

binding chimeric antigen receptors (CARs), and cells expressing such CARs, are available. However, there remains a need for improved GPRC5D-binding CARs and engineered GPRC5D-CAR expressing targeting cells, such as for use in adoptive cell therapy. Provided herein are embodiments that meet such needs.

Summary

[0005] Provided herein are chimeric antigen receptors containing: (1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPRC5D), wherein the extracellular antigen-binding domain contains: (i) a variable heavy chain (V_H) region containing an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_H region amino acid sequence set forth in any of SEQ ID NO:21, 23, 25, 27, 29, 31 or 33; and (ii) a variable light chain (V_L) region containing an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_L region amino acid sequence set forth in any of SEQ ID NO:22, 24, 26, 28, 30, 32 or 34; (2) a spacer of at least 125 amino acids in length; (3) a transmembrane domain; and (4) an intracellular signaling region.

[0005a] In one example, the present disclosure refers to a chimeric antigen receptor comprising: (1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPRC5D), wherein the extracellular antigen-binding domain comprises: (i) a variable heavy chain (V_H) region comprising an amino acid sequence having at least at or about 90%, at or about 91%, at or about 92%, at or about 93%, at or about 94%, at or about 95%, at or about 96%, at or about 97%, at or about 98%, or at or about 99% sequence identity to the amino acid sequence set forth in SEQ ID NO:27; and (ii) a variable light chain (V_L) region comprising an amino acid sequence having at least at or about 90%, at or about 91%, at or about 92%, at or about 93%, at or about 94%, at or about 95%, at or about 96%, at or about 97%, at or about 98%, or at or about 99% sequence identity to the amino acid sequence set forth in SEQ ID NO:66; (2) a spacer of at least 125 amino acids comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge, an IgG2/4 chimeric CH2 region, and an IgG4 CH3 region; (3) a transmembrane domain; and (4) an intracellular signaling region.

[0006] Also provided are chimeric antigen receptors containing: (1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPRC5D), wherein the extracellular antigen-binding domain contains: (i) a variable heavy chain (V_H) containing a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence selected from any one of SEQ ID NOs: 21, 23, 25, 27, 29, 31 or 33; and (ii) a variable light chain (V_L) region containing a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence selected from any one of SEQ ID NOs: 22, 24, 26, 28, 30, 32 or 34; (2) a spacer of at least 125 amino acids in length; (3) a transmembrane domain; and (4) an intracellular signaling region.

[0006a] In one aspect, the present disclosure refers to a chimeric antigen receptor comprising: (1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPRC5D), wherein the extracellular antigen-binding domain comprises: (i) a variable heavy chain (V_H) region comprising a heavy chain complementarity determining region 1 (CDR-H1), heavy chain complementarity determining region 2 (CDR-H2) and heavy chain complementarity determining region 3 (CDR-H3) contained within the V_H region amino acid sequence of SEQ ID NO: 27; and (ii) a variable light chain (V_L) region comprising a light chain complementarity determining region 1 (CDR-L1), light chain complementarity determining region 2 (CDR-L2) and light chain complementarity determining region 3 (CDR-L3) contained within the V_L region amino acid sequence of SEQ ID NO: 66; (2) a spacer with the amino acid sequence set forth in SEQ ID NO: 17; (3) a transmembrane domain; and (4) an intracellular signaling region.

[0007] Also provided are chimeric antigen receptors containing: (1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPRC5D) wherein the extracellular antigen-binding domain contains: (i) a variable heavy chain (V_H) containing a heavy chain complementarity determining region 1 (CDR-H1) containing the amino acid sequence selected from any one of SEQ ID NOs: 75, 78, 80, 82, 90, 93, 95, 97, 105, 108, 110, 112, 120, 123, 125, 127, 135, 138, 140, 142, 135, 152, 162, 165, 167 or 169; (b) a heavy chain complementarity determining region 2 (CDR-H2) containing the amino acid sequence selected from any one of SEQ ID NOs: 76, 79, 81, 83, 91, 94, 96, 98, 106, 109, 111, 113, 121, 124, 126, 128, 136, 139, 141, 143, 150, 153, 154, 155, 163, 166, 169 or 170; and (c) a heavy chain complementarity determining region 3 (CDR-H3) containing the amino acid sequence selected from any one of SEQ ID NOs: 77, 84, 92, 99, 107, 114, 133, 129, 137, 144, 151, 156, 164 or 171; and (ii) a variable light chain (V_L) region containing a light chain complementarity determining region 1 (CDR-L1) containing the amino acid sequence selected from any one of SEQ ID NOs: 85, 88, 100, 103, 115, 118, 130, 133, 145, 148, 157, 160, 172 or 174; (b) a light

chain complementarity determining region 2 (CDR-L2) containing the amino acid sequence selected from any one of SEQ ID NOs: 86, 89, 101, 104, 116, 119, 131, 134, 146, 149, 158 or 161; and (c) a light chain complementarity determining region 3 (CDR-L3) containing the amino acid sequence selected from any one of SEQ ID NOs: 87, 102, 117, 132, 147, 159, 173, 175, or 297; (2) a spacer of at least 125 amino acids in length; (3) a transmembrane domain; and (4) an intracellular signaling region.

[0007a] In another aspect, the present disclosure refers to a chimeric antigen receptor comprising: (1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPCR5D), wherein the extracellular antigen-binding domain comprises: (i) a variable heavy chain (V_H) region comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 125; (b) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 126; and (c) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 122; and (ii) a variable light chain (V_L) region comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 130; (b) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 131; and (c) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 132; (2) a spacer with the amino acid sequence set forth in SEQ ID NO: 17; (3) a transmembrane domain; and (4) an intracellular signaling region.

[0008] In some of any of the provided examples, the extracellular antigen-binding domain of the chimeric antigen receptor contains: (i) a variable heavy chain (V_H) containing: an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_H region amino acid sequence set forth in any of SEQ ID NO: 21, 23, 25, 27, 29, 31 or 33; and (ii) a variable light chain (V_L) region containing an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_L region amino acid sequence set forth in any of SEQ ID NO: 22, 24, 26, 28, 30, 32 or 34.

[0009] In some of any of the provided examples, the spacer has a length from or from about 125 to 300 amino acids in length, 125 to 250 amino acids in length, 125 to 230 amino acids in length, 125 to 200 amino acids in length, 125 to 180 amino acids in length, 125 to 150 amino acids in length, 150 to 300 amino acids in length, 150 to 250 amino acids in length, 150 to 230 amino acids in length, 150 to 200 amino acids in length, 150 to 180 amino acids in length, 180 to 300 amino acids in length, 180 to 250 amino acids in length, 180 to 230 amino acids in length, 180 to 200 amino acids in length, 200 to 300 amino acids in length, 200 to 250 amino acids in length, 200 to 230 amino acids in length, 230 to 300 amino acids in length, 230 to 250 amino acids in length or 250 to 300 amino acids in length. In some of any of the provided examples, the spacer is at least or at least about or is or is about 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 221, 222, 223, 224, 225, 226, 227, 228 or 229 amino acids in length, or has a length between any of the foregoing.

[0010] In some of any of the provided examples, the spacer is derived from an immunoglobulin. In some of any of the provided examples, the spacer contains a sequence of a hinge region, a CH2 and CH3 region. In some of any of the provided examples, one of more of the hinge, CH2 and CH3 is derived all or in part from IgG4 or IgG2, optionally human IgG4 or human IgG2. In some of any of the provided examples, the hinge, CH2 and CH3 is derived from IgG4. In some of any of the provided examples, one or more of the hinge, CH2 and CH3 is chimeric and contains a sequence derived from IgG4 and IgG2. In some of any of the provided examples, the spacer contains an IgG4/2 chimeric hinge or a modified IgG4 containing at least one amino acid replacement compared to human IgG4, an IgG2/4 chimeric CH2, and an IgG4 CH3 region.

[0011] In some of any of the provided examples, the spacer is or contains (i) the sequence set forth in SEQ ID NO: 17; (ii) a functional variant of SEQ ID NO: 17 that has at least 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 17; or (iii) a contiguous portion of (i) or (ii) that is at least 125 amino acids in length. In some of any of the provided examples, the spacer is or contains the

of a zeta chain of a human CD3-zeta (CD3 ζ) chain and an intracellular signaling domain of a human CD28. Also provided are chimeric antigen receptors containing: (1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor (GPCR5D), wherein the extracellular antigen-binding domain contains: a variable heavy (V_H) region containing a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence set forth in SEQ ID NO: 27; and a variable light (V_L) region containing a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence set forth in SEQ ID NO: 28; or a V_H region containing a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOS:125, 126 and 122, respectively, and a V_L region containing a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOS:130, 131 and 132, respectively; (2) a spacer set forth in SEQ ID NO:17; (3) a transmembrane domain derived from a human CD28; and (4) an intracellular signaling region containing a cytoplasmic signaling domain of a zeta chain of a human CD3-zeta (CD3 ζ) chain and an intracellular signaling domain of a human 4-1BB.

[0033a] In one aspect, the present disclosure refers to a chimeric antigen receptor comprising: (1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPCR5D), wherein the extracellular antigen-binding domain comprises: (i) a variable heavy chain (V_H) region comprising an amino acid sequence set forth in SEQ ID NO: 27; and (ii) a variable light chain (V_L) region comprising an amino acid sequence set forth in SEQ ID NO: 66; (2) a spacer with the amino acid sequence set forth in SEQ ID NO: 17; (3) a transmembrane domain from human CD28; and (4) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3 ζ) chain and an intracellular signaling domain of 4-1BB.

[0033b] In one aspect, the present disclosure refers to a chimeric antigen receptor comprising: (1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPCR5D), wherein the extracellular antigen-binding domain comprises: a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:125, 126 and 122, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:130, 131 and 132, respectively; (2) a spacer with the amino acid sequence set forth in SEQ ID NO: 17; (3) a transmembrane domain from human CD28; and (4) an intracellular signaling region comprising a cytoplasmic signaling domain of a human CD3-zeta (CD3 ζ) chain and an intracellular signaling domain of a human 4-1BB.

[0034] In some of any of the provided examples, the extracellular antigen-binding domain contains the V_H region amino acid sequence set forth in SEQ ID NO:27 and the V_L region amino acid sequence set forth in SEQ ID NO:28; and/or in some of any of the provided examples, the extracellular antigen-binding domain contains an scFv set forth in SEQ ID NO:7 or SEQ ID NO:8. In some of any of the provided examples, the extracellular antigen-binding domain contains the V_H region amino acid sequence set forth in SEQ ID NO:27 and the V_L region amino acid sequence set forth in SEQ ID NO:28; and the extracellular antigen-binding domain contains an scFv set forth in SEQ ID NO:7. In some of any of the provided examples, the extracellular antigen-binding domain contains the V_H region amino acid sequence set forth in SEQ ID NO:27 and the V_L region amino acid sequence set forth in SEQ ID NO:28; and the extracellular antigen-binding domain contains an scFv set forth in SEQ ID NO:8.

[0035] In some of any of the provided examples, the transmembrane domain is or contains the sequence set forth in SEQ ID NO:18 or a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:18. In some of any of the provided examples, the transmembrane domain is or contains the sequence set forth in SEQ ID NO:18.

[0036] In some of any of the provided examples, the intracellular signaling region contains the sequence set forth in SEQ ID NO:20 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:20 and the sequence set forth in SEQ ID NO:46 or a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 46. In some of any of the provided examples, the intracellular signaling region is or contains the sequences set forth in SEQ ID NO:20 and SEQ ID NO:46. In some of any of the provided examples, the intracellular

[0178] In some of any of the provided examples, the engineered cell was engineered from a primary cell obtained from a subject.

[0179] In some of any of the provided examples, the engineered cell is among a plurality of the engineered cells, where less than or less than about 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2% or 1% of the cells in the plurality contain a chimeric antigen receptor that exhibits tonic signaling and/or antigen-independent activity or signaling.

[0180] Also provided are compositions containing any of the chimeric receptors provided herein. In some of any of the provided examples, the composition contains CD4⁺ and CD8⁺ T cells and the ratio of CD4⁺ to CD8⁺ T cells is from or from about 1:3 to 3:1. In some examples, the ratio of CD4⁺ and CD8⁺ T cells in the composition is 1:2 to 2:1. In some examples, the ratio of CD4⁺ and CD8⁺ T cells in the composition is 1:1. In some of any of the provided examples, the composition further contains a pharmaceutically acceptable excipient. In some of any of the provided examples, the composition is sterile.

[0180c] In yet another aspect, the present disclosure refers to a composition comprising: a plurality of T cells comprising a chimeric antigen receptor that is the chimeric antigen receptor disclosed herein or encoded by the polynucleotide disclosed herein.

[0180d] In one aspect, the present disclosure refers to the use of a composition comprising the cells disclosed herein or the composition disclosed herein for the manufacture of a medicament for treatment of a cancer.

[0181] Also provided herein are methods of treatment, including administering any of the compositions provided herein containing any of the engineered cells provided herein or any of the compositions provided herein containing any of the chimeric antigen receptors provided herein to a subject having a disease or disorder. In some of any of the provided examples, the dose of cells contains between at or about 2.5×10^7 CAR-expressing T cells and 1.2×10^9 CAR-expressing T cells, between at or about 5.0×10^7 CAR-expressing T cells and 4.5×10^8 CAR-expressing T cells, between at or about 1.5×10^8 CAR-expressing T cells and 3.0×10^8 CAR-expressing T cells. In some of any examples, the dose of cells contains between at or about 1×10^7 CAR-expressing T cells and at or about 2×10^9 CAR-expressing T cells. In some of any examples, the dose of cells contains between at or about 2.5×10^7 CAR-expressing T cells and at or about 1.2×10^9 CAR-expressing T cells, between at or about 5.0×10^7 CAR-expressing T cells and at or about 4.5×10^8 CAR-expressing T cells, or between at or about 1.5×10^8 CAR-expressing T cells and at or about 3.0×10^8 CAR-expressing T cells. In some of any examples, the dose of cells contains at or about 1.5×10^7 , at or about 2.5×10^7 , at or about 5.0×10^7 , at or about 7.5×10^7 , at or about 1.5×10^8 , at or about 2.25×10^8 , at or about 3.0×10^8 , at or about 4.5×10^8 , at or about 6.0×10^8 , at or about 8.0×10^8 or at or about 1.2×10^9 CAR-expressing T cells. In some of any examples, the dose of cells contains at or about 5.0×10^7 , at or about 1.5×10^8 , at or about 3.0×10^8 or at or about 4.5×10^8 CAR-expressing T cells. In some of any examples, the dose of cells contains at or about 5.0×10^7 , at or about 1.5×10^8 , at or about 3.0×10^8 or at or about 4.5×10^8 CAR-expressing T cells. In some of any examples, the dose of the cells contains at or about 5.0×10^7 CAR-expressing T cells.

[0182] Also provided herein are methods of treatment, including administering any of the compositions provided herein containing any of the engineered cells provided herein or any of the compositions provided herein containing any of the chimeric antigen receptors provided herein to a

subject having a disease or disorder. In some of any of the provided examples, the dose of cells contains contain between at or about 1.0×10^7 CAR-expressing T cells and 1.2×10^9 CAR-expressing T cells, between at or about 1.5×10^7 CAR-expressing T cells and 4.5×10^8 CAR-expressing T cells, between at or about 2.0×10^7 CAR-expressing T cells and 3.0×10^8 CAR-expressing T cells.

[0183] Also provided here in are methods of treatment, that include: administering a composition containing a plurality of engineered cells containing a first chimeric antigen receptor and a second chimeric antigen receptor, wherein each is any chimeric antigen receptor provided herein or encoded by any of the polynucleotides provided herein, to a subject having a disease or disorder; and administering to the subject a composition containing a plurality of second engineered cells containing a second chimeric antigen receptor. In some of any of the provided examples, the dose of the plurality of first engineered cells and the dose of the plurality of second engineered cells independently contain between at or about 1.0×10^7 CAR-expressing T cells and 1.5×10^9 CAR-expressing T cells, between at or about 1.25×10^7 CAR-expressing T cells and 0.6×10^8 CAR-expressing T cells, between at or about 2.5×10^7 CAR-expressing T cells and 2.25×10^8 CAR-expressing T cells, between at or about 7.5×10^7 CAR-expressing T cells and 1.5×10^8 CAR-expressing T cells, between at or about 2.5×10^7 CAR-expressing T cells and 1.2×10^9 CAR-expressing T cells, between at or about 5.0×10^7 CAR-expressing T cells and 4.5×10^8 CAR-expressing T cells, between at or about 1.5×10^8 CAR-expressing T cells and 3.0×10^8 CAR-expressing T cells.

[0183a] In one aspect, the present disclosure refers to a composition comprising a first dose of a plurality of first T cells comprising a first chimeric antigen receptor that is the chimeric antigen receptor disclosed herein or encoded by the polynucleotide disclosed herein and a composition comprising a second dose of a plurality of second T cells comprising a second chimeric antigen receptor for use in the treatment of a cancer.

[0183b] In another aspect, the present disclosure refers to the use of a composition comprising a first dose of a plurality of first cells comprising a first chimeric antigen receptor that is the chimeric antigen receptor disclosed herein or encoded by the polynucleotide disclosed herein and a composition comprising a second dose of a plurality of second cells comprising a second chimeric antigen receptor for the manufacture of a medicament for treatment of a cancer.

[0183c] In yet another example, the present disclosure refers to a combination comprising: a plurality of first T cells comprising a first chimeric antigen receptor that is the chimeric antigen receptor disclosed herein and/or encoded by the polynucleotide disclosed herein; and a plurality of second T cells comprising a second chimeric antigen receptor.

[0184] In some of any of the provided examples of the provided methods, the disease or disorder is associated with expression of G protein-coupled receptor class C group 5 member D (GPRC5D).

[0185] In some of any of the provided examples of the provided methods, the disease or disorder is further associated with expression of B cell maturation antigen (BCMA).

[0186] In some of any of the provided examples of the provided methods, the disease or disorder is a B cell-related disorder. In some of any of the provided examples of the provided methods, the disease or disorder associated with BCMA is an autoimmune disease or disorder. In some of any of the provided examples of the provided methods, the autoimmune disease or disorder is systemic lupus erythematosus (SLE), lupus nephritis, inflammatory bowel disease, rheumatoid arthritis, ANCA associated vasculitis, idiopathic thrombocytopenia purpura (ITP), thrombotic thrombocytopenia purpura (TTP), autoimmune thrombocytopenia, Chagas' disease, Grave's disease, Wegener's granulomatosis, poly-arteritis nodosa, Sjogren's syndrome, pemphigus vulgaris, scleroderma, multiple sclerosis, psoriasis, IgA nephropathy, IgM polyneuropathies, vasculitis, diabetes mellitus, Reynaud's syndrome, anti-phospholipid syndrome, Goodpasture's disease, Kawasaki disease, autoimmune hemolytic anemia, myasthenia gravis, or progressive glomerulonephritis.

[0187] In some of any of the provided examples of the provided methods, the disease or disorder is a cancer. In some of any of the provided examples of the provided methods, the cancer is

some embodiments, the CARs include activating or stimulatory CARs, and costimulatory CARs, both expressed on the same cell (see WO 2014/055668). In some aspects, the GPRC5D-targeting CAR is the stimulatory or activating CAR; in other aspects, it is the costimulatory CAR. In some embodiments, the cells further include inhibitory CARs (iCARs, see Fedorov *et al.*, Sci. Transl. Medicine, 5(215) (December, 2013), such as a CAR recognizing an antigen other than GPRC5D, whereby a stimulatory or an activating signal delivered through the GPRC5D -targeting CAR is diminished or inhibited by binding of the inhibitory CAR to its ligand, *e.g.*, to reduce off-target effects.

[0369] In certain embodiments, the intracellular signaling region comprises a CD28 transmembrane and signaling domain linked to a CD3 (*e.g.*, CD3-zeta) intracellular domain. In some embodiments, the intracellular signaling domain comprises a chimeric CD28 and 4-1BB (CD137; TNFRSF9) co-stimulatory domains, linked to a CD3 zeta intracellular domain.

[0370] In some embodiments, the CAR encompasses one or more, *e.g.*, two or more, costimulatory domains and a stimulatory or an activation domain, *e.g.*, primary activation domain, in the cytoplasmic portion. Exemplary CARs include intracellular components of CD3-zeta, CD28, and 4-1BB.

[0371] In some embodiments, provided embodiments of anti-GPRC5D CAR contains an extracellular antigen-binding domain containing any of the anti-GPRC5D antibody or antigen-binding fragments described herein, such as in Section I.1a; a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, such as one that is about 228 amino acids in length, or a spacer set forth in SEQ ID NO:17, such as encoded by the nucleotide sequence set forth in any of SEQ ID NOS: 73, 74, 283 or 284; a transmembrane domain, such as a transmembrane domain from a human CD28 ; and an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and an intracellular signaling domain of a T cell costimulatory molecule. Also provided are polynucleotides encoding such a chimeric antigen receptor. In some embodiments, the transmembrane domain is or comprises the sequence set forth in SEQ ID NO:18. In some embodiments, the intracellular signaling domain of a T cell costimulatory molecule is an intracellular signaling domain of human CD28, human 4-1BB or human ICOS or a signaling portion thereof. In particular embodiments, the intracellular signaling domain is an intracellular signaling domain of human 4-1BB. In some embodiments, the intracellular signaling domain is or comprises the sequence set forth in SEQ ID NO:19. In some embodiments, the cytoplasmic signaling domain is a human CD3-zeta cytoplasmic signaling domain, such as set forth in SEQ ID NO:20. In some embodiments, the intracellular signaling region comprises the sequences set forth in SEQ ID NO:20 and SEQ ID NO:19.

[0372] In some embodiments, provided embodiments of an anti-GPRC5D CARs has an amino acid sequence set forth in SEQ ID NO:290, or an amino acid sequence that is at least at or about 90%, at or about 91%, at or about 92%, at or about 93%, at or about 94%, at or about 95%, at or about 96%, at or about 97%, at or about 98% or at or about 99% sequence identity to the amino acid sequence set forth in

SEQ ID NO:290. In some embodiments, provided embodiments of an anti-GPRC5D CAR is encoded by the nucleotide sequence set forth in SEQ ID NO:289 or a nucleotide sequence that has at least at or about 90%, at or about 91%, at or about 92%, at or about 93%, at or about 94%, at or about 95%, at or about 96%, at or about 97%, at or about 98% or at or about 99% sequence identity to the sequence set forth in any of SEQ ID NO:289.

2. *Exemplary Features*

[0373] In some of any of the provided embodiments, the anti-GPRC5D CAR and/or anti-GPRC5D antigen-binding domain, specifically binds to GPRC5D, such as GPRC5D on the surface of a multiple myeloma plasma cell. In any of the embodiments, an antibody or antigen binding fragment, in the provided CARs, that specifically binds GPRC5D. In some embodiments binding can be to a human GPRC5D, a mouse GPRC5D protein, or a non-human primate (*e.g.*, cynomolgus monkey) GPRC5D protein. In some embodiments, among provided anti-GPRC5D CAR and/or anti-GPRC5D antigen-binding domain are those that bind human GPRC5D protein. The observation that an antibody or other binding molecule binds to GPRC5D protein or specifically binds to GPRC5D protein does not necessarily mean that it binds to a GPRC5D protein of every species. For example, in some embodiments, features of binding to GPRC5D protein, such as the ability to specifically bind thereto and/or to compete for binding thereto with a reference antibody, and/or to bind with a particular affinity or compete to a particular degree, in some embodiments, refers to the ability with respect to a human GPRC5D protein and the antibody may not have this feature with respect to a GPRC5D protein of another species, such as mouse.

[0374] In some embodiments, the antibodies specifically bind to human GPRC5D protein, such as to an epitope or region of human GPRC5D protein, such as the human BCMA protein comprising the amino acid sequence of SEQ ID NO:49 (Uniprot Q9NZD1), or an allelic variant or splice variant thereof.

[0375] In one embodiment, the extent of binding of an anti- GPRC5D antibody or antigen-binding domain or CAR to an unrelated, non- GPRC5D protein, such as a non-human GPRC5D protein or other non- GPRC5D protein, is less than at or about 10% of the binding of the antibody or antigen-binding domain or CAR to human GPRC5D protein or human membrane-bound GPRC5D as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, among the antibodies or antigen-binding domains in the provided CARs, are antibodies or antigen-binding domains or CARs in which binding to mouse GPRC5D protein is less than or at or about 10% of the binding of the antibody to human GPRC5D protein. In some embodiments, among the antibodies or antigen-binding domains in the provided CARs, are antibodies in which binding to cynomolgus monkey GPRC5D protein is less than or at or about 10% of the binding of the antibody to human GPRC5D protein. In some embodiments, among the antibodies or antigen-binding domains in the provided CARs, are antibodies in which binding to cynomolgus

Claims

1. A chimeric antigen receptor comprising:

(1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPRC5D), wherein the extracellular antigen-binding domain comprises:

(i) a variable heavy chain (V_H) region comprising a heavy chain complementarity determining region 1 (CDR-H1), heavy chain complementarity determining region 2 (CDR-H2) and heavy chain complementarity determining region 3 (CDR-H3) contained within the V_H region amino acid sequence of SEQ ID NO: 27; and

(ii) a variable light chain (V_L) region comprising a light chain complementarity determining region 1 (CDR-L1), light chain complementarity determining region 2 (CDR-L2) and light chain complementarity determining region 3 (CDR-L3) contained within the V_L region amino acid sequence of SEQ ID NO: 66;

(2) a spacer with the amino acid sequence set forth in SEQ ID NO: 17;

(3) a transmembrane domain; and

(4) an intracellular signaling region.

2. A chimeric antigen receptor comprising:

(1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPRC5D), wherein the extracellular antigen-binding domain comprises:

(i) a variable heavy chain (V_H) region comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO:125; (b) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:126; and (c) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:122; and

(ii) a variable light chain (V_L) region comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 130; (b) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 131; and (c) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 132;

(2) a spacer with the amino acid sequence set forth in SEQ ID NO: 17;

(3) a transmembrane domain; and

(4) an intracellular signaling region.

3. The chimeric antigen receptor of claim 2, wherein the extracellular antigen-binding domain comprises:

(i) a variable heavy chain (V_H) region comprising: an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 27; and

(ii) a variable light chain (V_L) region comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO: 66.

4. The chimeric antigen receptor of any of claims 1-3, wherein the V_H region and the V_L region comprise the amino acid sequence set forth in SEQ ID NOs: 27 and 66, respectively.

5. The chimeric antigen receptor of any of claims 1-4, wherein the extracellular antigen-binding domain comprises a single chain variable fragment (scFv).

6. The chimeric antigen receptor of any of claims 2-5, wherein the extracellular antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 8 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 8.

7. The chimeric antigen receptor of any of claims 1-6, wherein the extracellular antigen-binding domain is encoded by the nucleotide sequence of SEQ ID No: 264 or a nucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the nucleotide sequence of SEQ ID No: 264.

8. The chimeric antigen receptor of any of claims 1-7, wherein the intracellular signaling region comprises an intracellular signaling domain that comprises a cytoplasmic signaling domain of a CD3-zeta ($CD3\zeta$) chain or a functional variant or signaling portion thereof.

9. The chimeric antigen receptor of claim 8, wherein the cytoplasmic signaling domain of the $CD3\zeta$ chain is set forth in SEQ ID NO:20.

10. The chimeric antigen receptor of claim 8, wherein the intracellular signaling region further comprises a costimulatory signaling region.

11. The chimeric antigen receptor of claim 10, wherein the costimulatory signaling region comprises an intracellular signaling domain of CD28, 4-1BB, or ICOS, or a signaling portion thereof.

12. The chimeric antigen receptor of claim 11, wherein the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

13. The chimeric antigen receptor of claim 11 or 12, wherein the intracellular signaling domain of 4-1BB is set forth in SEQ ID NO:19.

14. The chimeric antigen receptor of any of claims 1-13, wherein the transmembrane domain comprises a transmembrane domain from CD4, CD28, or CD8.

15. The chimeric antigen receptor of claim 14, wherein the transmembrane domain comprises a transmembrane domain from CD28.

16. The chimeric antigen receptor of claim 14 or 15, wherein the intracellular signaling domain of CD28 is set forth in SEQ ID NO:18.

17. A chimeric antigen receptor comprising:

(1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPCR5D), wherein the extracellular antigen-binding domain comprises:

(i) a variable heavy chain (V_H) region comprising an amino acid sequence set forth in SEQ ID NO: 27; and (ii) a variable light chain (V_L) region comprising an amino acid sequence set forth in SEQ ID NO: 66;

(2) a spacer with the amino acid sequence set forth in SEQ ID NO: 17;

(3) a transmembrane domain from human CD28; and

(4) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta ($CD3\zeta$) chain and an intracellular signaling domain of 4-1BB.

18. A chimeric antigen receptor comprising:

(1) an extracellular antigen-binding domain that specifically binds human G-protein coupled receptor class C group 5 member D (GPCR5D), wherein the extracellular antigen-binding domain comprises:

a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:125, 126 and 122, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:130, 131 and 132, respectively;

(2) a spacer with the amino acid sequence set forth in SEQ ID NO: 17;

(3) a transmembrane domain from human CD28; and

(4) an intracellular signaling region comprising a cytoplasmic signaling domain of a human CD3-zeta (CD3 ζ) chain and an intracellular signaling domain of a human 4-1BB.

19. The chimeric antigen receptor of any one of claims 5-21, wherein:

- (a) the scFv is set forth in SEQ ID NO:8;
- (b) the spacer is set forth in SEQ ID NO:17;
- (c) the transmembrane domain is set forth in SEQ ID NO:18;
- (d) the cytoplasmic domain of the CD3 ζ chain is set forth in SEQ ID NO:20; and
- (e) the intracellular signaling domain of 4-1BB is set forth in SEQ ID NO:19.

20. The chimeric antigen receptor of any of claims 1-19, wherein the spacer is encoded by the nucleotide sequence set forth in SEQ ID NO:74.

21. The chimeric antigen receptor of any of claims 2-20, wherein:
the chimeric antigen receptor comprises the amino acid sequence set forth in SEQ ID NO:290, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO:290.

22. The CAR of claim 21, wherein the CAR has the sequence set forth in SEQ ID NO: 290.

23. The chimeric antigen receptor of any of claims 2-22, wherein the chimeric antigen receptor is encoded by the nucleotide sequence set forth in SEQ ID NO:289, or a nucleotide sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO:289.

24. The CAR of claim 23, wherein the CAR is encoded by the nucleotide sequence set forth in SEQ ID NO: 289.

25. A polynucleotide comprising a nucleotide sequence encoding the chimeric antigen receptor of any of claims 1-24.

26. The polynucleotide of claim 25, wherein the nucleic acid encoding the spacer of the chimeric antigen receptor comprises at least one modified splice donor and/or splice acceptor site, said modified splice donor and/or acceptor site comprising one or more nucleotide modifications corresponding to a reference splice donor site and/or reference splice acceptor site contained in the sequence set forth in SEQ ID NO:73, wherein the modified splice donor site is set forth in

tcaactggtatgtgg (SEQ ID NO:183) and/or the modified acceptor site is set forth in
cagtttcttctgtatagtagactaccgtggataaatcaa (SEQ ID NO:186) and/or
cgccttgctcctctgtcccgctcctctgttgccggacct (SEQ ID NO:188).

27. The polynucleotide of claim 26, wherein the modified splice donor site is set forth in
tcaactggtatgtgg (SEQ ID NO:183) and/or the modified acceptor site is set forth in
cagtttcttctgtatagtagactaccgtggataaatcaa (SEQ ID NO:186).

28. The polynucleotide of claim 26 or 27, wherein the nucleotide sequence encoding the
chimeric antigen receptor is set forth in SEQ ID NO:289, or a nucleotide sequence that has at least 90%,
91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the nucleotide sequence set
forth in SEQ ID NO:289.

29. The polynucleotide of claim 28, wherein the nucleotide sequence encoding the chimeric
antigen receptor is set forth in SEQ ID NO: 289.

30. The polynucleotide of any of claims 25-29, wherein the nucleic acid encoding the spacer
of the chimeric antigen receptor is set forth in SEQ ID NO:74.

31. The polynucleotide of any of claims 25-30, wherein the polynucleotide is codon-
optimized for expression in a human cell.

32. The polynucleotide of any of claims 25-31, wherein the chimeric antigen receptor is a
first chimeric antigen receptor and the polynucleotide further comprises a nucleotide sequence encoding
a second chimeric antigen receptor.

33. The polynucleotide of claim 32, wherein the second chimeric antigen receptor (CAR)
comprises an extracellular antigen-binding domain that specifically binds a second antigen, wherein the
second antigen is selected from B cell maturation antigen (BCMA), CD38, CD138, CS-1, BAFF-R,
TACI, and FcRH5.

34. A vector comprising the polynucleotide of any of claims 25-33.

35. The vector of claim 34, which is a viral vector.

36. A T cell comprising the chimeric antigen receptor of any of claims 1-24.

37. The T cell of claim 36, wherein the chimeric antigen receptor is a first chimeric receptor and the cell further comprises a polynucleotide comprising a nucleotide encoding a second chimeric antigen receptor.

38. A T cell comprising the polynucleotide of any of claims 25-33.

39. A T cell comprising the polynucleotide of any of claims 25-31, which is the first polynucleotide, wherein the T cell further comprises a second polynucleotide comprising a nucleotide sequence encoding a second chimeric antigen receptor (CAR).

40. The T cell of claim 39, wherein the second chimeric antigen receptor (CAR) comprises an extracellular antigen-binding domain that specifically binds a second antigen, wherein the second antigen is selected from B cell maturation antigen (BCMA), CD38, CD138, CS-1, BAFF-R, TACI, and FcRH5.

41. A composition comprising:
a plurality of T cells comprising a chimeric antigen receptor that is the chimeric antigen receptor of any of claims 1-24 or encoded by the polynucleotide of any of claims 25-31.

42. The composition of claim 41, further comprising a plurality of second T cells comprising a second chimeric antigen receptor (CAR).

43. Use of a composition comprising the cells of any of claims 36-40 or the composition of claim 41 or 42 for the manufacture of a medicament for treatment of a cancer.

44. A composition comprising a first dose of a plurality of first T cells comprising a first chimeric antigen receptor that is the chimeric antigen receptor of any of claims 1-24 or encoded by the polynucleotide of any of claims 25-31 and a composition comprising a second dose of a plurality of second T cells comprising a second chimeric antigen receptor for use in the treatment of a cancer.

45. Use of a composition comprising a first dose of a plurality of first cells comprising a first chimeric antigen receptor that is the chimeric antigen receptor of any of claims 1-24 or encoded by the polynucleotide of any of claims 25-31 and a composition comprising a second dose of a plurality of second cells comprising a second chimeric antigen receptor for the manufacture of a medicament for treatment of a cancer.

46. The composition of any one of claims 41, 42, and 44 or the use of claim 43 or 45, wherein the cancer is a plasma cell malignancy and the plasma cell malignancy is multiple myeloma (MM) or plasmacytoma.

47. The composition or the use of claim 46, wherein the cancer is multiple myeloma (MM).

48. The composition of any one of claims 41, 42, 44, 46, and 47 or the use of any one of claims 45-47, wherein the cancer is a relapsed/refractory multiple myeloma.

49. The compositions of any one of claims 42, 44 and 46-48 or the use of any one of claims 45-48, wherein the second chimeric antigen receptor comprises an extracellular antigen binding domain that specifically binds a second antigen expressed on or associated with multiple myeloma, optionally wherein the second antigen is selected from B cell maturation antigen (BCMA), CD38, CD138, CS-1, BAFF-R, TACI, and FcRH5.