

GENERATION OF CAR MODIFIERS FOR TUMOR TREATMENT

CROSS-REFERENCE TO RELATED APPLICATION

The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 63/489,979, filed March 13, 2023, which is hereby incorporated by reference in its entirety herein.

BACKGROUND OF THE INVENTION

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 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 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 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 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 $\alpha 2$ (IL13R $\alpha 2$) is expressed in different human tumor types but no expression is seen on normal human tissues, except adult testes. IL13 signaling through IL13R $\alpha 2$ 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, which is
5 expressed in some normal human tissues and have demonstrated adverse, off-target effects.

Tumor heterogeneity and the immunosuppressive tumor microenvironment (TME) are major obstacles to chimeric antigen receptor (CAR) T cell therapy in GBM. The site-specific reduction in EGFRvIII following CAR T therapy is consistent with the observed intratumoral heterogeneity of this alteration in GBM. Importantly, immunohistochemical analysis of tissue
10 demonstrated the presence of an adaptive response within the GBM TME that closely followed the temporal sequence of CAR T activation. IDO1, PD-L1, IL10, and TGF β were all increased within tumor tissue proximal to CAR T cells following treatment. These immunoregulatory pathways play important roles in the evasion of tumor immunity in a number of settings, and suggest an adaptive resistance develops within GBM that further
15 blunts immune responses within the tumor.

There is a need in the art for improved CAR T therapies that target multiple antigens as well as disrupt the immunosuppressive signals within the TME. The present invention addresses and satisfies this need.

20 SUMMARY OF THE INVENTION

As described herein, the current invention relates to modified immune cells or precursors thereof (*e.g.*, T cells) comprising a first chimeric antigen receptor (CAR) capable of binding human IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII). Compositions and methods
25 of treatment are also provided.

In one aspect, the invention includes a nucleic acid comprising
a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) comprising a first antigen-binding domain that binds human IL13R α 2, a transmembrane domain, and an intracellular domain,
30 a second polynucleotide sequence encoding a second CAR comprising a second antigen-binding domain that binds epidermal growth factor receptor (EGFR) or an isoform thereof, a transmembrane domain, and an intracellular domain, and

a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII).

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

In certain embodiments, the first 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
10 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
15 (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID
NO: 7).

In certain embodiments, the first 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/or a light chain variable region
20 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 first 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.

25 In certain embodiments, the first polynucleotide sequence encodes a CAR comprising
an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%
identical to SEQ ID NO: 23, 24, 42, or 43.

In certain embodiments, the second antigen-binding domain comprises:

a heavy chain variable region that comprises three heavy chain complementarity
30 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 certain embodiments, the second 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: 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 second 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: 33 or 71.

In certain embodiments, the second polynucleotide sequence encodes a CAR comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35 or 75.

In certain embodiments, the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

In certain embodiments, the nucleic acid encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 77 or 79.

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 (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 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 B7-5 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.

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 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 current invention includes a nucleic acid comprising a first polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, and a second polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII).

wherein the CAR comprises 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 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 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: 8; 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: 9.

In certain embodiments, the antigen-binding domain is an 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 certain embodiments, 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, 24, 42, or 43.

In certain embodiments, the DN-TGFβRII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

In another aspect, the current invention includes a nucleic acid comprising a first polynucleotide sequence encoding a CAR comprising an antigen-binding domain that binds epidermal growth factor receptor (EGFR) or an isoform thereof, a transmembrane domain, and an intracellular domain, and

a second polynucleotide sequence encoding a dominant negative TGFβ type II receptor (DN-TGFβRII).

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: 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 is an scFv comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 71.

In certain embodiments, 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, 24, 35, 42, 43, or 75.

In certain embodiments, the DN-TGFβRII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

In certain embodiments, the nucleic acid encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 81, 83, or 85.

5 In another aspect, the current invention 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 a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

the first CAR comprises an antigen-binding domain comprising:

10 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 GVKWAGGSTDYNSALMS (SEQ ID NO: 3), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and

15 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), and

20 wherein the second CAR comprises an antigen-binding domain comprising:

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

25 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), and

wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

In another aspect, the current invention includes a nucleic acid comprising a first polynucleotide sequence encoding a first CAR capable of binding IL13R α 2, a second polynucleotide sequence encoding a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a third polynucleotide sequence encoding a DN-TGF β RII wherein:

5 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: 44 or 54; and

a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%,

10 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 48 or 58; 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: 73; and

a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%,

15 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 74.

In another aspect, the current invention 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 a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a third polynucleotide sequence encoding a DN-TGF β RII wherein:

20 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: 64, 65, 66, or 69; and

the second CAR comprises a single-chain variable fragment (scFv) encoded by a

25 polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 70.

In another aspect, the current invention includes a nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof,

30 and a third polynucleotide sequence encoding a DN-TGF β RII 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: 52 or SEQ ID NO: 53 or SEQ ID NO: 62 or SEQ ID NO: 63; 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: 34.

In certain embodiments, the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2 or is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 14.

In certain embodiments, the first polynucleotide sequence and the second polynucleotide sequence is separated by a linker.

In certain embodiments, the second polynucleotide sequence and the third polynucleotide sequence is separated by a linker.

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

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

In another aspect, the current invention includes a vector comprising the nucleic acid of any of the preceding claims.

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 of the above aspects or embodiments or any aspect for embodiment disclosed herein further comprises an EF-1 promoter.

In certain embodiments, the vector of the above aspects or embodiments or any aspect for embodiment disclosed herein further comprises a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE).

In certain embodiments, the vector of the above aspects or embodiments or any aspect for embodiment disclosed herein further comprises a rev response element (RRE).

In certain embodiments, the vector of the above aspects or embodiments or any aspect for embodiment disclosed herein further comprises a cPPT sequence.

In certain embodiments, the vector is a self-inactivating vector.

In another aspect, the current invention includes a modified immune cell or precursor cell thereof comprising the nucleic acid of any one of claims 1-38, or the vector of any one of claims 39-46.

5 In another aspect, the current 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
10 domain capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof;
and a dominant negative TGF β type II receptor (DN-TGF β RII).

In another aspect, the current invention includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-
15 TGF β RII), 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
20 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
25 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:

30 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
- 5 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 current invention includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of
- 10 binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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/or
- 15 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/or
- 20 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 current invention includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-
- 25 TGF β RII), 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
- 30 amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 71.

In another aspect, the current invention includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of

binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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, 24, 42, or 43; and

5 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: 35 or 75.

In another aspect, the current invention includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-

10 TGF β RII), 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: 44 or 54; and

15 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: 48 or 58; 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: 73; and

20 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: 74.

In another aspect, the current invention includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

25 the first CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 64, 65, 66, or 69; and

the second CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 70.

30 In another aspect, the current invention includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 52 or SEQ ID NO: 53 or SEQ ID NO: 62 or SEQ ID NO: 63; 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: 34.

In certain embodiments, the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2 or is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 14.

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.

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 certain embodiments, the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

In another aspect, the current invention includes a pharmaceutical composition comprising a therapeutically effective amount of the modified cell of any one of claims 47-61.

In another aspect, the current invention includes 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 47-61, or the pharmaceutical composition of claim 62.

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 current 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:

5 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; and a dominant negative TGF β type II receptor (DN-TGF β RII).

10 In another aspect, the current 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 CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein

the first CAR comprises:

15 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

the second CAR comprises:

25 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

30 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 current 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 CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

the first CAR comprises:

5 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:

10 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 another aspect, the current invention includes a method of treating glioblastoma in
15 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

the first CAR comprises an scFv comprising an amino acid sequence at least 80%,
20 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 an scFv comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 71.

In another aspect, the current invention includes a method of treating glioblastoma in
25 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

the first CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%,
30 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or SEQ ID NO: 24 or SEQ ID NO: 42 or SEQ ID NO: 43; 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: 35 or 75.

In certain embodiments, the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2 or is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 14.

5 In another aspect, the current 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 CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

10 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: 44 or 54; 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: 48 or 58; and

15 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: 73; 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: 74.

20 In another aspect, the current 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 CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

25 the first CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 64, 65, 66, or 69; and

the second CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 70.

30 In another aspect, the current 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 CAR capable of binding IL13R α 2, a second CAR capable

of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

the first CAR comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 52 or SEQ ID NO: 53 or SEQ ID NO: 62 or SEQ ID NO: 63; and

the second CAR comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34.

In certain embodiments, the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2 or is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 14.

In another aspect, the current 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 polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 76 or 78.

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.

FIG. 1: Schematic for dominant-negative TGF β -RII CART-EGFR-IL13R α 2 construct. To inhibit the repressive signaling pathway, a truncated TGF- β receptor II was generated, which lacks the intracellular kinase domain at residue 199as, rendering it incapable of phosphorylating downstream signals. This was integrated into a construct comprising a parallel CAR targeting EGFR and IL13R α 2, to overcome the dual challenges of antigen heterogeneity and the suppressive tumor microenvironment.

FIGs. 2A-2D: TGF β 1 is highly expressed in GBM. FIG. 2A: TGF β 1 expression segregated by glioma histology based on RNA-seq data from the Cancer Genome Atlas (TCGA) database. The y-axis indicates the relative expression levels of each case. FIG. 2B: Overall survival curve for high and low TGF β 1 expression group in accordance with the Cancer Genome Atlas (TCGA) database, calculated by upper and lower quartile of the TGF β 1 expression on Kaplan-Meier analysis. FIG. 2C: TGF β 1 is expressed in GBM cell lines. The different glioblastoma cell lines were cultured at a density of 3e6 cells per flask for

3 days. The conditioned medium was collected to test the latent TGF β secretion by glioblastoma cell lines. PC3 is one of the cell lines that has been demonstrated having high level secretion to be compared with GBM cell lines as a positive control. FIG. 2D:

Immunohistochemical staining with anti-TGF- β 1 antibody on tissue sections from NSG mice intracranially implanted with U87 and D270 gliomas. The spleen and cerebral cortex sections were used as positive and negative controls, respectively. Statistically significant differences were calculated by Kruskal-Wallis Test, **p<0.01, ***p<0.001, ****p<0.0001.

FIGs. 3A-3D: T cells expressing dnTGF β RII construct block immunosuppressive TGF- β signaling. FIG. 3A: Schematic vector maps of 806-Hu07-dnTGF β RII, 806-Hu07-mCherry, and dnTGF β RII-M5 CAR constructs. 806Hu07mCherry served as a corresponding control, and the dnTGF β RII-M5 is a mesothelin CAR containing the dnTGF β RII that was used as a relevant negative control. FIG. 3B: Flow cytometric detection of T cell transduction. T cells were transduced with lentiviral vectors. CAR T cells had approximate equal expression among three groups. TGF receptor II expression was screened on CAR T cells with anti TGF β antibody. The expression was around 35% of the CAR with dnTGF β RII. This confirmed that the CAR and dominant negative TGF β RII both expressed on T cells successfully. Upper row is stained for CAR expression by biotinylated protein L; lower row is stained for TGF β RII. FIG. 3C and D: TGF- β signal induction of each group was assessed via intracellular phosphorylated Smad2/3 and quantified in (D). dnTGF β RII blocks immunosuppressive TGF β signaling in CAR-806-Hu07.

FIGs. 4A-4B: Short-term CAR cytotoxicity is unaffected by dnTGF β RII *in vitro*. FIG. 4A: U87vIII-CBG luciferase target cells were co-cultured with effector T cells at different effector: target ratios for 16 hours, with either 20ng/ml of hTGF β 1 or media only. FIG. 4B: D270 target cells were co-cultured with effector T cells for 37 hours, screened by axion impedance.

FIG. 5: 806Hu07dnTGF β RII CAR T cells exhibit reduced PD1 expression in co-culture *in vitro*. Transduced or UTD T cells (2×10^5 cells per well in 100 μ L R10 media) were co-cultured with target cells (2×10^5 cells per well in 100 μ L R10 media) in 96-well round bottom tissue culture plates, at 37°C with 5% CO₂ for 20hrs. T cells were collected and stained with CD3+PD1+, using MFI to analyze.

FIGs. 6A-6B: Human TGF β 1 does not reduce the activation of 806Hu07dnTGF β CAR T cells *in vitro*. Transduced or UTD T cells (2×10^5 cells per well in 100 μ L R10 media) were co-cultured with target cells (2×10^5 cells per well in 100 μ L R10 media) in 96-well

round bottom tissue culture plates, at 37°C with 5% CO₂ for 16hrs. T cells were collected and stained with CAR+CD69+ or CAR+CD25+.

FIG. 7: dnTGFβRII boosted proliferation of CAR T cells with repeated stimulation. CAR T cells were restimulated by U87vIII tumor cell lines with either conditioned media or media only every week. The comparison of proliferation between 806-Hu07-dnTGFβRII and 806-Hu07-mCherry CAR T cells that were either in conditioned media or media only.

FIG. 8: Collected the supernatant from proliferation coculture plates on days 7, 14, and 21, then stored it at -80 degrees celsius for future analysis of cytokine secretion. In addition to improved proliferation, the 806-Hu07-dnTGFβRII CAR T cells also secreted a higher quantity of effector cytokines than the 806-Hu07-mCherry CAR T cells, including IFN-γ, TNF-α, IL-12, GM-CSF, and IL-2.

FIG. 9: 806-Hu07-dnTGFβRII changes to an effector cell phenotype. FIG. 9A: In vitro long-term restimulation assay was performed weekly, with T cells being collected and stained to assess their phenotypic evolution over time, specifically on Day 0, Day 14, and Day 21. These changes were evaluated within different subsets: Central Memory (CM), Naive (N), Effector (E), and Effector Memory (EM) T cells. FIG. 9B: The most notable alteration occurred in the cohort subjected to 20ng/ml of TGF-β1. On Day 14, the 806-Hu07-dnTGFβRII cells demonstrated a significantly more pronounced effector T cell phenotype than the 806-Hu07-mCherry CAR T cells (p=0.0015). FIG. 9C: By Day 21, the final time point, the 806-Hu07-dnTGFβRII cells exhibited an enhanced “effector memory” phenotype (p=0.0009). FIG. 9D: The CAR expression in both 806-Hu07-dnTGFβRII and 806-Hu07-mCherry CAR T cells was compared. These cells were subjected to either conditioned media (+) or media alone (-), and this comparison was conducted at the same time points as above. The CAR expression was normalized to 41% at Day 0 to start later assays.

FIG. 10A-10D: dnTGFβRII construct is safe *in vivo*. FIG. 10A: Schematic of the D270-CBG-GFP NSG mice model (n = 5 per cohort). 5e5 tumor cells were implanted subcutaneously and treated by intravenous infusion of CAR T cells into each mouse, 7 days after tumor implantation. The BLI of tumor burden was performed 3-4 days. FIG. 10B: Mice weight were measured every 3-4 days. FIG. 10C: Tumor size was measured by the calipers in length and width as the area of tumor. FIG. 10D: Tumor regression was compared between each treated mouse by using BLI.

FIG. 11A-11D: 806-Hu07-dnTGFβRII CAR T cells enhance eradication of GBM tumors *in vivo*. FIG. 11A: Schematic of the U87vIII CBG-GFP NSG mice model (n =

5 per cohort). 5e5 tumor cells were implanted intracranially and treated by intravenous infusion of CAR T cells into each mouse, 8 days after tumor implantation. The BLI of tumor burden was performed 3-4 days. FIG. 11B: Survival curves of treated mice were computed using the log-rank test with P values adjusted by the Bonferroni correction for multiple comparisons survival, based on time to experimental endpoint, was plotted using a Kaplan-Meier curve. FIG. 11C-11D: Tumor size was compared between each treated mouse by using BLI.

DETAILED DESCRIPTION

10 The present invention provides compositions and methods comprising modified immune cells or precursors thereof (*e.g.*, modified T cells) comprising a first chimeric antigen receptor (CAR) capable of binding human IL13R α 2, a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β R). The provided compositions and methods are useful for
15 treating cancer (*e.g.*, glioma, high-grade astrocytoma, and glioblastoma).

Transforming Growth Factor β (TGF β), the ligand for TGF β RII and a cytokine with a well-established role in cancer suppression, is highly expressed in GBM and increases following CART-EGFRvIII therapy. A number of immunosuppressive features within the tumor microenvironment (TME) have been attributed to TGF β including M2 polarization of
20 macrophages, iTregs and direct effects on naïve and effector T cells' proliferation, differentiation and function. Expression of a truncated type II TGF β (TGF β RII) lacking the kinase domain serves as a dominant negative TGF β type II receptor (DN-TGF β R) to inhibit TGF β signaling in cells. Expression of DN-TGF β R in human T cells with a CAR through use of a lentiviral vector enhanced anti-tumor effects *in vivo* in a xenograft model of prostate
25 cancer. Given the high TGF β expression within GBM, and in order to mitigate TGF- β mediated suppressive activity, a dominant-negative TGF- β receptor II (dnTGF β RII) was combined with a bicistronic CART-EGFR-IL13R α 2 construct, to generate CART-EGFR-IL13R α 2-dnTGF β RII, a tri-modular construct. Without wishing to be bound by theory, it was hypothesized that this approach would more effectively subvert resistance mechanisms
30 observed with GBM. The data disclosed herein demonstrates that CART-EGFR-IL13R α 2-dnTGF β RII significantly augments T cell proliferation and enhances functional responses, particularly in TGF β -rich tumor environments. Additionally, *in vivo* studies validated the

safety and efficacy of the dnTGFβRII cooperating with CARs in targeting and eradicating GBM in a NSG mouse model.

As overcoming the adaptive changes in the local tumor microenvironment and addressing antigen heterogeneity is required to improve the clinical efficacy of CAR T-directed strategies. The current disclosure demonstrates that bicistronic CART constructs efficiently cooperate with truncated TGFβ receptors II. There are multiple benefits to using the dominant negative TGFβRII in conjunction with CAR constructs, including CART-EGFR-IL13Rα2, including the suppression of immunosuppressive TGFβ signaling and the reduction in PD-1 expression by immune effector cells. In certain embodiments, DN-TGFβRII receptors enhance the proliferative ability of CAR T cells in situations of chronic antigen stimulation without significant side effects *and* have result in enhanced eradication of tumors. In certain embodiments, the tri-modular CAR T constructs disclosed herein, including TGFβRII CART-EGFR-IL13Rα2, address the clinical challenges of antigenic heterogeneity and the immunosuppressive TME in GBM.

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 sequence 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.

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.

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 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.

“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

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 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).

5 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;
10 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.

15 "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
20 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
25 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
30 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, or can exist in a non-native environment such as, for example, a host cell.

5 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 lentiviruses. Vectors derived from lentiviruses offer the means to achieve significant levels of gene transfer *in vivo*.

10 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.

15 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.

20 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.”

25 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 versions contain an intron(s).

30 “Parenteral” administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), intracerebroventricular, intracranial, or intrasternal injection, or infusion techniques. In certain embodiments, the immunogenic compositions disclosed herein can be delivered to the CNS by way of intracerebroventricular administration (e.g., with an Ommaya catheter).

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.

5 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.

10 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
15 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.

20 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.

25 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.

30 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,
5 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
10 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
15 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
20 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.
25 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
30 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 chimeric antigen receptors (CARs) capable of binding IL13R α 2 and/or epidermal growth factor receptor (EGFR) or an isoform thereof. CARs of the invention are used in conjunction with a dominant negative TGF β type II receptor (DN-TGF β RII). In certain embodiments, the CAR comprises an antigen binding domain capable of binding IL13R α 2, a transmembrane domain, and an intracellular domain. In certain embodiments, the CAR comprises an antigen binding domain capable of binding EGFR or an isoform thereof, 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.

A subject CAR of the invention comprises an antigen binding domain capable of binding IL13R α 2 and/or epidermal growth factor receptor (EGFR), 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 and/or epidermal growth factor receptor (EGFR), a hinge domain, a transmembrane domain, and an intracellular domain.

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 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 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
5 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 embodiment, the CAR comprises an antigen-binding domain capable of
10 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 GVKWAGGSTDYNSALMS (SEQ ID NO: 3), and HCDR3 comprises the amino acid
15 sequence DHRDAMDY (SEQ ID NO: 4); and/or 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 embodiment, the CAR comprises an antigen-binding domain capable of
20 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
25 TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence 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 KASQDVGTA (SEQ ID NO: 16), LCDR2 comprises the amino acid sequence SASYRST (SEQ ID NO: 17), and LCDR3 comprises the amino acid
30 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.

5 In certain embodiments, the CAR capable of binding IL13R α 2, comprises 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/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: 9.

10 In certain embodiments, the CAR capable of binding IL13R α 2, comprises 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 certain embodiments, the CAR capable of binding IL13R α 2 comprises an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises an scFv comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10, 11, 21, or 22.

20 In certain embodiments, the CAR capable of binding IL13R α 2, 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: 42 or SEQ ID NO: 43 or SEQ ID NO: 52 or SEQ ID NO: 53 or SEQ ID NO: 62 or SEQ ID NO: 63 or SEQ ID NO: 75.

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

25 In another embodiment, the CAR comprises an antigen-binding domain capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, 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 GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNP SLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGPYW (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 embodiment, the CAR capable of binding EGFR or an isoform thereof comprises an antigen-binding domain comprising a heavy chain variable region comprising
 5 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 another embodiment, the CAR capable of binding EGFR or an isoform thereof
 10 comprises an antigen-binding domain comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 71.

In another embodiment, the CAR capable of binding EGFR or an isoform thereof comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35 or 75.

15 *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
 20 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 is capable of binding EGFR or an isoform thereof. In certain embodiments, the antigen-binding domain is capable of binding an EGFR isoform selected from the group consisting of wild type EGFR (wtEGFR),
 25 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.

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
 30 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: 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.

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: 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 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.

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-
 5 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,
 10 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
 15 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: 86), (GGGS)_n (SEQ ID NO: 87), and (GGGGS)_n (SEQ ID NO: 88), where n represents an integer of at least 1. Exemplary linker sequences can
 20 comprise amino acid sequences including, without limitation, GGSG (SEQ ID NO: 89), GGSGG (SEQ ID NO: 90), GSGSG (SEQ ID NO: 91), GSGGG (SEQ ID NO: 92), GGGSG (SEQ ID NO: 93), GSSSG (SEQ ID NO: 94), GGGGS (SEQ ID NO: 95), GGGGSGGGGSGGGGS (SEQ ID NO: 68) 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
 25 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 (SEQ ID NO: 68), which may be encoded by the nucleic acid sequence
 GGTGGCGGTGGCTCGGGCGGTGGTGGGTGGGTGGCGGCGGATCT (SEQ ID NO:
 30 96).

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 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., *Hybridoma (Larchmt)* 2008 27(6):455-51; Peter et al., *J Cachexia Sarcopenia Muscle* 2012 August 12; Shieh et al., *J Immunol* 2009 183(4):2277-85; 5 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 Biol Chem* 2003 278(38):36740-7; Xie et al., *Nat Biotech* 1997 15(8):768-71; Ledbetter et al., *Crit Rev Immunol* 1997 17(5-6):427-55; Ho et al., *Biochim* 10 *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 constant region; Fc region that does not bind to an antigen).

15 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 20 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 25 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 30 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
5 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
10 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.

15 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.

20 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
25 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,
30 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 triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain.

5 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.

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
10 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 include a domain selected from Fc fragments of antibodies, hinge regions of antibodies, CH2
15 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
20 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
25 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).

30 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: 86) and (GGGS)_n (SEQ ID NO: 87), 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, GSGG (SEQ ID NO: 89), GSGGG (SEQ ID NO: 90), GSGSG (SEQ ID NO: 91), GSGGG (SEQ ID NO: 92), GGGSG (SEQ ID NO: 93), GSSSG (SEQ ID NO: 94), 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.*, 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: 97); CPPC (SEQ ID NO: 98); CPEPKSCDTPPPCPR (SEQ ID NO: 99) (see, *e.g.*, Glaser et al., *J. Biol. Chem.* (2005) 280:41494-41503); ELKTPLGDTTHT (SEQ ID NO: 100); KSCDKTHTCP (SEQ ID NO: 101); KCCVDCP (SEQ ID NO: 102); KYGPPCP (SEQ ID NO: 103); EPKSCDKTHTCPPCP (SEQ ID NO: 104) (human IgG1 hinge); ERKCCVECP (SEQ ID NO: 105) (human IgG2 hinge); ELKTPLGDTTHTCPRCP (SEQ ID NO: 106) (human IgG3 hinge); SPNMVPHAHHAQ (SEQ ID NO: 107) (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 EPKSCDKTYTCTPPCP (SEQ ID NO: 104); 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.

5

Intracellular Domain

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
 10 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 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
 15 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
 20 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
 25 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
 30 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 (KLRF1), 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, CDlib, 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-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 (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, 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 (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. In certain embodiments, the intracellular domain comprises an intracellular domain of CD3ζ.

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 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, 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 include 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 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 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.

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.

- 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
- 5 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
2	DN-TGFβRII	MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNN GAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCV AVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKIMKEKKK PGETFFMCSSSDECDNIIFSEEYNTSNPDLLL VIFQVTGISLLP PLGVAISVIIIIFYCYRVNRQQLSS
14	DN-TGFβRII	ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACAT CGTCCTGTGGACGCGTATCGCCAGCACGATCCCACCGCACG TTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAAC AACGGTGCAGTCAAGTTTCCACAACGTGTAAATTTTGTGA TGTGAGATTTTCCACCTGTGACAACCAGAAATCCTGCATGA GCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAA GTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAA CACTAGAGACAGTTTGCCATGACCCCAAGCTCCCCTACCAT GACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATG AAGGAAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGTTT CTGTAGCTCTGATGAGTGCAATGACAACATCATCTTCTCAG AAGAATATAACACCAGCAATCCTGACTTGTGTGTCATATA TTTCAAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAGT TGCCATATCTGTCATCATCATCTTCTACTGCTACCGCGTTAA CCGGCAGCAGAAGCTGAGTTCATAG
1	Hu07 HCDR1	TKYGVH
3	Hu07 HCDR2	GVKWAGGSTDYNSALMS
4	Hu07 HCDR3	DHRDAMDY
5	Hu07 LCDR1	TASLSVSSTYLH
6	Hu07 LCDR2	STSNLAS
7	Hu07 LCDR3	HQYHRSPLT
8	Hu07 VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLTKEYGVHWVRQAPG KGLEWVGKWKWAGGSTDYNSALMSRFTISKDNAKNSLYLQMN SLRAEDTAVYYCARDHRDAMDYWGQGTLLTVSS
9	Hu07 VL	DIQMTQSPSSLSASVGDRTITCTASLSVSSTYLHWYQQKPGSS PKLWIYSTSNLASGVPSRFSGSGSGTSYTLTISSLQPEDFATYYC HOYHRSPLTFGGGKVEIK
10	Hu07 scFv (VL>VH)	DIQMTQSPSSLSASVGDRTITCTASLSVSSTYLHWYQQKPGSS PKLWIYSTSNLASGVPSRFSGSGSGTSYTLTISSLQPEDFATYYC HOYHRSPLTFGGGKVEIKGGGGSGGGSGGGGSEVQLVESG GGLVQPGGSLRLSCAASGFSLTKEYGVHWVRQAPGKGLEWVG VKWAGGSTDYNSALMSRFTISKDNAKNSLYLQMN SLRAEDTAVYYCARDHRDAMDYWGQGTLLTVSS
11	Hu07 scFv (VH>VL)	EVQLVESGGGLVQPGGSLRLSCAASGFSLTKEYGVHWVRQAPG KGLEWVGKWKWAGGSTDYNSALMSRFTISKDNAKNSLYLQMN

		SLRAEDTAVYYCARDHRDAMDYWGQGTLLTVSSGGGGSGG GGSGGGGSDIQTQSPSSLSASVGDRVITITCTASLSVSSTYLH WYQQKPGSSPKLWIYSTSNLASGVPSRFSGSGSGTSYTLTISSL QPEDFATYYCHQYHRSPLTFGGGKVEIK
23	Hu07-BBz CAR (VH>VL)	MALPVTALLPLALLHAARPGSEVQLVESGGGLVQPGGSLRL SCAASGFSLTKEYGVHWVRQAPGKGLEWVGKWAAGSTDYN SALMSRFTISKDNAKNSLYLQMNSLRAEDTAVYYCARDHRDA MDYWGQGTLLTVSSGGGGSGGGGSGGGGSDIQTQSPSSLSA SVGDRVITITCTASLSVSSTYLHWYQQKPGSSPKLWIYSTSNLA SGVPSRFSGSGSGTSYTLTISSLQPEDFATYYCHQYHRSPLTFG GGTKVEIKSGTTTPAPRPPTPAPTASQPLSLRPEACRPAAGGA VHTRGLDFACDIYIWAPLAGTCGVLLSLVITLYCKRGRKKLL YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYKQGQNQLYNELNLGRREEYDVLDRRGRDPGEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR
55	Hu07-BBz CAR (VL>VH)	MALPVTALLPLALLHAARPGSDIQTQSPSSLSASVGDRVIT TCTASLSVSSTYLHWYQQKPGSSPKLWIYSTSNLASGVPSRFS GSGSGTSYTLTISSLQPEDFATYYCHQYHRSPLTFGGGKVEIK GGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGF SLTKYGVHWVRQAPGKGLEWVGKWAAGSTDYNSALMSRF TISKDNAKNSLYLQMNSLRAEDTAVYYCARDHRDAMDYWG QGTLLTVSSSGTTTPAPRPPTPAPTASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLSLVITLYCKRGRKKLL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYKQGQNQLYNELNLGRREEYDVLDRRGRDPGEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR
12	Hu08 HCDR1	SRNGMS
13	Hu08 HCDR2	TVSSGGSYIYYADSVKG
15	Hu08 HCDR3	QGTALATRFDDV
16	Hu08 LCDR1	KASQDVGTAVA
17	Hu08 LCDR2	SASYRST
18	Hu08 LCDR3	QHHYSAPWT
19	Hu08 VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQTPD KRLEWVATVSSGGSYIYYADSVKGRFTISRDNANKNSLYLQMS SLRAEDTAVYYCARQGTALATRFDDVWGQGTLLTVSS
20	Hu08 VL	DIQTQSPSSLSASVGDRVITITCKASQDVGTAVAWYQQIPGKA PKLLIYSASYRSTGVPDRFSGSGSGTDFSFHISLQPEDFATYYC QHHYSAPWTFGGGKVEIK
21	Hu08 scFv (VH>VL)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQTPD KRLEWVATVSSGGSYIYYADSVKGRFTISRDNANKNSLYLQMS SLRAEDTAVYYCARQGTALATRFDDVWGQGTLLTVSSGGG GSGGGGSGGGGSDIQTQSPSSLSASVGDRVITITCKASQDVGT AVAWYQQIPGKAPKLLIYSASYRSTGVPDRFSGSGSGTDFSFHIS SLQPEDFATYYCQHHYSAPWTFGGGKVEIK
22	Hu08 scFv (VL>VH)	DIQTQSPSSLSASVGDRVITITCKASQDVGTAVAWYQQIPGKA PKLLIYSASYRSTGVPDRFSGSGSGTDFSFHISLQPEDFATYYC QHHYSAPWTFGGGKVEIKGGGGSGGGGSGGGGSEVQLVES GGGLVQPGGSLRLSCAASGFTFSRNGMSWVRQTPDKRLEWV ATVSSGGSYIYYADSVKGRFTISRDNANKNSLYLQMSSLRAEDT AVYYCARQGTALATRFDDVWGQGTLLTVSS
24	Hu08 CAR (VH>VL)	MALPVTALLPLALLHAARPGSEVQLVESGGGLVQPGGSLRL SCAASGFTFSRNGMSWVRQTPDKRLEWVATVSSGGSYIYYAD

		SVKGRFTISRDNAKNSLYLQMSSLRAEDTAVYYCARQGTAL ATRFDDVWQGTLLVTVSSGGGGSGGGGGSGGGGSDIQMTQSPS SLSASVGDRTTITCKASQDVGTAVAWYQQIPGKAPKLLIYSAS YRSTGVDPDRFSGSGSGTDFSFHSSLQPEDFATYYCQHHYSAPW TFGGGKTKVEIKSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYKQGQNLYNELNLGRREEYDVLDRRRGRDPMEGGKRP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGDGLY QGLSTATKDTYDALHMQALPPR
25	806 HCDR1	GYSITSDFAWN
26	806 HCDR2	GYISYSGNTRYNP SLK
27	806 HCDR3	VTAGRGFPYW
28	806 LCDR1	HSSQDINSNIG
29	806 LCDR2	HGTNLDD
30	806 LCDR3	VQY AQFPWT
31	806 VH	DVQLQESGPSLVKPSQSLSLTCTVTGYISITSDFAWN WIRQFPG NKLEWMGYISYSGNTRYNP SLKSRISITRDTSKNQFFLQNSVT IEDTATYYCVTAGRGFPYWQGTLLVTVSA
73	806 VH	GACGTACAAC TGCAAGAATCCGGGCCGAGTTTGGTCA AGCCCTCTCAATCTCTTTCTCTCACTTGACGGTCACC GGATACTCCATAACCAGCGATTTTGCGTGGAATTGGAT TCGACAATTTCCAGGGAATAAATTGGAATGGATGGGA TATATCAGTTATTCTGGTAATACCAGATACAACCCGTC ATTGAAAAGTCGCATCTCTATAACACGAGACACTTCA AAGAATCAGTTCTTCTTCCTTCAGCTCAATTCTGTAAACCAT CGAAGATACTGCTACTTATTACTGTGTAAACGGCGGGTC GAGGATTCCTTACTGGGGCCAGGGTACACTGGTTACT GTTTCCGCC
32	806 VL	DILMTQSPSSMSVSLGDTVSI TCHSSQDINSNIGWLQQRPGKSF KGLIYHGTNLDDDEVPSRFSGSGADYSLTISSESEDFADYYC VQY AQFPWTFGGGKLEIKR
74	806 VL	GATATTCTGATGACTCAATCTCCGCTCTTCTATGAGCGTGAGC TTGGGTGACACCGTCAGCATCACCTGTCATTCCAGCCAGGA TATAAACTCAAATATCGGCTGGCTCCAGCAACGCCAGGCA AGTCATTCAAGGGGCTTATTTATCATGGCACCAATCTTGAC GATGAAGTCCCATCACGCTTCAGCGGATCAGGCTCAGGTGC GGACTATTCCTTGACTATAAGTTCCCTCGAATCTGAGGATTT CGCCGACTATTATTGCGTACAATACGCCAGTTTCCCTGGA CCTTCGGAGGCGGCACCAAATTGGAGATAAAAAGG
70	806 scFv (VL>VH)	GATATTCTGATGACTCAATCTCCGCTCTTCTATGAGCGTGAGC TTGGGTGACACCGTCAGCATCACCTGTCATTCCAGCCAGGA TATAAACTCAAATATCGGCTGGCTCCAGCAACGCCAGGCA AGTCATTCAAGGGGCTTATTTATCATGGCACCAATCTTGAC GATGAAGTCCCATCACGCTTCAGCGGATCAGGCTCAGGTGC GGACTATTCCTTGACTATAAGTTCCCTCGAATCTGAGGATTT CGCCGACTATTATTGCGTACAATACGCCAGTTTCCCTGGA CCTTCGGAGGCGGCACCAAATTGGAGATAAAAAGGGGTGG AGGAGGATCAGGCGGGGTGGAAGCGGCGGAGGAGGCAG CGACGTACAAC TGCAAGAATCCGGGCCGAGTTTGGTCAAGC CCTCTCAATCTCTTTCTCTCACTTGACGGTCACCGGATACT CCATAACCAGCGATTTTGCGTGGAATTGGATTTCGACAATTT CCAGGGAATAAATTGGAATGGATGGGATATATCAGTTATTC

		TGGTAATACCAGATACAACCCGTCATTGAAAAGTCGCATCT CTATAACACGAGACACTTCAAAGAATCAGTTCTTCCTTCAG CTCAATTCTGTAACCATCGAAGATACTGCTACTTATTACTGT GTAACGGCGGGTCGAGGATTCCCCTACTGGGGCCAGGGTAC ACTGGTTACTGTTTCCGCC
33	806 scFv (VH>VL)	DVQLQESGPSLVKPSQSLSLTCTVTGYSTSDFAWNWIRQFPG NKLEWMGYISYSGNTRYNP SLKSRISITRDT SKNQFFLQLNSVT IEDTATYYCVTAGRGFPYWGQGLVTVSAGGGSGGGSGG GGSDILMTQSPSSMSVSLGDTV SITCHSSQDINSNIGWLQQRPG KSFKGLIYHGTNLDDEVPSRFSGSGSGADYSLTISSLESEDFAD YYCVQY AQFPWTFGGG TKLEIKR
71	806 scFv (VL>VH)	DILMTQSPSSMSVSLGDTV SITCHSSQDINSNIGWLQQRPGKSF KGLIYHGTNLDDEVPSRFSGSGSGADYSLTISSLESEDFADYYC VQY AQFPWTFGGG TKLEIKRGGGGSGGGSGGGGSDVQLQES GPSLVKPSQSLSLTCTVTGYSTSDFAWNWIRQFPGNKLEWMG YISYSGNTRYNP SLKSRISITRDT SKNQFFLQLNSVTIEDTATYY CVTAGRGFPYWGQGLVTVSA
34	806-BBZ-CAR (VL>VH)	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTT CTGCTCCACGCCGCTCGGCCCGATATTCTGATGACTCAATCT CCGTCTTCTATGAGCGTGAGCTTGGGTGACACCGTCAGCAT CACCTGTCATTCCAGCCAGGATATAAACTCAAATATCGGCT GGCTCCAGCAACGCCAGGCAAGTCATTCAAGGGGCTTATT TATCATGGCACCAATCTTGACGATGAAGTCCCATCACGCTT CAGCGGATCAGGCTCAGGTGCGGACTATTCCTTGACTATAA GTTCCCTCGAATCTGAGGATTTCCCGGACTATTATTGCGTAC AATACGCCCAGTTTCCCTGGACCTTCGGAGGCGGCACCAAA TTGGAGATAAAAAGGGGTGGAGGAGGATCAGGCGGGGGTG GAAGCGGCGGAGGAGGCAGCGACGTACAACCTGCAAGAATC CGGGCCGAGTTTGGTCAAGCCCTCTCAATCTCTTTCTCTCAC TTGCACGGTCACCGGATACTCCATAACCAGCGATTTTGCGT GGAATTGGATTTCGACAATTTCAGGGAATAAATTGGAATGG ATGGGATATATCAGTTATTCTGGTAATACCAGATACAACCC GTCATTGAAAAGTCGCATCTCTATAACACGAGACACTTCAA AGAATCAGTTCTTCTTCAGCTCAATTCTGTAACCATCGAAG ATACTGCTACTTATTACTGTGTAACGGCGGGTCCGAGGATT CCCTACTGGGGCCAGGGTACACTGGTTACTGTTTCCGCCAC CACTACCCAGCACCGAGGCCACCCACCCCGGCTCCTACCA TCGCCTCCAGCCTCTGTCCCTGCGTCCGAGGCATGTAGA CCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTT CGCCTGCGATATCTACATTTGGGCCCCCTCTGGCTGGTACTTG CGGGGTCTGCTGCTTTCAGTCTGATCACTCTTTACTGTAA GCGCGGTCCGAAGAAGCTGCTGTACATCTTTAAGCAACCCT TCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGT TCATGCCGGTTCCAGAGGAGGAGGAAGGCGGCTGCGAAC TGCGCGTGAAATTCAGCCGACGCGCAGATGCTCCAGCCTAC AAGCAGGGGAGAAACAGCTCTACAACGAACTCAATCTTG GTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGG ACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAAT CCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGA TGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACG CAGAAGAGGCAAAGGCCACGACGACTGTACCAGGGACTC AGCACCGCCACCAAGGACACCTATGACGCTCTTACATGCA GGCCCTGCCGCTCGG

35	806-BBZ-CAR (VL>VH)	MALPVTALLLPLALLLHAARPDILMTQSPSSMSVSLGDTV SITC HSSQDINSNIGWLQQRPGKSFGLIYHGTNLDDEVPSRFSGSGS GADYSLTISSESEDFADYYCVQY A QFPWTFGGG TKLEIKRGG GGSGGGSGGGGSDVQLQESGPSLVKPSQSLTCTVTGY SIT SDFAWN WIRQFPGNKLEWMGYISYSGNTRYNP SLKSRISITRD TSKNQFFLQLNSVTIEDTATYYCVTAGRGFPYWGQGLVTVS ATTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRSADAPAYKQGGN QLYNELNLGRREEYDVL DKRRGRDP EMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR
75	806-BBZ-CAR (VH>VL)	MALPVTALLLPLALLLHAARPDVQLQESGPSLVKPSQSLTCT TVTGY SITSDFAWN WIRQFPGNKLEWMGYISYSGNTRYNP SL KSRI SITRDTSKNQFFLQLNSVTIEDTATYYCVTAGRGFPYWGQ GTLVTVSAGGGSGGGSGGGSDILMTQSPSSMSVSLGDTV SITCHSSQDINSNIGWLQQRPGKSFGLIYHGTNLDDEVPSRFS GSGSGADYSLTISSESEDFADYYCVQY A QFPWTFGGG TKLEI KRTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRP RPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRSADAPAYKQGGN NQLYNELNLGRREEYDVL DKRRGRDP EMGGKPRRKNPQEGLY YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
36	CD8 hinge	ACCACTACCCAGCACCAGGCCACCCACCCCGGCTCCTAC CATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTA GACCCGAGCTGGTGGGGCCGTGCATACCCGGGTCTTGAC TTCGCCTGCGAT
37	CD8 trans- membrane domain	ATCTACATTTGGGCCCCCTCTGGCTGGTACTTGCGGGGTCTG CTGCTTTCACTCGTGATCACTCTTTACTGT
38	4-IBB intra- cellular domain	AAGCGCGGTTCGGAAGAAGCTGCTGTACATCTTTAAGCAACC CTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCT GTTTCATGCCGTTCCAGAGGAGGAGGAAGGCGGCTGCGA ACTG
39	CD3-zeta	CGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAA GCAGGGGCAGAACAGCTCTACAACGAACCTCAATCTTGGTC GGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACG GGACCCAGAAATGGGCGGGAAGCCGCCAGAAAGAATCCC CAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGG CAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAG AAGAGGCCAAAGGCCACGACGGACTGTACCAGGGACTCAGC ACCGCCACCAAGGACACCTATGACGCTCTTCACATGCAGGC CCTGCCGCTCGG
40	T2A	GGCAGCGGAGAGGGCAGAGGAAGTCTTCTAACATGCGGTG ACGTGGAGGAGAATCCCGGC
41	P2A	GGCTCCGGCGCTACAACTTTAGTCTGCTGAAACAGGCTGG AGATGTGGAGGAAAACCCCGCCCT
43	Hu08 CAR (VL>VH)	MALPVTALLLPLALLLHAARPGSDIQMTQSPSSLSASVGDRTI TCKASQDVGTAVAWYQQIPGKAPKLLIYSASYRSTGVPDRFSG SGSGTDFSFHSSLPEDFATYYCQHHYSAPWTFGGG TKVEIKG GGSGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFT FSRNGMSWVRQTPDKRLEWVATVSSGGSYIYYADSVKGRFTI SRDNAKNSLYLQMSSLRAEDTAVYYCARQGTALATRFDFV

		WGQGTLVTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKRGRK KLLYIFKQPFMRPVQTTQEEEDGCSCRFPEEEEGGCELRVKFSRS ADAPAYKQGGNQLYNELNLGRREEYDVLDKRRGRDPENGGK PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL YQGLSTATKDTYDALHMQALPPR
44	Hu07 VH	GAAGTACAGCTGGTTGAGAGTGGCGGGGGTCTCGTACAGCC CGCGGGGTCTCTTAGGCTCTCTGTGCTGCTTCTGGTTCTC CTTGACTAAATACGGGGTACATTGGGTTCCGACGGCCCCTG GCAAAGGTCTTGAATGGGTGGGCGTCAAGTGGGCTGGCGG AAGCACTGATTATAATTCCGCATTGATGTCCCGATTCACTAT TTCTAAGGATAATGCCAAGAACAGTCTCTATTGCAAATGA ACTCCCTGAGAGCGGAGGATACTGCCGTTTACTACTGTGCA CGGGATCACCGAGACGCTATGGATTACTGGGGTCAAGGTAC CCTGGTGACCGTAAGCTCC
45	Hu07 HCDR1	ACTAAATACGGGGTACAT
46	Hu07 HCDR2	GGCGTCAAGTGGGCTGGCGGAAGCACTGATTATAATTCCGC ATTGATGTCC
47	Hu07 HCDR3	GATCACCGAGACGCTATGGATTAC
48	Hu07 VL	GACATACAAATGACACAGTCCCCCTCATCCTTGCTGCTTCC GTAGGAGACCGGGTTACCATCACGTGCACCGCTTCTTTGTC CGTTTCAAGTACCTACCTCCACTGGTACCAGCAAAAACCCG GCAGCAGCCCCAAGTTGTGGATTTACTCAACTTCTAACTTG GCCTCAGGGGTACCGTCAAGATTTAGCGGATCTGGCAGTGG CACGAGTTATACTTTGACGATATCAAGCCTTCAACCGGAGG ATTTCCGCACCTATTACTGTATCATCATCGAAGCCCCCT TGACCTTTGGGGGAGGGACAAAAGTGAAATAAAA
49	Hu07 LCDR1	ACCGCTTCTTTGTCCGTTTCAAGTACCTACCTCCAC
50	Hu07 LCDR2	TCAACTTCTAACTTGGCCTCA
51	Hu07 LCDR3	CATCAGTATCATCGAAGCCCCCTTGACC
52	Hu07 CAR (VL>VH)	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTG CTGCTCCACGCCGCCAGGCCGGGATCCGACATACAAATGAC ACAGTCCCCCTCATCCTTGCTGCTTCCGTAGGAGACCGGGT TACCATCACGTGCACCGCTTCTTTGTCCGTTTCAAGTACCTA CCTCCACTGGTACCAGCAAAAACCCGGCAGCAGCCCCAAGT TGTGGATTTACTCAACTTCTAACTTGGCCTCAGGGGTACCGT CAAGATTTAGCGGATCTGGCAGTGGCACGAGTTATACTTTG ACGATATCAAGCCTTCAACCGGAGGATTTCCGCACCTATTA CTGTATCAGTATCATCGAAGCCCCCTTGACCTTTGGGGGAG GGACAAAAGTGGAATAAAAAGGGGGAGGTGGAAGTGGTGG CGGTGGATCTGGTGGCGGCGGGTCAGAAGTACAGCTGGTTG AGAGTGGCGGGGGTCTCGTACAGCCCGCGGGTCTCTTAGG CTCTCCTGTGCTGCTTCTGGTTTCTCCTTGACTAAATACGGG GTACATTGGGTTCCGCCAGGCCCTGGCAAAGGTCTTGAATG GGTGGGCGTCAAGTGGGCTGGCGGAAGCACTGATTATAATT CCGCATTGATGTCCCGATTCACTATTCTAAGGATAATGCCA AGAACAGTCTCTATTTGCAAAATGAACTCCCTGAGAGCGGAG GATACTGCCGTTTACTACTGTGCACGGGATCACCGAGACGC TATGGATTACTGGGGTCAGGGTACCCTGGTGACCGTAAGCT CCTCCGGAACCACGACGCCAGCGCCGCGACCACCAACACC GGCGCCACCATCGCGTCGCAGCCCCCTGTCCCTGCGCCAG AGGCGTGCCGCCAGCGGCGGGGGCGCAGTGCACACGAG GGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCT TGGCCGGGACTTGTGGGGTCCCTTCTCCTGTCACTGGTTATCA

		CCTTTACTGCAAACGGGGCAGAAAGAACTCCTGTATATA TTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGA GGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAA GGAGGATGTGAAGTGAAGTTCAGCAGGAGCGCAG ACGCCCCCGCTACAAGCAGGGCCAGAACCAGCTCTATAAC GAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGG ACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCC GAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTG CAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGA TGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCT TTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACG CCTTCACATGCAGGCCCTGCCCCCTCGC
53	Hu07 CAR (VH>VL)	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTG CTGCTCCACGCCGCCAGGCCGGGATCCGAAGTACAGCTGGT TGAGAGTGGCGGGGGTCTCGTACAGCCCCGGCGGGTCTCTTA GGCTCTCCTGTGCTGCTTCTGGTTTTCTCCTTGACTAAATACG GGGTACATTGGGTTCGCCAGGCCCTGGCAAAGGTCTTGAA TGGGTGGGCGTCAAGTGGGCTGGCGGAAGCACTGATTATAA TTCCGCATTGATGTCCCATTCACTATTTCTAAGGATAATGC CAAGAACAGTCTCTATTTGCAAATGAACTCCCTGAGAGCGG AGGATACTGCCGTTTACTACTGTGCACGGGATCACCGAGAC GCTATGGATTACTGGGGTCAGGGTACCCTGGTGACCGTAAG CTCCGGGGGAGGTGGAAGTGGTGGCGGTGGATCTGGTGGC GCGGGTTCAGACATACAAATGACACAGTCCCCCTCATCCTT GTCTGCTTCCGTAGGAGACCGGGTTACCATCACGTGCACCG CTTCTTTGTCCGTTTCAAGTACCTACCTCCACTGGTACCAGC AAAAACCCGGCAGCAGCCCCAAGTTGTGGATTTACTCAACT TCTAACTTGGCCTCAGGGGTACCGTCAAGATTTAGCGGATC TGGCAGTGGCACGAGTTATACTTTGACGATATCAAGCCTTC AACCGGAGGATTTGCCACCTATTACTGTCATCAGTATCAT CGAAGCCCCTGACCTTTGGGGGAGGGACAAAAGTGGAAA TAAAAATCCGGAACCACGACGCCAGCGCCGCGACCAAC ACCGGCGCCACCATCGCGTCGCAGCCCCGTGCCCTGCGCC CAGAGGCGTGCCGGCCAGCGCGGGGGGCGCAGTGCACAC GAGGGGGTGGACTTCGCCTGTGATATCTACATCTGGGCGC CTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTA TCACCCTTTACTGCAAACGGGGCAGAAAGAACTCCTGTAT ATATTCAAACAACCATTTATGAGACCAGTACAACTACTCA AGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAA GAAGGAGGATGTGAAGTGAAGTTCAGCAGGAGCG CAGACGCCCCCGCTACAAGCAGGGCCAGAACCAGCTCTAT AACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTT GGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAG CCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAAC TGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGG GATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGC CTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGA CGCCCTTCACATGCAGGCCCTGCCCCCTCGC
54	Hu08 VH	GAGGTTCAAGTTGGTAGAGTCAGGCGGTGGTCTGGTGCAGCC AGGTGGGTCCCTGCGCCTCAGCTGTGCAGCTTCCGGCTTTA CTTTCTCAAGGAATGGTATGTCCTGGGTACGGCAAACGCCG GACAAACGCCCTTGAGTGGGTAGCTACCGTATCCTCTGGGGG CTCTTACATATACTATGCAGACTCTGTGAAAGGAAGATTTA CAATTTACGCGACAATGCAAAAAATAGTTTGTACCTCCAA ATGTCTAGTCTTAGGGCCGAGGATACTGCCGTCTACTACTG

		TGCACGCCAGGGAACGACGGCTCTTGCTACCCGATTTTTCG ACGTTTGGGGCCAAGGAACGTTGGTGACAGTTAGCAGT
55	Hu08 HCDR1	TCAAGGAATGGTATGTCC
56	Hu08 HCDR2	ACCGTATCCTCTGGGGGCTCTTACATATACTATGCAGACTCT GTGAAAGGA
57	Hu08 HCDR3	CAGGGAAACGACGGCTCTTGCTACCCGATTTTTTCGACGTT
58	Hu08 VL	GACATCCAAATGACTCAGAGCCCCCTCTAGCCTCAGTGC AAG CGTCGGAGACCCGGGTGACCATCACCTGTAAAGCGTCCCAGG ATGTTGGAACGGCAGTAGCTTGGTATCAACAAATCCCAGGG AAGGCTCCAAAGCTCCTTATATACTCTGCTAGTTACAGGTC CACCGGGGTGCCCAGCCGATTCTCTGGCTCCGGGAGCGGCA CTGACTTTTCATTTCATCATTAGTAGTCTTCAACCTGAGGACT TTGCCACCTATTATTGCCAGCACCCTACTCTGCGCCGTGGA CTTTCGGAGGAGGCACGAAGGTTGAAATTA
59	Hu08 LCDR1	AAAGCGTCCCAGGATGTTGGAACGGCAGTAGCT
60	Hu08 LCDR2	TCTGCTAGTTACAGGTCCACC
61	Hu08 LCDR3	CAGCACCACTACTCTGCGCCGTGGACT
62	Hu08 CAR (VH>VL)	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTG CTGCTCCACGCCGCCAGGCCGGGATCCGAGGTTTCAAGTGGT AGAGTCAGGCGGTGGTCTGGTGCAGCCAGGTGGGTCCCTGC GCCTCAGCTGTGCAGCTTCCGGCTTTACTTTCTCAAGGAATG GTATGTCTGGGTACGGCAAACGCCGGACAAACGCCTTGAG TGGGTAGCTACCGTATCCTCTGGGGGCTCTTACATATACTAT GCAGACTCTGTGAAAGGAAGATTTACAATTTACGCGACAA TGCAAAAAATAGTTTGTACCTCCAAATGTCTAGTCTTAGGG CCGAGGATACTGCCGTCTACTACTGTGCACGCCAGGGAACG ACGGCTCTTGCTACCCGATTTTTTCGACGTTTGGGGCCAAGG AACGTTGGTGACAGTTAGCAGTGGTGGAGGTGGGTCTGGCG GAGGTGGAAGTGGTGGAGGCGGGTCCGACATCCAAATGAC TCAGAGCCCCCTCTAGCCTCAGTGCAAGCGTCGGAGACCGGG TGACCATCACCTGTAAAGCGTCCCAGGATGTTGGAACGGCA GTAGCTTGGTATCAACAAATCCCAGGGAAGGCTCCAAAGCT CCTTATATACTCTGCTAGTTACAGGTCCACCGGGGTGCCCG ACCGATTCTCTGGCTCCGGGAGCGGCACTGACTTTTCATTCA TCATTAGTAGTCTTCAACCTGAGGACTTTGCCACCTATTATT GCCAGCACCACTACTCTGCGCCGTGGACTTTCCGAGGAGGC ACGAAGGTTGAAATTAATCCGGAACACGACGCCAGCGC CGCGACCACCAACACCGGGCGCCACCATCGCGTCGACGCC CTGTCCCTGCGCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGACACAGAGGGGGCTGGACTTCGCCTGTGATATC TACATCTGGGCGCCCTTGCCCGGGACTTGTGGGGTCCCTCTC CTGTCACTGGTTATCACCTTTACTGCAAACGGGGCAGAAA GAAACTCCTGTATATATTCAAACAACCATTTATGAGACCCAG TACAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTT CCAGAAGAAGAAGAAGGAGGATGTGAAGTGAAGTGAAGT TCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCA GAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAG GAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCCTGA GATGGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGC CTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCCT ACAGTGAGATTGGGATGAAAGGCGAGCGCCGAGGGGGCAA GGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCA AGGACACCTACGACGCCCTTACATGCAGGCCCTGCCCCCT CGC

63	Hu08 CAR (VL>VH)	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTG CTGCTCCACGCCGCCAGGCCGGGATCCGACATCCAAATGAC TCAGAGCCCTCTAGCCTCAGTGCAAGCGTCGGAGACCGGG TGACCATCACCTGTAAAGCGTCCCAGGATGTTGGAACGGCA GTAGCTTGGTATCAACAAATCCCAGGGAAGGCTCCAAAGCT CCTTATATACTCTGCTAGTTACAGGTCCACCGGGGTGCCCG ACCGATTCTCTGGCTCCGGGAGCGGCACTGACTTTTCATTCA TCATTAGTAGTCTTCAACCTGAGGACTTTGCCACCTATTATT GCCAGCACCACTACTCTGCGCCGTGGACTTTCGGAGGAGGC ACGAAGGTTGAAATTAAGGTGGAGGTGGGTCTGGCGGAG GTGGAAGTGGTGGAGGCGGGTCCGAGGTTCAAGTTGGTAGA GTCAGGCCGTGGTCTGGTGCAGCCAGGTGGGTCCCTGCGCC TCAGCTGTGCAGCTTCCGGCTTTACTTTCTCAAGGAATGGTA TGTCTGGGTACGGCAAACGCCGGACAAACGCCTTGAGTGG GTAGCTACCGTATCCTCTGGGGGCTCTTACATATACTATTGCA GACTCTGTGAAAGGAAGATTTACAATTTACGCGACAATGC AAAAAATAGTTTGTACCTCCAAATGTCTAGTCTTAGGGCCG AGGATACTGCCGTCTACTACTGTGCACGCCAGGGAACGACG GCTCTTGCTACCCGATTTTTTCGACGTTTGGGGCCAAGGAAC GTTGGTGACAGTTAGCAGTTCCGGAACACGACGCCAGCGC CGCGACCACCAACACCGGCGCCACCATCGCGTCGACGCC CTGTCCCTGCGCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGACACAGAGGGGGCTGGACTTCGCCTGTGATATC TACATCTGGGCGCCCTTGCCCGGACTTGTGGGTCTCTCTC CTGTCACTGGTTATCACCTTTACTGCAAACGGGCGAGAAA GAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAG TACAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTT CCAGAAGAAGAAGAAGGAGGATGTGAAGTGAAGTGAAGT TCAGCAGGAGCGCAGACGCCCCCGGTACAAGCAGGGCCA GAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAG GAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGA GATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGC CTGTACAATGAAGTGCAGAAAGATAAGATGGCGGAGGCCCT ACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAA GGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCA AGGACACCTACGACGCCCTTACATGCAGGCCCTGCCCCCT CGC
64	Hu07 scFv (VL > VH)	GACATACAAATGACACAGTCCCCCTCATCCTTGCTCTGCTTCC GTAGGAGACCGGGTTACCATCACGTGCACCGCTTCTTTGTC CGTTTCAAGTACCTACCTCCACTGGTACCAGCAAAAACCCG GCAGCAGCCCCAAGTTGTGGATTTACTCAACTTCTAACTTG GCCTCAGGGGTACCGTCAAGATTTAGCGGATCTGGCAGTGG CACGAGTTATACTTTGACGATATCAAGCCTTCAACCGGAGG ATTTGCGCACCTATTACTGTCATCAGTATCATCGAAGCCCCCT TGACCTTTGGGGGAGGGACAAAAGTGGAAATAAAAGGGGG AGGTGGAAGTGGTGGCGGTGGATCTGGTGGCGGCGGGTCA GAAGTACAGCTGGTTGAGAGTGGCGGGGGTCTCGTACAGCC CGCGGGGTCTCTTAGGCTCTCTGTGCTGCTTCTGGTTTCTC CTTGACTAAATACGGGGTACATTGGGTTTCGCCAGGCCCTTG GCAAAGGTCTTGAATGGGTGGGCGTCAAGTGGGCTGGCGG AAGCACTGATTATAATTCCGCATTGATGTCCCGATTCACTAT TTCTAAGGATAATGCCAAGAACAGTCTCTATTTGCAAATGA ACTCCCTGAGAGCGGAGGATACTGCCGTTTACTACTGTGCA CGGGATACCGAGACGCTATGGATTACTGGGGTCAGGGTAC CCTGGTGACCGTAAGCTCC

65	Hu08 scFv (VH > VL)	GAGGTTCAAGTTGGTAGAGTCAGGCGGTGGTCTGGTGCAGCC AGGTGGGTCCCTGCGCCTCAGCTGTGCAGCTTCCGGCTTIA CTTTCTCAAGGAATGGTATGTCCTGGGTACGGCAAACGCCG GACAAACGCCTTGAGTGGGTAGCTACCGTATCCTCTGGGGG CTCTTACATATACTATGCAGACTCTGTGAAAGGAAGATTTA CAATTTACGCGACAATGCAAAAAATAGTTTGTACCTCCAA ATGTCTAGTCTTAGGGCCGAGGATACTGCCGTCTACTACTG TGCACGCCAGGGAACGACGGCTCTTGCTACCCGATTTTCG ACGTTTGGGGCCAAGGAACGTTGGTGACAGTTAGCAGTGGT GGAGGTGGGTCTGGCGGAGGTGGAAGTGGTGGAGGCGGGT CCGACATCCAATGACTCAGAGCCCCCTCTAGCCTCAGTGCA AGCGTCGGAGACCGGGTGACCATCACCTGTAAAGCGTCCCA GGATGTTGGAACGGCAGTAGCTTGGTATCAACAAATCCCAG GGAAGGCTCCAAAGCTCCTTATATACTCTGCTAGTTACAGG TCCACCGGGGTGCCCGACCGATTCTCTGGCTCCGGGAGCGG CACTGACTTTTCATTTCATCATTAGTAGTCTTCAACCTGAGGA CTTTGCCACCTATTATTGCCAGCACCACTACTCTGCGCCGTG GACTTTCGGAGGAGGCACGAAGGTTGAAATTA
66	Hu08 scFv (VL > VH)	GACATCCAAATGACTCAGAGCCCCCTCTAGCCTCAGTGCAAG CGTCGGAGACCGGGTGACCATCACCTGTAAAGCGTCCCAGG ATGTTGGAACGGCAGTAGCTTGGTATCAACAAATCCCAGGG AAGGCTCCAAAGCTCCTTATATACTCTGCTAGTTACAGGTC CACCGGGGTGCCCGACCGATTCTCTGGCTCCGGGAGCGGCA CTGACTTTTCATTTCATCATTAGTAGTCTTCAACCTGAGGACT TTGCCACCTATTATTGCCAGCACCACTACTCTGCGCCGTGGA CTTTCGGAGGAGGCACGAAGGTTGAAATTAAGGTGGAGG TGGGTCTGGCGGAGGTGGAAGTGGTGGAGGCGGGTCCGAG GTTCAAGTTGGTAGAGTCAGGCGGTGGTCTGGTGACGCCAGG TGGGTCCCTGCGCCTCAGCTGTGCAGCTTCCGGCTTTACTTT CTCAAGGAATGGTATGTCCTGGGTACGGCAAACGCCGGACA AACGCCTTGAGTGGGTAGCTACCGTATCCTCTGGGGGCTCT TACATATACTATGCAGACTCTGTGAAAGGAAGATTTACAAT TTCACGCGACAATGCAAAAAATAGTTTGTACCTCCAAATGT CTAGTCTTAGGGCCGAGGATACTGCCGTCTACTACTGTGCA CGCCAGGGAACGACGGCTCTTGCTACCCGATTTTCGACGT TTGGGGCCAAGGAACGTTGGTGACAGTTAGCAGT
67	Linker	GGGGGAGGTGGAAGTGGTGGCGGTGGATCTGGTGGCGGCG GGTCA
68	Linker	GGGGSGGGSGGGGS
69	Hu07 scFv (VH > VL)	GAAGTACAGCTGGTTGAGAGTGGCGGGGGTCTCGTACAGCC CGGCGGGTCTCTTAGGCTCTCCTGTGCTGCTTCTGGTTTCTC CTTGACTAAATACGGGGTACATTGGGTTCCGCCAGGCCCTG GCAAAGGTCTTGAATGGGTGGGCGTCAAGTGGGCTGGCGG AAGCACTGATTATAATTCCGCATTGATGTCCCGATTCACTAT TTCTAAGGATAATGCCAAGAACAGTCTCTATTGTCAAATGA ACTCCCTGAGAGCGGAGGATACTGCCGTTTACTACTGTGCA CGGGATCACCGAGACGCTATGGATTACTGGGGTCAGGGTAC CCTGGTGACCGTAAGCTCCGGGGGAGGTGGAAGTGGTGGC GGTGGATCTGGTGGCGGCGGGTCAGACATACAAATGACAC AGTCCCCCTCATCCTTGTCTGCTTCCGTAGGAGACCGGGTAA CCATCACGTGCACCGCTTCTTTGTCCGTTTCAAGTACCTACC TCCACTGGTACCAGCAAAAACCCGGCAGCAGCCCCAAGTTG TGGATTTACTCAACTTCTAACTTGGCCTCAGGGGTACCGTCA AGATTTAGCGGATCTGGCAGTGGCACGAGTTATACTTTGAC

		GATATCAAGCCTTCAACCGGAGGATTTGCGCCACCTATTACT GTCATCAGTATCATCGAAGCCCCTTGACCTTTGGGGGAGGG ACAAAAGTGGAAATAAAA
70	806Hu07dnTGFβ RII	GCCACCATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTG GCTCTTCTGCTCCACGCCGCTCGGCCCCGATATTCTGATGACT CAATCTCCGTCTTCTATGAGCGTGAGCTTGGGTGACACCGT CAGCATCACCTGTCATTCCAGCCAGGATATAAACTCAAATA TCGGCTGGCTCCAGCAACGCCAGGCAAGTCATTCAAGGGG CTTATTTATCATGGCACCAATCTTGACGATGAAGTCCCATCA CGCTTCAGCGGATCAGGCTCAGGTGCGGACTATTCCTTGAC TATAAGTTCCCTCGAATCTGAGGATTTGCGCGACTATTATTG CGTACAATACGCCAGTTTCCCTGGACCTTCGGAGGCGGCA CCAAATTGGAGATAAAAAGGGGTGGAGGAGGATCAGGCGG GGGTGGAAGCGGCGGAGGAGGCAGCGACGTACAACGTCAA GAATCCGGGGCGAGTTTGGTCAAGCCCTCTCAATCTCTTTCT CTCACTTGACGGTCAACGGATACTCCATAACCAGCGATTT TGCGTGGAATTGGATTTCGACAATTTCCAGGGAATAAATTGG AATGGATGGGATATATCAGTTATTCTGGTAATACCAGATAC AACCCGTCATTGAAAAGTCGCATCTCTATAACACGAGACAC TTCAAAGAATCAGTTCTTCTTCAGCTCAATTCTGTAACCAT CGAAGATACTGCTACTTATTACTGTGTAACGGCGGGTCGAG GATTCCCCTACTGGGGCCAGGGTACACTGGTTACTGTTTCC GCCACCACTACCCAGCACCAGGCGCCACCCACCCCGGCTCC TACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCAT GTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTT GACTTCGCCTGCGATATCTACATTTGGGCCCCCTCTGGCTGGT ACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTAC TGTAAGCGCGGTGCGGAAGAAGCTGCTGTACATCTTTAAGCA ACCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACG GCTGTTTCATGCCGTTTCCCAGAGGAGGAGGAAGGCGGCTGC GAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGC CTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAAT CTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGA GAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAA GAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGAT AAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGG AACGCAGAAGAGGCAAAGGCCACGACGACTGTACCAGGG ACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACA TGCAGGCCCTGCCGCCTCGGGTTAACGGCTCCGGCGCTACA AACTTTAGTCTGCTGAAACAGGCTGGAGATGTGGAGGAAA ACCCCGGCCCTTCTAGAATGGCCTTACCAGTGACCGCCTTG CTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGG ATCCGACATACAAATGACACAGTCCCCCTCATCCTTGCTGTG CTTCCGTAGGAGACCGGGTTACCATCACGTGCACCGCTTCT TTGTCCGTTTCAAGTACCTACCTCCACTGGTACCAGCAAAA ACCCGGCAGCAGCCCCAAGTTGTGGATTTACTCAACTTCTA ACTTGGCCTCAGGGGTACCGTCAAGATTTAGCGGATCTGGC AGTGGCACGAGTTATACTTTGACGATATCAAGCCTTCAACC GGAGGATTTGCCCACCTATTACTGTCATCAGTATCATCGAA GCCCCTTGACCTTTGGGGGAGGGACAAAAGTGGAAATAAA AGGGGGAGGTGGAAGTGGTGGCGGTGGATCTGGTGGCGGC GGGTCAGAAGTACAGCTGGTTGAGAGTGGCGGGGGTCTCGT ACAGCCCCGGCGGTCTCTTAGGCTCTCCTGTGCTGCTTCTGG TTTCTCCTTGACTAAATACGGGGTACATTGGGTTCCGCCAGG CCCCTGGCAAAGGTCTTGAATGGGTGGGCGTCAAGTGGGCT

		GGCGGAAGCACTGATTATAATTCCGCATTGATGTCCCGATT CACTATTTCTAAGGATAATGCCAAGAACAGTCTCTATTTGC AAATGAACTCCCTGAGAGCGGAGGATACTGCCGTTTACTAC TGTGCACGGGATCACCGAGACGCTATGGATTACTGGGGTCA GGGTACCCTGGTGACCGTAAGCTCCTCCGGAACCACGACGC CAGCGCCGCGACCACCAACACCGGGCGCCACCATCGCGTCG CAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGC GGGGGGCGCAGTGCACACGAGGGGGCTGGAATTCGCCCTGT GATATCTACATCTGGGCGCCCTTGCGCCGGGACTTGTGGGGT CCTTCTCCTGTCACTGGTTATCACCCTTTACTGCAAACGGGG CAGAAAGAACTCCTGTATATATTCAAACAACCATTTATGA GACCAGTACAACTACTCAAGAGGAAGATGGCTGTAGCTG CCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGA GTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCTACAAGC AGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACG AAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGG GACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTC AGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGC GGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGG AGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTAC AGCCACCAAGGACACCTACGACGCCCTTACATGCAAGGCC TGCCCCCTCGCACTAGTGGCAGCGGAGAGGGCAGAGGAAG TCTTCTAACATGCGGTGACGTGGAGGAGAATCCCCGGCCCTA GGATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCAC ATCGTCCTGTGGACGCGTATCGCCAGCACGATCCCACCGCA CGTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACA ACAACGGTGCAGTCAAGTTTCCACAACGTGTGTAATTTGT GATGTGAGATTTTCCACCTGTGACAACCAGAAATCCTGCAT GAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGG AAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACAT AACACTAGAGACAGTTTGCCATGACCCCAAGCTCCCCCTACC ATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTA TGAAGGAAAAAAAAAGCCTGGTGAGACTTTCTTCATGTGT TCCTGTAGCTCTGATGAGTGCAATGACAACATCATCTTCTCA GAAGAATATAACACCAGCAATCCTGACTTGTGTAGTCAT ATTTCAAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAG TTGCCATATCTGTCATCATCATCTTCTACTGCTACCGCGTTA ACCGGCAGCAGAAGCTGAGTTCATAG
77	806Hu07dnTGFβ RII	ATMALPVTALLLPLALLLHAARPDILMTQSPSSMSVSLGDTVSI TCHSSQDINSNIGWLQQRPGKSFKGLIYHGTNLDDEVPSRFSGS GSGADYSLTISSLESEDFADYYCVQYAQFPWTFGGGKLEIKR GGGGSGGGSGGGGSDVQLQESGPSLVKPSQSLSLTCTVTGYS ITSDFAWNWIRQFPGNKLEWMGYISYSGNTRYNP SLKSRISITR DTSKNQFFLQLNSVTIEDTATYYCVTAGRGFPYWQGTLVTV SATTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKLLYIFKQPFM RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQMGQ NQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPRRKNPQEGL YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPRVNGSGATNFSLLKQAGDVEENPGPSRMAL PVTALLLPLALLLHAARPGSDIQMTQSPSSLSASVGDRTITCT ASLSVSSTYLHWYQKPGSSPKLWIYSTSNLASGVPSRFSGSGS GTSYTLTISSLQPEDFATYCHQYHRSPLTFGGGTKVEIKGGG GSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSKAASGFSLT KYGVHWRQAPGKGLEWVGKWAAGGSTDYNSALMSRFTIS

		KDNAKNSLYLQMNSLRAEDTAVYYCARDHRDAMDYWGQGT LVTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKLLYIF KQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPA YKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS TATKDTYDALHMQALPPRTSGSGEGRGSLLTCGDVEENPGPR MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNN GAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPOEVCV AVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKICIMKEKKK PGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLL VIFQVTGISLLP PLGVAISVIIIIFYCYRVNRQQKLSS
78	806Hu08dnTGFβ RII	GCCACCATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTG GCTCTTCTGCTCCACGCCGCTCGGCCGATATTCTGATGACT CAATCTCCGTCTTCTATGAGCGTGAGCTTGGGTGACACCGT CAGCATCACCTGTCATTCCAGCCAGGATATAAACTCAAATA TCGGCTGGCTCCAGCAACGCCCAGGCAAGTCATTCAAGGGG CTTATTTATCATGGCACCAATCTTGACGATGAAGTCCCATCA CGCTTCAGCGGATCAGGCTCAGGTGCGGACTATTCCCTTGAC TATAAGTTCCTCGAATCTGAGGATTTGCGCGACTATTATTG CGTACAATAACGCCCAGTTTCCCTGGACCTTCGGAGCGGCA CCAAATTGGAGATAAAAAGGGGTGGAGGAGGATCAGGCGG GGGTGGAAGCGGCGGAGGAGGCAGCGACGTACAACCTGCAA GAATCCGGGCGGAGTTTGGTCAAGCCCTCTCAATCTCTTTCT CTCACTTGACGGGTCACCGGATACTCCATAACCAGCGATT TGCGTGGAATTGGATTGACAATTTCCAGGGAATAAATTGG AATGGATGGGATATATCAGTTATTCTGGTAATACCAGATAC AACCCGTCATTGAAAAGTCGCATCTCTATAACACGAGACAC TTCAAAGAATCAGTTCTTCCTTCAGCTCAATTCTGTAACCAT CGAAGATACTGCTACTTATTACTGTGTAACGGCGGGTGCAG GATTCCCCTACTGGGGCCAGGGTACACTGGTTACTGTTCC GCCACCACTACCCAGCACCGAGGCCACCCACCCCGGCTCC TACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCAT GTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTT GACTTCGCCTGCGATATCTACATTTGGGCCCTCTGGCTGGT ACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTAC TGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCA ACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACG GCTGTTTCATGCCGTTCCCAGAGGAGGAGGAAGGCGGCTGC GAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGC CTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAAT CTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGA GAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAA GAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGAT AAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAAGGGG AACGCAGAAGAGGCAAAGGCCACGACGGACTGTACAGGG ACTCAGCACCCGCCACCAAGGACACCTATGACGCTCTTCACA TGCAGGCCCTGCCGCTCGGGTTAACGGCTCCGGCGCTACA AACTTTAGTCTGCTGAAACAGGCTGGAGATGTGGAGGAAA ACCCCGGCCCTTCTAGAATGGCCTTACCAGTGACCGCCTTG CTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGG ATCCGACATCCAAATGACTCAGAGCCCCTCTAGCCTCAGTG CAAGCGTCGGAGACCGGGTGACCATCACCTGTAAGCGTCC CAGGATGTTGGAACGGCAGTAGCTTGGTATCAACAATCCC AGGGAAGGCTCCAAAGCTCCTTATATACTCTGCTAGTTACA

		GGTCCACCGGGGTGCCCCACCGATTCTCTGGCTCCGGGAGC GGCACTGACTTTTCATTCATCATTAGTAGTCTTCAACCTGAG GACTTTGCCACCTATTATTGCCAGCACCACTACTCTGCGCCG TGGACTTTCGGAGGAGGCACGAAGGTTGAAATTAAGGTG GAGGTGGGTCTGGCGGAGGTGGAAGTGGTGGAGGCGGGTC CGAGGTTCAGTTGGTAGAGTCAGGCGGTGGTCTGGTGCAGC CAGGTGGGTCCCTGCGCCTCAGCTGTGCAGCTTCCGGCTTT ACTTTCTCAAGGAATGGTATGTCTGGGTACGGCAAACGCC GGACAAACGCCTTGAGTGGGTAGCTACCGTATCCTCTGGGG GCTCTTACATATACTATGCAGACTCTGTGAAAGGAAGATTT ACAATTTACGCGACAATGCAAAAAATAGTTTGTACCTCCA AATGTCTAGTCTTAGGGCCGAGGATACTGCCGTCTACTACT GTGCACGCCAGGGAACGACGGCTCTTGCTACCCGATTTTC GACGTTTGGGGCCAAGGAACGTTGGTGACAGTTAGCAGTTC CGGAACCACGACGCCAGCGCCGCGACCAACACCGGCG CCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGC GTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGG CTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGCC GGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCTT TACTGCAAACGGGGCAGAAAGAACTCCTGTATATATTCAA ACAACCATTTATGAGACCAGTACAACTACTCAAGAGGAA GATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAG GATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGACGC CCCCGCGTACAAGCAGGGCCAGAACCAGCTCTATAACGAG CTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAA GAGACGTGGCCGGGACCCTGAGATGGGGGAAAGCCGAGA AGGAAGAACCCTCAGGAAGGCCTGTACAATGAATGCAGA AAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAA AGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTAC CAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCT TCACATGCAGGCCCTGCCCCCTCGCACTAGTGGCAGCGGAG AGGGCAGAGGAAGTCTTCAACATGCGGTGACGTGGAGGA GAATCCCGGCCCTAGGATGGGTGCGGGGCTGCTCAGGGGCC TGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGCCAGC ACGATCCCACCGCACGTTTCAGAAGTCGGTTAATAACGACAT GATAGTCACTGACAACAACGGTGCAGTCAAGTTTCCACAAC TGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACAACC AGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGT GAGAAGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGA ATGACGAGAACATAACACTAGAGACAGTTTGCCATGACCCC AAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCT CCAAAGTGCATTATGAAGGAAAAAAAAAAGCCTGGTGAGA CTTTCTTCATGTGTTCTGTAGCTCTGATGAGTGCAATGACA ACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGAC TTGTTGCTAGTCATATTTCAAGTGACAGGCATCAGCCTCCTG CCACCACTGGGAGTTGCCATATCTGTATCATCTCTCTAC TGCTACCGCGTTAACCAGCAGCAGAAGCTGAGTTCATAG
79	806Hu08dnTGFβ RII	ATMALPVTALLLPLALLHAARPDILMTQSPSSMSVSLGDTVSI TCHSSQDINSNIGWLQQRPGKSFKGLIYHGTNLDDEVPSRFSGS GSGADYSLTISSLESEDFADYYCVQYAQFPWTFGGGKLEIKR GGGGSGGGSGGGGSDVQLQESGPSLVKPSQSLSLTCTVTGY ITSDFAWNWIQFPGNKLEWMGYISYSGNTRYNPSLKSRSITR DTSKNQFFLQLNSVTIEDTATYYCVTAGRGFPYWQGTLVT SATTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM

		RPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPRVNGSGATNFSLLKQAGDVEENPGPSRMAL PVTALLPLALLLHAARPGSDIQMTQSPSSLSASVGDRVITCK ASQDVGTAVAWYQIPGKAPKLLIYSASYRSTGVPDRFSGSGS GTDFSFISSLQPEDFATYYCQHHSAPWTFGGGKTVEIKGGG GSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSR NGMSWVRQTPDKRLEWVATVSSGGSYIYYADSVKGRFTISR NAKNSLYLQMSLRAEDTAVYYCARQGTTALATRFDDVWGQ GTLVTVSSSGTTTPAPRPPTPAPTASQPLSLRPEACRPAAGGA VHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL YIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSAD APAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPRTSGSGEGRGSLLTCGDVEENP GPRMGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTD NNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEV CVAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEK KKPGETFFMCSSSDECNDNIIFSEEYNTSNPDLLLVIQVGTGIS LLPPLGVAISVIIIFYCYRVNRQQKLSS
80	806dnTGFβRII	GCCACCATGGCCCTCCCTGTACCCGCCCTGCTGCTTCCGCTG GCTCTTCTGCTCCACGCCGCTCGGCCGATATTCTGATGACT CAATCTCCGTCTTCTATGAGCGTGAGCTTGGGTGACACCGT CAGCATCACCTGTCATTCCAGCCAGGATATAAACTCAAATA TCGGCTGGCTCCAGCAACGCCCAGGCAAGTCATTCAAGGGG CTTATTTATCATGGCACCAATCTTGACGATGAAGTCCCATCA CGCTTCAGCGGATCAGGCTCAGGTGCGGACTATTCCCTTGAC TATAAGTTCCTCGAATCTGAGGATTTCCGCCGACTATTATTG CGTACAATACGCCAGTTTCCCTGGACCTTCGGAGGCGGCA CCAAATTGGAGATAAAAAGGGGTGGAGGAGGATCAGGCGG GGGTGGAAGCGGCGGAGGAGGCAGCGACGTACAACGTCAA GAATCCGGGCGGAGTTTGGTCAAGCCCTCTCAATCTCTTTCT CTCACTTGACCGGTACCCGATACTCCATAACCAAGCGATT TGCGTGGAATTGGATTTCGACAATTTCCAGGGAATAAATTGG AATGGATGGGATATATCAGTTATTCTGGTAATACCAGATAC AACCCGTCATTGAAAAGTCGCATCTCTATAACACGAGACAC TTCAAAGAATCAGTTCTTCTTCAGCTCAATTCTGTAACCAT CGAAGATACTGCTACTTATTACTGTGTAACGGCGGGTTCGAG GATTCCCCTACTGGGGCCAGGGTACACTGGTTACTGTTTCC GCCACCACTACCCACGACCCGAGGCCACCCACCCGCGTCC TACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCAT GTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTT GACTTCGCCTGCGATATCTACATTTGGGCCCTCTGGCTGGT ACTTGCGGGTCTCTGCTGCTTTCACTCGTGATCACTCTTTAC TGTAAGCGCGGTGGAAGAAGCTGCTGTACATCTTTAAGCA ACCCCTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACG GCTGTTTCATGCCGTTTCCCAGAGGAGGAGGAAGGCGGCTGC GAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGC CTACAAGCAGGGGCAGAACCAGCTCTACAACGAACCTCAAT CTTGGTTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGA GAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAA GAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGAT AAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGG AACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGG

		ACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACA TGCAGGCCCTGCCGCCTCGGGTTAACGGCTCCGGCGCTACA AACTTTAGTCTGCTGAAACAGGCTGGAGATGTGGAGGAAA ACCCCGGCCCTATGGGTCGGGGGCTGCTCAGGGGCTGTGG CCGCTGCACATCGTCCTGTGGACGCGTATCGCCAGCACGAT CCCACCGCACGTTTCAAGTTCGGTTAATAACGACATGATAG TCACTGACAACAACGGTGCAGTCAAGTTTCCACAACGTGTGT AAATTTTGTGATGTGAGATTTTCCACCTGTGACAACAGAA ATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGA AGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGA CGAGAACATAACACTAGAGACAGTTTGCCATGACCCCAAGC TCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAA AGTGCATTATGAAGGAAAAAAAAAGCCTGGTGAGACTTT CTTTCATGTGTTCTGTAGCTCTGATGAGTGCAATGACAACAT CATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGT TGCTAGTCATATTTCAAGTGACAGGCATCAGCCTCCTGCCA CCACTGGGAGTTGCCATATCTGTCATCATCATCTTCTACTGC TACCGCGTTAATCGGCAGCAGAAGCTGAGTTCATAG
81	806dnTGFβRII	ATMALPVTALLPLALLLHAARPDILMTQSPSSMSVSLGDTVSI TCHSSQDINSNIGWLQQRPGKSFKGLIYHGTNLDDEVSPSRFSGS GSGADYSLTISSLESEDFADYVCVQYAQFPWTFGGGKLEIKR GGGGSGGGGSGGGGSDVQLQESGPSLVKPSQSLSLTCTVTGYS ITSDFAWNWIQFPGNKLEWMGYISYSGNTRYNPGLSRISITR DTSKNQFFLQLNSVTIEDTATYYCVTAGRGFPYWGQGLTVTV SATTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKLLYIFKQPFM RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG YNELQKDKMAEAYSEIGMKGERRRGKGHDLGLYQGLSTATKD TYDALHMQALPPRVNGSGATNFSLLKQAGDVEENPGPMGRG LLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKF PQLCKFCDFVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRK NDENITLETVCHDPKLPYHDFILEDAAAPKIMKEKKKPGETFF MCSSSDECNDNIIFSEYNTSNPDLLLVIQVGTGISLLPLGVAI SVIIFCYRVRNRQQLSS
82	Hu07dnTGFβRII	GCCACCATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCT GGCTTGCTGCTCCACGCCGCCAGGCCGGGATCCGACATAC AAATGACACAGTCCCCCTCATCCTTGCTGCTTCCGTAGGA GACCGGGTTACCATCACGTGCACCGCTTCTTTGTCCGTTTCA AGTACCTACCTCCACTGGTACCAGCAAAAACCCGGCAGCAG CCCCAGTTGTGGATTACTCAACTTCTAACTTGGCCTCAGG GGTACCGTCAAGATTAGCGGATCTGGCAGTGGCAGCAGATT ATACTTTGACGATATCAAGCCTTCAACCGGAGGATTTCCGCC ACCTATTACTGTCATCAGTATCATCGAAGCCCCCTTGACCTTT GGGGGAGGGACAAAAGTGGAATAAAAGGGGGAGGTGGA AGTGGTGGCGGTGGATCTGGTGGCGGCGGGTCAGCAAGTAC AGCTGGTTGAGAGTGGCGGGGTCTCGTACAGCGGAGCGG GTCTCTTAGGCTCTCCTGTGCTGCTTCTGGTTTCTCCTTGACT AAATACGGGGTACATTGGGTTCGCCAGGCCCTTGCAAAAGG TCTTGAATGGGTGGGCGTCAAGTGGGCTGGCGGAAGCACTG ATTATAATTCCGCATTGATGTCCCGATTCACTATTTCTAAGG ATAATGCCAAGAACAGTCTCTATTTGCAAATGAACTCCCTG AGAGCGGAGGATACTGCCGTTTACTACTGTGCACGGGATCA CCGAGACGCTATGGATTACTGGGGTCAGGGTACCTCGGTGA CCGTAAGCTCCTCCGGAACCACGACGCCAGCGCCGCGACCA

		CCAACACCGGCGCCACCATCGCGTCGCAGCCCCTGTCCCT GCGCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTG CACACGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTG GCGCCCTTGCCGGGACTTGTGGGGTCTTCTCCTGTCACT GGTATCACCCCTTACTGCAAACGGGGCAGAAAGAACTCC TGTATATATTCAAACAACCATTTATGAGACCAGTACAAACT ACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGA AGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGG AGCGCAGACGCCCCCGCGTACAAGCAGGGCCAGAACCAGC TCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGA TGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGG GGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACA ATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGA GATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCAC GATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACAC CTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCGTTA ACGGCTCCGGCGCTACAACTTTAGTCTGCTGAAACAGGCT GGAGATGTGGAGGAAAACCCCGGCCCTATGGGTCTGGGGGC TGCTCAGGGGCCTGTGGCCGCTGCACATCGTCTGTGGACG CGTATCGCCAGCACGATCCACCGCACGTTTCAAGTTCGGT TAATAACGACATGATAGTCACTGACAACAACGGTGCAGTCA AGTTTCCACAACCTGTGTAAATTTTGTGATGTGAGATTTTCCA CCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATC ACCTCCATCTGTGAGAAGCCACAGGAAGTCTGTGTGGCTGT ATGGAGAAAAGAATGACGAGAACATAACACTAGAGACAGTT TGCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAA GATGCTGCTTCTCCAAAGTGCATTATGAAGGAAAAAAGAA GCCTGGTGAGACTTTCTTCATGTGTTCTGTAGCTGTGATGA GTGCAATGACAACATCATCTTCTCAGAAGAATATAACACCA GCAATCCTGACTTGTTGCTAGTCATATTTCAAGTGACAGGC ATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTGTGCATC ATCATCTTCTACTGCTACCGCGTTAATCGGCAGCAGAAGCT GAGTTCATAG
83	Hu07dnTGFβRII	ATMALPVTALLPLALLHAARPGSDIQMTQSPSSLSASVGD VTITCTASLSVSSTYLHWYQQKPGSSPKLWIYSTNLASGVPSR FSGSGGTSYTLTISSLQPEDFATYYCHQYHRSPLTFGGGTKE IKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAAS GFSLTKEYGVHWVRQAPGKLEWVGKWAAGSTDYNSALMS RFTISKDNKNSLYLQMNSLRAEDTAVYYCARDHRDAMDYW GQGLTVTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLSLVITLYCKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPRVNGSGATNFSLLKQAGDVEE NPGPMGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVT DNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKE KKKPGETFFMCSSSDECNDNIIFSEYNTSNPDLLLVIQVTGI SLLPPLGVAISVIIIIFYCYRVNRQQLSS
84	Hu08dnTGFβRII	GCCACCATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCT GGCCTTGCTGCTCCACGCCGCCAGGCCGGGATCCGACATCC AAATGACTCAGAGCCCCCTTAGCCTCAGTGCAAGCGTCGGA GACCGGGTGACCATCACCTGTAAAGCGTCCCAGGATGTTGG AACGGCAGTAGCTTGGTATCAACAAATCCCAGGGAAGGCTC

		CAAAGCTCCTTATATACTCTGCTAGTTACAGGTCCACCGGG GTGCCCCGACCGATTCTCTGGCTCCGGGAGCGGCACCTGACTT TTCATTTCATCATTAGTAGTCTTCAACCTGAGGACTTTGCCAC CTATTATTGCCAGCACCCTACTCTGCGCCGTGGACTTTTCGG AGGAGGCACGAAGGTTGAAATTAAGGTGGAGGTGGGTCT GGCGGAGGTGGAAGTGGTGGAGGCGGGTCCGAGGTTTCAGT TGGTAGAGTCAAGCGGTGGTCTGGTGCAGCCAGGTGGGTCC CTGCGCCTCAGCTGTGCAGCTTCCGGCTTTACTTTCTCAAGG AATGGTATGTCCTGGGTACGGCAAACGCCGACAAACGCCT TGAGTGGGTAGCTACCGTATCCTCTGGGGGCTCTTACATAT ACTATGCAGACTCTGTGAAAGGAAGATTTACAATTTACGC GACAAATGCAAAAAATAGTTTGTACCTCCAAATGTCTAGTCT TAGGGCCGAGGATACTGCCGTCTACTACTGTGCACGCCAGG GAACGACGGCTCTTGCTACCCGATTTTTCGACGTTTGGGGC CAAGGAACGTTGGTGACAGTTAGCAGTTCCGGAACACGAC GCCAGCGCCGCGACCACCAACACCGGCGCCACCATCGCGT CGCAGCCCCTGTCCCTGCGCCCGAGGGCGTGCCGGCCAGCG GCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCT GTGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGG GTCCTTCTCCTGTCACCTGGTTATCACCTTTACTGCAACCG GGCAGAAAGAACTCCTGTATATATTCAAACAACCATTTAT GAGACCAGTACAACTACTCAAGAGGAAGATGGCTGTAGC TGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAAGTGA GAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAA GCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGAC GAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCG GGACCTTGAGATGGGGGGAAGCCGAGAAGGAAGAACCTT CAGGAAGGCCTGTACAATGAAGTGCAGAAAGATAAGATGG CGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCG GAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGT ACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGC CCTGCCCCCTCGCGTTAACGGCTCCGGCGCTACAACTTTA GTCTGCTGAAACAGGCTGGAGATGTGGAGGAAAACCCCG CCCTATGGGTGCGGGGGCTGCTCAGGGGGCTGTGGCCGCTG ACATCGTCTGTGGACGCGTATCGCCAGCACGATCCACCG CACGTTTCAGAAAGTCGGTTAATAACGACATGATAGTCACTGA CAACAACGGTGCAGTCAAGTTTCCACAAGTGTGTAAATTTT GTGATGTGAGATTTTCCACCTGTGACAACCAGAAATCCTGC ATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACA GGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAAC ATAACACTAGAGACAGTTTGCCATGACCCCAAGCTCCCTA CCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCAT TATGAAGGAAAAAAAAAGCCTGGTGAGACTTTCTTCATGT GTTCTGTAGCTCTGATGAGTGCAATGACAACATCATCTTCT CAGAAGAATATAACACCAGCAATCCTGACTTGTGTAGTC ATATTTCAAGTGACAGGCATCAGCCTCCTGCCACCACTGGG AGTTGCCATATCTGTCATCATCATCTTCTACTGCTACCGCGT TAATCGGCAGCAGAAGCTGAGTTCATAG
85	Hu08dnTGFβRII	ATMALPVTALLLPLALLLHAARPGSDIQMTQSPSSLSASVGD VTITCKASQDVGTAVAWYQQIPGKAPKLLIYSASYRSTGVPDR FSGSGSGTDFSFIISSLQPEDFATYYCQHHYSAPWTFGGGKVE IKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLS CAASGFTFSRNGMSWVRQTPDKRLEWVATVSSGGSYIYYADSVKGR FTISRDNAKNSLYLQMSSLRAEDTAVYYCARQGTALATRF FDVWQGTLTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEACRPA

		AGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKRGR KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS RSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPRVNGSGATNFSLLKQAG DVEENPGPMGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNND MIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAPKC IMKEKKKPGETFFMCSSDECNDNIIFSEYNTSNPDLLLVIQ VTGISLLPPLGVAISVIIIIFYCYRVNRQQLSS
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C. Tandem and Parallel Bi-Specific CARs

Also provided herein is a tandem CAR, a cell (*e.g.*, T cell) comprising a tandem CAR,
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). 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 biding domain (*e.g.*, a second scFv),
followed by a transmembrane domain and an intracellular domain (*e.g.* 4-1BB and/or CD3 ζ).
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. 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: 77 and may be encoded by the nucleotide sequence of SEQ ID NO: 76. 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:
79 and may be encoded by the nucleotide sequence of SEQ ID NO: 78. 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: 81 and
may be encoded by the nucleotide sequence of SEQ ID NO: 80.

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.

D. Nucleic Acids and Expression Vectors

The present disclosure provides nucleic acids encoding a first CAR capable of binding IL13R α 2a, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII). The nucleic acid of the present disclosure may comprise a polynucleotide sequence encoding any one or more of the CARs disclosed herein.

In one embodiment, a nucleic acid of the present disclosure comprises a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) comprising a first antigen-binding domain that binds human IL13R α 2, a transmembrane domain, and an intracellular domain; a second polynucleotide sequence encoding a second CAR comprising a second antigen-binding domain that binds epidermal growth factor receptor (EGFR) or an isoform thereof, a transmembrane domain, and an intracellular domain; and a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII).

In certain embodiments, a nucleic acid of the present disclosure comprises a first polynucleotide sequence encoding a CAR capable of binding IL13R α 2 and a second polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII). In certain embodiments, the CAR comprises 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

GVKWAGGSTDYNSALMS (SEQ ID NO: 3), and HCDR3 comprises the amino acid sequence DHRDAMDY (SEQ ID NO: 4); and/or 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 nucleic acid encodes a CAR and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the 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: 44 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: 48.

In certain embodiments, the nucleic acid encodes a CAR and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the antigen-binding domain of the 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: 64 or 69.

Also provided is a nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the CAR comprises 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 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 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 nucleic acid encodes a CAR and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the antigen-binding domain of the 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: 54; 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: 58.

In certain embodiments, the nucleic acid encodes a CAR and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the antigen-binding domain of the 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: 65 or 66.

In another aspect, the disclosure provides a nucleic acid comprising a polynucleotide sequence encoding a CAR capable of binding IL13R α 2, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the CAR comprises 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: 44; 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: 48.

In another aspect, the disclosure provides a nucleic acid comprising a polynucleotide sequence encoding a CAR capable of binding IL13R α 2, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the CAR comprises 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: 54; 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: 58.

In another aspect, the invention provides a nucleic acid comprising a first 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: 62 or SEQ ID NO: 63 and a second polynucleotide sequence that encodes a DN-TGF β RII.

In certain embodiments, the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

Also provided is a nucleic acid comprising a first polynucleotide sequence encoding a CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof and a second polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII).

In certain embodiments, the nucleic acid encodes a CAR and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the antigen-binding domain of 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: 31; and/or a light
5 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 nucleic acid encodes a CAR and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the antigen-binding domain is an scFv comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or
10 100% identical to SEQ ID NO: 33 or 71.

In certain embodiments, the nucleic acid encodes a CAR and a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 35 or
15 75.

In certain embodiments, the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

Also provided is a nucleic acid comprising a first polynucleotide sequence encoding a first CAR capable of binding IL13R α 2, a second polynucleotide sequence encoding a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and
20 a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII), 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
25 regions (HCDRs), wherein HCDR1 comprises the amino acid sequence TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence 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
30 (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 TVSSGGSYIYYADSVKG (SEQ ID NO: 13), and HCDR3 comprises the amino acid sequence 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 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 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: 44; 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: 48.

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: 54; 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: 58.

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: 64 or SEQ ID NO: 65 or SEQ ID NO: 66.

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: 52 or SEQ ID NO: 53 or SEQ ID NO: 62 or SEQ ID NO: 63.

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

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: 8 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: 9. 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: 19 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: 20.

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: 70 or SEQ ID NO: 71. 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: 65 or SEQ ID NO: 66 or SEQ ID NO: 64.

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: 34. 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: 35 or SEQ ID NO: 75.

Also provided is a nucleic acid comprising a first polynucleotide sequence encoding a first CAR capable of binding IL13R α 2, a second polynucleotide sequence encoding a CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII), 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 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 GYISYSGNTRYNP SLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFY (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 HGTLNDD (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 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, and a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β R II), 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: 44, or 54; 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: 48, or 58; 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: 73 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: 74.

Also provided is a nucleic acid comprising a first polynucleotide sequence encoding a first CAR capable of binding IL13R α 2, a second polynucleotide sequence encoding a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 69, 64, 65, or 66; 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: 70.

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, and a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 52 or 53 or 62 or 63; 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: 34.

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. In some embodiments, a nucleic acid of the present disclosure comprises a first polynucleotide sequence, a second polynucleotide sequence, and a third polynucleotide sequence. The first and second polynucleotide sequence may be separated by a linker and/or the second and third 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 2 CAR 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, a linker, and the second polynucleotide sequence. In certain embodiments, the nucleic acid comprises from 5' to 3' the second polynucleotide sequence, a linker, and the first polynucleotide sequence. In certain embodiments, the nucleic acid comprises from 5' to 3' the first polynucleotide sequence, a linker, the second polynucleotide sequence, a linker and the third polynucleotide sequence. In certain embodiments, the nucleic acid comprises from 5' to 3' the second polynucleotide sequence, a linker, the first polynucleotide sequence, a linker, and the third polynucleotide sequence.

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.*, cardiovirus, rhinovirus, aphthovirus, HCV, Friend murine leukemia virus (FrMLV), and 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 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), Thosia 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. 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 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 or Arg-X1-Arg-Arg, X2-Arg-X1-X3-Arg (SEQ ID NO:108) and Arg-X1-
5 X1-Arg, such as an Arg-Gln-Lys-Arg (SEQ ID NO:109), where X1 is any naturally occurring amino acid, X2 is Lys or Arg, and X3 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,
10 a linker comprising a nucleic acid sequence encoding Furin and F2A, a linker comprising a 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
15 sequence between the Furin and 2A peptide. Various spacer sequences are known in the art, including, without limitation, glycine serine (GS) spacers such as (GS)_n, (GSGGS)_n (SEQ ID NO: 86) and (GGGS)_n (SEQ ID NO: 87), where n represents an integer of at least 1. Exemplary spacer sequences can comprise amino acid sequences including, without
20 limitation, GGSG (SEQ ID NO: 89), GGS GG (SEQ ID NO: 90), GSGSG (SEQ ID NO: 91), GSGGG (SEQ ID NO: 92), GGGSG (SEQ ID NO: 93), GSSSG (SEQ ID NO: 94), 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
25 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,
30 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 Ncr1 (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.

5 Examples of inducible promoters include, but are not limited to a metallothionine 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, tandem CAR, parallel CAR, and the like) comprising an inducible promoter.

10 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 some embodiments, the locus or construct or transgene containing the suitable promoter is irreversibly switched through the induction of an inducible system. Suitable

15 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,

20 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.

25 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

30 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 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; 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 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

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.

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 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).

E. Modified Immune Cells

The present invention provides modified immune cells or precursors thereof (*e.g.*, T cells) comprising a chimeric antigen receptor (CAR) capable of binding IL13R α 2 (*e.g.*, human IL13R α 2), a CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII). 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 includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII). 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 QGTTALATRFFDV (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 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 that comprises three heavy chain complementarity determining regions (HCDRs). HCDR1 comprises the amino acid sequence GYSITSDFAWN (SEQ ID NO: 25), HCDR2
 5 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:
 10 143) or HGTLNDD (SEQ ID NO: 29), and LCDR3 comprises the amino acid sequence VQYAQFPWT (SEQ ID NO: 30).

Another aspect of the invention includes a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a dominant
 15 negative TGF β type II receptor (DN-TGF β R2), 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
 20 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: 31 or SEQ ID NO: 19 or 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: 32 or SEQ ID NO: 9 or SEQ ID NO: 20.

Also provided is a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β R2), wherein the first CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence
 25 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%,
 30 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or SEQ ID NO: 71.

Also provided is a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 35 or 75.

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.

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

F. 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
 5 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,
 10 *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,
 15 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
 20 (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,
 25 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
 30 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
5 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
10 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,
15 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,
20 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
30 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
5 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
10 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
15 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
20 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 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
25 cells' expression or expression level of one or more markers, typically cell surface markers, 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.

30 Such separation steps can be based on positive selection, in which the cells having 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 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 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.

10 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 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 subpopulation, *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 sub-

population, 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 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 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.

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

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
5 controlled freezing may be used as well as uncontrolled freezing immediately at -20°C or in 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
10 of T cells. In yet another embodiment, purified T cells comprise the population of T cells.

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
15 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.

G. Methods of Treatment

20 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.

In one aspect, the invention includes a method of treating a disease or condition in a
25 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 the present invention. In another aspect, the invention includes a method for adoptive cell
30 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, 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
5 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.

10 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
15 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.

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
20 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 embodiments, the administration effectively treats the subject despite the subject having
25 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 over time. In some embodiments, the subject has not relapsed. In some such embodiments,
30 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 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, 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, 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.

5 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, 10 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, 15 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, 20 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 25 conditions related thereto include, without limitation, light chain myeloma, non-secretory myeloma, monoclonal gamopathy of undertermined 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 30 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.

- 5 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

- 10 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
15 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.

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

- 20 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 or transplantation. The compositions described herein may be administered to a patient transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary,
25 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.

- 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
30 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 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⁺ 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 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.

5 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

10 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

15 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

20 (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,

25 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.

30 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
5 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
10 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.
15 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
20 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
25 (*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
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.

5 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.

10 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.

15 In an exemplary embodiment, for a subject having cancer, the subject receives lymphodepleting chemotherapy including 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 chemotherapy including fludarabine at a dose of 30 mg/m² for 3
20 days.

In an exemplary embodiment, for a subject having cancer, the subject receives 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
25 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 cyclophosphamide at a dose of about 300 mg/m²/day, and fludarabine at a dose of 30 mg/m² for 3 days.

30 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. 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 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 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 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 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 atlizumab (marketed as Actemra, or RoActemra). Tocilizumab is 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.

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 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 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 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; and a dominant negative TGF β type II receptor (DN-TGF β RII).

- 5 In certain embodiments, 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 QGTTALATRFFDV (SEQ ID NO: 15); and/or 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).

- In certain embodiments, 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/or 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).

- 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 CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a DN-TGF β RII, 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/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: 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/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.

5 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 first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a DN-TGF β RII, wherein the first CAR comprises a single-chain variable fragment (scFv) comprising an amino acid sequence at least
10 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 an 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
15 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, a second CAR capable of binding EGFR or an isoform thereof, and a DN-TGF β RII, 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 SEQ ID NO: 24 or SEQ ID NO: 55 or SEQ ID NO: 56,
20 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 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
25 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 certain embodiments, the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

H. Expansion of Immune Cells

Whether prior to or after modification of cells to express a CAR, the cells can be
30 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 prior to expansion. In yet another embodiment, the cryopreserved T cells are thawed for electroporation with the RNA encoding the chimeric membrane protein.

5 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, *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
10 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 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
15 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 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
20 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 measured by the amount of time required for the cells to double in number, otherwise known as the doubling time.

25 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 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
30 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-acetylcysteine 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
5 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.

I. Methods of Producing Genetically Modified Immune Cells

10 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(s) is/are introduced into a cell by an expression vector. Expression vectors comprising a nucleic acid sequence encoding a CAR of the present
15 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
20 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
25 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.

30 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
5 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.

10 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
15 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
20 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
25 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
30 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 is 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
5 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
10 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
15 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.

20 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
25 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
30 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.

5 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
10 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
15 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
20 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
25 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.

30 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 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)).

10 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.

15 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.

20 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.

25 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.

30 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
5 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
10 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
15 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
20 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
25 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
30 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 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.

5 **J. Pharmaceutical compositions and Formulations**

Also provided are populations of immune cells of the invention, compositions 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
10 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 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
15 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 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
20 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 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
25 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, 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
30 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; 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 polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; 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).

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 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 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, 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.

- 5 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
- 10 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
- 15 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
- 20 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

25 *Cell lines and culture:*

- The human GSC line (5077) was derived from excised tumor tissue obtained from the University of Pennsylvania Institutional Review Board with written informed consent from the patients (Department of Neurosurgery, Perelman School of Medicine, Philadelphia, PA). It was maintained in DMEM F12 Ham supplemented with penicillin/streptomycin, GlutaMAX-1,
- 30 B27 minus A, epidermal growth factor, and basic fibroblast growth factor (Corning, Corning, NY). The U87MG cell line was obtained from the American Type Culture Collection (ATCC HTB-14) and cultured in MEM containing GlutaMAX-1, HEPES, pyruvate, penicillin/streptomycin (Thermo Fisher Scientific, Carlsbad, CA), and supplemented with 10%

fetal bovine serum (FBS). This cell line was engineered to express the EGFRvIII protein, click beetle green luciferase, and green fluorescent protein through single-cell purification and expansion. The PC3 prostate cancer cell line was obtained from the ATCC (CRL-1435) and cultured in D10 media consisting of DMEM supplemented with 10% FBS, HEPES, penicillin, and streptomycin. The D270 glioma cells were grown and passaged in the flanks of NSG mice. The human glioma cell line U251 was provided by Dr. Jay Dorsey (Department of Radiation Oncology, University of Pennsylvania). The cells were routinely screened for identity and mycoplasma contamination.

10 ***Immunohistochemical Stain:***

Immunohistochemical staining used a recombinant anti-TGF β 1 antibody (ab215715, Abcam, Waltham, Boston) and DAPI on tissue sections derived from NSG mice. These mice had been intracranially implanted with U87 and D270 gliomas, a procedure facilitated by Daniel Martinez (Pathology Core Laboratory of the Children's Hospital of Philadelphia Research Institute, PA). Tissue sections of the spleen and cerebral cortex of mice were utilized as positive and negative controls, respectively.

Vector constructs:

The 806-Hu07-mCherry CAR was assembled by combining an EGFR-targeting scFv (806) and an IL13R α 2-targeting scFv (Hu07), which were synthesized and ligated into a pTRPE lentiviral vector with the EF1 α promoter. The construct ended with an mCherry (Twist Bioscience, San Francisco, CA). The dnTGF β RII structure was digested using AvrII and SalI enzymes and ligated into the 806-Hu07-mCherry construct at the same enzyme sites, replacing the mCherry gene to create the 806-Hu07-dnTGF β RII CAR construct. The CAR-dnTGF β RII-M5 was a gift from Joseph A. Fraietta at the Perelman School of Medicine, University of Pennsylvania. The control CAR-CD19 was a gift from Carl H. June lab, University of Pennsylvania. All CARs have the same second-generation CAR structure, except for the scFv components.

30 ***Human T cell transduction and culture in vitro:***

Lentiviruses were packaged in HEK 293 T cells using a split genome approach and tittered using SUPT1 cells from ATCC (CRL-1942). Normal human T cells were isolated from the PBMC of the Human Immunology Core at the University of Pennsylvania and were transduced using lentiviral vectors. T cells were stimulated for 5 days with Dynabeads Human

T-Activator CD3/CD28 (Life Technologies, Carlsbad, CA) at a bead-to-cell ratio of 3:1. The cell concentration was determined using a Coulter Multisizer (Beckman Coulter, Brea, CA) and maintained at 0.7×10^6 cells per mL until fully rested at approximately 300fl in volume. The cells were cultured in R10 media (RPMI-1640 supplemented with GlutaMAX-1, HEPES, pyruvate, penicillin/streptomycin, and 10% FBS) with 30 IU/mL rhIL-2 (Thermo Fisher Scientific, Carlsbad, CA). The CAR T cells were then cryopreserved in a mixture of 90% FBS and 10% DMSO for future use.

TGF- β ELISA:

The tumor cell lines were cultured at a density of 3×10^6 cells per flask for 3 days. The conditioned medium was collected by centrifugation, and stored at -80°C . The culture medium containing 10% fetal bovine serum is expected to have extra TGF- β 1 secretion. Thus, a control medium should be used as a blank and subtracted from the samples. The thawed conditioned medium was processed using the TGF- β 1 ELISA kit (R&D, DY240-05), and the samples were diluted four-fold prior to activation of latent TGF- β 1 into an immunoreactive form.

Bioluminescence Cytotoxicity Assay:

The U87vIII-CBG luciferase target cells were co-cultured with effector T cells at different effector: target ratios for 16 hours, with either 20ng/ml of hTGF- β 1 or media only. Prior to measuring the luminescence, 15 μ g of D-Luciferin (Gold Biotechnology, St. Louis, MO) was added and incubated at room temperature for 10mins. The luminescence was measured using a BioTek Synergy H4 hybrid multi-mode microplate reader. When calculating the cytotoxicity results, it was established that the target cells alone exhibited 0% lysis, the maximum lysis was observed in the treatment with 5% SDS Solution (Thermo Fisher Scientific, Carlsbad, CA).

Proliferation Assay:

The U87vIII cells were subjected to 10,000 rad of irradiation for 40min. Subsequently, triplicated cocultured of 0.2×10^6 tumor cells and 1×10^6 T cells in 12-well plates containing 4 ml of R10 media, with the addition of either 20 ng/ml of human TGF- β 1 or media alone. The media was replenished if it became yellowed around day 4 or 5. Supernatant of 1-2 ml was collected from each well at day 7, 14, 21 centrifuged and stored at -80°C for subsequent cytokine analysis

(Eve technologies, Calgary, Canada) The T cells were then resuspended, counted using a Beckman Coulter Multisizer 3 Cell Counter, and 1e6 T cells were transferred to the new 0.2e6 irradiated U87vIII cells to continue performing the proliferation assay weekly, the rest T cells were stained for phenotype and CAR expression.

5

Impedance Cytotoxicity Assays:

2e5 target tumor cells were seeded into Axion Biosystems microelectrode-containing 96-well plates (Axion Biosystems, Atlanta, GA). Each impedance plate was prepared prior to experimentation by coating with 20 µg/mL laminin overnight at 37°C. After coating was complete, wells were rinsed thrice with diH2O and then overlaid with 100 µL of cell culture media. The plate was placed into the Axion Biosystems ZHT analyzer (Axion Biosystems, Atlanta, GA) to record baseline readings of the background impedance without cells present. After baseline was established, the plate was removed from the analyzer and was seeded with 5e5 target cells in a volume of 200 µL/well. After cell plating, the plate was left in the cell culture hood for 1h at room temperature to ensure settling and 1 attachment of the cells down to the microelectrodes on the bottom surface. The plate was then returned to the analyzer and data collection began. Data were collected every 1 min for 24 h for cell monolayer growth measurement. For cytotoxicity assessment, the instrument was paused at 24h, and media was exchanged for media containing 1:1 dosages of effector cells or UTD control T cells, or media alone. Changes in impedance are reported as the resistive component of the complex impedance, as described previously. Using AxIS Z software (Axion Biosystems, Atlanta, GA), all data are corrected for “media alone” to remove small changes in media only impedance over time and then normalized to the impedance at the time of addition of effector cells. The % cytotoxicity calculations utilize the no treatment control and full lysis controls to determine % of target cell cytotoxicity as follows:

$$\%Cytotoxicity_{sample}(t) = \left[\frac{Z_{sample}(t) - \overline{Z_{FullLysis}}(t)}{Z_{TargetOnly}(t) - \overline{Z_{FullLysis}}(t)} \right] \times 100\%$$

Flow cytometry:

A 5-laser LSRFortessa flow cytometer was used for analysis. The cells were first stained with a live/dead viability stain (Thermo Fisher Scientific, Carlsbad, CA) in PBS. Then, they were stained with appropriate antibodies in FACS buffer (0.5% BSA in PBS) at 4°C for 30 minutes. The expression of the CAR was detected using a biotinylated protein L

(GenScript) antibody and streptavidin-coupled PE (BD Biosciences). The PE anti-human TGF- β Receptor II Antibody (399703) was used to detect dnTGF β RII expression on the CAR. The phosphorylation-SMAD2/3 ability of CAR T cells was detected using BD Phosflow antibodies (562586). CD69-FITC (clone FN50, BioLegend) and CD25-PerCP/Cyanine5.5 (clone BC96, BioLegend) were used to detect T cell activation.

Coculture Assay:

In co-culture experiments, transduced or UTD T cells (2e5 cells per well in 100 μ L R10 media) were co-cultured with target cells (2e5 cells per well in 100 μ L R10 media) in 96-well round bottom tissue culture plates, at 37°C with 5% CO₂ for 16 or 20hrs. In PD-1 marker staining assay, transduced or UTD T cells were distinguished with live/dead viability stain (Thermo Fisher Scientific, Carlsbad, CA), followed by human CD3 (clone OKT3, BioLegend, San Diego, CA) stain. BV711-conjugated anti-human PD-1 (clone EH12.2H7, BioLegend, San Diego, CA) was used to detect the expression of PD-1. In T cell phenotype assay, transduced or UTD T cells (5e5 cells per well in 500 μ L R10 media) were co-cultured with target cells (2.5e5 cells per well in 500 μ L R10 media) in 48-well flat bottom tissue culture plates. Target cells were irradiated with 10,000 rad ahead of co-culture with T cells. On day 2 and 4, 2.5e5 irradiated target cells were added in each well. After 5 days co-culture, remove 500ul supernatant from each well, gently resuspend and shift 250ul cells to 96well round bottom plate to stain. Human T cells were distinguished with live/dead viability stain (Thermo Fisher Scientific, Carlsbad, CA), followed by human CD3 and CD8 (clone OKT3 and clone SK1, BioLegend, San Diego, CA) stain. BV711-conjugated anti-human CD45RA (clone HI100, BioLegend, San Diego, CA), APC-conjugated anti-human CCR7 (clone G043H7, BioLegend, San Diego, CA) were used to detect T cell phenotypes. Staining was properly controlled with isotype antibodies. Before and after each staining, cells were washed twice with PBS containing 2% fetal bovine serum (FACS buffer). Fluorescence was assessed using a BD LSRFortessa flow cytometer and data were analyzed with FlowJo software.

Mouse models:

All mouse experiments were conducted according to Institutional Animal Care and Use Committee (IACUC)-approved protocols. In orthotopic tumor model, 5 $\times$ 10⁵ U87MG-CBG-GFP cells were implanted intracranially or 5 $\times$ 10⁵ D270MG-CBG-GFP cells subcutaneously into 6- to 8- week-old NSG mice. The intracranial surgical implants were done using a stereotactic surgical setup with tumor cells implanted 2mm right and 2mm anterior to the

lambda and 2mm into the brain. In subcutaneous models, NSG mice were injected with 5×10^5 D270 tumors subcutaneously in 100 μ L PBS on day 0. Tumor progression was evaluated by luminescence emission on a Xenogen IVIS spectrum after intraperitoneal injection of D-luciferin (Gold Biotechnology, St. Louis, MO) injection according to the manufacturer's directions. Subcutaneous tumor was measured by calipers in length and width for the duration of the experiment. Tumor size was calculated as the area of tumor by multiplying the two dimensions. T cells were injected in a total volume of 100 μ L of PBS intravenously via the tail vein 7-8 days after tumor implantation. Survival was followed over time until a predetermined IACUC-approved endpoint was reached.

Statistical analysis:

Data are presented as means $\pm$ SEM. RNA-seq data from TCGA was analyzed with Kruskal-Wallis test. Proliferation assay was analyzed with unpaired t test. Impedance and cytotoxicity assay were analyzed by ordinary one-way Analysis of Variance (ANOVA) with Tukey test to compare the differences in each group. The flow experiments were analyzed with two-way ANOVA with Tukey test to compare the differences in each group. Survival curves were analyzed with Kaplan-Meier (log-rank test). For the *in vivo* tumor study, linear regression was used to test for significant differences between the experimental groups. Survival, based on time to experimental endpoint, was plotted using a Kaplan-Meier curve. All statistical analyses were performed with Prism software version 9 (GraphPad, La Jolla, CA).

Example 1: Armored Bicistronic CAR T Cells with Dominant-negative TGF- β Receptor II to Alleviate Antigenic Heterogeneity and the Suppressive Immune Microenvironment in Glioblastoma

CAR T cell clinical trials for glioblastoma (GBM) have identified several key challenges to therapeutic efficacy, including the inherently heterogeneous genomic landscape and the immunosuppressive tumor microenvironment (TME) found in GBM. A previous study showed that EGFR variant III (EGFRvIII)-targeting monovalent CAR T cells reduced target-positive tumor cell populations, but tumor recurrence resulted from target-negative tumor cells, highlighting the limitation of single-target approaches in heterogeneous tumors (O'Rourke *et al.*, (2017). *Sci Transl Med* 9. 10.1126/scitranslmed.aaa0984). With regards to the highly immunosuppressive TME in GBM, transforming growth factor- β (TGF β) was present in the GBM TME as a major driver of suppression of the anti-GBM response in

clinical samples. TGF β is consistently highly expressed in both GBM tumor cell lines and patient tumor tissues (FIGs. 2A-2D).

A trivalent construct (CART-EGFR-IL13R α 2-dnTGF β) was designed using two parallel scFv constructs that independently target both IL13R α 2 and EGFRvIII, and a truncated dominant negative (dn) TGF β receptor II (FIG. 1, FIGs. 3A-3B). This trivalent construct was designed to explore possible additive effects in both *in vitro* and *in vivo* GBM model systems to limit tumor escape and overcome the immunosuppressive GBM TME. The CART-EGFR-IL13R α 2-dnTGF β construct broadened the targeted tumor cell repertoire, blocked TGF β signaling (FIG. 3), and served as a sink for free TGF β in the GBM TME to overcome the suppressive function of TGF β .

The tri-modular CAR T construct (*i.e.*, trivalent construct (CART-EGFR-IL13R α 2-dnTGF β)) had an enhanced proliferative response when compared with the CART-EGFR-IL13R α 2 construct, *in vitro* (FIG. 7). In co-culture assays, this construct led to reduced PD-1 expression (FIG. 5) and increased effector phenotype (FIGs. 9A-D), when compared to the bicistronic CAR T construct, which suggested a lower fraction of exhausted T cells. Tri-modular CAR T cells blocked the suppressive pSmad2/3 signaling pathway, leading to the unimpeded activation (FIGs. 6A-6B) and uninhibited proliferative response, albeit without any significant enhancement in tumor killing activity in short-term co-culture (FIGs. 4A-4B). In an immunodeficient mouse model, tri-modular CAR T cells eradicated tumor cells safely, efficiently and mice had a longer median survival when compared those treated with the bicistronic CART-EGFR-IL13R α 2 cells, lacking the dnTGF β receptor II.

Overcoming the adaptive changes in the local TME and addressing antigen heterogeneity is required to improve the clinical efficacy of CAR T-directed strategies. The present work showed that bicistronic CART constructs cooperate with truncated TGF β receptor II efficiently. There are multiple benefits to using the dominant negative TGF β RII in conjunction with CART-EGFR-IL13R α 2, including CAR T cells suppress the immunosuppressive TGF β signal and have reduced PD-1 expression (FIG. 5), have a remarkable proliferation ability in the chronic stimulation model *in vitro* (FIG. 7), have no side effects *in vivo*, and have enhanced eradication of GBM tumors *in vivo* (FIGs. 10A-D, FIG. 11). In summary, the tri-modular CAR T construct, TGF β RII CART-EGFR-IL13R α 2, described herein, addresses the clinical challenges of antigenic heterogeneity and the immunosuppressive TME in GBM.

Enumerated Embodiments

The following enumerated embodiments are provided, the numbering of which is not to be construed as designating levels of importance.

Embodiment 1 provides a nucleic acid comprising a) a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) comprising a first antigen-binding domain that binds human IL13R α 2, a transmembrane domain, and an intracellular domain; b) a second polynucleotide sequence encoding a second CAR comprising a second antigen-binding domain that binds epidermal growth factor receptor (EGFR) or an isoform thereof, a transmembrane domain, and an intracellular domain, and c) a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII).

Embodiment 2 provides the nucleic acid of embodiment 1, wherein the first and/or second 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.

Embodiment 3 provides the nucleic acid of any preceding embodiment, wherein the first 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 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).

Embodiment 4 provides the nucleic acid of any preceding embodiment, wherein the first 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/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: 9.

Embodiment 5 provides the nucleic acid of any preceding embodiment, wherein the first 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.

Embodiment 6 provides the nucleic acid of any preceding embodiment, wherein the first polynucleotide sequence encodes a CAR comprising an amino acid sequence at least
5 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 24.

Embodiment 7 provides the nucleic acid of any preceding embodiment, wherein the second 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 GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid
10 sequence GYISYSGNTRYNP SLK (SEQ ID NO: 26), and HCDR3 comprises the amino acid sequence VTAGRGFY (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
15 acid sequence VQYAQFPWT (SEQ ID NO: 30).

Embodiment 8 provides the nucleic acid of any preceding embodiment, wherein the second 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: 31; and/or a light chain variable region comprising an amino acid sequence at
20 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 32.

Embodiment 9 provides the nucleic acid of any preceding embodiment, wherein the second 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: 33 or 71

25 Embodiment 10 provides the nucleic acid of any preceding embodiment, wherein the second polynucleotide sequence encodes a CAR comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35 or 75.

Embodiment 11 provides the nucleic acid of any preceding embodiment, wherein the DN-TGFβRII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%,
30 98%, 99%, or 100% identical to SEQ ID NO: 2.

Embodiment 12 provides the nucleic acid of any preceding embodiment, wherein the nucleic acid encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 77 or 79.

Embodiment 13 provides the nucleic acid of any preceding embodiment, 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,
 5 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).

Embodiment 14 provides the nucleic acid of any preceding embodiment, wherein the transmembrane domain comprises a transmembrane domain of CD8.

Embodiment 15 provides the nucleic acid of embodiment 13, wherein the
 10 transmembrane domain of CD8 is a transmembrane domain of CD8 alpha.

Embodiment 16 provides the nucleic acid of any preceding embodiment, wherein the intracellular domain comprises a costimulatory signaling domain and an intracellular signaling domain.

Embodiment 17 provides the nucleic acid of any preceding embodiment, wherein the
 15 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).

Embodiment 18 provides the nucleic acid of any preceding embodiment, wherein the
 20 intracellular domain comprises a costimulatory domain of 4-1BB.

Embodiment 19 provides the nucleic acid of any preceding embodiment, 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,
 25 Fc ϵ RI, a cytoplasmic tail of an Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, Fc γ gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or a variant thereof.

Embodiment 20 provides the nucleic acid of any preceding embodiment, wherein the intracellular signaling domain comprises an intracellular domain of CD3 ζ .

Embodiment 21 provides a nucleic acid comprising a first polynucleotide sequence
 30 encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, and a second polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the CAR comprises 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 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).

Embodiment 22 provides the nucleic acid of embodiment 20, 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: 8; 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: 9.

Embodiment 23 provides the nucleic acid of embodiment 20, wherein the antigen-binding domain is an 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.

Embodiment 24 provides the nucleic acid of embodiment 20, 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, 24, 42, or 43.

Embodiment 25 provides the nucleic acid of any of embodiments 20-23, wherein the DN-TGFβRII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

Embodiment 26 provides a nucleic acid comprising a first polynucleotide sequence encoding a CAR comprising an antigen-binding domain that binds epidermal growth factor receptor (EGFR) or an isoform thereof, a transmembrane domain, and an intracellular domain, and a second polynucleotide sequence encoding a dominant negative TGFβ type II receptor (DN-TGFβRII).

Embodiment 27 provides the nucleic acid of embodiment 25, 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: 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.

Embodiment 28 provides the nucleic acid of embodiment 25, wherein the antigen-binding domain is an scFv comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 71.

Embodiment 29 provides the nucleic acid of embodiment 25, wherein the CAR
5 comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23, 24, 35, 42, 43, or 75.

Embodiment 30 provides the nucleic acid of embodiment 25, wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

10 Embodiment 31 provides the nucleic acid of embodiment 26, wherein the nucleic acid encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 81, 83, or 85.

Embodiment 32 provides a nucleic acid comprising a first polynucleotide sequence encoding a first CAR capable of binding IL13R α 2, and a second polynucleotide sequence
15 encoding a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII), wherein: the first CAR comprises an antigen-binding domain comprising: a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence
20 TKYGVH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence 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
25 acid sequence STSNLAS (SEQ ID NO: 6), and LCDR3 comprises the amino acid sequence HQYHRSPLT (SEQ ID NO: 7), and wherein the second CAR comprises an antigen-binding domain comprising: 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
30 GYISYSGNTRYNP SLK (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), and wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

Embodiment 33 provides a nucleic acid comprising a first polynucleotide sequence
5 encoding a first CAR capable of binding IL13R α 2, a second polynucleotide sequence
encoding a second CAR capable of binding epidermal growth factor receptor (EGFR) or an
isoform thereof, and a third polynucleotide sequence encoding a DN-TGF β RII 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: 44 or
10 54; 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: 48 or 58; 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: 73;
and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%,
15 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 74.

Embodiment 34 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, and a third polynucleotide sequence encoding a DN-TGF β RII wherein: the
20 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: 64, 65, 66, or 69; 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: 70.

Embodiment 35 provides a nucleic acid comprising a first polynucleotide sequence
25 encoding a first chimeric antigen receptor capable of binding IL13R α 2, a second
polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of
binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a third
polynucleotide sequence encoding a DN-TGF β RII wherein: the first polynucleotide sequence
30 comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%
identical to SEQ ID NO: 52 or SEQ ID NO: 53 or SEQ ID NO: 62 or SEQ ID NO: 63; 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: 34.

Embodiment 36 provides the nucleic acid of any of embodiments 31-33, wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

Embodiment 37 provides the nucleic acid of any preceding embodiment, wherein the first polynucleotide sequence and the second polynucleotide sequence is separated by a linker.

Embodiment 38 provides the nucleic acid of any preceding embodiment, wherein the second polynucleotide sequence and the third polynucleotide sequence is separated by a linker.

Embodiment 39 provides the nucleic acid of any preceding embodiment, wherein the nucleic acid comprises from 5' to 3' the second polynucleotide sequence, a linker, and the first polynucleotide sequence.

Embodiment 40 provides the nucleic acid of any preceding embodiment, wherein the nucleic acid comprises from 5' to 3' the second polynucleotide sequence, a linker, the first polynucleotide sequence, a linker, and the third polynucleotide sequence.

Embodiment 41 provides a vector comprising the nucleic acid of any of the preceding embodiments.

Embodiment 42 provides the vector of embodiment 39, wherein the vector is an expression vector.

Embodiment 43 provides the vector of embodiment 39 or 40, 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.

Embodiment 44 provides the vector of any one of embodiments 39-41, further comprising an EF-1 promoter.

Embodiment 45 provides the vector of any one of embodiments 39-42, further comprising a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE).

Embodiment 46 provides the vector of any one of embodiments 39-43, further comprising a rev response element (RRE).

Embodiment 47 provides the vector of any one of embodiments 39-44, further comprising a cPPT sequence.

Embodiment 48 provides the vector of any one of embodiments 39-45, wherein the vector is a self-inactivating vector.

Embodiment 49 provides a modified immune cell or precursor cell thereof comprising the nucleic acid of any one of embodiments 1-38, or the vector of any one of embodiments 39-46.

Embodiment 50 provides a modified immune cell or precursor cell thereof,
 5 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; and a dominant negative TGF β type II receptor (DN-TGF β RII).

10 Embodiment 51 provides a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein: the first CAR comprises: a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino
 15 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
 20 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
 25 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),
 30 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).

Embodiment 52 provides a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding

EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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/or a light chain variable region comprising an amino acid sequence at least
 5 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/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.

10 Embodiment 53 provides a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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
 15 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: 33 or 71.

Embodiment 54 provides a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding
 20 EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 35 or 75.

25 Embodiment 55 provides a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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
 30 NO: 44 or 54; 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: 48 or 58; 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: 73; 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: 74.

Embodiment 56 provides a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding
 5 EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the first CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 64, 65, 66, or 69; and the second CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 70.

10 Embodiment 57 provides a modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF type II receptor (DN-TGF β RII), 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: 52 or SEQ ID NO: 53 or SEQ
 15 ID NO: 62 or SEQ ID NO: 63; 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: 34.

Embodiment 58 provides the modified immune cell or precursor cell thereof of any of embodiments 53-55 wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2 or is
 20 encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 14.

Embodiment 59 provides the modified cell of any one of embodiments 47-52, 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,
 25 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.

Embodiment 60 provides the modified cell of any one of embodiments 49-59, wherein the modified cell is a modified T cell.

30 Embodiment 61 provides the modified cell of any one of embodiments 49-60, wherein the modified cell is an autologous cell.

Embodiment 62 provides the modified cell of any one of embodiments 49-61, wherein the modified cell is an autologous cell obtained from a human subject.

Embodiment 63 provides the modified cell of any one of embodiments 49-62, wherein the DN-TGF β R_{II} comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

Embodiment 64 provides a pharmaceutical composition comprising a therapeutically effective amount of the modified cell of any one of embodiments 49-63.

Embodiment 65 provides 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 embodiments 49-63, or the pharmaceutical composition of embodiment 64.

Embodiment 66 provides the method of embodiment 65, wherein the disease is a cancer.

Embodiment 67 provides the method of embodiment 66, wherein the cancer is a glioma.

Embodiment 68 provides the method of embodiment 65 or 66, wherein the cancer is an astrocytoma.

Embodiment 69 provides the method of any one of embodiments 65-68, wherein the cancer is a high-grade astrocytoma.

Embodiment 70 provides the method of any one of embodiments 63-67, wherein the cancer is glioblastoma.

Embodiment 71 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 (CAR) comprising a first antigen-binding domain capable of binding IL13R α 2; a second chimeric antigen receptor (CAR) comprising a second antigen-binding domain capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof; and a dominant negative TGF β type II receptor (DN-TGF β R_{II}).

Embodiment 72 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β R_{II}), 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 QGTTALATRFFDV (SEQ ID NO: 15); 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 GYISYSGNTRYNP SLK (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).

Embodiment 73 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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/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.

Embodiment 74 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein: the first CAR comprises an 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 an scFv comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 71.

Embodiment 75 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII),

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 SEQ ID NO: 24 or SEQ ID NO: 42 or SEQ ID NO: 43; 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: 35 or 75.

5 Embodiment 76 provides the method of any of embodiments 71-75, wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

 Embodiment 77 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
10 comprising: a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 44 or 54; and a light chain variable region encoded by a polynucleotide sequence at least
15 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 48 or 58; 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: 73; 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: 74.

20 Embodiment 78 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the first CAR comprises a scFv encoded by a polynucleotide sequence at least 80%,
25 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 64, 65, 66, or 69; and the second CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 70.

 Embodiment 79 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
30 comprising: a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein the first CAR comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 52 or SEQ ID NO: 53 or SEQ ID NO: 62 or SEQ ID

NO: 63; and the second CAR comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34.

Embodiment 80 provides the method of any of embodiments 75-77 wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2 or is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 14.

Embodiment 81 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 polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 76 or 78.

Other Embodiments

The recitation of a listing of elements in any definition of a variable herein includes 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 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 nucleic acid comprising
a first polynucleotide sequence encoding a first chimeric antigen receptor (CAR) comprising a first antigen-binding domain that binds human IL13R α 2, a transmembrane domain, and an intracellular domain,
a second polynucleotide sequence encoding a second CAR comprising a second antigen-binding domain that binds epidermal growth factor receptor (EGFR) or an isoform thereof, a transmembrane domain, and an intracellular domain, and
a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII).
2. The nucleic acid of claim 1, wherein the first and/or second 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.
3. The nucleic acid of any preceding claim, wherein the first 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 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).
4. The nucleic acid of any preceding claim, wherein the first 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/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: 9.

5. The nucleic acid of any preceding claim, wherein the first 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.

6. The nucleic acid of any preceding claim, wherein the first polynucleotide sequence encodes a CAR comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 23, 24, 42, or 43.

7. The nucleic acid of any preceding claim, wherein the second 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 GYSITSDFAWN (SEQ ID NO: 25), HCDR2 comprises the amino acid sequence GYISYSGNTRYNP SLK (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).

8. The nucleic acid of any preceding claim, wherein the second 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: 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.

9. The nucleic acid of any preceding claim, wherein the second 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: 33 or 71.

10. The nucleic acid of any preceding claim, wherein the second polynucleotide sequence encodes a CAR comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35 or 75.
11. The nucleic acid of any preceding claim, wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.
12. The nucleic acid of any preceding claim, wherein the nucleic acid encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 77 or 79.
13. The nucleic acid of any preceding claim, 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).
14. The nucleic acid of any preceding claim, wherein the transmembrane domain comprises a transmembrane domain of CD8.
15. The nucleic acid of claim 9, wherein the transmembrane domain of CD8 is a transmembrane domain of CD8 alpha.
16. The nucleic acid of any preceding claim, wherein the intracellular domain comprises a costimulatory signaling domain and an intracellular signaling domain.
17. The nucleic acid of any preceding claim, 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).

18. The nucleic acid of any preceding claim, wherein the intracellular domain comprises a costimulatory domain of 4-1BB.
19. The nucleic acid of any preceding claim, 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.
20. The nucleic acid of any preceding claim, wherein the intracellular signaling domain comprises an intracellular domain of CD3 ζ .
21. A nucleic acid comprising a first polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding IL13R α 2, and a second polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII),
wherein the CAR comprises 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 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).
22. The nucleic acid of claim 21, 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: 8; 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: 9.

23. The nucleic acid of claim 21, wherein the antigen-binding domain is an 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.

24. The nucleic acid of claim 21, 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, 24, 42, or 43.

25. The nucleic acid of any of claims 21-24, wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

26. A nucleic acid comprising
a first polynucleotide sequence encoding a CAR comprising an antigen-binding domain that binds epidermal growth factor receptor (EGFR) or an isoform thereof, a transmembrane domain, and an intracellular domain, and
a second polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII).

27. The nucleic acid of claim 26, 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: 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.

28. The nucleic acid of claim 26, wherein the antigen-binding domain is an scFv comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 71.

29. The nucleic acid of claim 26, 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, 24, 35, 42, 43, or 75.

30. The nucleic acid of claim 26, wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

31. The nucleic acid of claim 26, wherein the nucleic acid encodes an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NOs: 81, 83, or 85.

32. 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, and a third polynucleotide sequence encoding a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

the first CAR comprises an antigen-binding domain comprising:

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 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), and

wherein the second CAR comprises an antigen-binding domain comprising:

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), and

wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

33. A nucleic acid comprising a first polynucleotide sequence encoding a first CAR capable of binding IL13R α 2, a second polynucleotide sequence encoding a second CAR capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a third polynucleotide sequence encoding a DN-TGF β RII 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: 44 or 54; 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: 48 or 58; 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: 73; 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: 74.

34. 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, and a third polynucleotide sequence encoding a DN-TGF β RII 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: 64, 65, 66, or 69; 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: 70.

35. A nucleic acid comprising a first polynucleotide sequence encoding a first chimeric antigen receptor capable of binding IL13R α 2, a second polynucleotide sequence encoding a second chimeric antigen receptor (CAR) capable of binding epidermal growth factor receptor (EGFR) or an isoform thereof, and a third polynucleotide sequence encoding a DN-TGF β RII 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: 52 or SEQ ID NO: 53 or SEQ ID NO: 62 or SEQ ID NO: 63; 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: 34.

36. The nucleic acid of any of claims 32-35, wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2 or is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 14.

37. The nucleic acid of any preceding claim, wherein the first polynucleotide sequence and the second polynucleotide sequence is separated by a linker.

38. The nucleic acid of any preceding claim, wherein the second polynucleotide sequence and the third polynucleotide sequence is separated by a linker.

39. The nucleic acid of any preceding claim, wherein the nucleic acid comprises from 5' to 3' the second polynucleotide sequence, a linker, and the first polynucleotide sequence.

40. The nucleic acid of any preceding claim, wherein the nucleic acid comprises from 5' to 3' the second polynucleotide sequence, a linker, the first polynucleotide sequence, a linker, and the third polynucleotide sequence.

41. A vector comprising the nucleic acid of any of the preceding claims.

42. The vector of claim 41, wherein the vector is an expression vector.

43. The vector of claim 41 or 42, 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.
44. The vector of any one of claims 41-43, further comprising an EF-1 promoter.
45. The vector of any one of claims 41-44, further comprising a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE).
46. The vector of any one of claims 41-45, further comprising a rev response element (RRE).
47. The vector of any one of claims 41-46, further comprising a cPPT sequence.
48. The vector of any one of claims 41-47, wherein the vector is a self-inactivating vector.
49. A modified immune cell or precursor cell thereof comprising the nucleic acid of any one of claims 1-40, or the vector of any one of claims 41-48.
50. 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;
and a dominant negative TGF β type II receptor (DN-TGF β RII).
51. A modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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 SASYST (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).

52. A modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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/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: 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/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.

53. A modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 33 or 71.

54. A modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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, 24, 42, or 43; 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: 35 or 75.

55. A modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 44 or 54; 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: 48 or 58; 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: 73; 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: 74.

56. A modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

the first CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 64, 65, 66, or 69; and

the second CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 70.

57. A modified immune cell or precursor cell thereof, comprising a first CAR capable of binding IL13R α 2, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 52 or SEQ ID NO: 53 or SEQ ID NO: 62 or SEQ ID NO: 63; 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: 34.

58. The modified immune cell or precursor cell thereof of any of claims 51-57 wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2 or is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 14.

59. The modified cell of any one of claims 49-58, 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.

60. The modified cell of any one of claims 49-59, wherein the modified cell is a modified T cell.
61. The modified cell of any one of claims 49-60, wherein the modified cell is an autologous cell.
62. The modified cell of any one of claims 49-61, wherein the modified cell is an autologous cell obtained from a human subject.
63. The modified cell of any one of claims 49-62, wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.
64. A pharmaceutical composition comprising a therapeutically effective amount of the modified cell of any one of claims 49-63.
65. 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 49-63, or the pharmaceutical composition of claim 64.
66. The method of claim 65, wherein the disease is a cancer.
67. The method of claim 66, wherein the cancer is a glioma.
68. The method of claim 65 or 66, wherein the cancer is an astrocytoma.
69. The method of any one of claims 65-68, wherein the cancer is a high-grade astrocytoma.
70. The method of any one of claims 65-68, wherein the cancer is glioblastoma.
71. 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; and a dominant negative TGF β type II receptor (DN-TGF β RII).

72. 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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

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 VTAGRGFPPYW (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).

73. 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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/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.

74. 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

the first CAR comprises an 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 an scFv comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 33 or 71.

75. 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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 SEQ ID NO: 24 or SEQ ID NO: 42 or SEQ ID NO: 43; 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: 35 or 75.

76. The method of any of claims 71-75, wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to

SEQ ID NO: 2 or is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2.

77. 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), 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: 44 or 54; 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: 48 or 58; 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: 73; 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: 74.

78. 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

the first CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 64, 65, 66, or 69; and

the second CAR comprises a scFv encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 70.

79. 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, a second CAR capable of binding EGFR or an isoform thereof, and a dominant negative TGF β type II receptor (DN-TGF β RII), wherein:

the first CAR comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 52 or SEQ ID NO: 53 or SEQ ID NO: 62 or SEQ ID NO: 63; and

the second CAR comprises a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 34.

80. The method of any of claims 77-79 wherein the DN-TGF β RII comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2 or is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 14.

81. 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 polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 76 or 78.