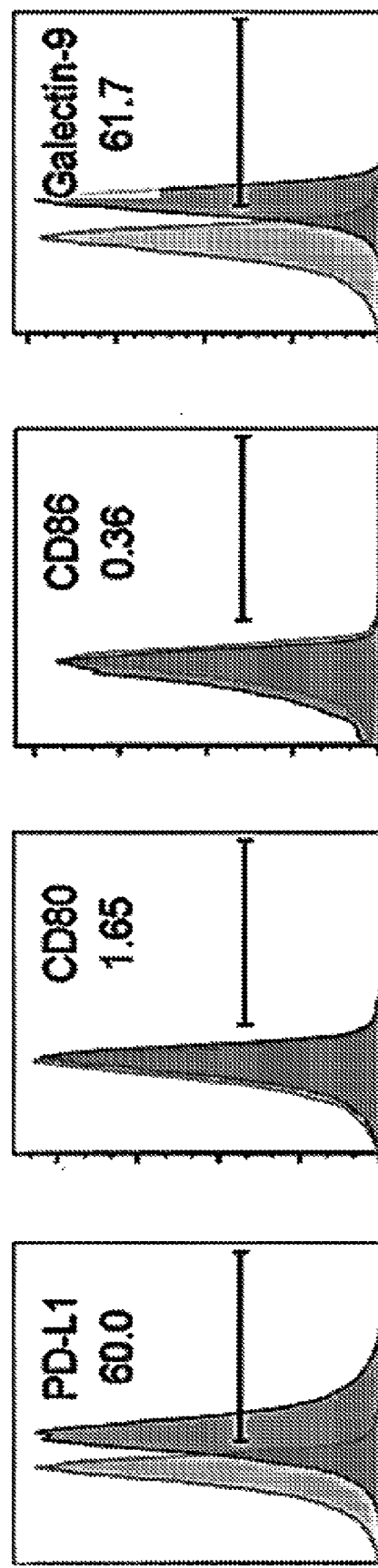


FIG. 12B



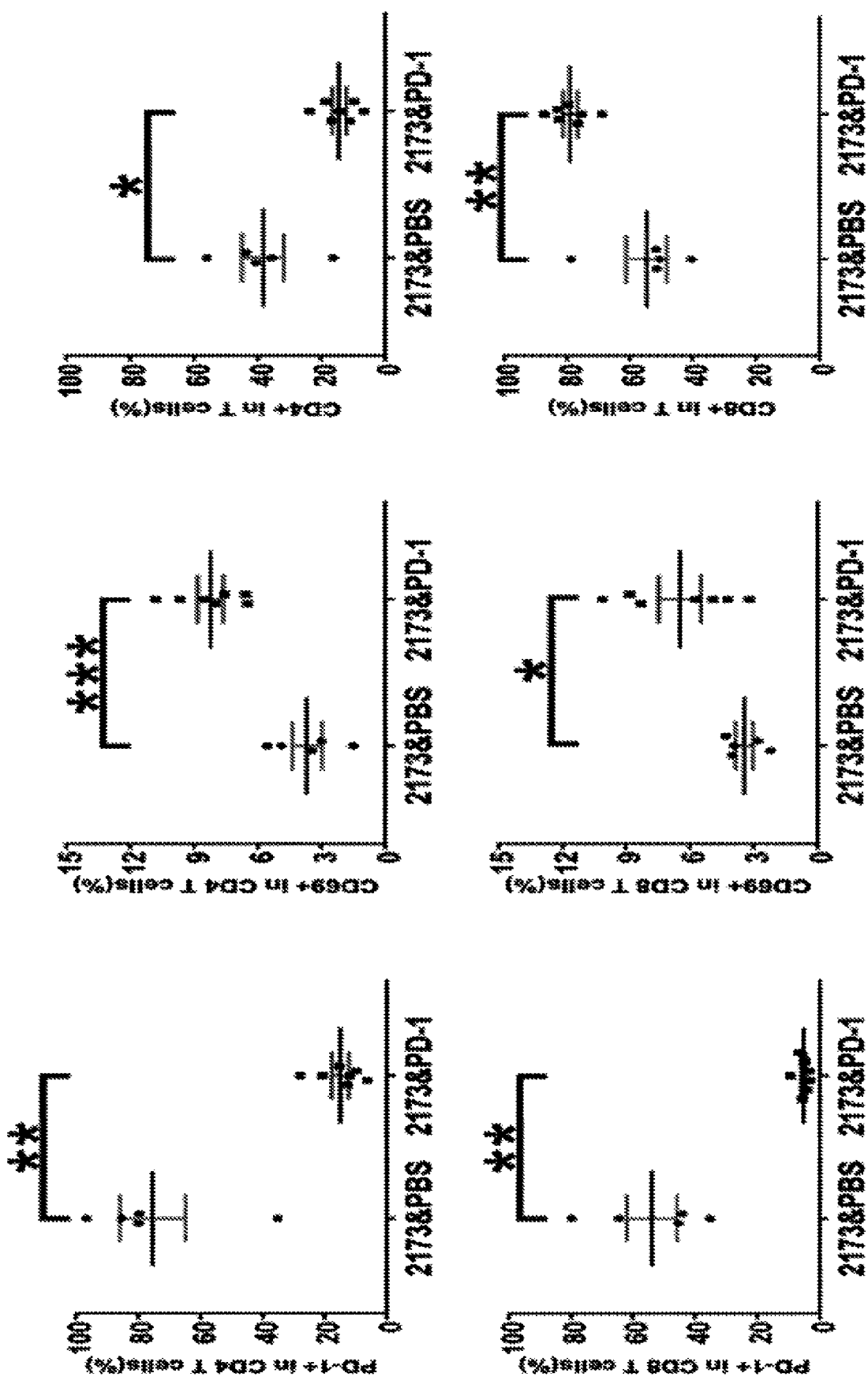
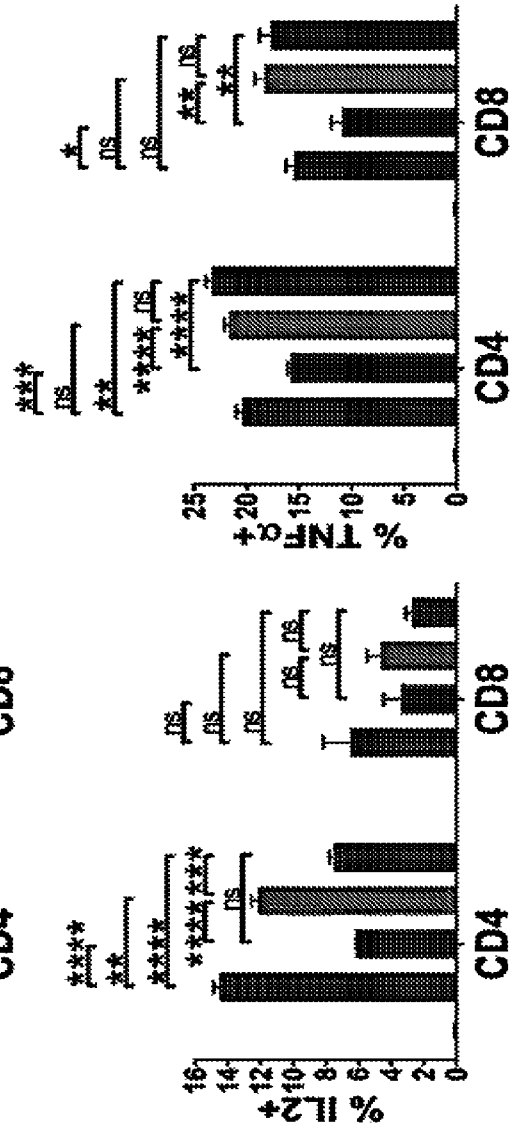
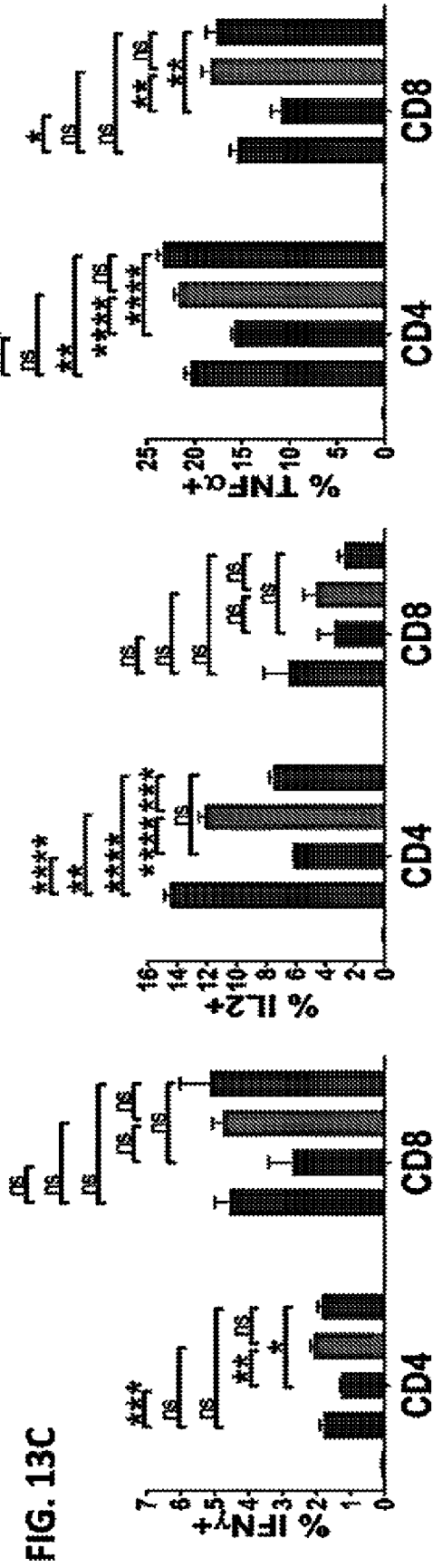
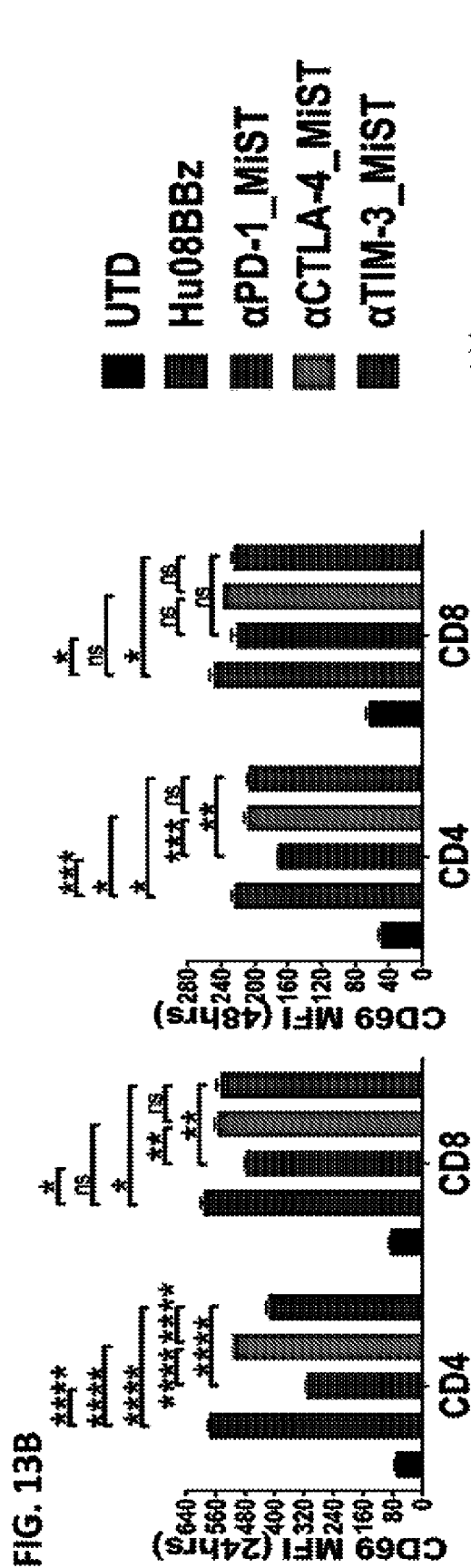
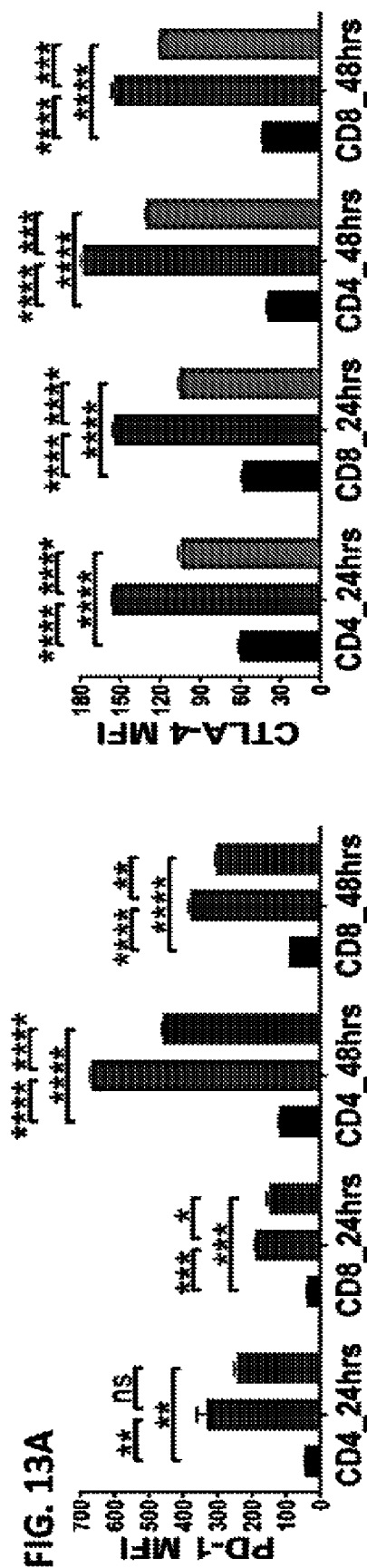


FIG. 12C



1	20	30	40	50																						
MAFIDHLDVGF	LYTLLVCTAFG	-SMLSNAEIKVNP	PQDFFIVDPGYLGYL	\$LOWQPP																						
MAFVCLAI	CCLYTFLIS	TTFGCTSSSDT	EIKVNP	PQDFFIVDPGYLGYL	YLOWQPP																					
60	70	80	90	100	110																					
LFPDNEFKECT	IXEYELKYRNI	DSENNWKTI	ITKNLHYKDG	FDLNKGI	EAKIN	TLLPAQ																				
LSLDHPEKRECT	VEYELKYRNI	CSHTWKTI	ITKNLHYKDG	FDLNKGI	EAKIH	TLLPWQ																				
120	130	140	150	160																						
CTNGSEVRSSWAETTYW	TSPOGNRETF	IXODMDCV	YYNNQYLV	CSWKPGM	GVHFDTN																					
CTNGSEVQSSWAETTYW	ISPOGIFETK	VODMDCV	YYNNQYLV	CSWKPGI	GVLLDTN																					
170	180	190	200	210	220																					
YOLFVWYEGLDHSAEC	TDYIEVNGKNM	GCRRFPY	LESSDYKDFY	ICVNGSS	ESQPIR																					
YNLFVWYEGLDHALQC	VDIYKADGQNI	GCRRFPY	LEASSDYKDFY	ICVNGSS	ENKPIR																					
230	240	250	260	270	280																					
PSYFI	EQLOQNI	VKPMPPFDYLS	LTVKNS	EEINL	KKWNMPKGP	IPAKCEI	YVEIEFT	EDC																		
SSYFT	EQLOQNI	VKPELPVYLT	FTRESS	CEIKL	KWSIFL	GPIPARCE	FYEIFEIR	EDD																		
290	300	310	320	330																						
TTWVYT	TVENE	IQITRTSNES	OKLCE	LVR	SKVNI	YCSDD	GINS	ENSD	EOCKKG	-DI																
TTLLVTA	TVENE	TYTLKT	TNETROL	CEV	VRS	KVNI	YCSDD	GINS	ENSD	KOCCEG	EDL															
340	350	360	370	380	390																					
WRETRIV	FELI	PEE	AVS	IFVL	VIT	CLL	YKOR	ALL	TL	FHT	KK	EV	F	SH	QD	TFC										
SKKTL	HR	WD	PE	GE	TL	IL	V	FG	LL	R	K	P	N	T	Y	P	K	M	I	P	E	E	F	C	D	T

FIG. 14A

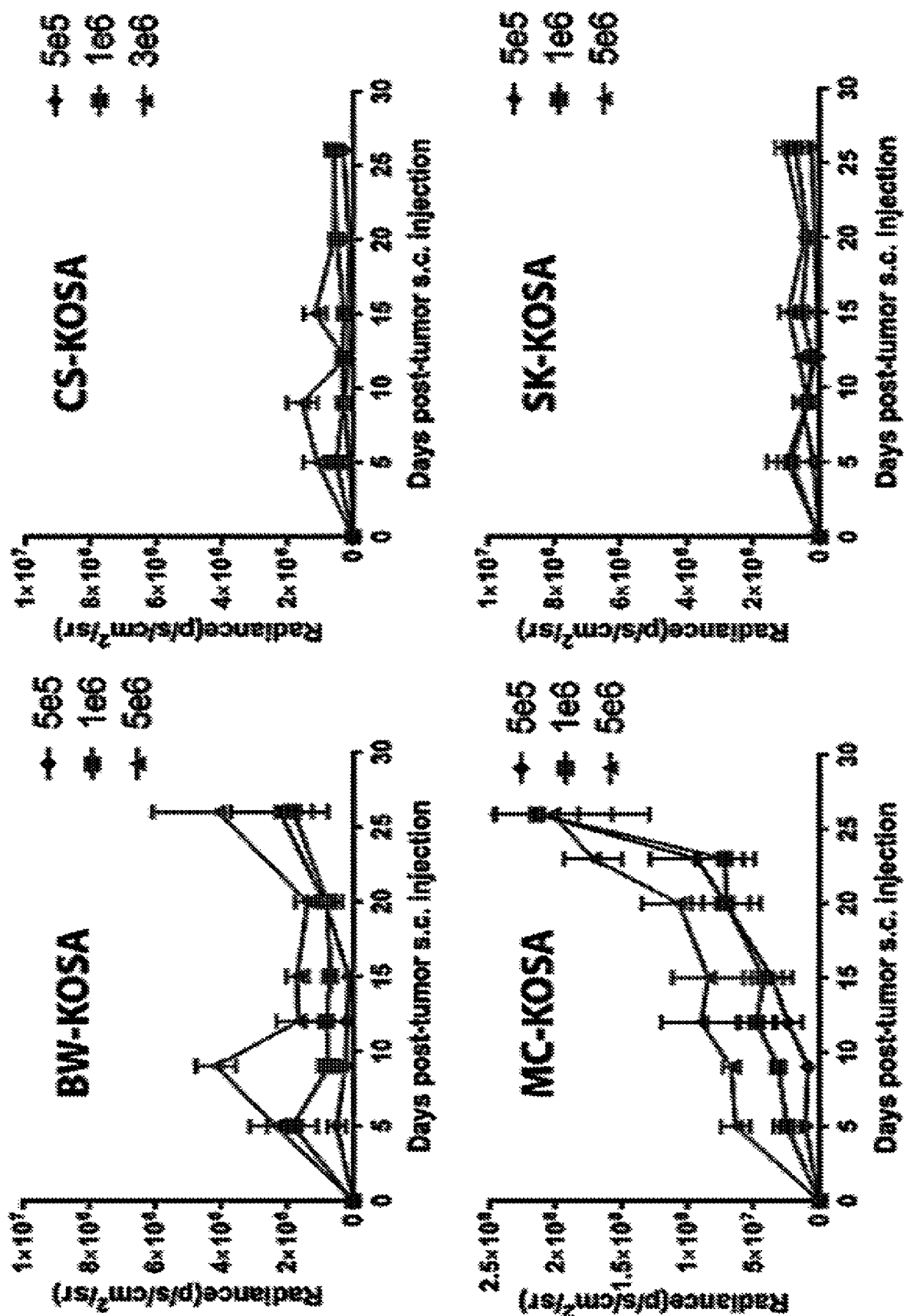


FIG. 14B

FIG. 15A

5'-

CCCGAIGTTTTCTGAGTTACTTTTGTATCCCCACCCCTCGAGGAGGAAAACTGTTTC
 ATACAGAGGCGTACGCCCTTCTGTAIGAACAGTTTTTCTCCACGCCCTTCTGTAIGAAA
 CAGTTTTTCCCTCCACGTGGACATTTTGACACCCCCATAATAATTTTCCAGAAATTAACAGTAT
 AAATTGCATCTCTTGTTC AAGAGTTCCTATC ACTCTCTTTAATCACTACTCACAGTAACCT
 CAACTCTGCCCAAGCTTGGCAATCCGGTACTGTTGGTAAA-3' (SEQ ID NO: 161)

FIG. 15B

5' -

CCCGAIGTTTTCTGAGTTACTTTTGTATCCCCACCCCTCGAGGAGGAAAACTGTTTC
ATACAGAGGCGTACGCCCTTCTGTAIGAACAGTTTTTCTCCACGCCCTTCTGTAIGAAA
CAGTTTTTCCCTCCACGTGGTTAACCCCGAIGTTTTCTGAGTTACTTTTGTATCCCCACCC
CCTCGAGGAGGAAAACTGTTTCATACAGAGGCGTACGCCCTTCTGTAIGAACAGTTT
TTCCTCCACGCCCTTCTGTAIGAACAGTTTTTCTCCACGTGGACATTTTGACACCCCCAT
 AATAATTTTCCAGAAATTAACAGTATAAATTGCATCTCTTGTTC AAGAGTTCCTATCACTCT
 CTTTAATCACTACTCACAGTAACTCAACTCTGCCCCAAGCTTGGCAATCCGGTACTGTTG
 GTAAA-3' (SEQ ID NO: 162)

FIG. 16A

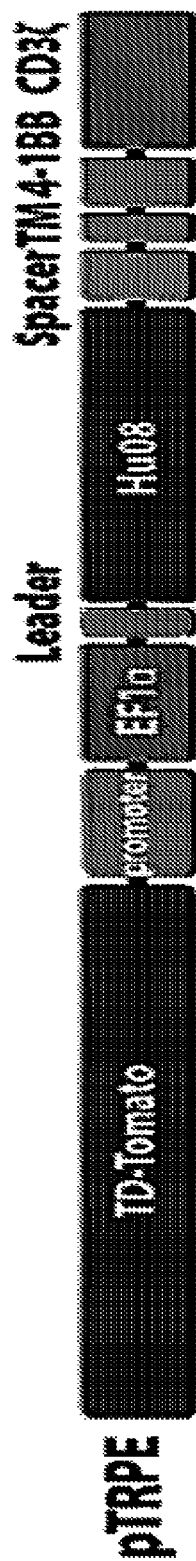
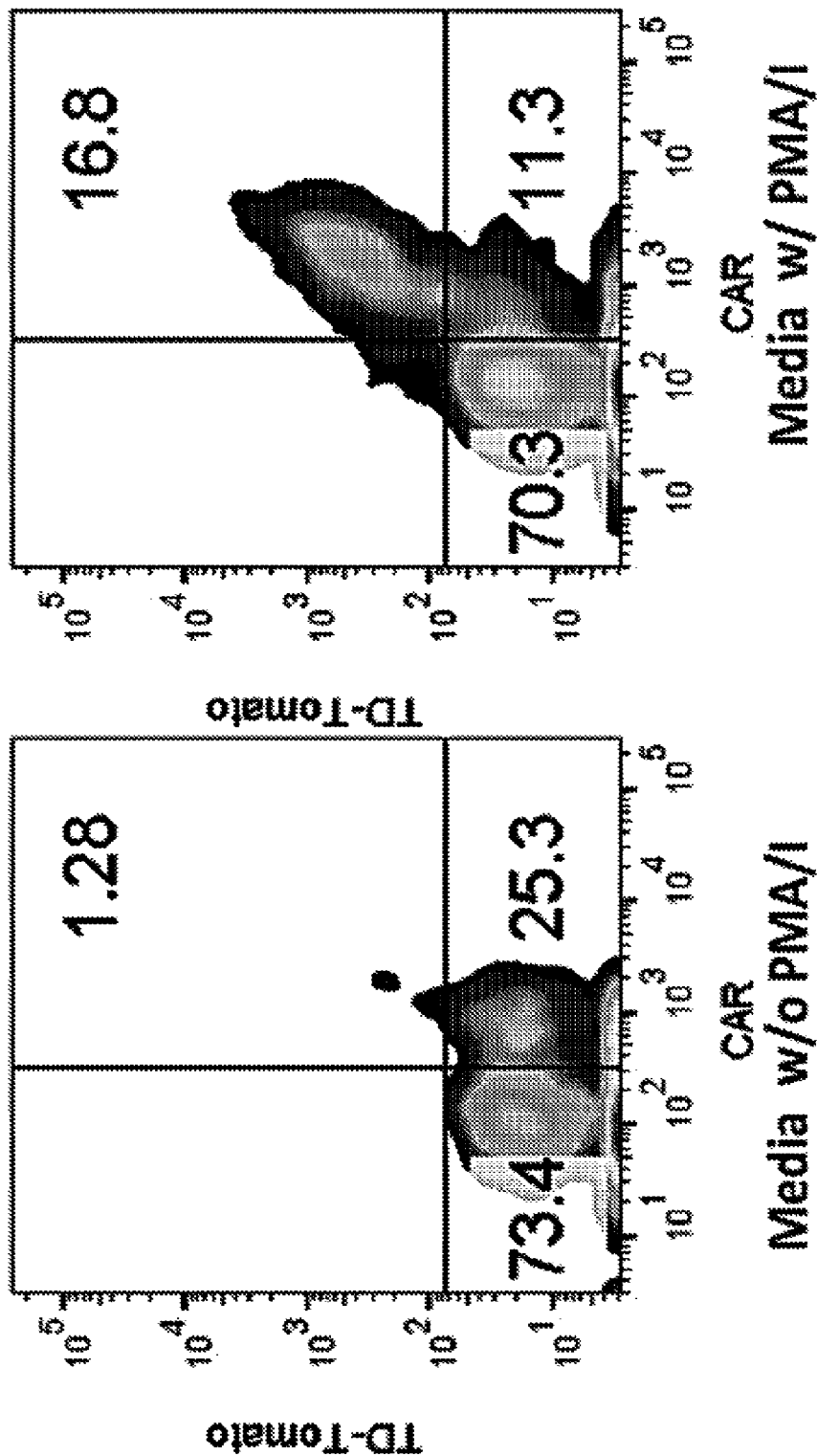


FIG. 16B



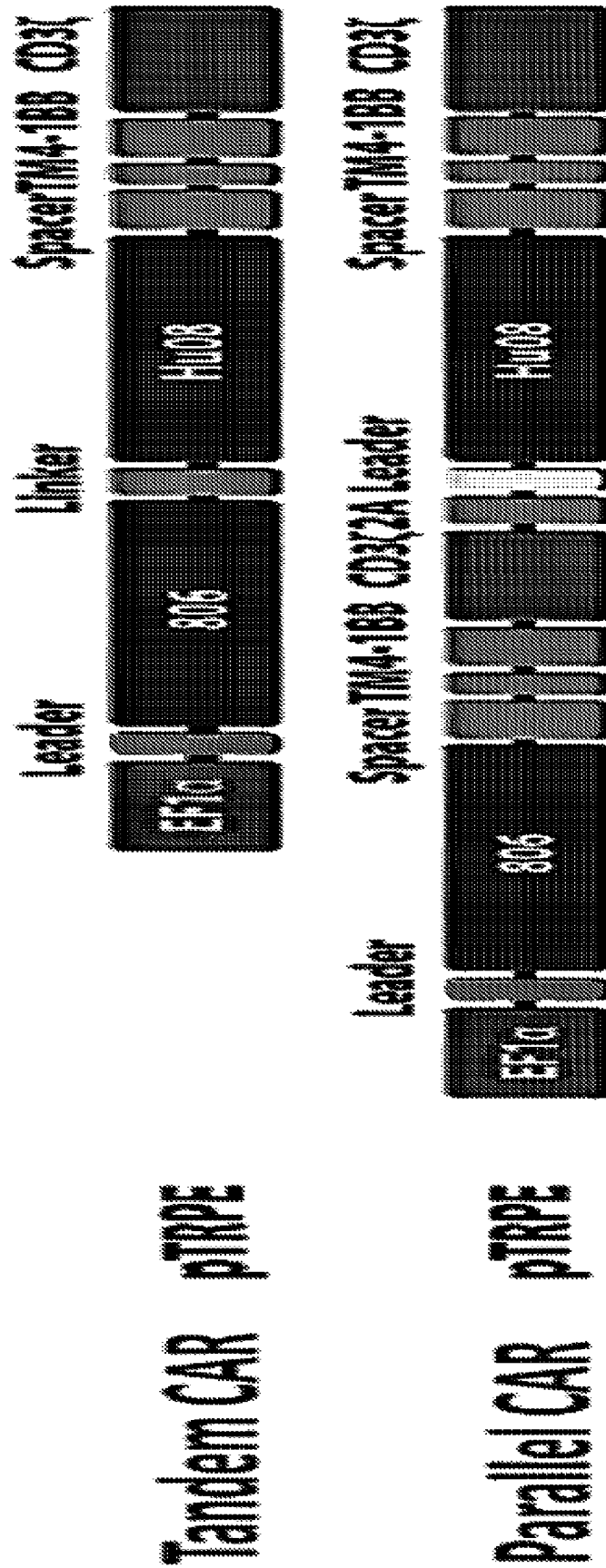


FIG. 17

(SEQ ID NO: 164)

CA 03149543 2022-2-25

(SEQ ID NO: 166)

FIG. 18B

Abstract

[illegible]

FIG. 18C

Amino acids

[illegible]

(SEQ ID NO: 169)

Nucleic acids:

[illegible]

(SEQ ID NO: 170)

FIG. 18D

[illegible]

ACATGCAAGCCCTGCCCCGCTGCTAA

FIG. 18E

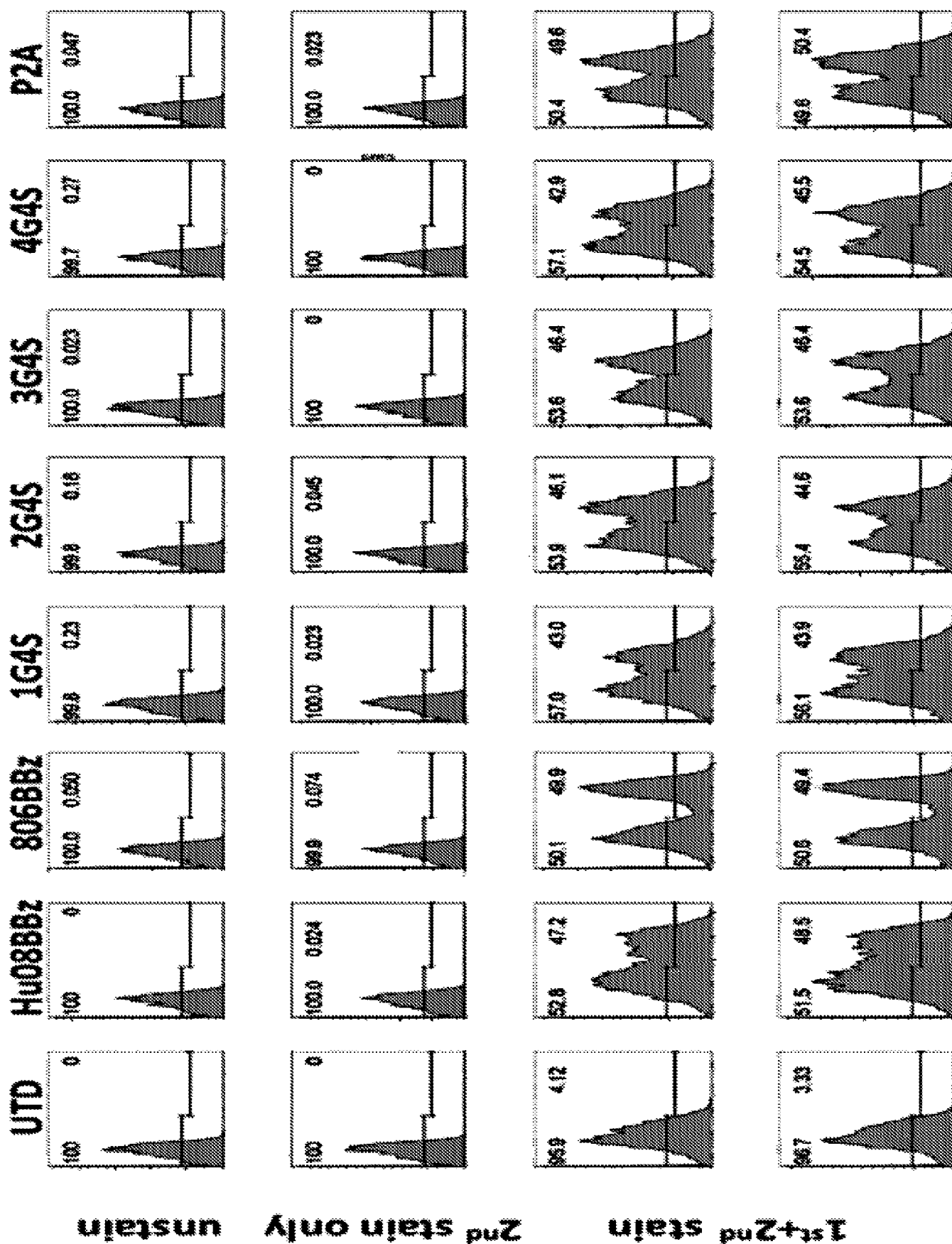


FIG. 19

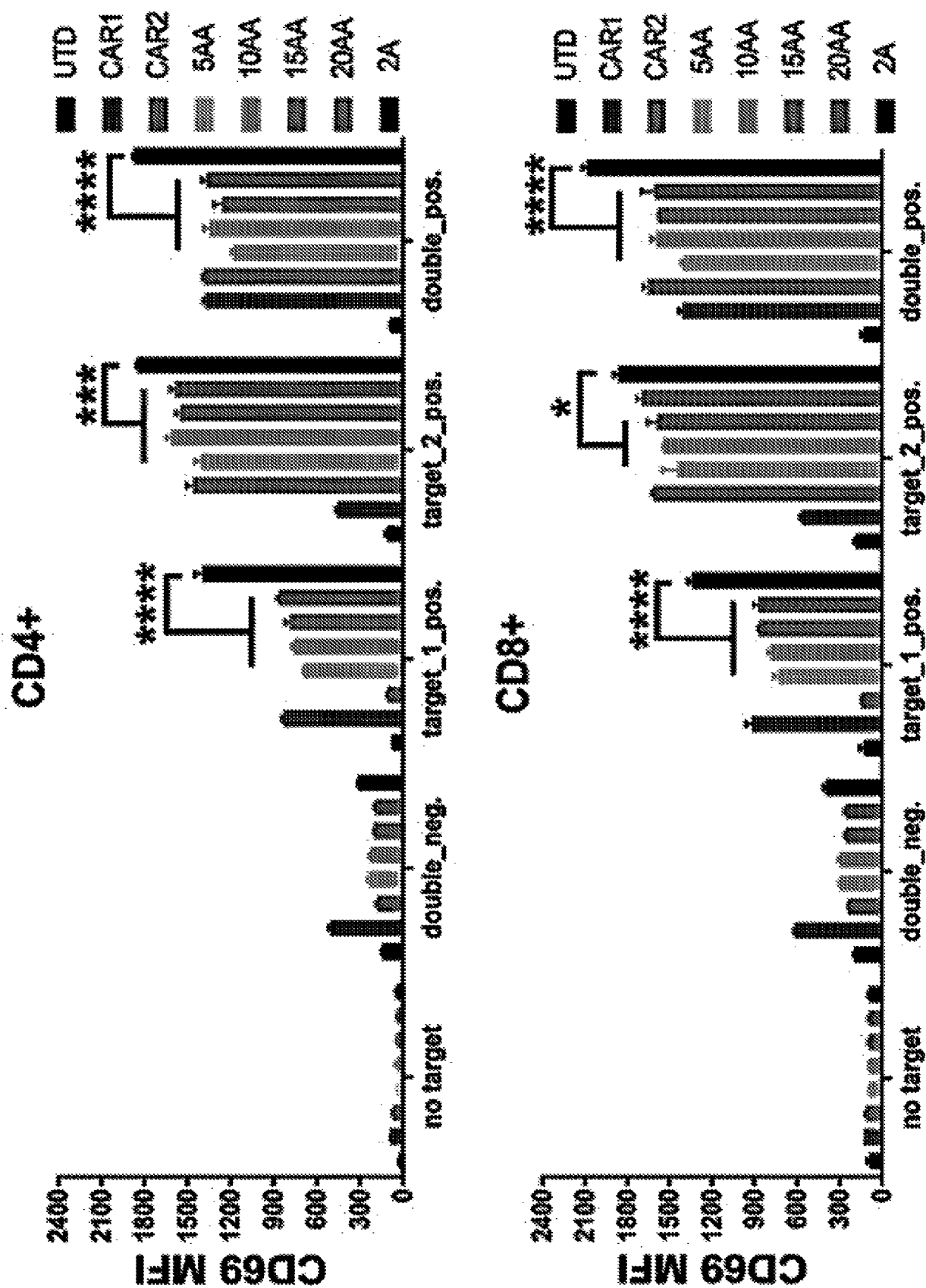
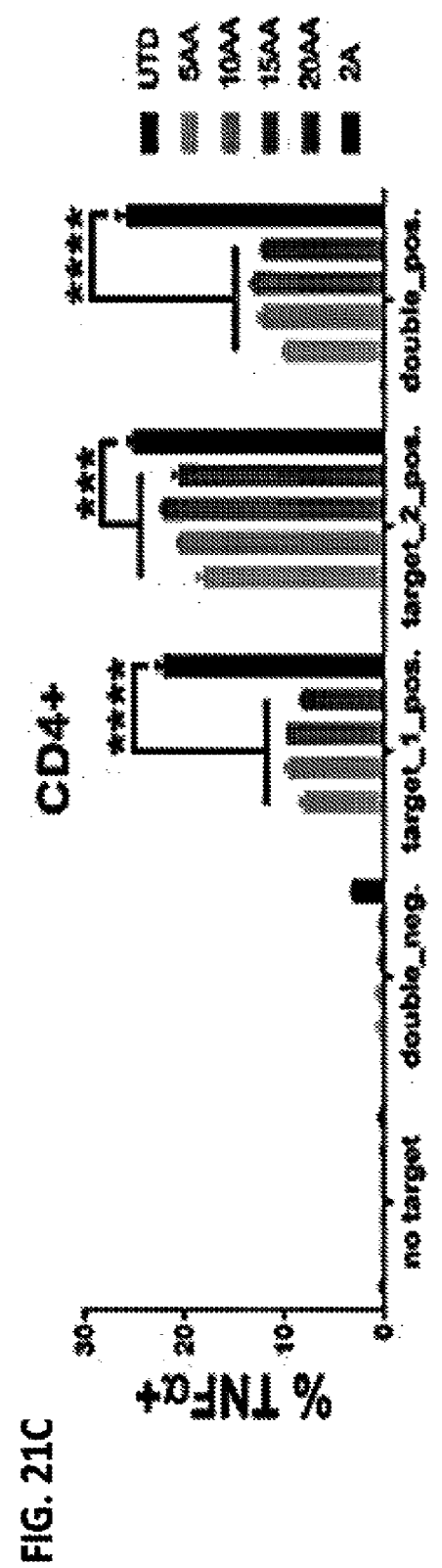
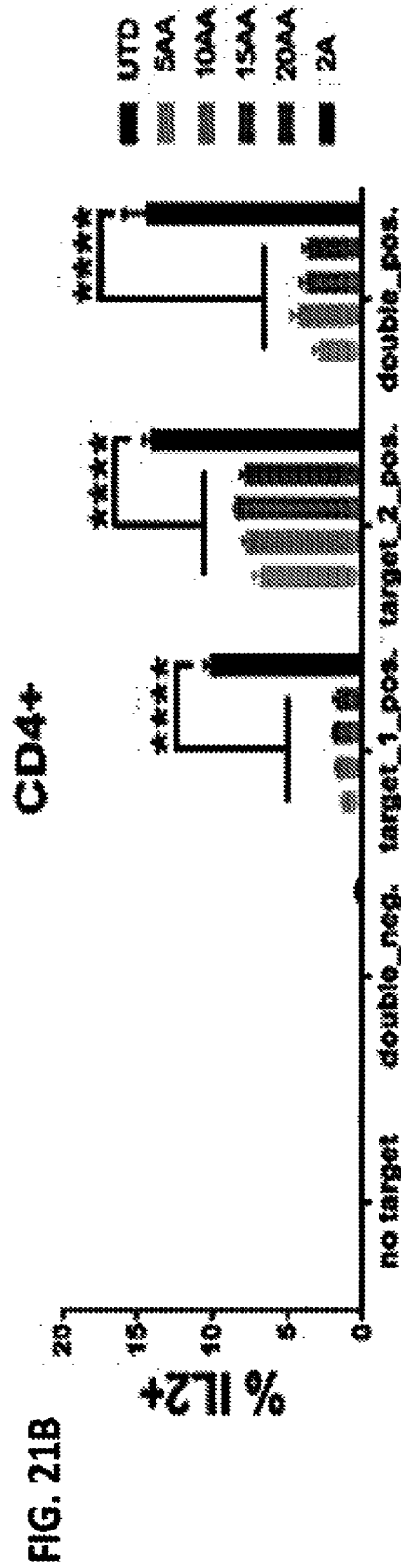
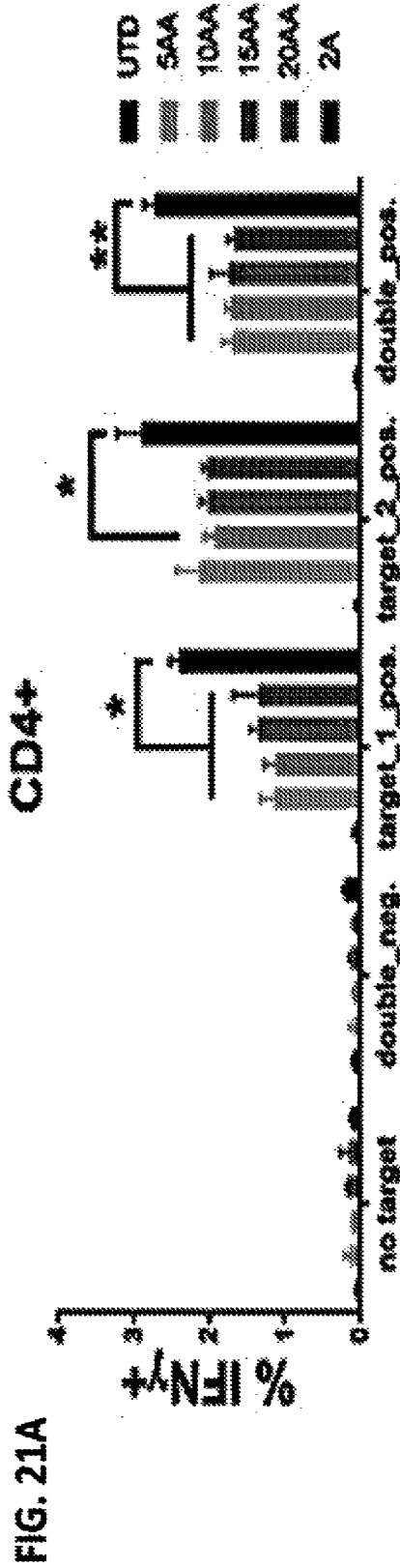
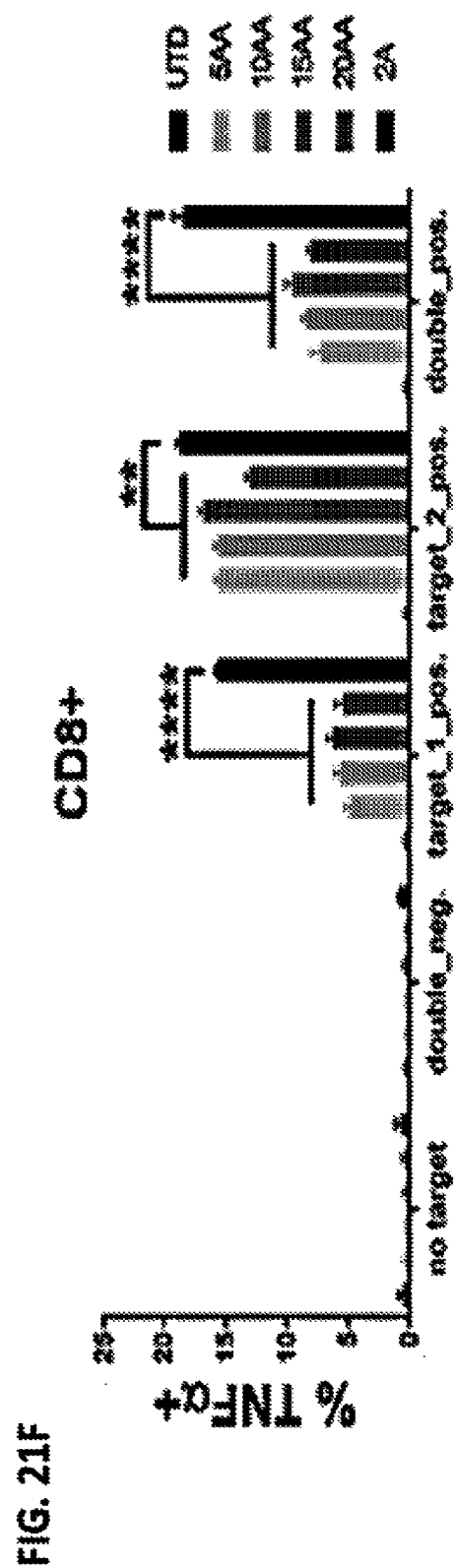
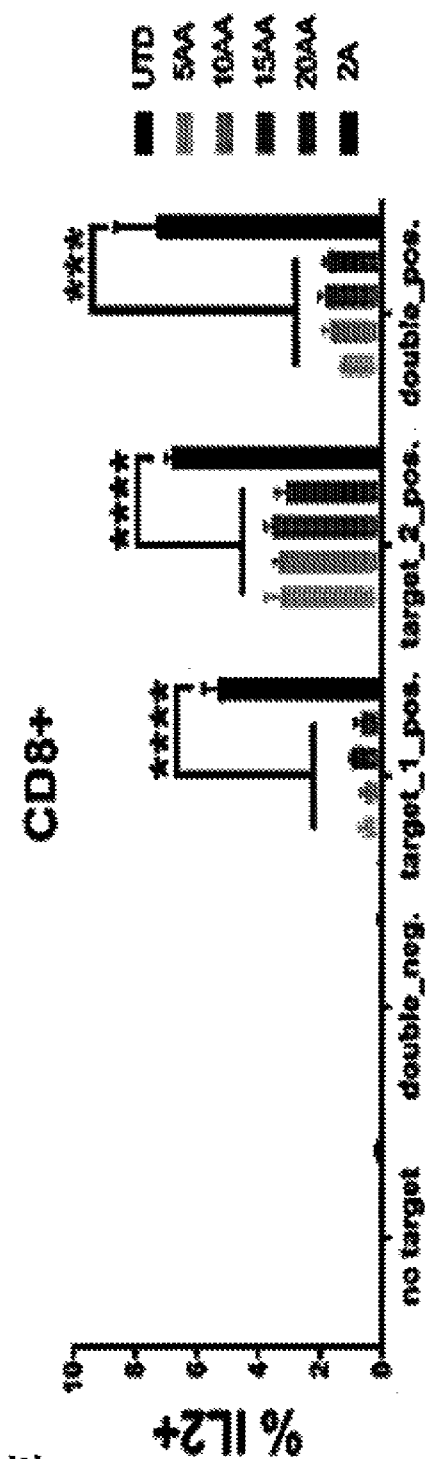
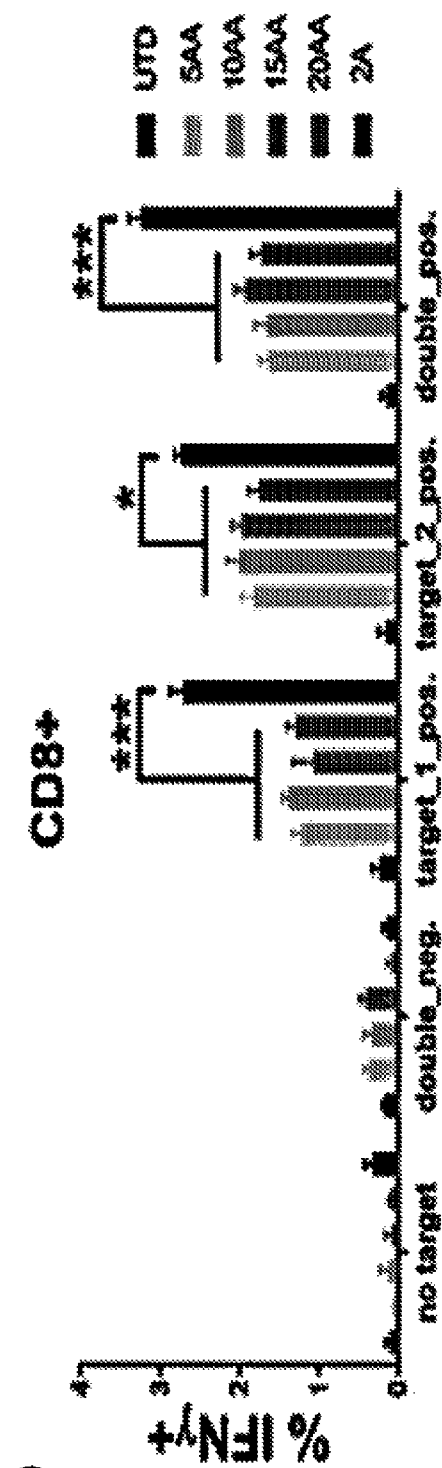


FIG. 20





1G4S

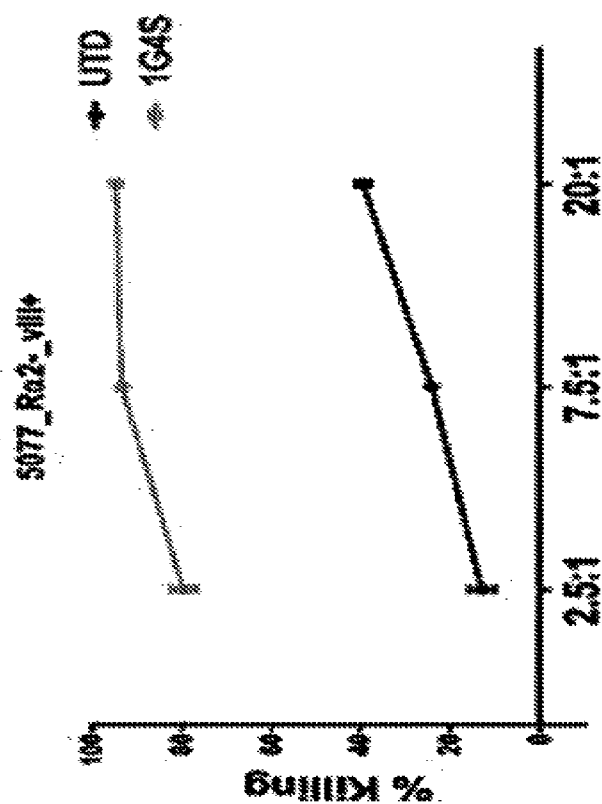
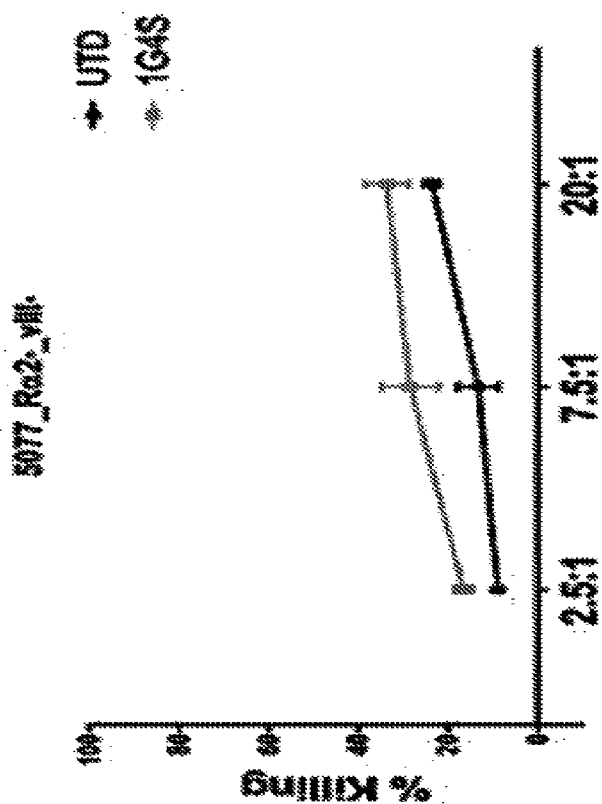
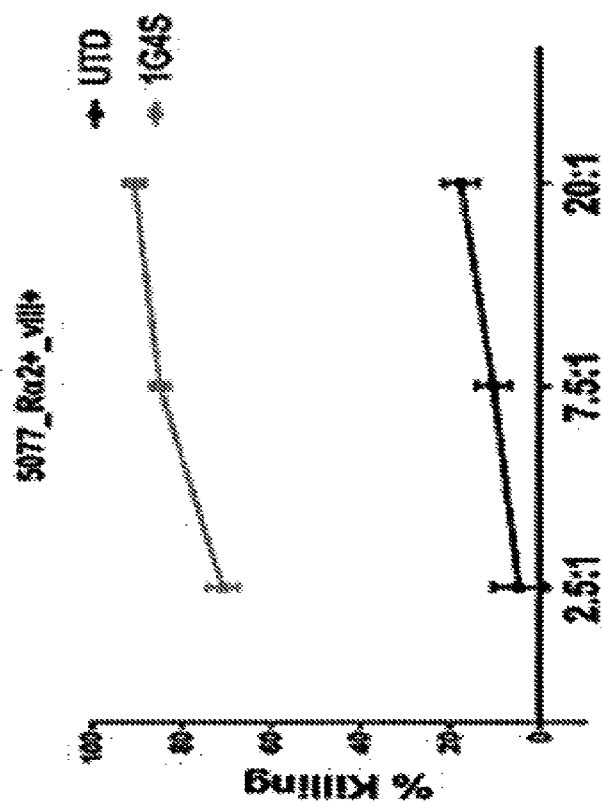
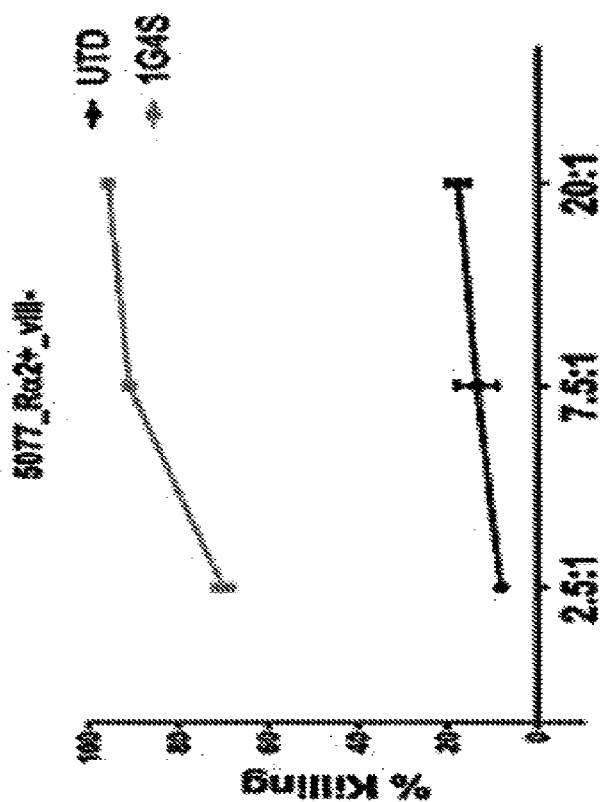


FIG. 22A

2G4S

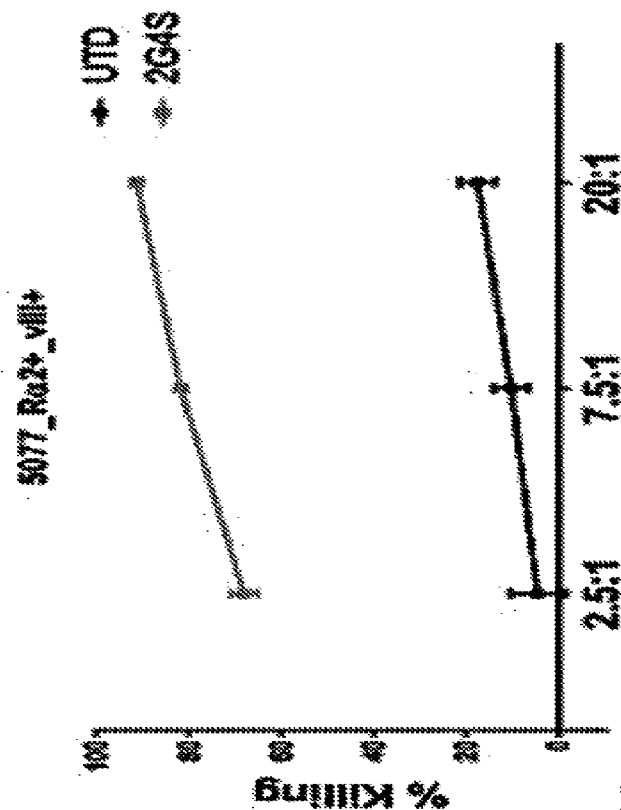
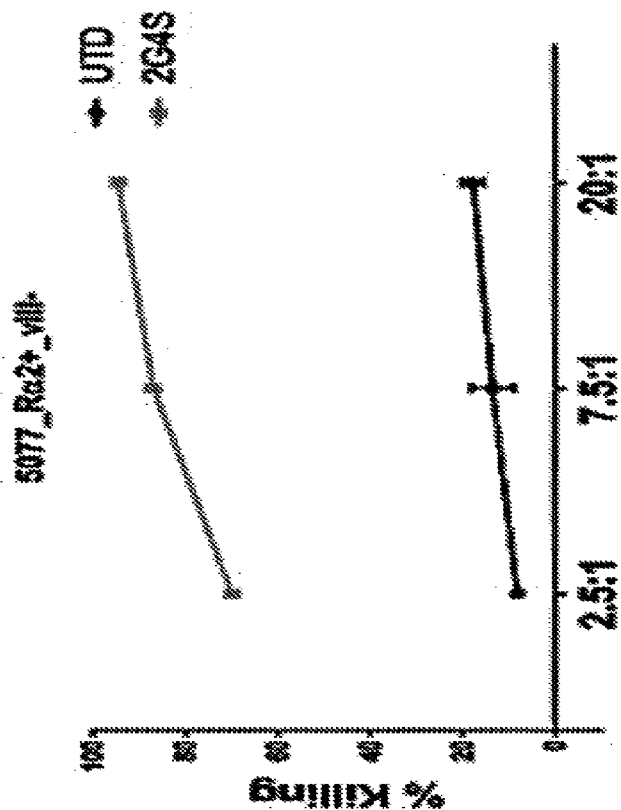
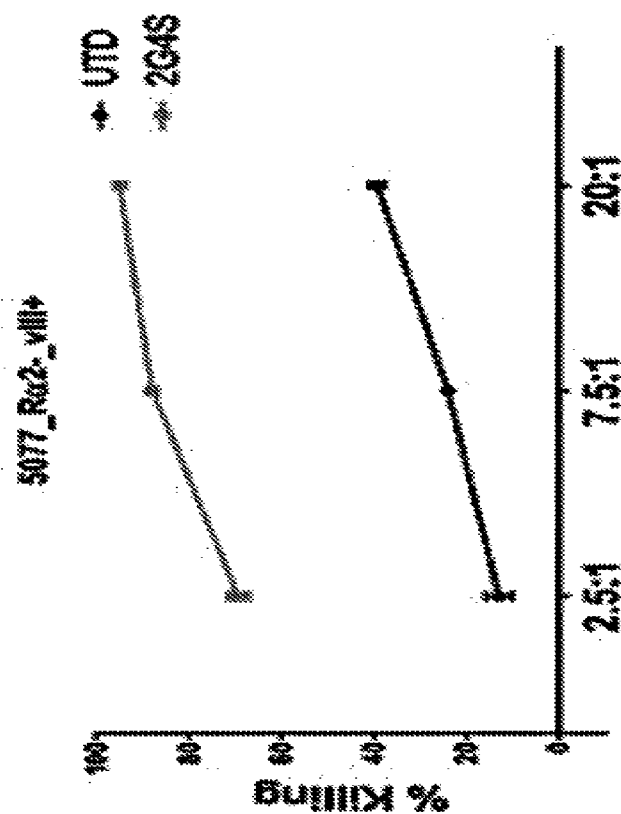
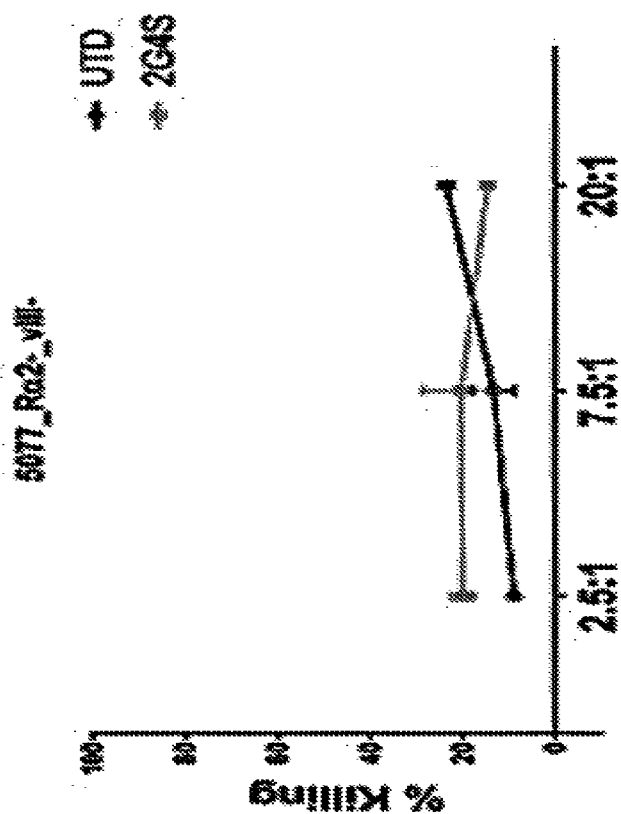


FIG. 22B



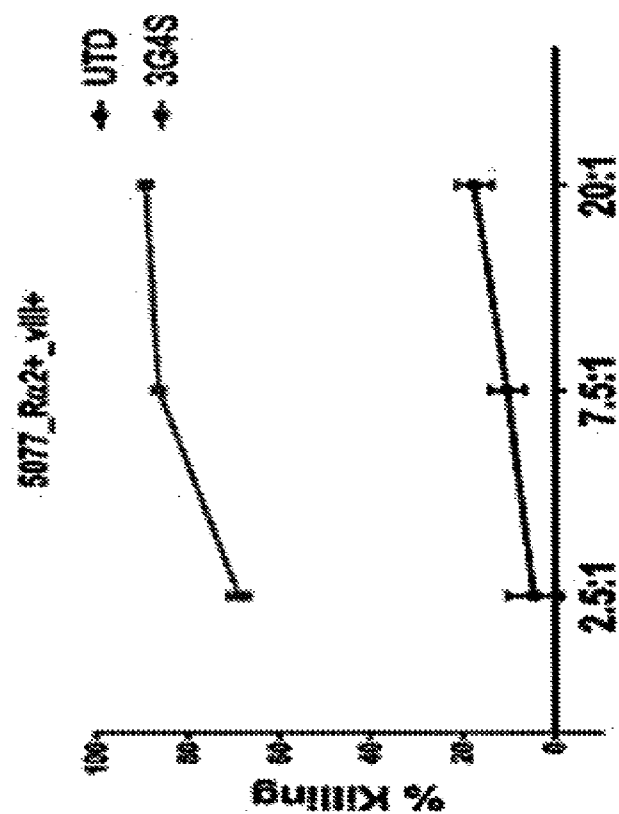
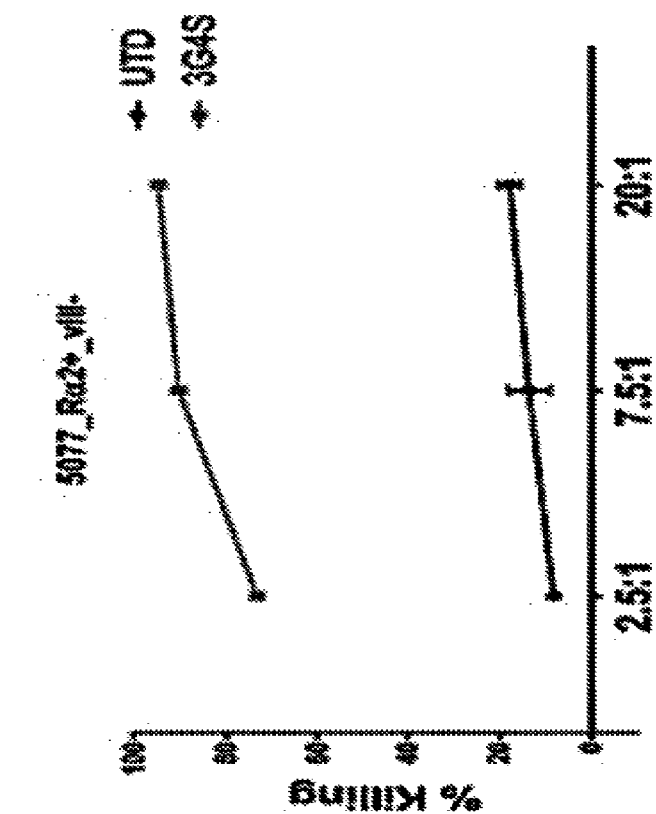
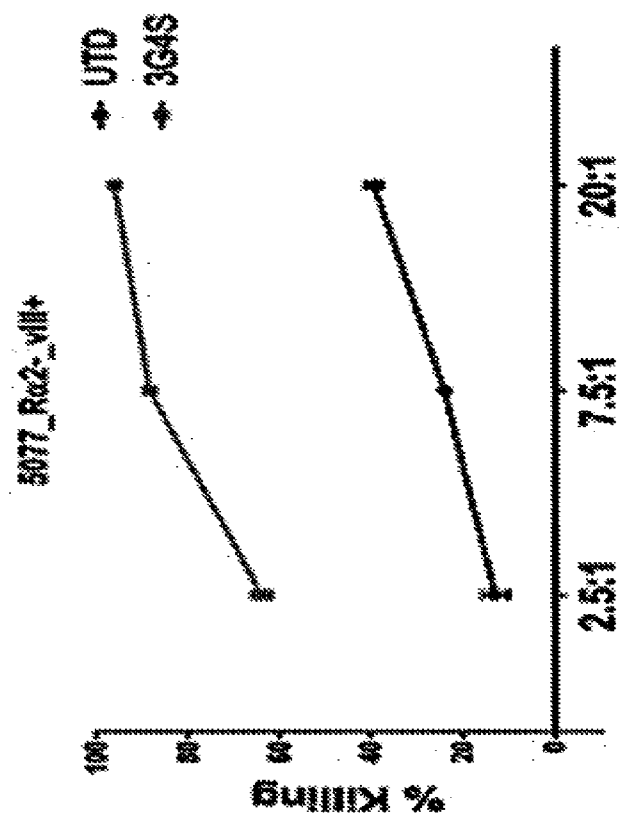
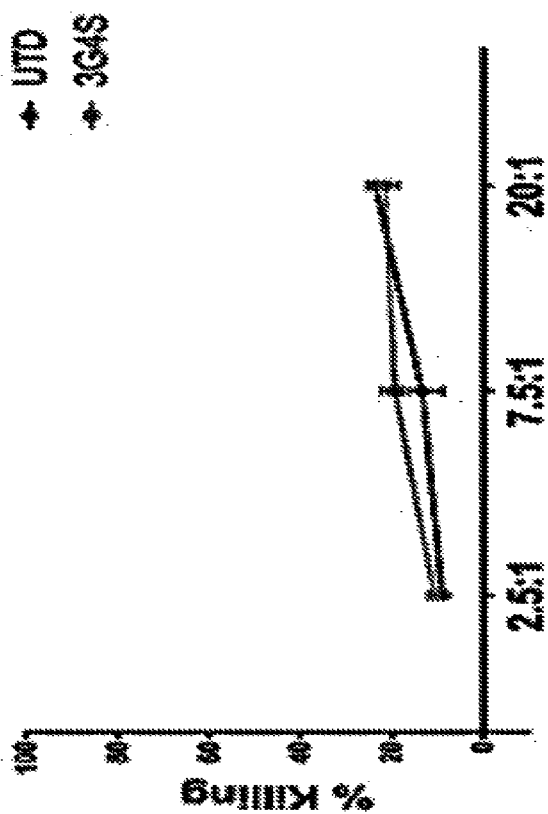


FIG. 22C



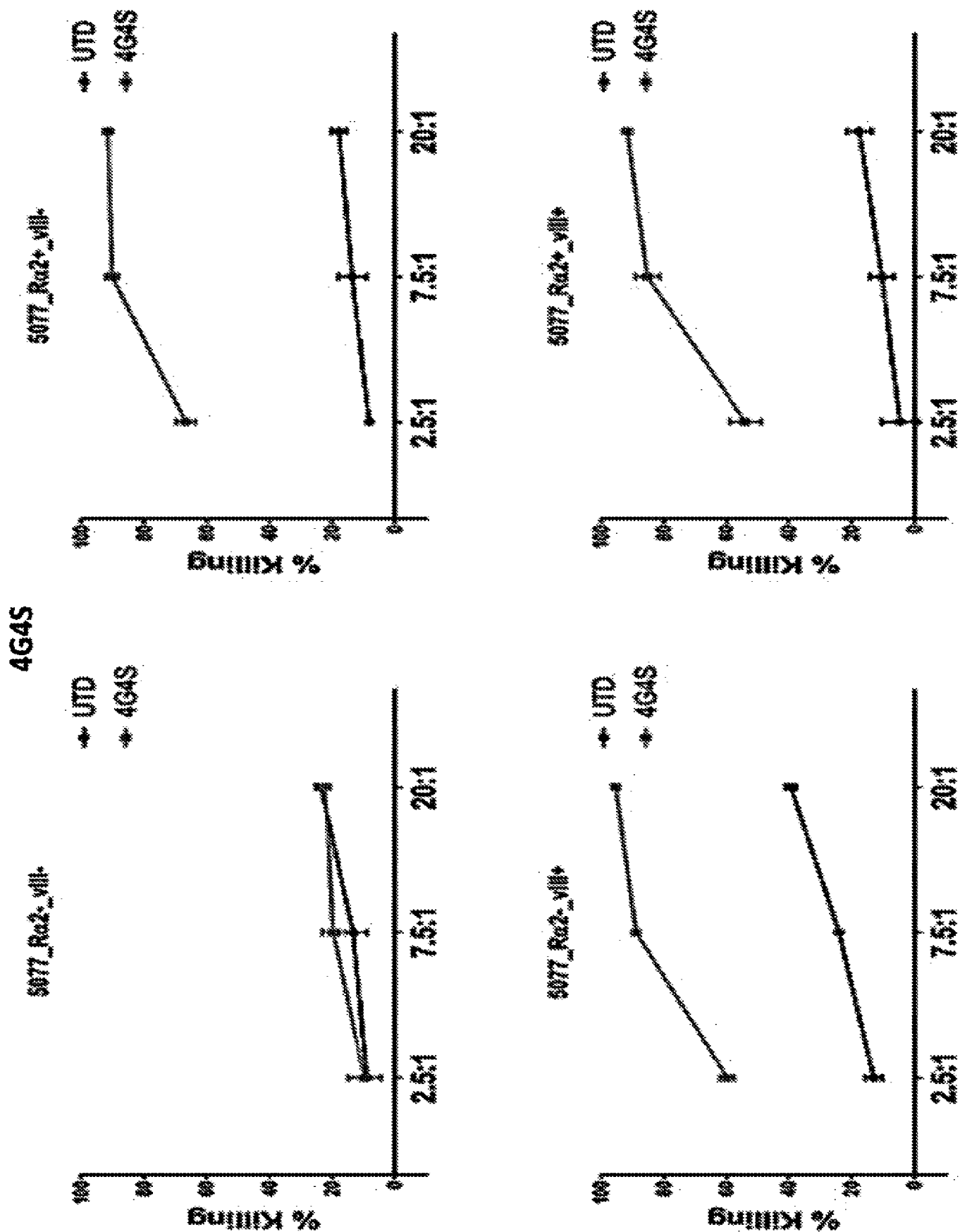


FIG. 22D

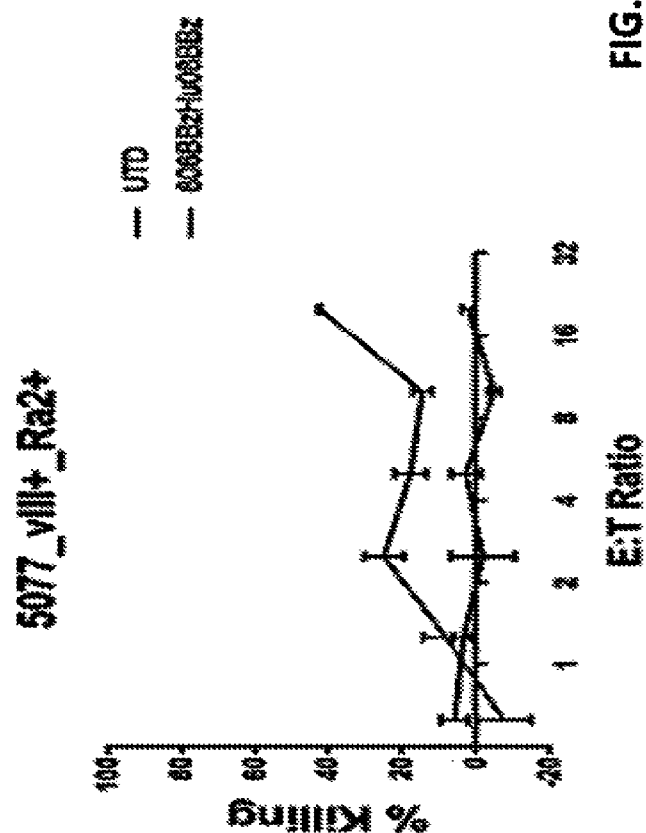
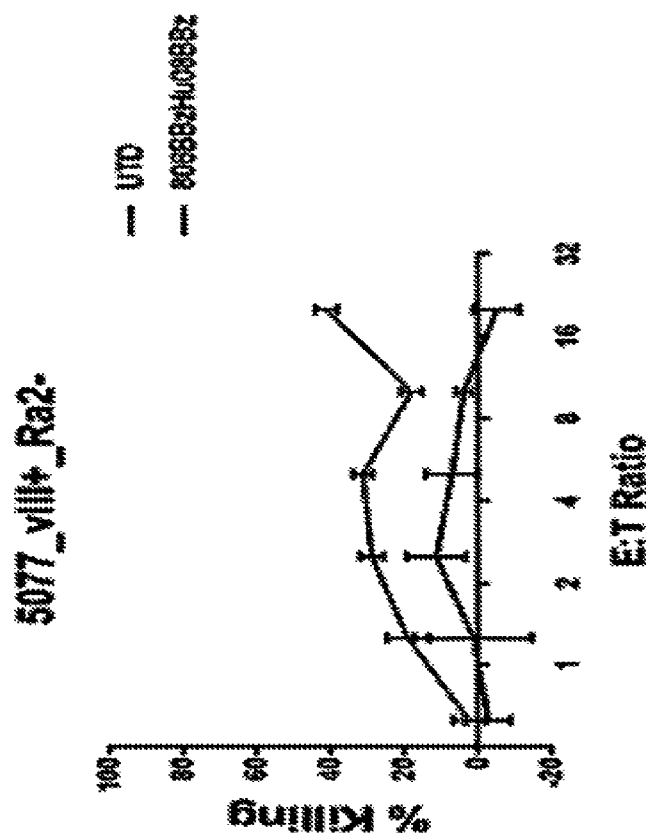
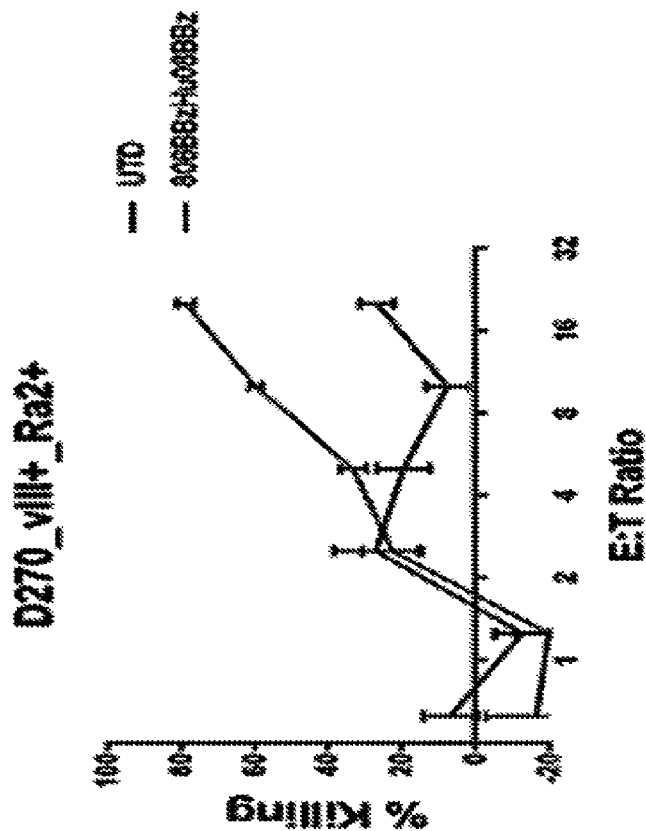
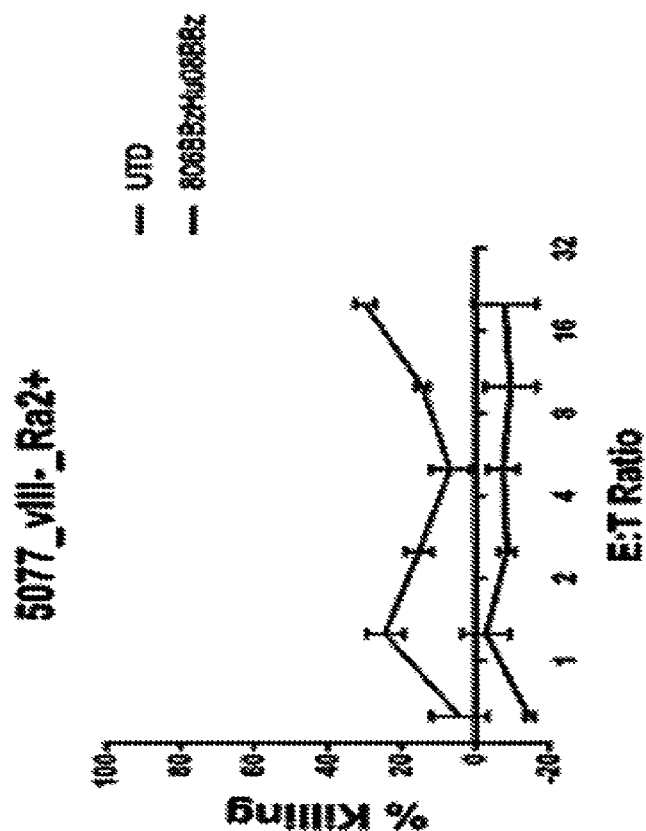


FIG. 23

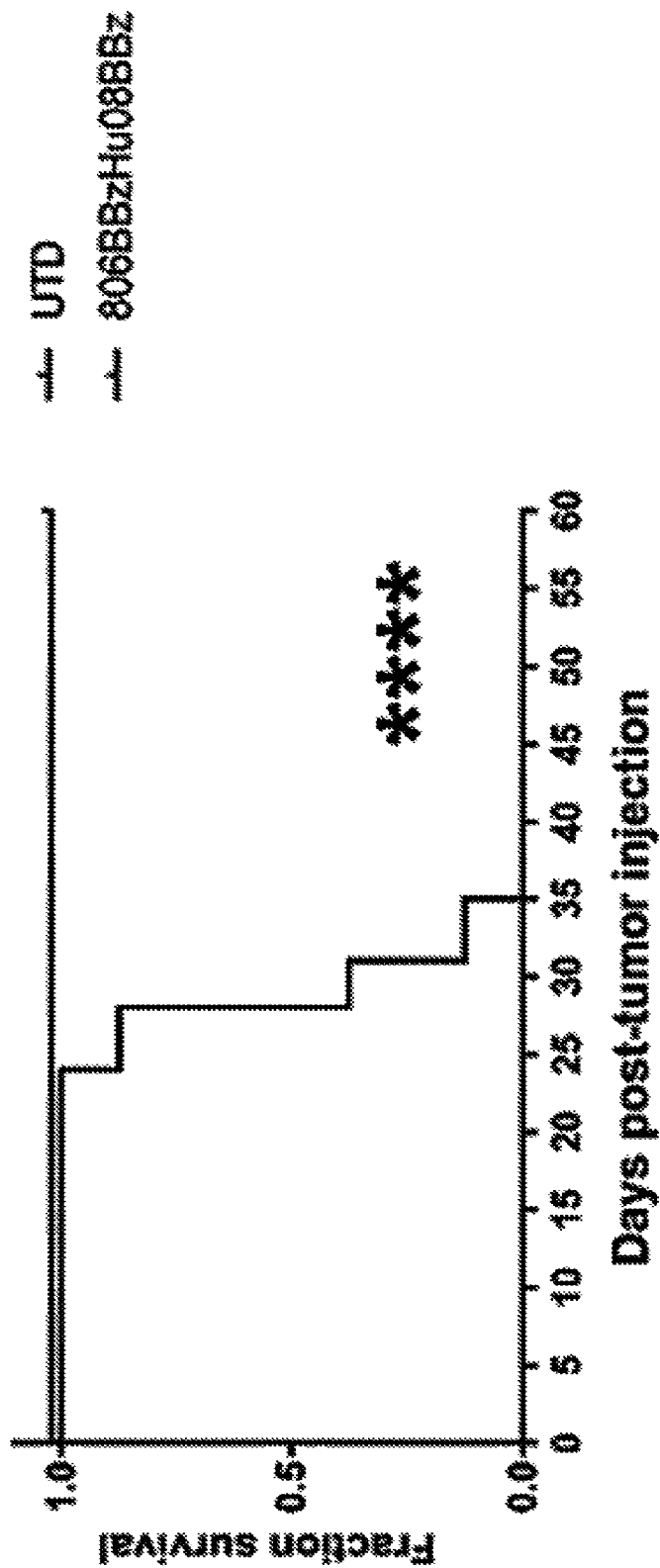
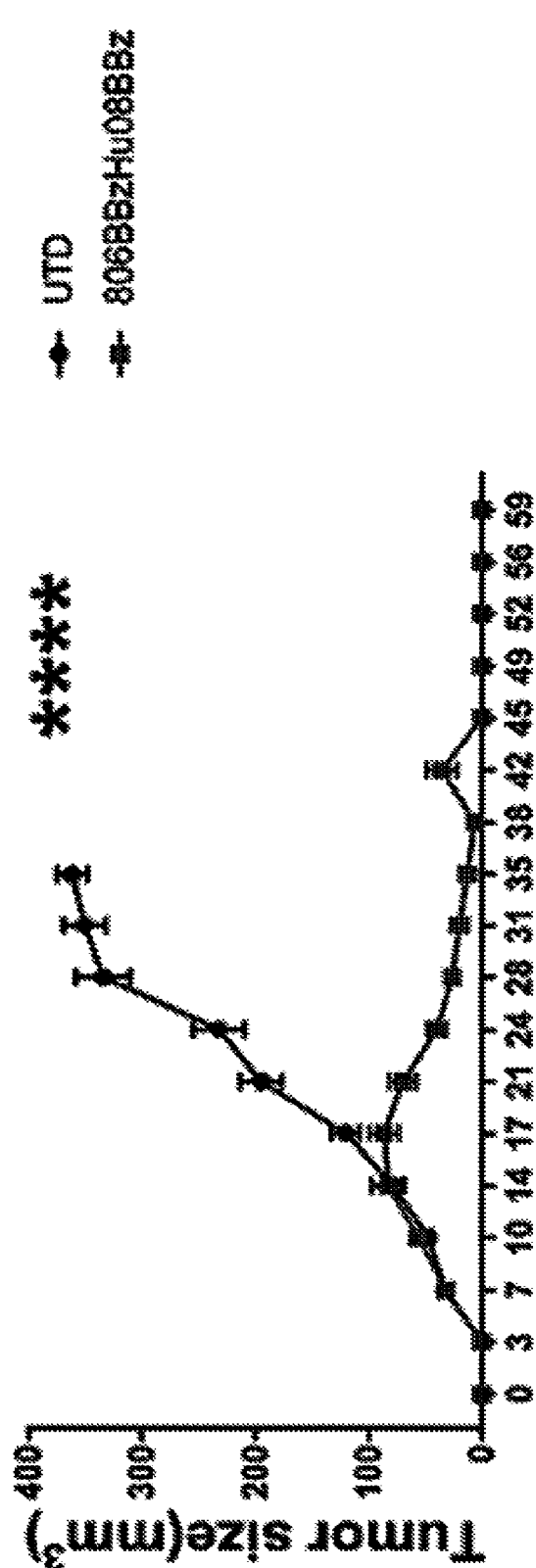


FIG. 24

FIG. 25A

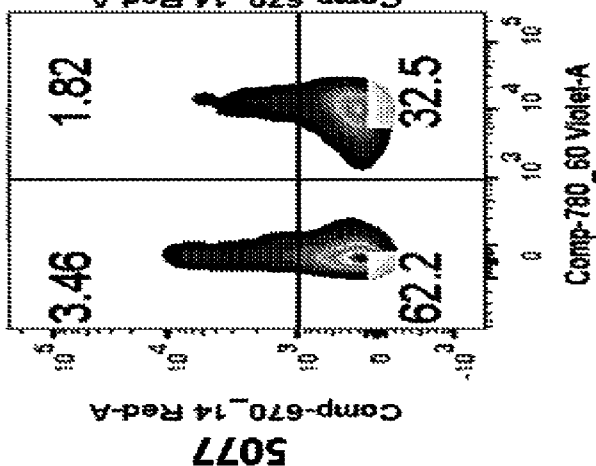


FIG. 25B

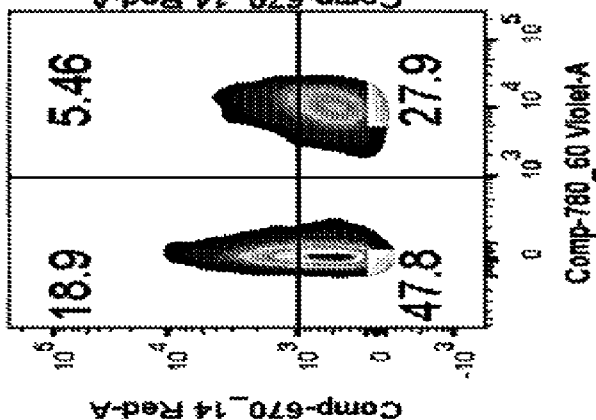


FIG. 25C

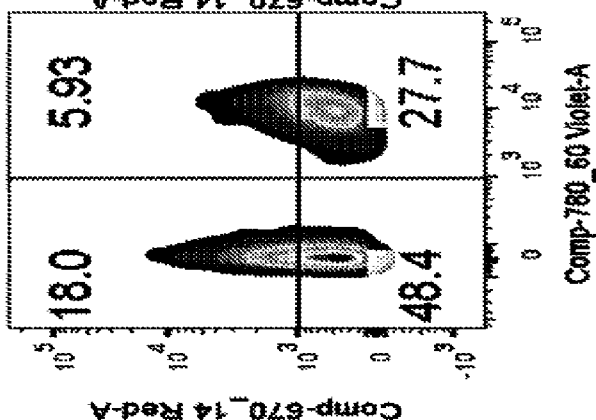
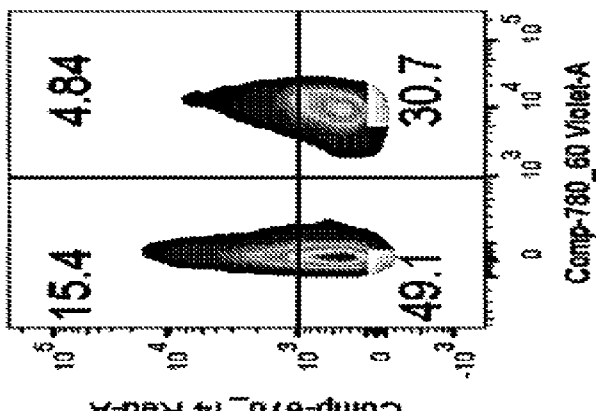
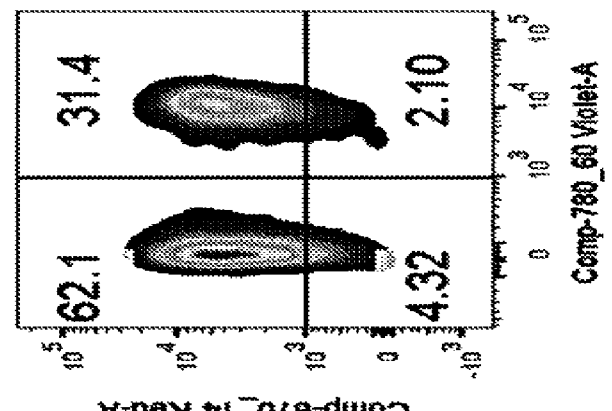
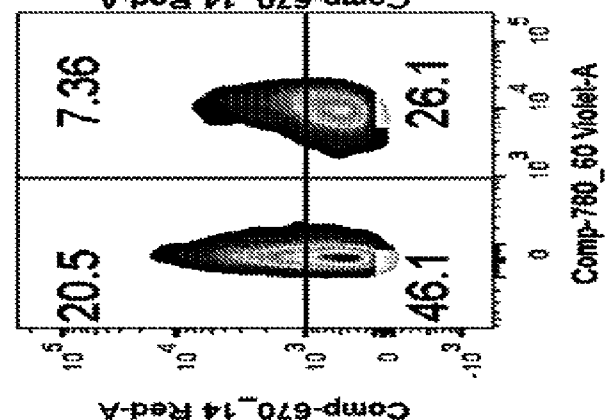
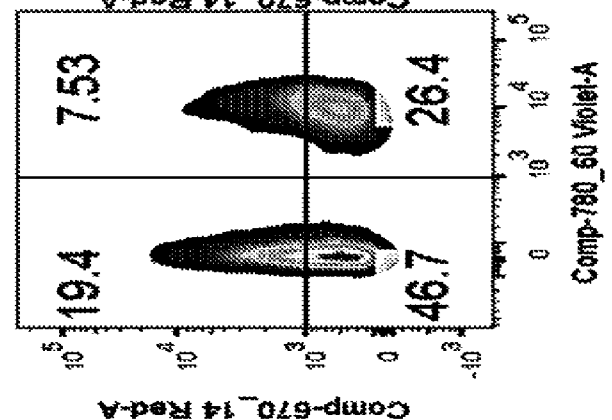
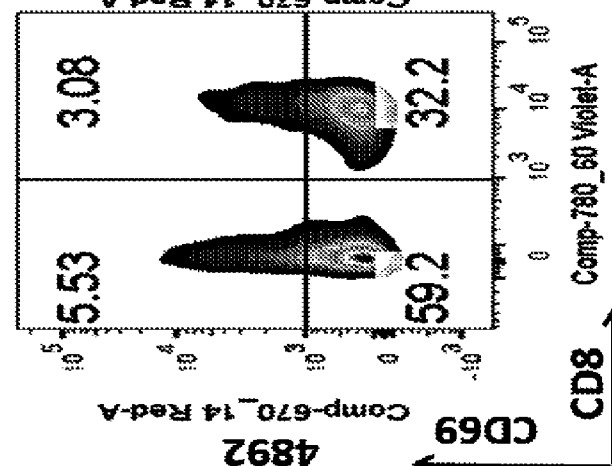


FIG. 25D



4892



Media:

Fresh

UTD

Hu08BBz

Hu07BiTE P2A Hu08TM

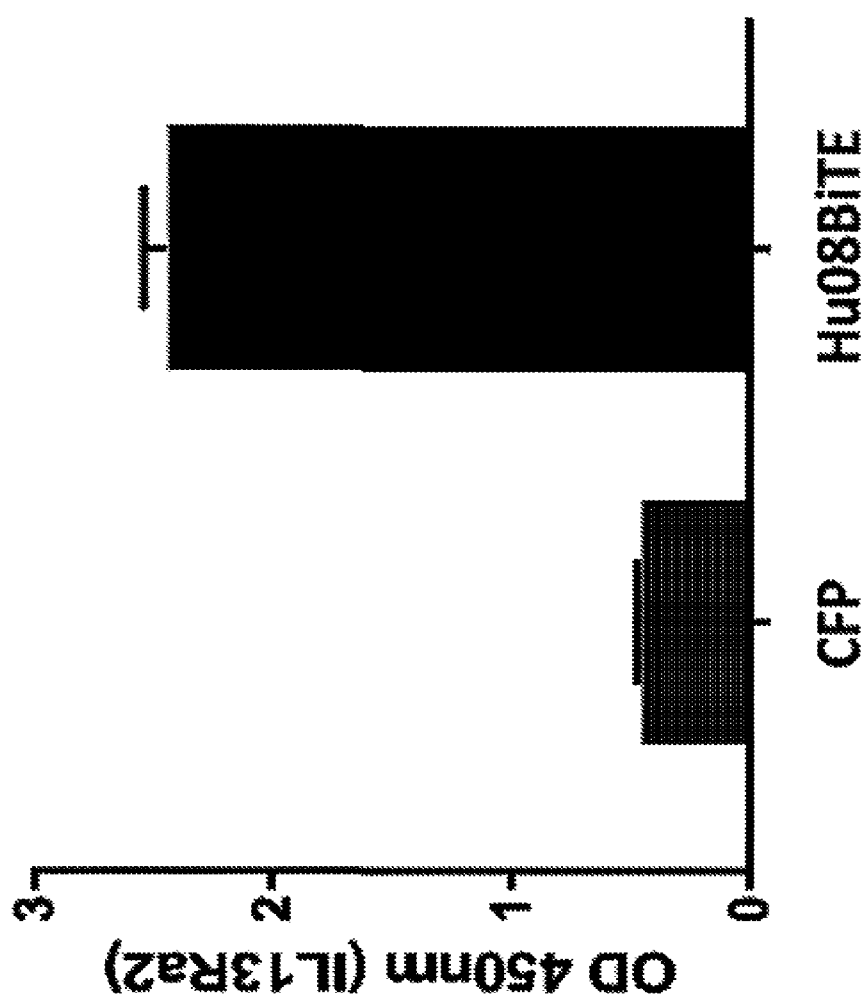


FIG. 26

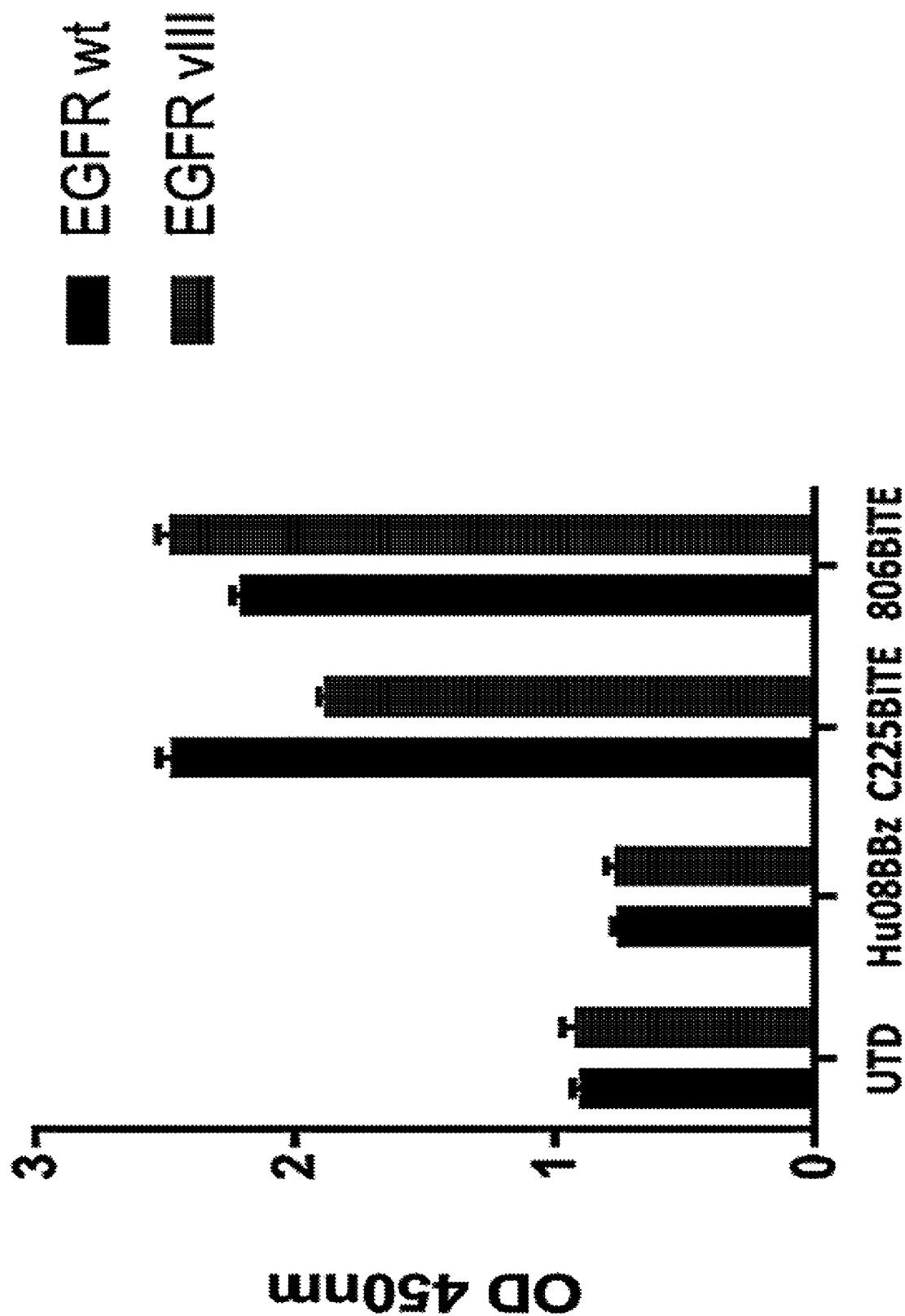


FIG. 27

FIG. 28A

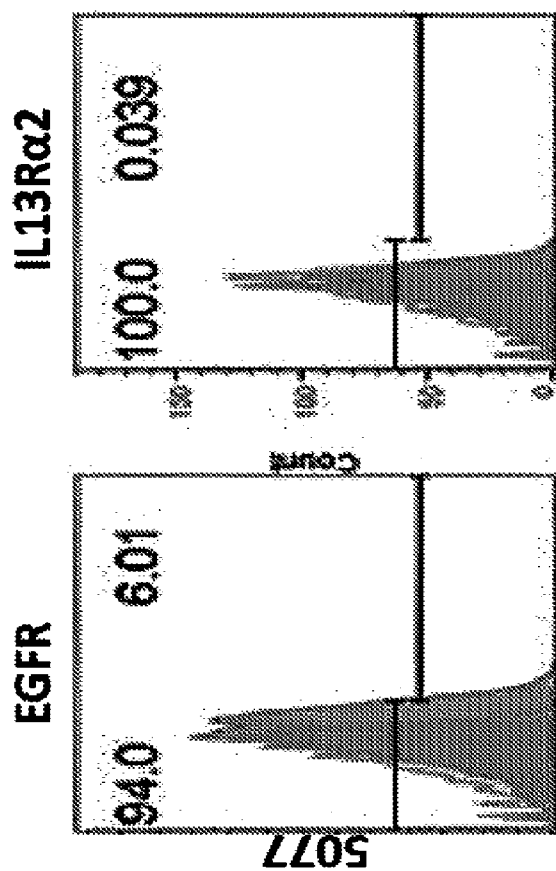
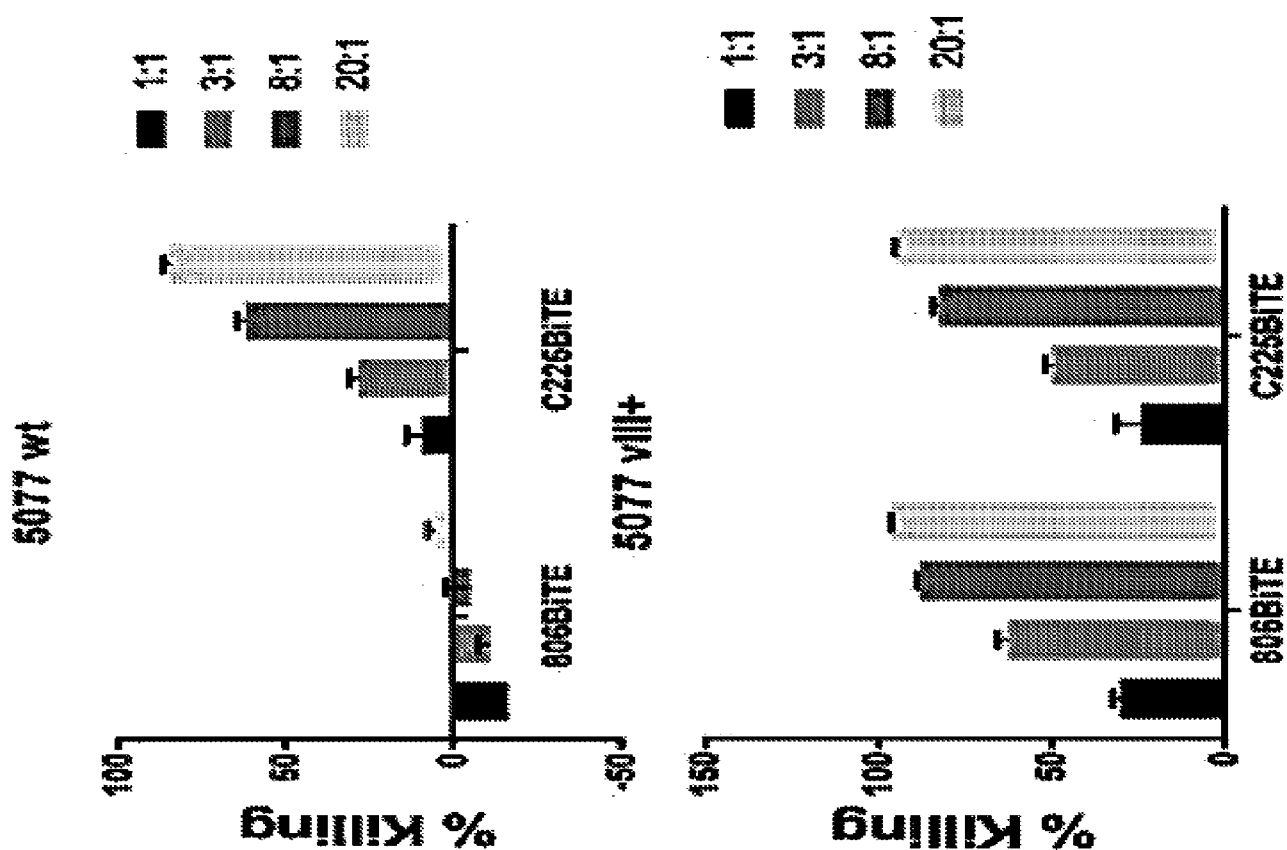
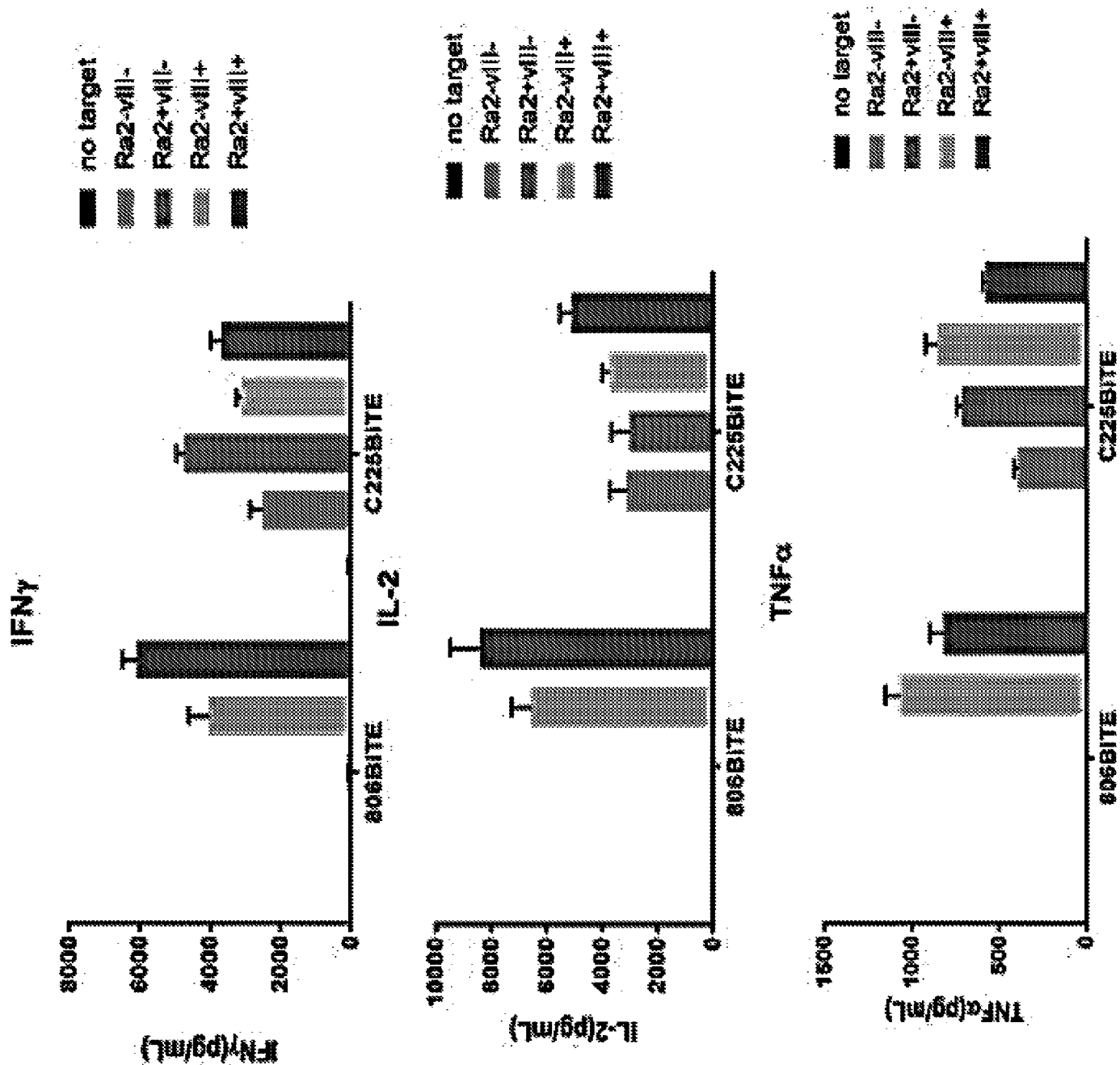


FIG. 28B



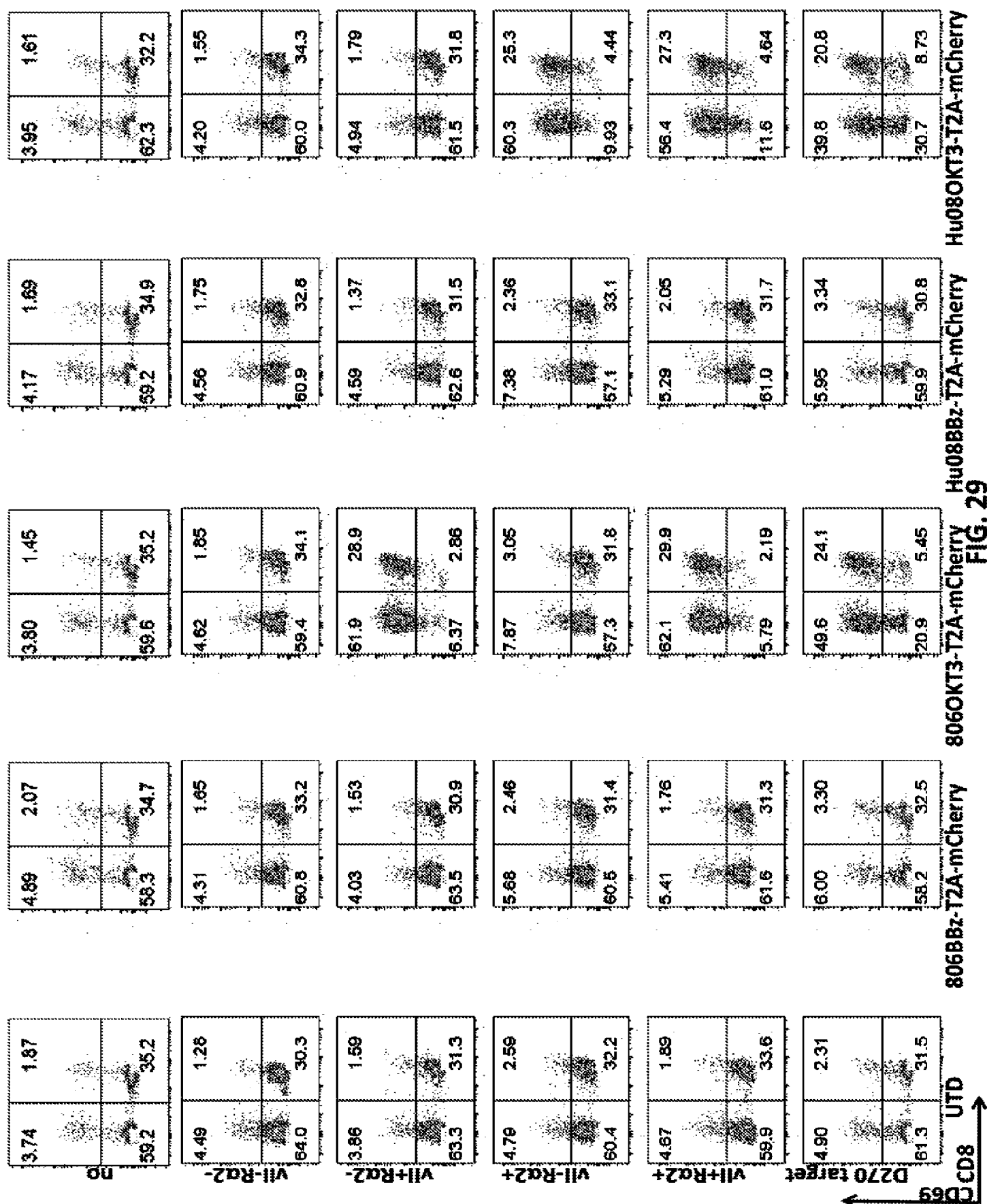


FIG. 29

FIG. 30A

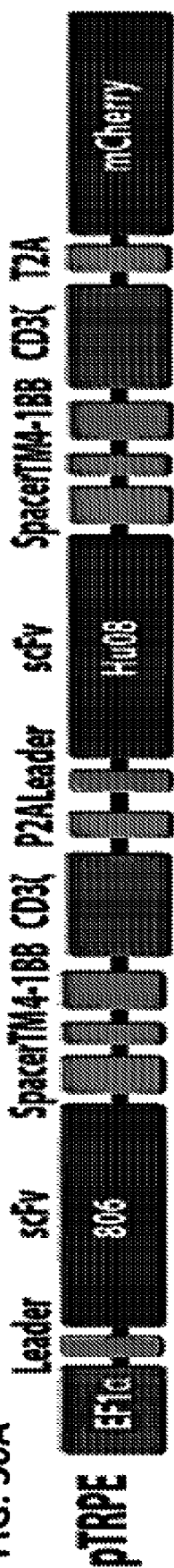


FIG. 30B

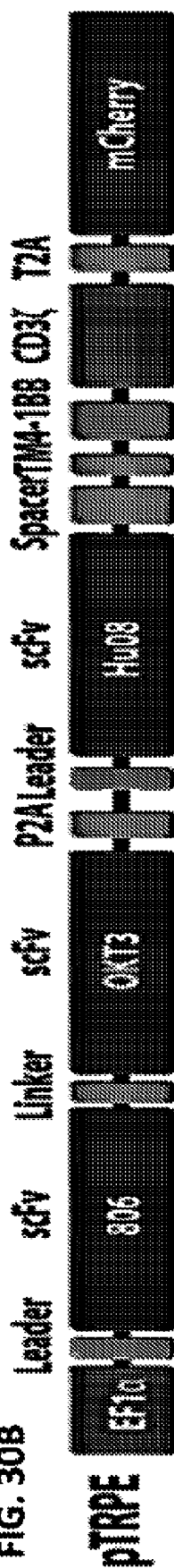
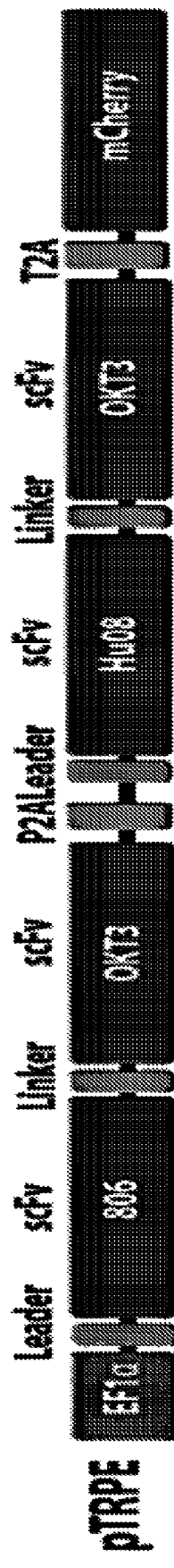


FIG. 30C



FIG. 30D



Amir

44-38861-1000

[illegible]

(SEQ ID NO: 174)

FIG. 31A

SECRET

(SEQ ID NO: 176)

316.7

Answered:

STOCK MARKET AND CREDIT MARKET

12345678910111213141516171819202122232425262728293031323334353637383940414243444546474849505152535455565758596061626364656667686970717273747576777879808182838485868788899091929394959697989910010110210310410510610710810911011111211311411511611711811912012112212312412512612712812913013113213313413513613713813914014114214314414514614714814915015115215315415515615715815916016116216316416516616716816917017117217317417517617717817918018118218318418518618718818919019119219319419519619719819920020120220320420520620720820921021121221321421521621721821922022122222322422522622722822923023123223323423523623723823924024124224324424524624724824925025125225325425525625725825926026126226326426526626726826927027127227327427527627727827928028128228328428528628728828929029129229329429529629729829930030130230330430530630730830931031131231331431531631731831932032132232332432532632732832933033133233333433533633733833934034134234334434534634734834935035135235335435535635735835936036136236336436536636736836937037137237337437537637737837938038138238338438538638738838939039139239339439539639739839940040140240340440540640740840941041141241341441541641741841942042142242342442542642742842943043143243343443543643743843944044144244344444544644744844945045145245345445545645745845946046146246346446546646746846947047147247347447547647747847948048148248348448548648748848949049149249349449549649749849950050150250350450550650750850951051151251351451551651751851952052152252352452552652752852953053153253353453553653753853954054154254354454554654754854955055155255355455555655755855956056156256356456556656756856957057157257357457557657757857958058158258358458558658758858959059159259359459559659759859960060160260360460560660760860961061161261361461561661761861962062162262362462562662762862963063163263363463563663763863964064164264364464564664764864965065165265365465565665765865966066166266366466566666766866967067167267367467567667767867968068168268368468568668768868969069169269369469569669769869970070170270370470570670770870971071171271371471571671771871972072172272372472572672772872973073173273373473573673773873974074174274374474574674774874975075175275375475575675775875976076176276376476576676776876977077177277377477577677777877978078178278378478578678778878979079179279379479579679779879980080180280380480580680780880981081181281381481581681781881982082182282382482582682782882983083183283383483583683783883984084184284384484584684784884985085185285385485585685785885986086186286386486586686786886987087187287387487587687787887988088188288388488588688788888989089189289389489589689789889990090190290390490590690790890991091191291391491591691791891992092192292392492592692792892993093193293393493593693793893994094194294394494594694794894995095195295395495595695795895996096196296396496596696796896997097197297397497597697797897998098198298398498598698798898999099199299399499599699799899910001001100210031004100510061007100810091010101110121013101410151016101710181019102010211022102310241025102610271028102910301031103210331034103510361037103810391040104110421043104410451046104710481049105010511052105310541055105610571058105910601061106210631064106510661067106810691070107110721073107410751076107710781079108010811082108310841085108610871088108910901091109210931094109510961097109810991100110111021103110411051106110711081109111011111112111311141115111611171118111911201121112211231124112511261127112811291130113111321133113411351136113711381139114011411142114311441145114611471148114911501151115211531154115511561157115811591160116111621163116411651166116711681169117011711172117311741175117611771178117911801181118211831184118511861187118811891190119111921193119411951196119711981199120012011202120312041205120612071208120912101211121212131214121512161217121812191220122112221223122412251226122712281229123012311232123312341235123612371238123912401241124212431244124512461247124812491250125112521253125412551256125712581259126012611262126312641265126612671268126912701271127212731274127512761277127812791280128112821283128412851286128712881289129012911292129312941295129612971298129913001

[illegible]

(SEQ ID NO: 178)

FIG. 31C

FIG. 32A

5077_vIII+_Ra2-

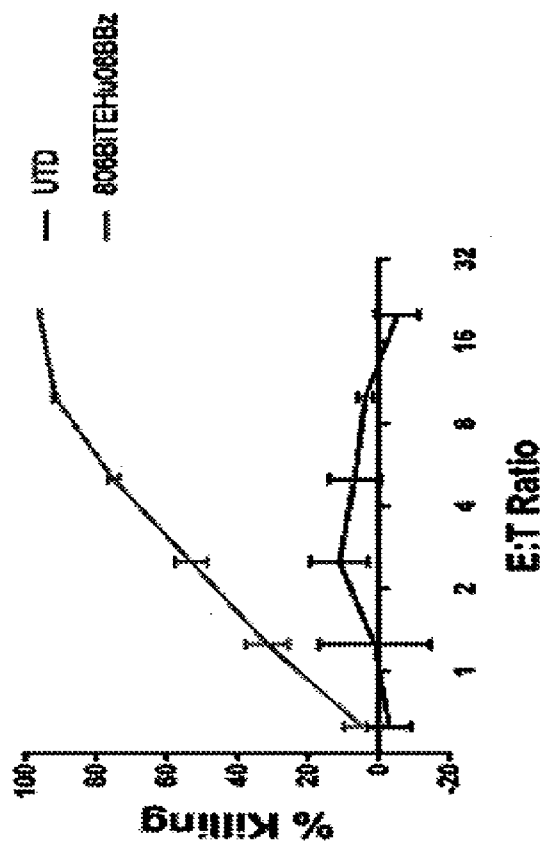


FIG. 32B

5077_vIII-_Ra2+

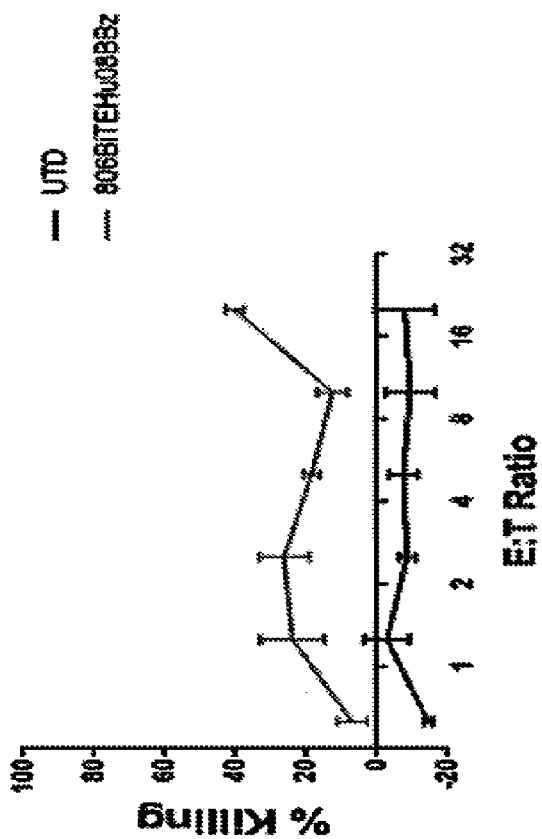


FIG. 32C

5077_vIII+_Ra2+

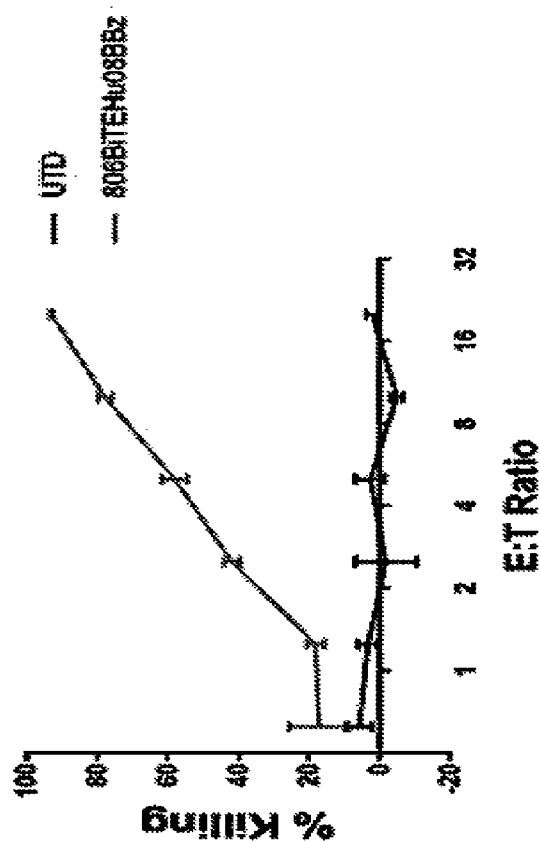


FIG. 32D

D270_vIII+_Ra2+

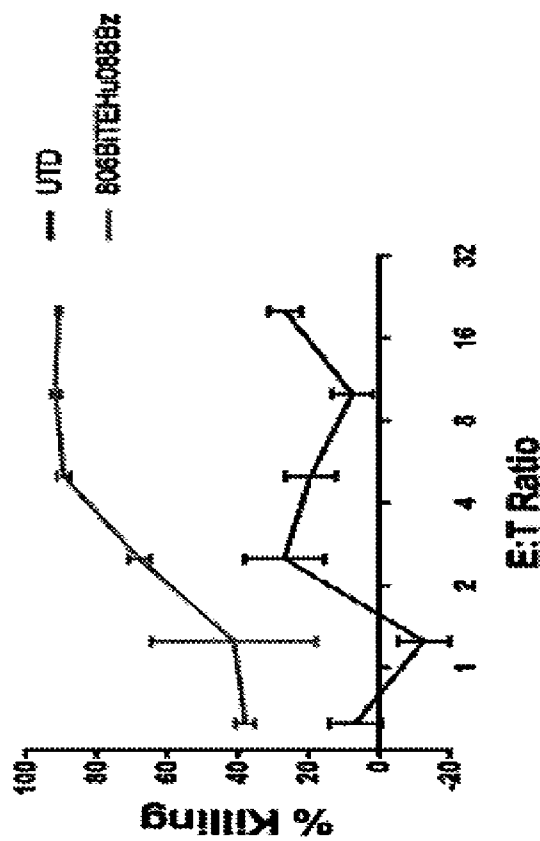


FIG. 33A

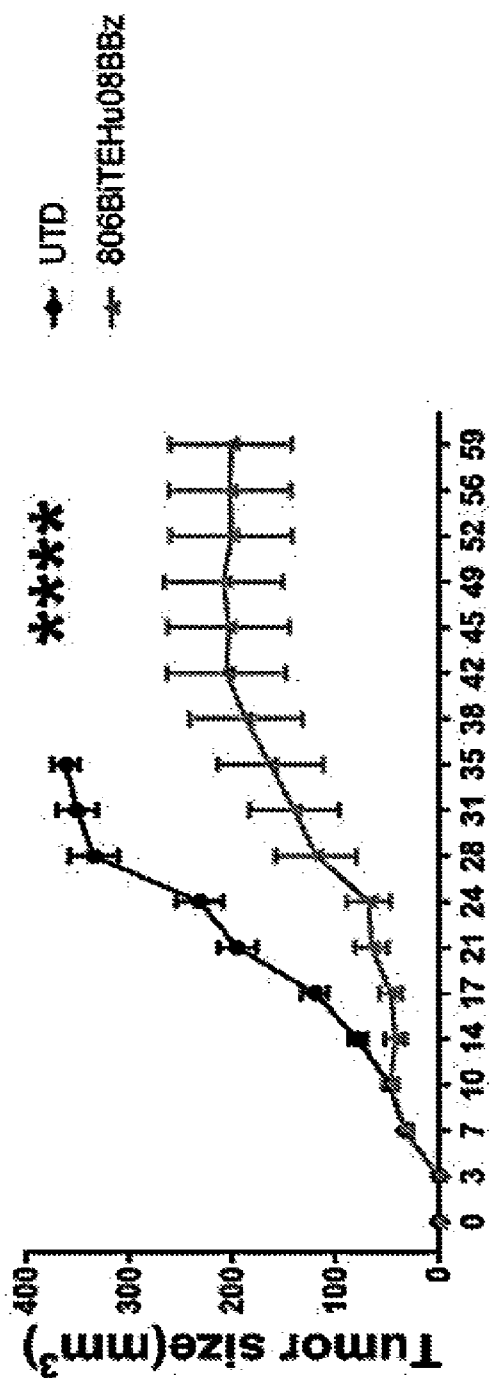


FIG. 33B

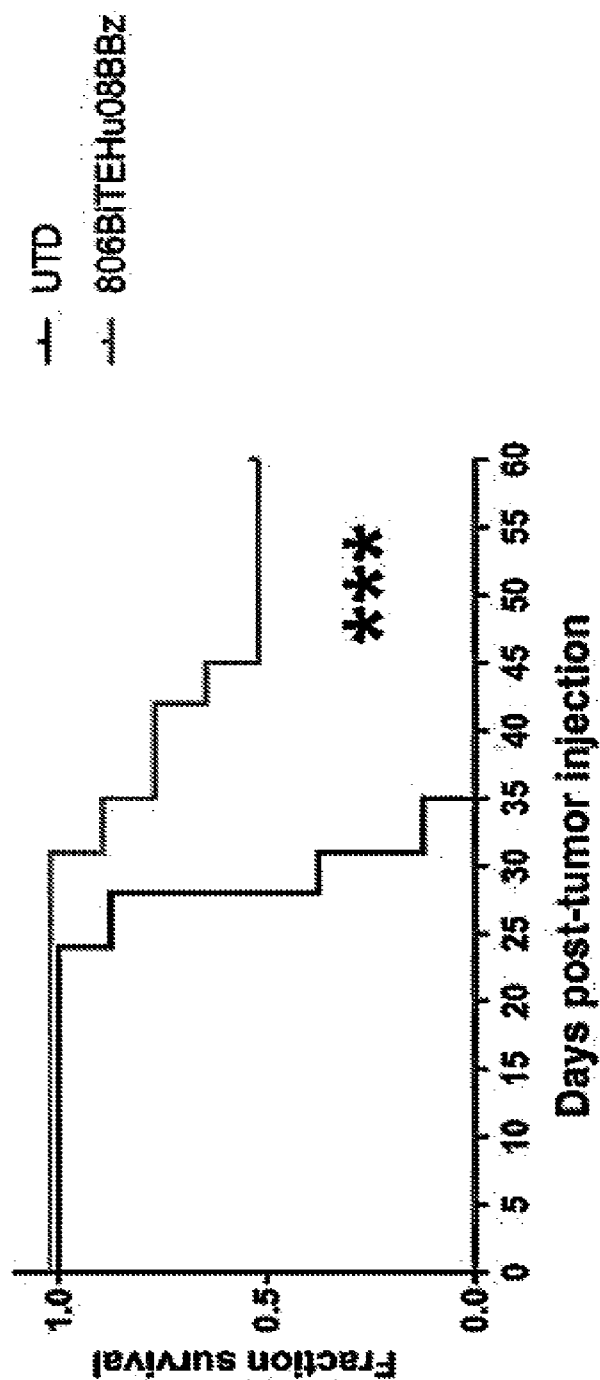


FIG. 34B

5077_vIII+Ra2+

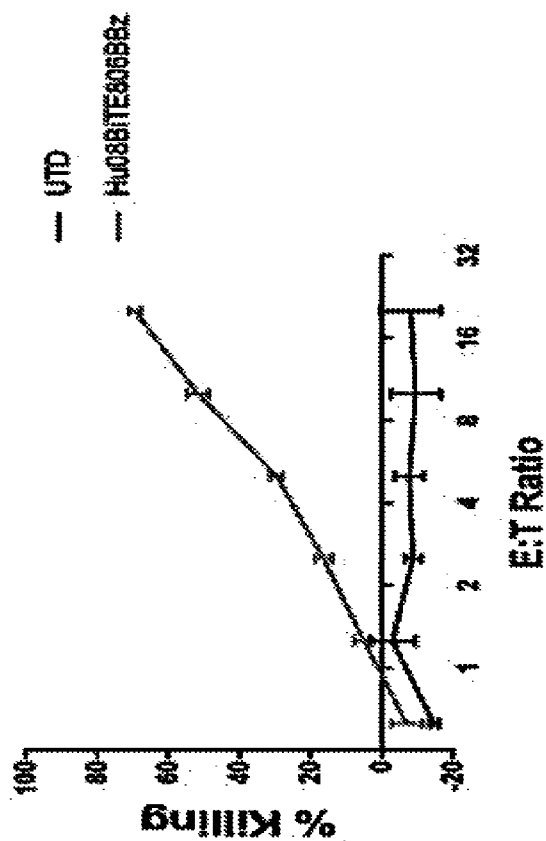


FIG. 34D

D270_vIII+Ra2+

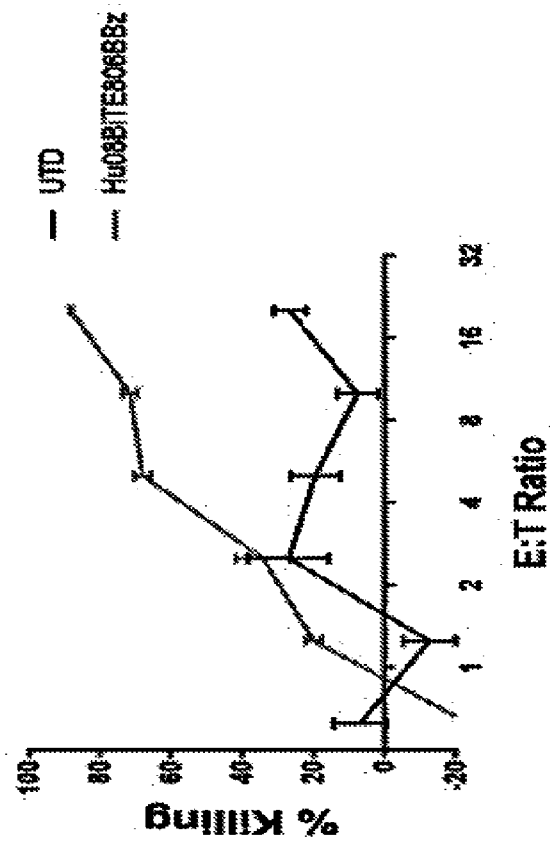


FIG. 34A

5077_vIII+Ra2-

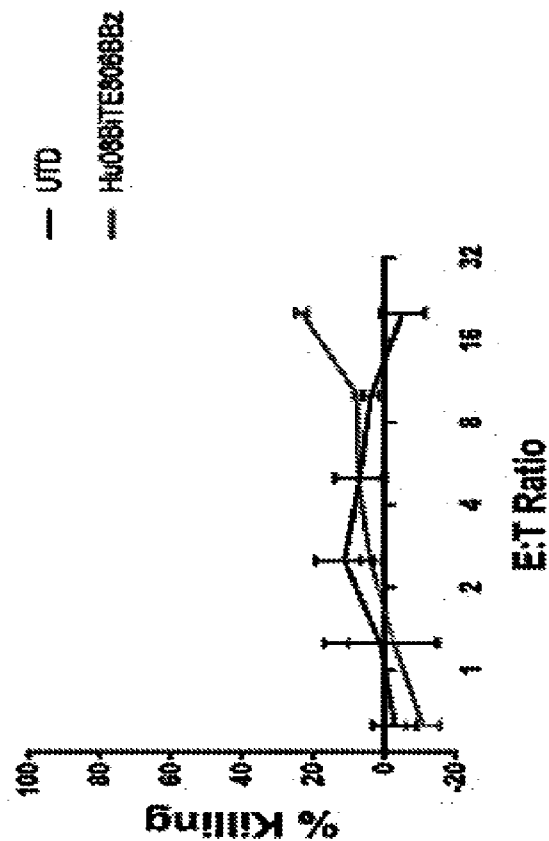


FIG. 34C

5077_vIII+Ra2+

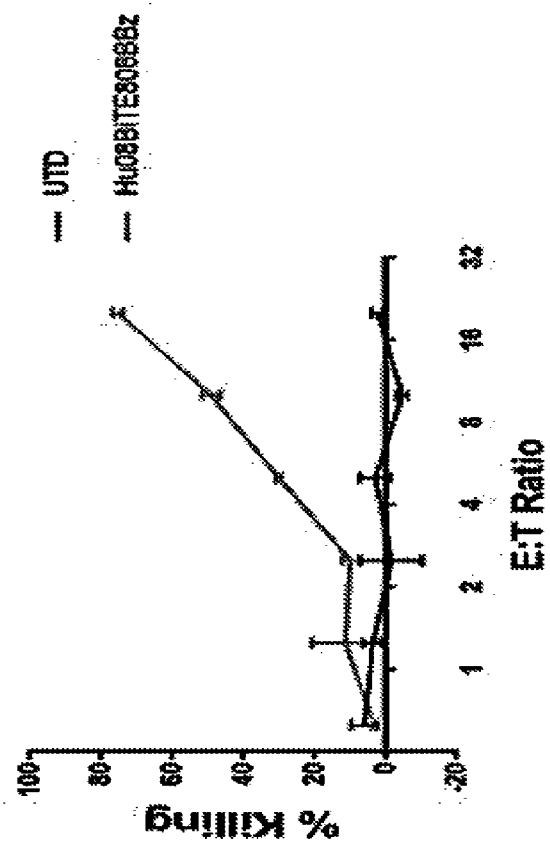


FIG. 35A

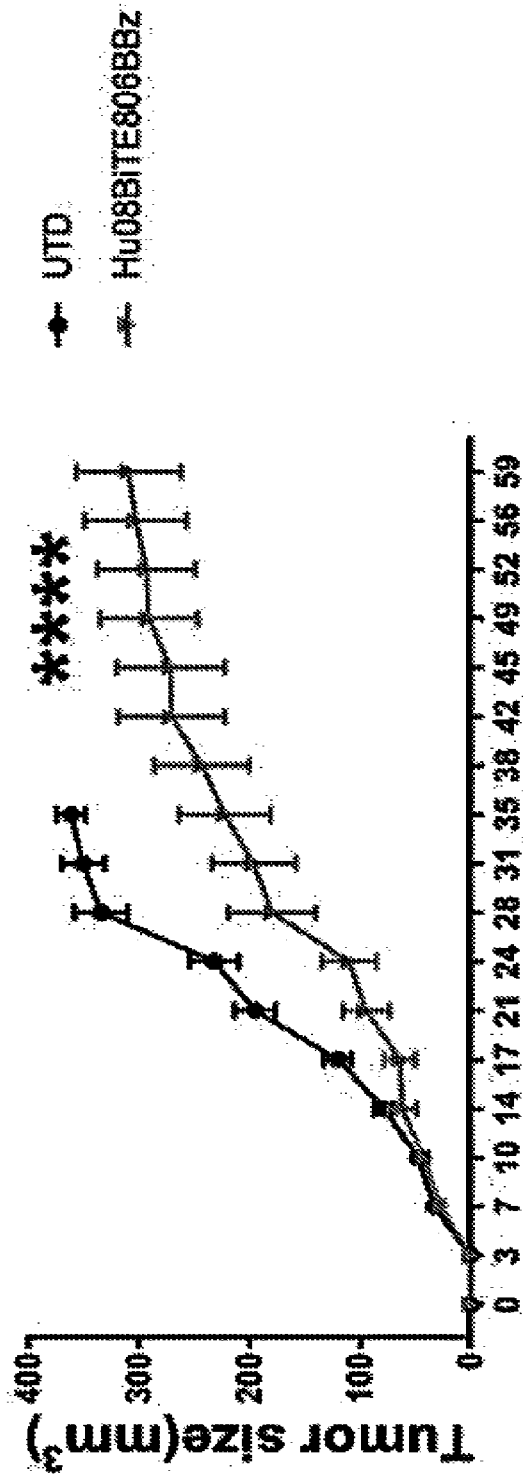


FIG. 35B

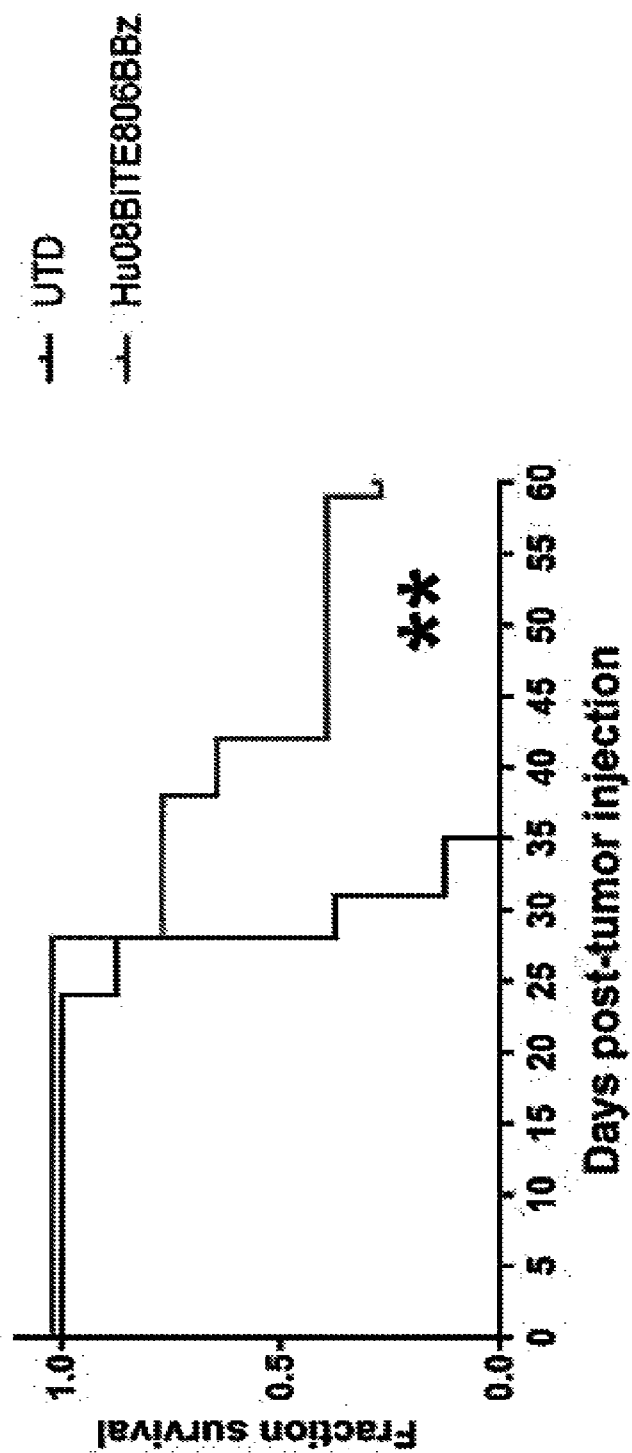


FIG. 36B

5077_vIII- _Ra2+

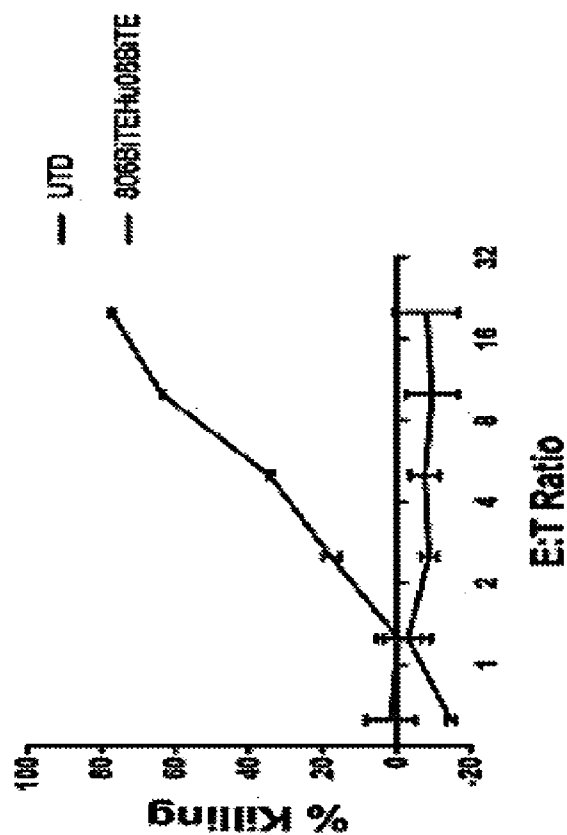


FIG. 36D

D270_vIII+ _Ra2+

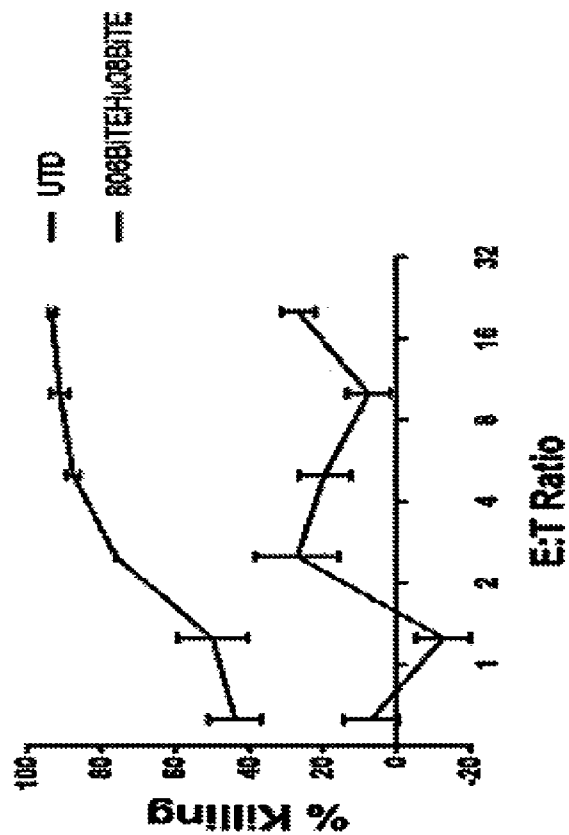


FIG. 36A

5077_vIII+ _Ra2-

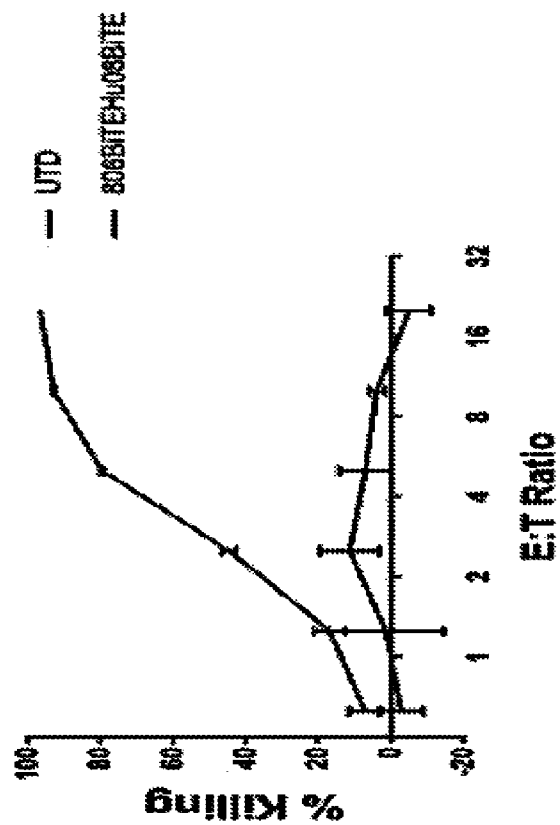


FIG. 36C

5077_vIII+ _Ra2+

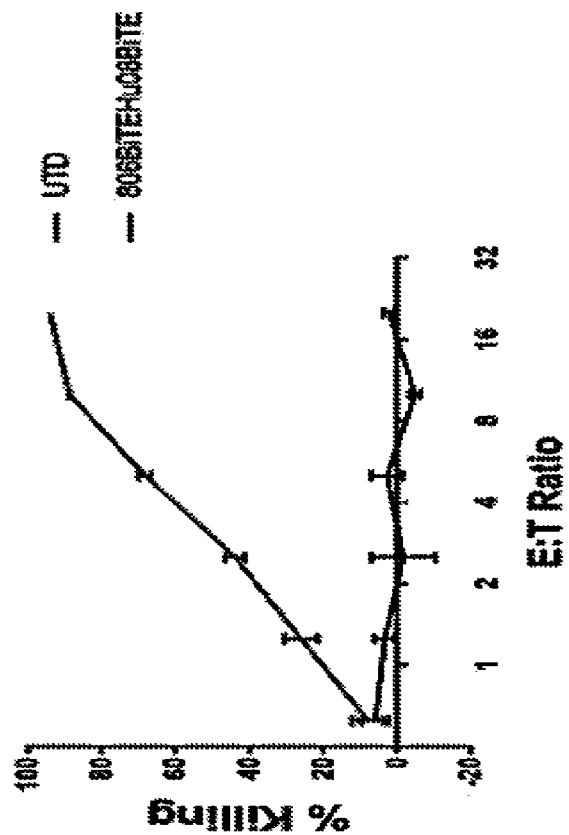


FIG. 37A

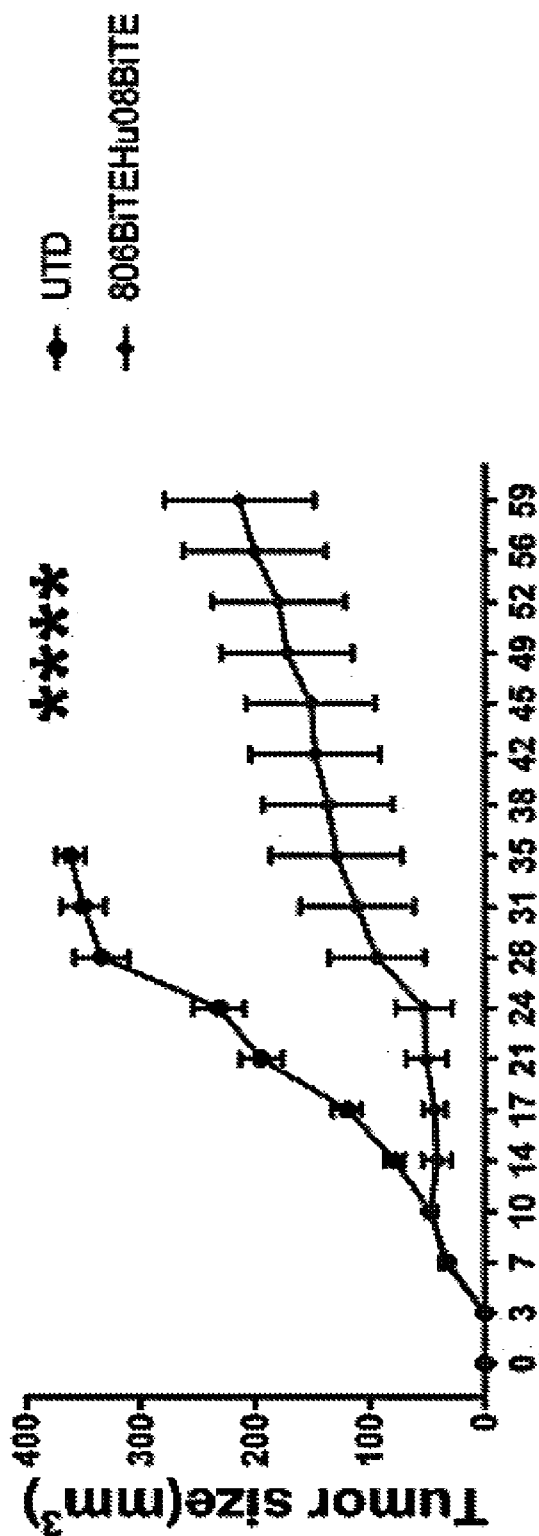
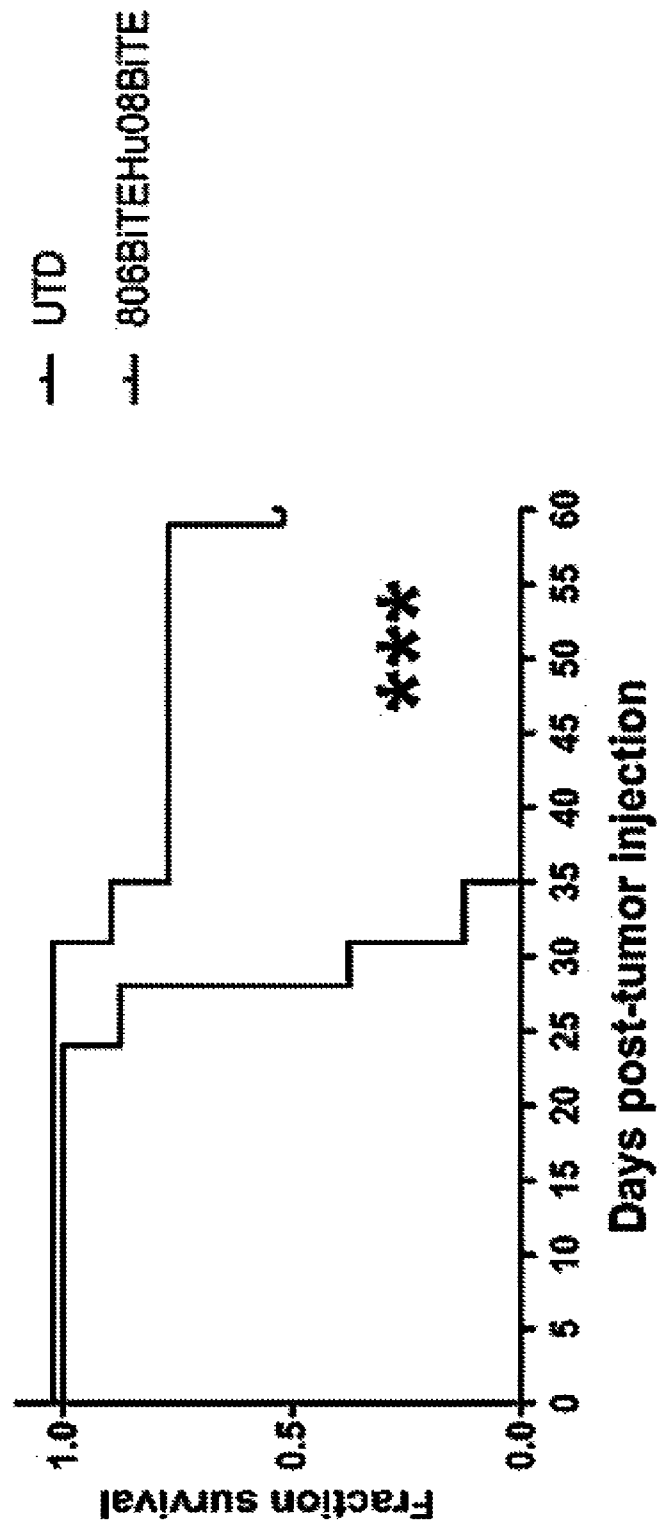


FIG. 37B



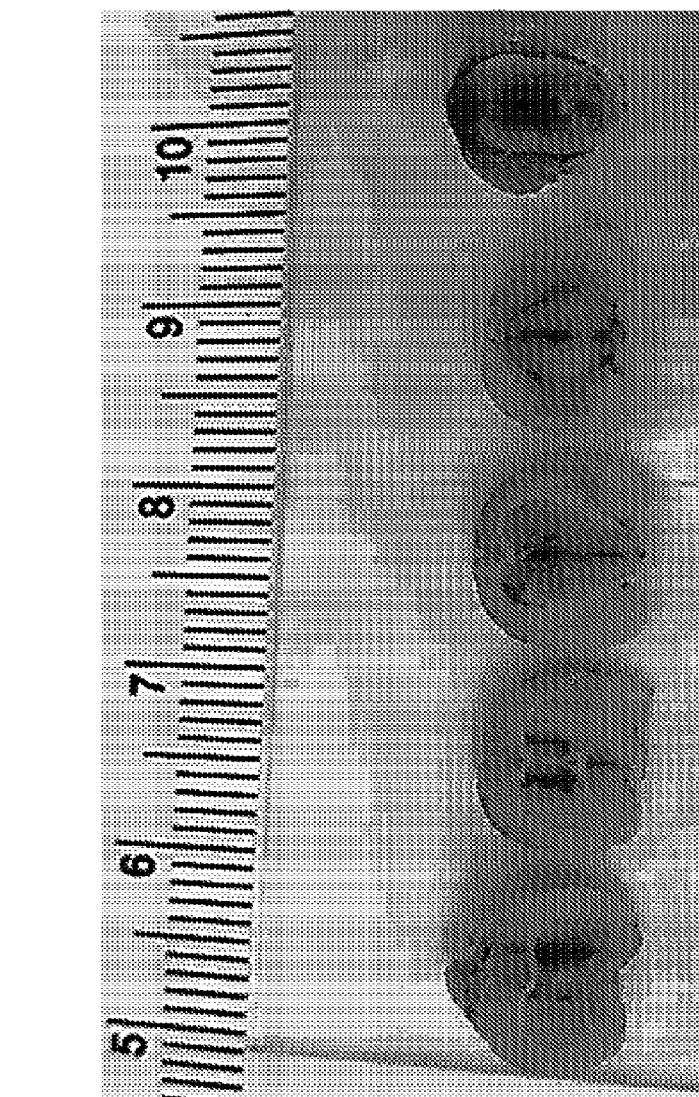


FIG. 38

