

CHIMERIC RECEPTORS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Application No. 63/231,626, filed August 10, 2021, and to U.S. Provisional Application No. 63/117,861, filed November 24, 2020, each of which is hereby incorporated by reference in its entirety for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on ~~Month~~ ~~XX~~January 13, 2022~~XX~~, is named ~~XXXXXXUS~~_sequencelistingSTB-023WO_SL.txt, and is ~~X,XXX,XXX-255,989~~ bytes in size.

FIELD OF THE INVENTION

[0003] The present application relates to solid tumor antigen targets for chimeric receptors and chimeric inhibitory receptors, and methods of using the same, such as for the treatment of cancer.

BACKGROUND

[0004] Immunotherapies, such as chimeric antigen receptor (CAR) based adoptive cell therapies used to redirect the specificity and function of immunoresponsive cells (*e.g.*, T cells and Natural Killer (NK) cells) have shown efficacy in patients with malignancies, with many prior studies focused on hematological malignancies (Pule *et al.*, *Nat. Med.* (14):1264-1270 (2008); Maude *et al.*, *N Engl J Med.* (371):1507-17 (2014); Brentjens *et al.*, *Sci Transl Med.* (5):177ra38 (2013)). For example, CAR T cells have been shown to induce complete remission in patients with CD19-expressing malignancies for whom chemotherapies have led to drug resistance and tumor progression.

[0005] Unlike T cells, natural killer (NK) cells are able to eliminate abnormal cells, such as cancer cells, without priming. NK cell activity is determined by a balance of external signals from inhibitory and activating NK cell receptors. Inhibitory receptors, such as killer immunoglobulin receptors (KIRs), interact with the major histocompatibility complex (MHC) class I antigens and protect normal cells from NK cell activity (*see* US20180057795A1).

[0006] One challenge to developing CAR therapy for solid tumors is a lack of suitable targets. The ability to identify appropriate CAR targets is important to effectively targeting and treating the tumor without damaging normal cells that express the same target antigen. Thus, there remains a need for CAR-NK cell-based tumor therapies that target tumor cells without targeting normal cells or tissues.

SUMMARY

[0007] In one [aspectexample](#), provided herein is an isolated cell comprising:

- (a) an inhibitory chimeric receptor comprising an extracellular antigen-binding domain that binds to an antigen, wherein the antigen is selected from the group consisting of: VSIG2, CPM, ITM2C, SLC26A2, SLC4A4, GPA33, PLA2G2A, ABCA8, ATP1A2, CHP2, and SLC26A3; and
- (b) a chimeric receptor comprising one or more extracellular antigen-binding domains, wherein the one or more extracellular antigen-binding domains bind one or more additional antigens selected from the group consisting of: CEA, CEACAM1, CEACAM5, and CEACAM6.

[0008] In some [aspectexamples](#), the antigen-binding domain of the inhibitory chimeric receptor comprises one or more single chain variable fragments (scFvs), optionally wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL), optionally wherein the VH and VL are separated by a peptide linker, optionally the peptide linker comprises the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 77, and/or optionally wherein each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0009] In some [aspectexample](#), the inhibitory chimeric receptor comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28 transmembrane domain, a CD25 transmembrane domain, a CD7 transmembrane domain, a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a LAX transmembrane domain, a LAT transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a TIM3 transmembrane domain, a KIR3DS1 transmembrane domain, a KIR3DL1 transmembrane domain, an NKG2D transmembrane domain, an NKG2A transmembrane domain, a TIGIT transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

[0010] In some [aspectexample](#), the inhibitory chimeric receptor comprises a spacer region between the antigen-binding domain and the transmembrane domain, optionally wherein the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 49-58; and/or

[0011] In some [aspectexample](#), the inhibitory chimeric receptor comprises one or more intracellular inhibitory domains selected from the group consisting of: PD-1, CTLA4, TIGIT, LAIR1, GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, BTLA, LIR1, NKG2A, KIR3DL1, GITR, PD-L1,

CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, RasGAP, CD94, and CD161.

[0012] In some [aspectexamples](#), the chimeric receptor is a CAR. In some [aspectexamples](#), the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of: a CD3zeta-chain intracellular signaling domain, a CD3epsilon-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, a 2B4 intracellular signaling domain, an NKp46 intracellular signaling domain, an NKp30 intracellular signaling domain, an NKp44 intracellular signaling domain, an NKG2D intracellular signaling domain, a CD226 intracellular signaling domain, and a CD160 intracellular signaling domain. In some [aspectexamples](#), the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28 transmembrane domain, a CD25 transmembrane domain, a CD7 transmembrane domain, a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a LAX transmembrane domain, a LAT transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a TIM3 transmembrane domain, a KIR3DS1 transmembrane domain, a KIR3DL1 transmembrane domain, an NKG2D transmembrane domain, an NKG2A transmembrane domain, a TIGIT transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

[0013] In some [aspectexamples](#), the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 49-58.

[0014] In some [aspectexamples](#), the antigen-binding domain of the inhibitory chimeric receptor and/or the chimeric receptor comprises one or more single chain variable fragments (scFvs), optionally wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL), optionally wherein the VH and VL are separated by a peptide

linker, optionally wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 77.

[0015] In some [aspectexamples](#), each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0016] In some [aspectexamples](#), the one or more additional antigen is CECAM1, optionally wherein the antigen-binding domain that binds to CEACAM1 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 1 and a light chain variable domain (VL) comprising the amino acid sequence of SEQ ID NO: 2.

[0017] In some [aspectexamples](#), the one or more additional antigen is CECAM5, optionally wherein the antigen-binding domain that binds to CEACAM5 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:

- (i) a VH comprising the amino acid sequence of SEQ ID NO: 3 and a VL comprising the amino acid sequence of SEQ. ID NO: 4;
- (ii) a VH comprising the amino acid sequence of SEQ ID NO: 9 and a VL comprising the amino acid sequence of SEQ. ID NO: 10;
- (iii) a VH comprising the amino acid sequence of SEQ ID NO: 11 and a VL comprising the amino acid sequence of SEQ. ID NO: 12;
- (iv) a VH comprising the amino acid sequence of SEQ ID NO: 13 and a VL comprising the amino acid sequence of SEQ. ID NO: 14;
- (v) a VH comprising the amino acid sequence of SEQ ID NO: 78 and a VL comprising the amino acid sequence of SEQ. ID NO: 79; and
- (vi) a VH comprising the amino acid sequence of SEQ ID NO: 15 and a VL comprising the amino acid sequence of SEQ. ID NO: 16.

[0018] In some [aspectexamples](#), the antigen-binding domain that binds to CEACAM5 comprises a heavy chain (HC) and a light chain (LC) selected from the group consisting of:

- (i) an HC comprising the amino acid sequence of SEQ ID NO: 5 and an LC comprising the amino acid sequence of SEQ. ID NO: 6; and
- (ii) an HC comprising the amino acid sequence of SEQ ID NO: 7 and an LC comprising the amino acid sequence of SEQ. ID NO: 8; or
- (c) the one or more additional antigen is CECAM6, optionally wherein the antigen-binding domain that binds to CEACAM6 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 17 and a light chain variable domain (VL) comprising the amino acid sequence of SEQ ID NO 18.

[0019] In some aspectexamples, the cell is an immunoresponsive cell. In some aspectexamples, binding of the inhibitory chimeric receptor to the antigen is capable of inhibiting the immunoresponsive cell and/or wherein binding of the chimeric receptor to the one or more additional antigens is capable of activating the immunoresponsive cell.

[0020] In some aspectexamples, the chimeric receptor binds to the one or more additional antigens with a low binding affinity.

[0021] In some aspectexamples, the chimeric receptor binds to the one or more additional antigens with a binding affinity that is lower than the binding affinity with which the inhibitory chimeric receptor binds to the antigen.

[0022] In some aspectexamples, the inhibitory chimeric receptor binds to the antigen with a low binding avidity.

[0023] In some aspectexamples, the chimeric receptor is recombinantly expressed, optionally wherein the chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.

[0024] In some aspectexamples, the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell. In some aspectexamples, the cell is autologous or the cell is allogeneic.

[0025] In some aspectexamples, provided herein is an isolated nucleic acid encoding an inhibitory chimeric receptor as provided herein.

[0026] In some aspectexamples, provided herein is a vector comprising a nucleic acid as provided herein.

[0027] In some aspectexamples, provided herein is a genetically modified cell comprising a nucleic acid as provided herein or vector as provided herein.

[0028] In some aspectexamples, provided herein is a pharmaceutical composition comprising an effective amount of an isolated cell as provided herein, an isolated nucleic acid as provided herein, or a vector as provided herein, and a pharmaceutically acceptable carrier, a pharmaceutically acceptable excipient, or a combination thereof.

[0029] In some aspectexamples, provided herein is a method of treating a subject in need thereof, the method comprising administering to the subject an effective amount of isolated cells as provided herein, an isolated nucleic acid as provided herein, a vector as provided herein, or the pharmaceutical composition as provided herein.

[0030] In some aspectexamples, provided herein is a method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor an effective amount of isolated cells as provided herein, an isolated nucleic acid as

provided herein, a vector as provided herein, or a pharmaceutical composition as provided herein.

[0031] In some [aspectexamples](#), provided herein is a method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof an effective amount of isolated cells as provided herein, an isolated nucleic acid as provided herein, a vector as provided herein, or a pharmaceutical composition as provided herein.

[0032] In some [aspectexamples](#), provided herein is a method of reducing tumor burden in a subject, comprising administering to the subject an effective amount of isolated cells as provided herein, an isolated nucleic acid as provided herein, a vector as provided herein, or a pharmaceutical composition as provided herein. In some [aspectexamples](#), the method reduces the number of tumor cells, the method reduces tumor size, the method reduces tumor volume, and/or the method eradicates the tumor in the subject.

[0033] In some [aspectexamples](#), provided herein is a method of treating a subject having a tumor, the method comprising administering an effective amount of isolated cells as provided herein, an isolated nucleic acid as provided herein, a vector as provided herein, or a pharmaceutical composition as provided herein.

[0034] In some [aspectexamples](#), provided herein is a method of treating or preventing a cancer in a subject, wherein the cancer is selected from the group consisting of: colorectal carcinoma, pancreatic cancer, a lung adenocarcinoma, and gastric cancer, the method comprising administering to the subject an effective amount of the isolated cells as provided herein, an isolated nucleic acid as provided herein, a vector as provided herein, or a pharmaceutical composition as provided herein.

[0035] In some [aspectexamples](#), the method increases progression free survival in the subject; and or the method increases survival in the subject.

[0036] In some [aspectexamples](#), provided herein is a kit for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer, comprising the isolated cells as provided herein, an isolated nucleic acid as provided herein, a vector as provided herein, or a pharmaceutical composition as provided herein. In some [aspectexamples](#), the kit further comprises written instructions for using the isolated cell or pharmaceutical composition for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer in a subject, or the kit further comprises instructions for using the nucleic acid or vector for producing one or more antigen-specific cells for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer in a subject.

[0037] In some [aspectexamples](#), provided herein is an inhibitory chimeric receptor comprising an extracellular antigen-binding domain that binds to an antigen selected from the group consisting of: VSIG2, CPM, ITM2C, SLC26A2, SLC4A4, GPA33, PLA2G2A, ABCA8, ATP1A2, CHP2, and SLC26A3.

[0038] In some [embodimentexamples](#), the antigen is VSIG2. In some [embodimentexamples](#), the antigen is CPM. In some [embodimentexamples](#), the antigen is ITM2C. In some [embodimentexamples](#), the antigen is SLC26A2. In some [embodimentexamples](#), the antigen is SLC4A4. In some [embodimentexamples](#), the antigen is GPA33. In some [embodimentexamples](#), the antigen is PLA2G2A. In some [embodimentexamples](#), the antigen is ABCA8. In some [embodimentexamples](#), the antigen is ATP1A2. In some [embodimentexamples](#), the antigen is CHP2. In some [embodimentexamples](#), the antigen is SLC26A3.

[0039] In some [embodimentexamples](#), when expressed on a cell, the inhibitory chimeric receptor inhibits one or more activities of the cell.

[0040] In some [embodimentexamples](#), the antigen is not expressed on a tumor cell, or the antigen is expressed on a tumor cell at a level that is lower than expression on a non-tumor cell.

[0041] In some [embodimentexamples](#), the antigen is expressed on a non-tumor cell, or the antigen is expressed on a non-tumor cell at a level that is higher than expression on a corresponding tumor cell.

[0042] In some [embodimentexamples](#), the antigen is expressed on a non-tumor cell derived from a tissue selected from the group consisting of: lung, pancreas, gastrointestinal tract, colon, brain, neuronal tissue, endocrine, bone, bone marrow, immune system, muscle, liver, gallbladder, pancreas, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

[0043] In some [embodimentexamples](#), the inhibitory chimeric receptor comprises one or more intracellular inhibitory domains selected from the group consisting of: PD-1, CTLA4, TIGIT, LAIR1, GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, BTLA, LIR1, NKG2A, KIR3DL1, GITR, PD-L1, CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, RasGAP, CD94, and CD161.

[0044] In some [embodimentexamples](#), the antigen-binding domain comprises one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs).

[0045] In some [embodimentexamples](#), the antigen-binding domain comprises one or more single chain variable fragments (scFvs). In some [embodimentexamples](#), each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some [embodimentexamples](#), the VH and VL are separated by a peptide linker. In some [embodimentexamples](#), the peptide linker comprises the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 77. In

some embodimentexamples, each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0046] In another aspectexample, provided herein is an isolated cell comprising the inhibitory chimeric receptor of any one of the above embodimentexamples. In some embodimentexamples, the inhibitory chimeric receptor is recombinantly expressed. In some embodimentexamples, the inhibitory chimeric receptor is expressed from a vector or a selected locus from the genome of the cell. In some embodimentexamples, the cell further comprises a chimeric receptor comprising one or more extracellular antigen-binding domains, wherein the one or more extracellular antigen-binding domains bind one or more antigens selected from the group consisting of: CEA, CEACAM1, CEACAM5, and CEACAM6.

[0047] In another aspectexample, provided herein is an isolated cell comprising:

(a) an inhibitory chimeric receptor comprising an extracellular antigen-binding domain that binds to an antigen, wherein the antigen is selected from the group consisting of: VSIG2, CPM, ITM2C, SLC26A2, SLC4A4, GPA33, PLA2G2A, ABCA8, ATP1A2, CHP2, and SLC26A3; and

(b) a chimeric receptor comprising one or more extracellular antigen-binding domains, wherein the one or more extracellular antigen-binding domains bind one or more additional antigens selected from the group consisting of: CEA, CEACAM1, CEACAM5, and CEACAM6.

[0048] In some embodimentexamples, the chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR). In some embodimentexamples, the chimeric receptor is a CAR. In some embodimentexamples, the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3zeta-chain intracellular signaling domain, a CD3epsilon-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP70 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, a 2B4 intracellular signaling domain, an NKp46 intracellular signaling domain, an NKp30 intracellular signaling domain, an NKp44 intracellular signaling domain, an NKG2D intracellular signaling domain, a CD226 intracellular signaling domain, and a CD160 intracellular signaling domain.

[0049] In some [embodimentexamples](#), the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane domain, a CD25 transmembrane domain, a CD7 transmembrane domain, a CD3 zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a LAX transmembrane domain, a LAT transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a TIM3 transmembrane domain, a KIR3DS1 transmembrane domain, a KIR3DL1 transmembrane domain, an NKG2D transmembrane domain, an NKG2A transmembrane domain, a TIGIT transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

[0050] In some [embodimentexamples](#), the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 49-58.

[0051] In some [embodimentexamples](#), the antigen-binding domain of the inhibitory chimeric receptor and/or the chimeric receptor comprises one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs).

[0052] In some [embodimentexamples](#), the antigen-binding domain of the inhibitory chimeric receptor and/or the chimeric receptor comprises one or more single chain variable fragments (scFvs). In some [embodimentexamples](#), each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some [embodimentexamples](#), the VH and VL are separated by a peptide linker. In some [embodimentexamples](#), the peptide linker comprises the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 77. In some [embodimentexamples](#), each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0053] In some [embodimentexamples](#), the cell is an immunoresponsive cell. In some [embodimentexamples](#), binding of the inhibitory chimeric receptor to the antigen is capable of inhibiting the immunoresponsive cell. In some [embodimentexamples](#), binding of the chimeric receptor to the one or more additional antigens is capable of activating the immunoresponsive cell.

[0054] In some [embodimentexamples](#), the chimeric receptor binds to the one or more additional antigens with a low binding affinity.

[0055] In some [embodimentexamples](#), the chimeric receptor binds to the one or more additional antigens with a binding affinity that is lower than the binding affinity with which the inhibitory chimeric receptor binds to the antigen. In some [embodimentexamples](#), the inhibitory chimeric receptor binds to the antigen with a low binding avidity.

[0056] In some [embodimentexamples](#), the chimeric receptor is recombinantly expressed. In some [embodimentexamples](#), the chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.

[0057] In some [embodimentexamples](#), the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

[0058] In some [embodimentexamples](#), the cell is autologous. In some [embodimentexamples](#), the cell is allogeneic.

[0059] In another [aspectexample](#), provided herein is an isolated nucleic acid encoding the inhibitory chimeric receptor of any one of the above inhibitory chimeric receptors.

[0060] In another [aspectexample](#), provided herein is a vector comprising the nucleic acid of the above [aspectexample](#).

[0061] In another [aspectexample](#), provided herein is a genetically modified cell comprising the nucleic acid or the vector of the above [aspectexamples](#).

[0062] In another [aspectexample](#), provided herein is a method of treating a subject in need thereof, the method comprising administering the isolated cells of any one of the above [embodimentexamples](#).

[0063] In another [aspectexample](#), provided herein is a method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor the isolated cells of any one of the above [embodimentexamples](#).

[0064] In another [aspectexample](#), provided herein is a method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof the isolated cells of any one of the above [embodimentexamples](#).

[0065] In another [aspectexample](#), provided herein is a method of reducing tumor burden in a subject, comprising administering to the subject the isolated cells of any one of the above [embodimentexamples](#).

In some [embodimentexamples](#), the method reduces the number of tumor cells. In some [embodimentexamples](#), the method reduces tumor size. In some [embodimentexamples](#), the method reduces tumor volume. In some [embodimentexamples](#), the method eradicates the tumor in the subject.

[0066] In another [aspectexample](#), provided herein is a method of treating a subject having a tumor, the method comprising administering the isolated cells of any one of the above [embodimentexamples](#).

[0067] In another [aspectexample](#), provided herein is a method of treating or preventing a colorectal carcinoma in a subject, comprising administering to the subject the isolated cells of any one of the above [embodimentexamples](#).

[0068] In another [aspectexample](#), provided herein is a method of treating or preventing pancreatic cancer in a subject, comprising administering to the subject the isolated cells of any one of the above

embodimentexamples.

[0069] In another aspectexample, provided herein is a method of treating or preventing a lung adenocarcinoma in a subject, comprising administering to the subject the isolated cells of any one of the above embodimentexamples.

[0070] In another aspectexample, provided herein is a method of treating or preventing gastric cancer in a subject, comprising administering to the subject the isolated cells of any one of the above embodimentexamples.

[0071] In some embodimentexamples of the above aspectexamples, the isolated cells are administered at an effective amount. In some embodimentexamples of the above aspectexamples, the method increases progression free survival in the subject. In some embodimentexamples of the above aspectexamples, the method increases survival in the subject.

[0072] In another aspect, the present disclosure provides a pharmaceutical composition comprising an effective amount of the isolated cell as described herein, the isolated nucleic acid as described herein, or the vector as described herein, and a pharmaceutically acceptable carrier, a pharmaceutically acceptable excipient, or a combination thereof. In another aspectexample, provided herein is a pharmaceutical composition comprising an effective amount of the isolated cell of any one of the above embodimentexamples, and a pharmaceutically acceptable carrier, a pharmaceutically acceptable excipient, or a combination thereof. In another aspect, the present disclosure provides a use of the isolated cells as described herein, the isolated nucleic acid as described herein, the vector as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating a subject in need thereof. In another aspect, the present disclosure provides a use of the isolated cells as described herein, the isolated nucleic acid as described herein, the vector as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for stimulating a cell-mediated immune response to a tumor cell in a subject. In another aspect, the present disclosure provides a use of the isolated cells as described herein, the isolated nucleic acid as described herein, the vector as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for providing an anti-tumor immunity in a subject. In another aspect, the present disclosure provides a use of the isolated cells as described herein, the isolated nucleic acid as described herein, the vector as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for reducing tumor burden in a subject, optionally wherein: (a) the use reduces the number of tumor cells, (b) the use reduces tumor size, (c) the use reduces tumor volume, and/or (d) the use eradicates the tumor in the subject. In another aspect, the present disclosure provides a use of the isolated cells as described herein, the isolated nucleic acid as described herein, the vector as described herein, or

the pharmaceutical composition as described herein in the manufacture of a medicament for treating a subject having a tumor. In another aspect, the present disclosure provides a use of the isolated cells as described herein, the isolated nucleic acid as described herein, the vector as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating or preventing a cancer in a subject, wherein the cancer is selected from the group consisting of: colorectal carcinoma, pancreatic cancer, a lung adenocarcinoma, and gastric cancer. In some **embodimentexamples**, the pharmaceutical composition is for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer.

[0073] In another aspect, the present disclosure provides a kit for use in treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer, comprising the isolated cell as described herein, the isolated nucleic acid as described herein, the vector as described herein, or the pharmaceutical composition as described herein, optionally wherein the kit further comprises written instructions for using the isolated cell or pharmaceutical composition for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer in a subject, or the kit further comprises instructions for using the nucleic acid or vector for producing one or more antigen-specific cells for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer in a subject. In another **aspectexample**, provided herein is a kit for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer, comprising the isolated cell of any one of the above **embodimentexamples**, or the pharmaceutical composition of the above **aspectexample**. In some **embodimentexamples**, the kit further comprises written instructions for using the isolated cell for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer in a subject.

[0074] In another **aspectexample**, provided herein is a kit for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer, comprising the isolated nucleic acid or the vector of the above **aspectexamples**. In some **embodimentexamples**, the kit further comprises written instructions for using the nucleic acid for producing one or more antigen-specific cells for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0075] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0076] These and other features, aspects, and advantages of the present disclosure will become better understood with regard to the following description, and accompanying drawings.

sequence (Gly-Gly-Gly-Gly-Ser)_n (SEQ ID NO: 139) or (Gly-Gly-Gly-Ser)_n (SEQ ID NO: 140), where n is a positive integer equal to or greater than 1. For example, n=1, n=2, n=3, n=4, n=5, n=6, n=7, n=8, n=9, or n=10. In some embodiments, the flexible polypeptide linkers include, but are not limited to, Gly₄Ser (SEQ ID NO: 37) or (Gly₄Ser)₃ (SEQ ID NO: 39). In other embodiments, the linkers include multiple repeats of (Gly₂Ser) (SEQ ID NO: 27), (GlySer) or (Gly₃Ser) (SEQ ID NO: 32). In some embodiments, the flexible polypeptide linkers include a Whitlow linker (*e.g.*, GSTSGSGKPGSGEGSTKG [SEQ ID NO:42]). Also included within the scope of the present disclosure are linkers described, for example, in WO2012/138475.

[0164] As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity and/or duration of a proliferative disorder (*e.g.*, cancer), or the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of a proliferative disorder resulting from the administration of one or more therapies (*e.g.*, one or more therapeutic agents such as a CAR of the present disclosure). In some embodiments, reduction or amelioration refers to the amelioration of at least one measurable physical parameter of a proliferative disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments, the terms "treat", "treatment", and "treating" refer to the inhibition of the progression of a proliferative disorder, either physically by, *e.g.*, stabilization of a discernible symptom, physiologically by, *e.g.*, stabilization of a physical parameter, or both. In some embodiments, reduction or amelioration include reduction or stabilization of tumor size or cancerous cell count.

[0165] As used herein, the term "subject" is intended to include living organisms in which an immune response can be elicited (*e.g.*, mammals, human).

Other interpretational conventions

[0166] Ranges recited herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50.

[0167] Unless otherwise indicated, reference to a compound that has one or more stereocenters intends each stereoisomer, and all combinations of stereoisomers, thereof.

Solid tumor antigens

[0168] Certain aspects of the present disclosure relate to chimeric receptors and cells, such as immunoresponsive cells, that have been genetically modified to express one or more of such chimeric receptors that bind to an antigen of interest, and to methods of using such receptors and cells to treat and/or prevent solid malignancies, such as lung cancer, pancreatic cancer,

VH₁-VH₂-VL₂. In some embodiments, a linker is disposed between the two antibodies or antibody fragments (*e.g.*, scFvs), for example, between VL₁ and VL₂ if the construct is arranged as VH₁-VL₁-VL₂-VH₂, or between VH₁ and VH₂ if the construct is arranged as VL₁-VH₁-VH₂-VL₂. The linker may be a linker as described herein, *e.g.*, a (Gly₄-Ser)_n linker ([SEQ ID NO: 138](#)), wherein n is 1, 2, 3, 4, 5, or 6. In general, the linker between the two scFvs should be long enough to avoid mispairing between the domains of the two scFvs. In some embodiments, a linker is disposed between the VL and VH of the first scFv. In some embodiments, a linker is disposed between the VL and VH of the second scFv. In constructs that have multiple linkers, any two or more of the linkers may be the same or different. Accordingly, in some embodiments, a bispecific CAR or tanCAR comprises VLs, VHs, and may further comprise one or more linkers in an arrangement as described herein.

[0219] In some embodiments, the bivalent receptor comprises a CEA CAR and a CEACAM1 CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and a CEACAM5 CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and a CEACAM6 CAR. In some embodiments, the bivalent receptor comprises a CEACAM1 CAR and a CEACAM5 CAR. In some embodiments, the bivalent receptor comprises a CEACAM1 CAR and a CEACAM6 CAR. In some embodiments, the bivalent receptor comprises a CEACAM5 CAR and a CEACAM6 CAR.

[0220] In some embodiments, the bivalent receptor comprises a CEA CAR and a VSIG2 CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and a CPM CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and a ITM2C CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and a SLC26A2 CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and a SLC4A4 CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and a GPA33 CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and a PLA2G2A CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and an ABCA8 CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and an ATP1A2 CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and a CHP2 CAR. In some embodiments, the bivalent receptor comprises a CEA CAR and an SLC26A3 CAR.

[0221] In some embodiments, the bivalent receptor comprises a CEACAM1 CAR and a VSIG2 CAR. In some embodiments, the bivalent receptor comprises a CEACAM1 CAR and a CPM CAR. In some embodiments, the bivalent receptor comprises a CEACAM1 CAR and a ITM2C CAR. In some embodiments, the bivalent receptor comprises a CEACAM1 CAR and a SLC26A2 CAR. In some embodiments, the bivalent receptor comprises a CEACAM1 CAR and a SLC4A4 CAR. In some embodiments, the bivalent receptor comprises a CEACAM1 CAR and

	ACTTCGCCGCCTACCGGTCCAGAGTGAAGTTCAGCAGATCCGCCGATGCTCCCGCCTA TAAGCAGGGCCAGAATCAGCTGTACAACGAGCTGAACCTGGGGCGCAGAGAAGAGTA CGACGTGCTGGACAAGAGAAGAGGCAGGGACCCTGAGATGGGGCGCAAGCCCAGAA GAAAGAACCCTCAAGAGGGCCTGTATAACGAACCTGCAGAAAGACAAGATGGCCGAGG CCTACAGCGAGATCGGAATGAAGGGCGAACGCAGAAAGAGGAAAGGGGCCACGACGGA CTGTATCAGGGCCTGAGCACAGCCACCAAGGACACCTATGATGCCCTGCACATGCAGG CCCTGCCTCCAAGA (SEQ ID NO: 87)
SB02476 A.A. Seq	MALPVTALLLPLALLLHAARPDQMOTQTTSSLSASLGDRVTISCRTSQDIGNYLNWYQQKP DGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGKSLPRTFGGGT KLEIKEQKLISEEDLESKYGPPCPSCPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRL HSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 88)
SB02779 & SB03174 DNA Seq	ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCCGCCAG ACCTGACATCCAGCTGACACAGAGCCCTAGCAGCCTGTCTGCCTCTGTGGGCGACAGA GTGACCATCACATGCAAGGCCTCTCAGGACGTGGGCACAAGCGTGGCATGGTATCAG CAGAAGCCTGGCAAGGCCCTAAGCTGCTGATCTACTGGACCAGCACCAGACACACA GGCGTGCCAGCAGATTTTCTGGCAGCGGCTCTGGCACCAGCTTCACCTTCACCATAA GCAGCCTGCAGCCTGAGGATATCGCCACCTACTACTGCCAGCAGTACAGCCTGTACAG AAGCTTCGGCCAGGGCACCAAGGTGGAATCAAAGGCGGATCTGGAAGCGGCGGTTT TGGATCTGGTGGAAGCGGATCTGAGGTGCAGCTGGTGGAATCTGGTGGCGGAGTTGTG CAGCCTGGCAGATCTCTGAGACTGAGCTGTAGCGCCAGCGGCTTCGATTTACCACCT ACTGGATGAGCTGGGTCCGACAGGCCCTGGCAAAGGACTGGAATGGATCGGCGAGA TTCACCCCGACAGCAGCACCATCAATTACGCCCTAGCCTGAAGGACCGGTTACCAT CTCCAGAGACAACGCCAAGAATACCCTGTTCTGCAGATGGACAGCCTCCGGCCTGAA GATACCGGCGTGTAATTTTGCGCCAGCCTGTATTTTCGGCTTCCCTTGGTTTGCCTACTG GGGCCAGGGAACACCTGTGACCGTTAGCTCTGAGCAGAAGCTGATCTCCGAAGAGGA CCTGACCACAACACCAGCTCCTAGACCACCTACACCAGCACCTACAATCGCCCTGCAG CCACTGTCTCTGAGGCCAGAGGCTTGTAAGACCTGCTGCTGGCGGAGCCGTGCATACAA GAGGACTGGATTTTCGCTGCGACGTGGCCGCCATTCTCGGACTGGGACTTGTTCTGGG ACTGCTGGGACCTCTGGCCATTCTGCTGGCTCTGTACCTGCTGAGAAGGGACCAGAGA CTGCCTCCTGACGCTCACAACCTCCAGGCGGAGGCAGCTTCAGAACCCCTATCCAAG AGGAACAGGCTGACGCCCACAGCACCTGGCCAAGATCAGAGTGAAGTTCAGCAGAT CCGCCGACGCTCCCGCCTATAAGCAGGGACAGAATCAGCTGTACAACGAGCTGAACC TGGGGCGCAGAGAAGAGTACGACGTGCTGGACAAGAGAAGAGGCAGGGATCCTGAA ATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAG AAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAACGCAGAAG AGGAAAGGGGCCACGACGAGCTGTATCAGGGCCTGAGCACAGCCACCAAGGACACCTA TGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA (SEQ ID NO: 13688)
SB02779 & SB03174 A.A. Seq	MALPVTALLLPLALLLHAARPDQLTQSPSSLSASVGDRTITCKASQDVGTSAWYQQKP GKAPKLLIYWTSTRHTGVPSRFSGSGSGTDFTFTISSLPEDIATYYCQQYSLYRSFGQGTK VEIKGSGSGSGSGSGSEVQLVESGGGVVQPGRSLRLSCSASGFDFTTYWMSWVRQAP GKGLEWIGEIHPSSTINYAPSLKDRFTISRDNKNTLFLQMDSLRPEDTGVIYFCASLYFGF PWFAYWGQGTPTVTSSEQKLISEEDLTTTPAPRPPTAPLIALQPLSLRPEACRPAAGGAVH TRGLDFACDVAAILGLGLVLGLLPLAILLALYLLRRDQRLPPDAHKPPGGGSFRTPIQEEQ ADAHSTLAKIRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHGGLYQGLSTATKDTYDALHMQ ALPPR (SEQ ID NO: 89)
SB02782 DNA Seq	ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCCGCTAG ACCCCAAGTGAAGCTGGAACAGTCTGGCGCCGAGGTTGTGAAACCTGGCGCCTCTGTG AAGCTGAGCTGTAAAGCCAGCGGCTTCAACATCAAGGACAGCTACATGCACTGGCTG CGGCAAGGCCCTGGACAGAGACTGGAATGGATCGGCTGGATCGACCCCGAGAATGGC GATACAGAGTACGCCCCTAAGTTCCAGGGCAAAGCCACCTTCACCACCGACACCTCTG CCAACACAGCCTACCTGGGACTGTCTAGCCTGAGGCCTGAAGATACCGCCGTGTACTA CTGCAACGAGGGCACACCTACAGGCCCTACTACTTCGATTATTGGGGCCAGGGCACC CTGGTCAACGTTTCTAGCGGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGC GGTTCTGAAAATGTGCTGACACAGAGCCCCAGCAGCATGTCCGTGTCTGTGGGCGACA GAGTGACAATCGCCTGTAGCGCCTTAGCAGCGTGCCCTATATGCATTGGCTGCAGCA GAAGCCCGGCAAGAGCCCTAAGCTGCTGATCTACCTGACCAGCAATCTGGCCAGCGG

	CACCTGCAGGCCGAGGACTCCGCCATCTACTATTGCACCAGAGACAGAGGCCTGCGG TTCTACTTTCGACTATTGGGGCCAGGGAACCCTGGTCACAGTGTCTAGCGCCTCTACAA AGGGCCCCAGCGTTTTTCCCACTGGCTCCCTGTAGCAGAAGCACCAGCGAATCTACAGC CGCTCTGGGCTGCCTGGTCAAGGACTACTTTCTGAGCCAGTGACAGTGCTGTATCTCC AGATGAATACTCTCCAAGCTGAGGACAGCGCCATCTATTACTGTACGCGCGATCGGGG CCTGAGATTCTACTTTGATTACTGGGGACAAGGCACCCTCGTGACCGTGTCTCTGAG CAGAAGCTGATCTCCGAAGAGGACCTGAATGGCGCCGCTACCACAACACCAGCTCCT AGACCTCCAACCTCCTGCTCCTACAATCGCCCTGCAGCCACTGTCTCTGAGGCCTGAAG CTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACCAGAGGACTGGATTTTCGCTGCGA CATCTACATCTGGGCCCCCTCTGGCTGGAACATGCGGAGTGTTGCTGCTGAGCCTGGTC ATCACCAAGCGGGGAGAGAAAGAGCTGCTGTACATCTTCAAGCAGCCCTTCATGCGGC CCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGCAGATTCCCCGAGGAAGAAG AAGGCGGCTGCGAGCTGAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCA GCAGGGACAGAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACG ACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCAGACGG AAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAGAAAGACAAGATGGCCGAGGCC TACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGACT GTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCAGGCC CTGCCTCCAAGA (SEQ ID NO: 106)
SB03100 A.A. Seq	MALPVTALLPLALLLHAARPQTVLTQSPSSLSVSVGDRVITITCRASSSVTYIHWYQQKPG LAPKSLIYATSNLASGVPSRFSGSGSGTDYFTISSLQPEDATYYCQHWSSKPPTFGQGTK VEVKRTVGGGGSGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCATSGFTFTDYYMNWV RQAPGKGLEWLGFIGNKANGYTTEYSASVKGRFTISRDKSKSTLYLQMNLTQAEDSAIYY CTRDRGLRFYFDYWGQGLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV TVLYLQMNLTQAEDSAIYYCTRDRGLRFYFDYWGQGLVTVSSEQLISEEDLNGAATTT PAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI TKRGRKKLLYIFKQPFMRPVQTTQEEDGCSRFPPEEEGGCELRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRGKGDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 107)
SB03173 DNA Seq	ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCCGCCAGACC TGACATCCAGCTGACACAGAGCCCTAGCAGCCTGTCTGCCTCTGTGGGCGACAGAGTGACC ATCACATGCAAGGCCTCTCAGGACGTGGGCACAAGCGTGGCATGGTATCAGCAGAAGCCT GGCAAGGCCCTAAGCTGCTGATCTACTGGACCAGCACCAGACACACAGGCGTGCCCAGC AGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACCTTCACCATAAGCAGCCTGCAGCCTG AGGATATCGCCACCTACTACTGCCAGCAGTACAGCCTGTACAGAAGCTTCGGCCAGGGCAC CAAGGTGGAATCAAAGGCGGATCTGGAAGCGGCGGTTCTGGATCTGGTGGAAGCGGATC TGAGGTGCAGCTGGTGGAATCTGGTGGCGGAGTTGTGCAGCCTGGCAGATCTCTGAGACTG AGCTGTAGCGCCAGCGGCTTCGATTTCACCACCTACTGGATGAGCTGGGTCCGACAGGCC CTGGCAAAGGACTGGAATGGATCGGCGAGATTACCCCGACAGCAGCACCATCAATTACG CCCCTAGCCTGAAGGACCGGTTACCATCTCCAGAGACAACGCCAAGAATACCCTGTTCTC GCAGATGGACAGCCTCCGGCCTGAAGATACCGGCGTGTACTTTTGCGCCAGCCTGTATTT GGCTTCCCTTGGTTTGCCTACTGGGGCCAGGGAACACCTGTGACCGTTAGCTCTGAGCAGA AGCTGATCTCCGAAGAGGACCTGACCACAACACCAGCTCCTAGACCACCTACACCAGCAC CTACAATCGCCCTGCAGCCACTGTCTCTGAGGCCTGAAGCTTGTAGACCTGCTGCTGGCGG AGCCGTGCATACAAGAGGACTGGATTTTCGCTGCGACTTCTGGGTGCTCGTTGTTGTTGGC GGCGTGCTGGCCTGTTATTCCCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGAAG CAAGCGGAGCAGACTGCTGCACTCCGACTACATGAACATGACCCCTAGACGGCCCGGACC AACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCCAGA GTGAAGTTCTCCAGATCCGCCGATGCTCCCGCCTATAAGCAGGGACAGAACCAGCTGTACA ACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGA GATCCTGAGATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAGAGGGCCTGTATAATGAG CTGCAAAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAG AAGAGGCAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTA TGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA (SEQ ID NO: 137407)
SB03173 A.A. Seq	MALPVTALLPLALLLHAARDIQLTQSPSSLSASVGDRTITCKASQDVGTSAWYQQKP GKAPKLLIYWTSTRHTGVPSRFSGSGSGTDFTFTISSLQPEDATYYCQQYSLYRSFGQGTK VEIKGSGSGSGSGSGSGSEVQLVESGGGVVQPGRSLRLSCASGFDFTTYWMSWVRQAP GKGLEWIGEIHPSSTINYAPSLKDRFTISRDNKNTLFLQMDSLRLPEDTGVIYFCASLYFGF

CLAIMS

What is claimed is:

1. An isolated cell comprising:
 - (a) an inhibitory chimeric receptor comprising an extracellular antigen-binding domain that binds to an antigen, wherein the antigen is selected from the group consisting of: VSIG2, CPM, ITM2C, SLC26A2, SLC4A4, GPA33, PLA2G2A, ABCA8, ATP1A2, CHP2, and SLC26A3; and
 - (b) a chimeric receptor comprising one or more extracellular antigen-binding domains, wherein the one or more extracellular antigen-binding domains bind one or more additional antigens selected from the group consisting of: CEA, CEACAM1, CEACAM5, and CEACAM6.
2. The isolated cell of claim 1, wherein:
 - (a) the antigen-binding domain of the inhibitory chimeric receptor comprises one or more single chain variable fragments (scFvs), optionally wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL), optionally wherein the VH and VL are separated by a peptide linker, optionally the peptide linker comprises the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 77, and/or optionally wherein each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain;
 - (b) the inhibitory chimeric receptor comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28 transmembrane domain, a CD25 transmembrane domain, a CD7 transmembrane domain, a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a LAX transmembrane domain, a LAT transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a TIM3 transmembrane domain, a KIR3DS1 transmembrane domain, a KIR3DL1 transmembrane domain, an NKG2D transmembrane domain, an NKG2A transmembrane domain, a TIGIT transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain;
 - (c) the inhibitory chimeric receptor comprises a spacer region between the antigen-binding domain and the transmembrane domain, optionally wherein the spacer region

has an amino acid sequence selected from the group consisting of SEQ ID NOs: 49-58; and/or

(d) the inhibitory chimeric receptor comprises one or more intracellular inhibitory domains selected from the group consisting of: PD-1, CTLA4, TIGIT, LAIR1, GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, BTLA, LIR1, NKG2A, KIR3DL1, GITR, PD-L1, CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, RasGAP, CD94, and CD161

3. The isolated cell of claim 1 or claim 2, wherein the chimeric receptor is a CAR, optionally wherein the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of: a CD3zeta-chain intracellular signaling domain, a CD3epsilon-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, a 2B4 intracellular signaling domain, an NKp46 intracellular signaling domain, an NKp30 intracellular signaling domain, an NKp44 intracellular signaling domain, an NKG2D intracellular signaling domain, a CD226 intracellular signaling domain, and a CD160 intracellular signaling domain, optionally wherein the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28 transmembrane domain, a CD25 transmembrane domain, a CD7 transmembrane domain, a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a LAX transmembrane domain, a LAT transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a TIM3 transmembrane domain, a KIR3DS1 transmembrane domain, a KIR3DL1 transmembrane domain, an NKG2D transmembrane domain, an NKG2A transmembrane domain, a TIGIT transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain,

optionally wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 49-58, and optionally wherein the antigen-binding domain of the inhibitory chimeric receptor and/or the chimeric receptor comprises one or more single chain variable fragments (scFvs), optionally wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL), optionally wherein the VH and VL are separated by a peptide linker, optionally wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 39 or SEQ ID NO: 77, optionally wherein each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

4. The isolated cell of any one of claims 1-3, wherein:
 - (a) the one or more additional antigen is CECAM1, optionally wherein the antigen-binding domain that binds to CECAM1 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 1 and a light chain variable domain (VL) comprising the amino acid sequence of SEQ ID NO: 2;
 - (b) the one or more additional antigen is CECAM5, optionally wherein the antigen-binding domain that binds to CECAM5 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
 - (i) a VH comprising the amino acid sequence of SEQ ID NO: 3 and a VL comprising the amino acid sequence of SEQ. ID NO: 4;
 - (ii) a VH comprising the amino acid sequence of SEQ ID NO: 9 and a VL comprising the amino acid sequence of SEQ. ID NO: 10;
 - (iii) a VH comprising the amino acid sequence of SEQ ID NO: 11 and a VL comprising the amino acid sequence of SEQ. ID NO: 12;
 - (iv) a VH comprising the amino acid sequence of SEQ ID NO: 13 and a VL comprising the amino acid sequence of SEQ. ID NO: 14;
 - (v) a VH comprising the amino acid sequence of SEQ ID NO: 78 and a VL comprising the amino acid sequence of SEQ. ID NO: 79; and
 - (vi) a VH comprising the amino acid sequence of SEQ ID NO: 15 and a VL comprising the amino acid sequence of SEQ. ID NO: 16; or
- the antigen-binding domain that binds to CECAM5 comprises a heavy chain (HC) and a light chain (LC) selected from the group consisting of:

- (i) an HC comprising the amino acid sequence of SEQ ID NO: 5 and an LC comprising the amino acid sequence of SEQ. ID NO: 6; and
 - (ii) an HC comprising the amino acid sequence of SEQ ID NO: 7 and an LC comprising the amino acid sequence of SEQ. ID NO: 8; or
 - (c) the one or more additional antigen is CECAM6, optionally wherein the antigen-binding domain that binds to CEACAM6 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 17 and a light chain variable domain (VL) comprising the amino acid sequence of SEQ ID NO 18.
5. The isolated cell of any one of claims 1-4, wherein the cell is an immunoresponsive cell, optionally wherein binding of the inhibitory chimeric receptor to the antigen is capable of inhibiting the immunoresponsive cell and/or wherein binding of the chimeric receptor to the one or more additional antigens is capable of activating the immunoresponsive cell.
 6. The isolated cell of any one of claims 1-5, wherein:
 - (a) the chimeric receptor binds to the one or more additional antigens with a low binding affinity;
 - (b) the chimeric receptor binds to the one or more additional antigens with a binding affinity that is lower than the binding affinity with which the inhibitory chimeric receptor binds to the antigen; and/or
 - (c) the inhibitory chimeric receptor binds to the antigen with a low binding avidity.
 7. The isolated cell of any one of claims 1-6, wherein the chimeric receptor is recombinantly expressed, optionally wherein the chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.
 8. The isolated cell of any one of claims 1-7, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell, optionally wherein the cell is autologous or the cell is allogeneic.
 9. An isolated nucleic acid encoding the inhibitory chimeric receptor of any one of claims 1-8.

10. A vector comprising the nucleic acid of claim 9.
11. A genetically modified cell comprising the nucleic acid of claim 9 or the vector of claim 10.
12. A pharmaceutical composition comprising an effective amount of the isolated cell of any one of claims 1-8 and 11, the isolated nucleic acid of claim 9, or the vector of claim 10, and a pharmaceutically acceptable carrier, a pharmaceutically acceptable excipient, or a combination thereof.
13. ~~A method of treating a subject in need thereof, the method comprising administering to the subject an effective amount~~ Use of the isolated cells of any one of claims 1-8 and 11, the isolated nucleic acid of claim 9, the vector of claim 10, or the pharmaceutical composition of claim 12 in the manufacture of a medicament for treating a subject in need thereof.
14. ~~A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor an effective amount of the isolated cells~~ Use of any one of claims 1-8 and 11, the isolated nucleic acid of claim 9, the vector of claim 10, or the pharmaceutical composition of claim 12 in the manufacture of a medicament for stimulating a cell-mediated immune response to a tumor cell in a subject.
15. ~~A method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof an effective amount~~ Use of the isolated cells of any one of claims 1-8 and 11, the isolated nucleic acid of claim 9, the vector of claim 10, or the pharmaceutical composition of claim 12 in the manufacture of a medicament for providing an anti-tumor immunity in a subject.
16. ~~A method of reducing tumor burden in a subject, comprising administering to the subject an effective amount~~ Use of the isolated cells of any one of claims 1-8 and 11, the isolated nucleic acid of claim 9, the vector of claim 10, or the pharmaceutical composition of claim 12 in the manufacture of a medicament for reducing tumor burden in a subject, optionally wherein:
 - (a) the ~~method~~ use reduces the number of tumor cells,
 - (b) the ~~method~~ use reduces tumor size,

- (c) the ~~method-use~~ reduces tumor volume, and/or
- (d) the ~~method-use~~ eradicates the tumor in the subject.
17. ~~A method of treating a subject having a tumor, the method comprising administering an effective amount~~ Use of the isolated cells of any one of claims 1-8 and 11, the isolated nucleic acid of claim 9, the vector of claim 10, or the pharmaceutical composition of claim 12 in the manufacture of a medicament for treating a subject having a tumor.
18. ~~A method of treating or preventing a cancer in a subject, wherein the cancer is selected from the group consisting of: colorectal carcinoma, pancreatic cancer, a lung adenocarcinoma, and gastric cancer, the method comprising administering to the subject an effective amount~~ Use of the isolated cells of any one of claims 1-8 and 11, the isolated nucleic acid of claim 9, the vector of claim 10, or the pharmaceutical composition of claim 12 in the manufacture of a medicament for treating or preventing a cancer in a subject, wherein the cancer is selected from the group consisting of: colorectal carcinoma, pancreatic cancer, a lung adenocarcinoma, and gastric cancer.
19. The ~~method-use~~ of claim 17 or claim 18, wherein:
- (a) the ~~method-use~~ increases progression free survival in the subject; and or
- (b) the ~~method-use~~ increases survival in the subject.
20. A kit for use in treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer, comprising the isolated cell of any one of claims 1-8 and 11, the isolated nucleic acid of claim 9, the vector of claim 10, or the pharmaceutical composition of claim 12,
- optionally wherein the kit further comprises written instructions for using the isolated cell or pharmaceutical composition for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer in a subject, or the kit further comprises instructions for using the nucleic acid or vector for producing one or more antigen-specific cells for treating and/or preventing a colorectal carcinoma, a pancreatic cancer, a lung adenocarcinoma, and/or a gastric cancer in a subject.