improved in vivo therapeutic activity in clinical applications of chimeric receptor-modified T cells.

There is a need to identify methods for determining elements of chimeric receptor design that are important for therapeutic activity and cell populations to genetically modify and adoptively transfer that provide enhanced survival and efficacy in vivo.

Summary

In one aspect, the present disclosure refers to a nucleic acid encoding a chimeric receptor, the nucleic acid comprising: a) a polynucleotide encoding a ligand binding domain consisting of a single chain variable fragment (scFv) that binds to a CD19; b) a polynucleotide encoding a transmembrane domain; c) a polynucleotide encoding a polypeptide spacer located between the ligand binding domain and the transmembrane domain, wherein the polypeptide spacer is about 15 amino acids or less in length and comprises an amino acid sequence of X_1PPX_2P, wherein X_1 is a cysteine, glycine, or arginine and X_2 is a cysteine or a threonine (SEQ ID NO:1); and d) a polynucleotide encoding an intracellular signaling domain that comprises a CD3ζ signaling domain and a costimulatory domain.

In another aspect, the present disclosure refers to a nucleic acid encoding a chimeric receptor, the nucleic acid comprising: a) a polynucleotide encoding a ligand binding domain that binds to a CD19, wherein the ligand binding domain comprises a single chain variable fragment (scFv) that comprises: a variable light chain (VL) domain comprising a VL domain present in an amino acid sequence encoded by SEQ ID NO:3 or a sequence having at least 90% identity thereto, and a variable heavy chain (VH) domain comprising a VH domain present in an amino acid sequence encoded by SEQ ID NO:3 or a sequence having at least 90% identity thereto; a VL domain and a VH domain comprising the VL domain and the VH domain amino acid sequences, respectively, present in the amino acid sequence encoded by SEQ ID NO:3; or an amino acid sequence encoded by SEQ ID NO:3; b) a polynucleotide encoding a transmembrane domain; c) a polynucleotide encoding a polypeptide spacer located between the ligand binding domain and the transmembrane domain, wherein the polypeptide spacer is about 15 amino acids or less in length and comprises an amino acid sequence of X_1PPX_2P, wherein X_1 is a

cysteine, glycine, or arginine and X_2 is a cysteine or a threonine (SEQ ID NO:1); and d) a polynucleotide encoding an intracellular signaling domain that comprises a CD3ζ signaling domain and a costimulatory domain.

In a further aspect, the present disclosure refers to a chimeric receptor polypeptide encoded by the nucleic acid as described herein. In yet another aspect, the present disclosure refers to a chimeric receptor polypeptide, the chimeric receptor comprising: a) a ligand binding domain consisting of a single chain variable fragment (scFv) that binds to a CD19; b) a transmembrane domain; c) a polypeptide spacer located between the ligand binding domain and the transmembrane domain, wherein the polypeptide spacer is about 15 amino acids or less in length and comprises an amino acid sequence of X_1PPX_2P, wherein X_1 is a cysteine, glycine, or arginine and X_2 is a cysteine or a threonine (SEQ ID NO:1); and d) an intracellular signaling domain that comprises a CD3ζ signaling domain and a costimulatory domain. In yet another aspect, the present disclosure refers to a chimeric receptor polypeptide, the chimeric receptor comprising: a) a ligand binding domain that binds to a CD19, wherein the ligand binding domain comprises a single chain variable fragment (scFv) that comprises: a variable light chain (VL) domain comprising a VL domain present in an amino acid sequence encoded by SEQ ID NO:3 or a sequence having at least 90% identity thereto, and a variable heavy chain (VH) domain comprising a VH domain present in an amino acid sequence encoded by SEQ ID NO:3 or a sequence having at least 90% identity thereto; a VL domain and a VH domain comprising the VL domain and the VH domain amino acid sequences, respectively, present in the amino acid sequence encoded by SEQ ID NO:3; or an amino acid sequence encoded by SEQ ID NO:3; b) a transmembrane domain; c) a polypeptide spacer located between the ligand binding domain and the transmembrane domain, wherein the polypeptide spacer is about 15 amino acids or less in length and comprises an amino acid sequence of X_1PPX_2P, wherein X_1 is a cysteine, glycine, or arginine and X_2 is a cysteine or a threonine (SEQ ID NO:1); and d) an intracellular signaling domain that comprises a CD3ζ signaling domain and a costimulatory domain.

In a further aspect, the present disclosure refers to a chimeric receptor polypeptide encoded by a nucleic acid construct comprising the sequence set forth in SEQ ID NO:10 or a sequence having at least 90% identity thereto. In yet another aspect, the present disclosure refers to an expression vector comprising the nucleic acid as described herein or a nucleic acid encoding the chimeric receptor polypeptide as described herein. In yet another aspect, the present disclosure refers to an expression vector encoding the amino acid sequence MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIAT YFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLV APSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSAL KSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQG TSVTVSSESKYGPPCPPCPMFWVLVVVGGVLACYSLLVTVAFIIFWVKRGR KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR LEGGGEGRGSLLTCGDVEENPGPRMLLLVTSLLLCELPHPAFLLIPRKVCNGI GIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDIL KTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSL GLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKA TGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVEN SECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGEN NTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGA LLLLLVVALGIGLFM.

In yet another aspect, the present disclosure refers to a host cell comprising the nucleic acid as described herein, the chimeric receptor polypeptide as described herein, or the expression vector as described herein. In yet another aspect, the present disclosure refers to a composition comprising the host cell as described herein in a pharmaceutically acceptable excipient. In yet another aspect, the present disclosure refers to an in vitro method for preparing a host cell, comprising:

introducing the nucleic acid as described herein or the expression vector as described herein into a T cell; and culturing the cells in the presence of anti-CD3 or anti CD28 or both, and at least one homeostatic cytokine. In yet another aspect, the present disclosure refers to the use of a pharmaceutical composition, comprising the chimeric receptor polypeptide as described herein, the host cell as described herein, or the composition as described herein in the manufacture of a medicament for the treatment of a tumor expressing CD19.

In yet another aspect, the present disclosure refers to the use of a population of immune cells comprising the nucleic acid as described herein or the chimeric receptor polypeptide as described herein in the manufacture of a medicament for treatment of a tumor expressing CD19.

In yet another aspect, the present disclosure refers to the use of a population of CD8+ T cells comprising the nucleic acid as described herein or the chimeric receptor polypeptide as described herein, in the manufacture of a medicament for treatment of a tumor expressing CD19.

In yet another aspect, the present disclosure refers to the use of a population of CD4+ T cells comprising the nucleic acid as described herein or the chimeric receptor polypeptide as described herein, in the manufacture of a medicament for treatment of a tumor expressing CD19.

In yet another aspect, the present disclosure refers to the use of a combination comprising a population of CD8+ T cells comprising the nucleic acid as described herein or the chimeric receptor polypeptide as described herein and a population of CD4+ T cells comprising the nucleic acid as described herein or the chimeric receptor polypeptide as described herein, in the manufacture of a medicament for treatment of a tumor expressing CD19.

In one example, the present disclosure relates to methods and compositions to confer and/or augment immune responses mediated by cellular immunotherapy, such as by adoptively transferring tumor-specific, genetically modified subsets of CD8+ or CD4+ T cells alone, or in combination. The disclosure provides for chimeric receptor nucleic acids, and vectors and host cells including such nucleic acids. The nucleic acid sequence that encodes the chimeric receptor links together a number of modular components that can be excised and replaced with other

components in order to customize the chimeric receptor for efficient T cell activation and recognition of a specific target molecule or an epitope on the target molecule.

In examples, a chimeric receptor nucleic acid comprises a polynucleotide coding for a ligand binding domain, wherein the ligand is a molecule expressed on malignant or infected cells, a polynucleotide coding for a polypeptide spacer wherein the polypeptide spacer is about 200 amino acids or less, a polynucleotide coding for a transmembrane domain; and a polynucleotide coding for intracellular signaling domains. In examples, the polypeptide spacer comprises a modified IgG4 hinge region containing an amino acid sequence X_1PPX_2P that may be linked to other amino acid sequences including but not limited to the CH2 and CH3 or CH3 only sequences of the Ig Fc. It has been surprisingly found that the length of the spacer region that is presumed not to have signaling capability affects the in vivo efficacy of the T cells modified to express the chimeric receptor and needs to be customized for individual target molecules for optimal tumor or target cell recognition.

Another example of the disclosure provides an isolated chimeric receptor nucleic acid comprising: a polynucleotide coding for a ligand binding domain, wherein the ligand is a tumor specific antigen, viral antigen, or any other molecule expressed on a target cell population that is suitable to mediate recognition and elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer wherein the polypeptide spacer is of a customized length that is specific for each targeted ligand, wherein the spacer provides for enhanced T cell proliferation and/or cytokine production as compared to a reference chimeric receptor; a polynucleotide coding for a transmembrane domain; and a polynucleotide coding for one or more intracellular signaling domains. In examples, a long spacer is employed if the epitope on the target ligand is in a membrane proximal position and a short spacer is employed if the epitope on the target ligand is in a membrane distal position. The disclosure includes expression vectors and host cells comprising the isolated chimeric receptor as described herein.

Another example of the disclosure provides a chimeric receptor polypeptide comprising a ligand binding domain, wherein the ligand is a tumor specific antigen, viral antigen or any other molecule that is expressed on a target cell population and

can be targeted to mediate recognition and elimination by lymphocytes; a polypeptide spacer wherein the polypeptide spacer is about 10-229 amino acids; a transmembrane domain; and one or more intracellular signaling domains. In examples, the polypeptide spacer comprises a modified IgG hinge region containing the amino acid sequence X_1PPX_2P.

In another example, the present disclosure provides compositions to confer and/or augment immune responses mediated by cellular immunotherapy, such as by adoptively transferring tumor-specific, subset specific genetically modified CD4+ T cells, wherein the CD4+ T cells confer and/or augment the ability of CD8+ T cells to sustain anti-tumor reactivity and increase and/or maximize tumor-specific proliferation. In examples, the CD4+ cells are genetically modified to express a chimeric receptor nucleic acid and/or chimeric receptor polypeptide as described herein.

In another example, the present disclosure provides compositions to confer and/or augment immune responses mediated by cellular immunotherapy, such as by adoptively transferring tumor-specific, subset specific genetically modified CD8+ T cells. In examples, the CD8+ cells express a chimeric receptor nucleic acid and/or chimeric receptor polypeptide as described herein.

In another example, the presentdisclosure provides an adoptive cellular immunotherapy composition having a genetically modified CD8+ cytotoxic T lymphocyte cell preparation to confer and/or augment immune responses, wherein the cytotoxic T lymphocyte cell preparation comprises CD8+ T cells that express a chimeric receptor comprising a ligand binding domain for a ligand associated with the disease or disorder, a customized spacer region, a transmembrane domain; and an intracellular signaling domain of a T cell or other receptors, such as a costimulatory domain, and/or a genetically modified helper T lymphocyte cell preparation, wherein the helper T lymphocyte cell preparation has CD4+ T cells that express a chimeric receptor comprising an antibody variable domain specific for the ligand associated with the disease or disorder, a customized spacer region, a transmembrane domain; and one or more intracellular signaling domains.

In one example, the present disclosure provides a method of performing cellular immunotherapy in a subject having a disease or disorder by administering to the subject a genetically modified cytotoxic T lymphocyte cell preparation that

provides a cellular immune response, wherein the cytotoxic T lymphocyte cell preparation comprises CD8+ T cells that have a chimeric receptor comprising a polynucleotide coding for a ligand binding domain, wherein the ligand is a tumor specific antigen, viral antigen, or any other molecule expressed on a target cell population that is suitable to mediate recognition and elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer wherein the polypeptide spacer is of a customized length that is specific for each targeted ligand, wherein the spacer provides for enhanced T cell proliferation and/or cytokine production as compared to a reference chimeric receptor; a polynucleotide coding for a transmembrane domain; and a polynucleotide coding for one or more intracellular signaling domains. In example, the ligand binding domain is an extracellular antibody variable domain specific for a ligand associated with the disease or disorder. An example includes a genetically modified helper T lymphocyte cell preparation that wherein the helper T lymphocyte cell preparation comprises CD4+ T cells that have a chimeric receptor comprising an a polynucleotide coding for a ligand binding domain, wherein the ligand is a tumor specific antigen, viral antigen, or any other molecule expressed on a target cell population that is suitable to mediate recognition and elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer wherein the polypeptide spacer is of a customized length that is specific for each targeted ligand, wherein the spacer provides for enhanced T cell proliferation and/or cytokine production as compared to a reference chimeric receptor; a polynucleotide coding for a transmembrane domain; and a polynucleotide coding for one or more intracellular signaling domains. In examples, the genetically modified CD8+ and genetically modified CD4+ cell population are coadministered. In examples, the T cells are autologous or allogeneic T cells.

Various modifications of the above method are possible. For example, the chimeric receptor that is expressed by the CD4+ T cell and the CD8+ T cell can be the same or different.

In another example, the present disclosure provides a method of manufacturing an adoptive immunotherapy composition by obtaining a chimeric receptor modified tumor-specific CD8+ cytotoxic T lymphocyte cell preparation that elicits a cellular immune response and expresses an antigen-reactive chimeric receptor, wherein the modified cytotoxic T lymphocyte cell preparation comprises

CD8+ T cells that have a chimeric receptor comprising a ligand binding domain, wherein the ligand is a tumor specific antigen, viral antigen, or any other molecule expressed on a target cell population that is suitable to mediate recognition and elimination by a lymphocyte; a polypeptide spacer wherein the polypeptide spacer is of a customized length that is specific for each targeted ligand, wherein the spacer provides for enhanced T cell proliferation and/or cytokine production as compared to a reference chimeric receptor; a transmembrane domain; and one or more intracellular signaling domains.; and/or obtaining a modified naïve or memory CD4+ T helper cell wherein the modified helper T lymphocyte cell preparation comprises CD4+ cells that have a chimeric receptor comprising a ligand binding domain, wherein the ligand is a tumor specific antigen, viral antigen, or any other molecule expressed on a target cell population that is suitable to mediate recognition and elimination by a lymphocyte; a polypeptide spacer wherein the polypeptide spacer is of a customized length that is specific for each targeted ligand, wherein the spacer provides for enhanced T cell proliferation and/or cytokine production as compared to a reference chimeric receptor; a transmembrane domain; and one or more intracellular signaling domains.

These and other examples of the disclosure are described further in the accompanying specification, drawings and claims.

Brief Description of the Drawings

Figure 1 Library of spacer sequences. We constructed a plasmid library that contain codon optimized DNA sequences that encode extracellular components including of the IgG4 hinge alone, IgG4 hinge linked to CH2 and CH3 domains, or IgG4 hinge linked to the CH3 domain. Any scFV sequence (VH and VL) can be cloned 5' to the sequences encoding this library of variable spacer domains. The spacer domains are in turn linked to CD28 transmembrane and intracellular signaling domains and to CD3 zeta. A T2A sequence in the vector separates the chimeric receptor from a selectable marker encoding a truncated human epidermal growth factor receptor (tEGFR).

Figure 2: *In vitro* cytotoxicity, cytokine production, and proliferation of T-cells modified to express 2A2 ROR1 chimeric receptors with modified spacer length. (A) Phenotype of purified $CD8^+$ T_{CM}-derived cell lines modified with each of the 2A2 ROR1 chimeric receptors with long, intermediate and short spacer

domain. Staining with anti-F(ab) antibody that binds to an epitope in the 2A2 scFV shows surface expression of ROR1 chimeric receptors with full length or truncated spacer. (B) Cytolytic activity of T-cells expressing the various 2A2 ROR1 chimeric receptors with long (●), intermediate (▲) and short spacer (♦), or a tEGFR control lentiviral vector against $ROR1^+$ (x) and control target cells. The bar diagram summarizes cytotoxicity data from 3 independent experiments (E:T = 30:1) normalized to cytolytic activity by 2A2 ROR1 chimeric receptor 'long' = 1, and analyzed by Student's t-test. (C) CFSE dye dilution was used to measure proliferation of 2A2 ROR1 chimeric receptor and tEGFR control T-cells, 72 hours after stimulation with Raji/ROR1 (left panel) and primary CLL cells (right panel) without addition of exogenous cytokines. For analysis, triplicate wells were pooled and the proliferation of live (PI^-), $CD8^+$ T-cells analyzed. Numbers above each histogram indicate the number of cell divisions the proliferating subset underwent, and the fraction of T-cells in each gate that underwent ≥4/3/2/1 cell divisions is provided next to each plot. (D) Multiplex cytokine assay of supernatants obtained after 24 hours from triplicate co-cultures of $5x10^4$ T-cells expressing the various

CLAIMS

1. A nucleic acid encoding a chimeric receptor, the nucleic acid comprising:

a) a polynucleotide encoding a ligand binding domain consisting of a single chain variable fragment (scFv) that binds to a CD19;

b) a polynucleotide encoding a transmembrane domain;

c) a polynucleotide encoding a polypeptide spacer located between the ligand binding domain and the transmembrane domain, wherein the polypeptide spacer is about 15 amino acids or less in length and comprises an amino acid sequence of X_1PPX_2P, wherein X_1 is a cysteine, glycine, or arginine and X_2 is a cysteine or a threonine (SEQ ID NO:1); and

d) a polynucleotide encoding an intracellular signaling domain that comprises a CD3ζ signaling domain and a costimulatory domain.

2. A nucleic acid encoding a chimeric receptor, the nucleic acid comprising:

a) a polynucleotide encoding a ligand binding domain that binds to a CD19, wherein the ligand binding domain comprises a single chain variable fragment (scFv) that comprises:

a variable light chain (VL) domain comprising a VL domain present in an amino acid sequence encoded by SEQ ID NO:3 or a sequence having at least 90% identity thereto, and a variable heavy chain (VH) domain comprising a VH domain present in an amino acid sequence encoded by SEQ ID NO:3 or a sequence having at least 90% identity thereto;

a VL domain and a VH domain comprising the VL domain and the VH domain amino acid sequences, respectively, present in the amino acid sequence encoded by SEQ ID NO:3; or

an amino acid sequence encoded by SEQ ID NO:3;

b) a polynucleotide encoding a transmembrane domain;

c) a polynucleotide encoding a polypeptide spacer located between the ligand binding domain and the transmembrane domain, wherein the polypeptide spacer is about 15 amino acids or less in length and comprises an amino acid sequence of X_1PPX_2P, wherein X_1 is a cysteine, glycine, or arginine and X_2 is a cysteine or a threonine (SEQ ID NO:1); and

d) a polynucleotide encoding an intracellular signaling domain that comprises a CD3ζ signaling domain and a costimulatory domain.

3. The nucleic acid of claim 1 or 2, wherein the ligand binding domain binds to an epitope of CD19 located distal to the membrane.

4. The nucleic acid of any one of claims 1-3, wherein the scFv comprises a variable heavy chain (VH) domain and a variable light chain (VL) domain separated by a peptide linker.

5. The nucleic acid of claim 4, wherein the peptide linker comprises the amino acid sequence set forth in SEQ ID NO:36.

6. The nucleic acid of claim 4 or 5, wherein the scFv comprises a linker, and has a VL-linker-VH orientation.

7. The nucleic acid of any one of claims 1-6, wherein the polypeptide spacer comprises a human IgG1, IgG2, or IgG4 hinge or a modified version thereof.

8. The nucleic acid of any one of claims 1-7, wherein the polypeptide spacer comprises the amino acid sequence set forth in SEQ ID NO:17, 18, 20, 21, 51, 52, or 53.

9. The nucleic acid of any one of claims 1-8, wherein the polypeptide spacer comprises a modified human IgG4 hinge region.

10. The nucleic acid of any one of claims 1-9, wherein the polypeptide spacer comprises the amino acid sequence set forth in SEQ ID NO:51.

11. The nucleic acid of any one of claims 1-10, wherein the polypeptide spacer is 10 to 15 amino acids in length.

12. The nucleic acid of any one of claims 1-11, wherein the polypeptide spacer is about 12 amino acids in length.

13. The nucleic acid of any one of claims 1-12, wherein the polypeptide spacer consists of the amino acid sequence set forth in SEQ ID NO:21.

14. The nucleic acid of any one of claims 1-13, wherein the ligand binding domain comprises a variable light chain (VL) domain comprising a CDRL1, a CDRL2, and a CDRL3 of the antibody FMC63 or the amino acid sequence encoded by SEQ ID NO:3, and a variable heavy chain (VH) domain comprising a CDRH1, a CDRH2, and a CDRH3 of the antibody FMC63 or the amino acid sequence encoded by SEQ ID NO:3.

15. The nucleic acid of any one of claims 1-14, wherein the ligand binding domain comprises a variable light chain (VL) domain having a least 90% sequence identity to the VL domain of the antibody FMC63 or the VL domain present in the amino acid sequence encoded by SEQ ID NO:3; and a variable heavy chain (VH) domain having at least 90% identity to the VH domain of the antibody FMC63 or the VH domain present in the amino acid sequence encoded by SEQ ID NO:3.

16. The nucleic acid of any one of claims 1-15, wherein the ligand binding domain comprises a variable light chain (VL) domain domain comprising a CDRL1 sequence of RASQDISKYLN, a CDRL2 sequence of SRLHSGV, a CDRL3 sequence of GNTLPYTFG, and a variable heavy chain (VH) domain comprising a CDRH1 sequence of DYGVS, a CDRH2 sequence of VIWGSETTYYNSALKS, and a CDRH3 sequence of YAMDYWG.

17. The nucleic acid of any one of claims 1-16, wherein the transmembrane domain comprises a transmembrane domain of a CD8 or of a CD28.

18. The nucleic acid of any one of claims 1-17, wherein the transmembrane domain comprises a transmembrane domain of a CD28 comprising the amino acid sequence encoded by SEQ ID NO:5, or the amino acid sequence. MFWVLVVVGGVLACYSLLVTVAFIIFWV.

19. The nucleic acid of any one of claims 1-18, wherein the costimulatory domain comprises:

the signaling domain of a 4-1BB or a modified version thereof; or

the signaling domain of a CD28 or a modified version thereof.

20. The nucleic acid of any one of claims 1-19, wherein:

the transmembrane domain comprises a transmembrane domain of a CD28; and

the costimulatory domain comprises all or a portion of a 4-1BB signaling domain or all or a portion of a CD28 signaling domain.

21. The nucleic acid of claim 19 or 20, wherein the 4-1BB signaling domain comprises amino acids 214-255 of SEQ ID NO:15 or comprises the amino acid sequence encoded by SEQ ID NO:6.

22. The nucleic acid of claim 19 or 20, wherein the CD28 signaling domain comprises amino acids 180-220 of SEQ ID NO:14 or comprises a modified version thereof comprising an LL → GG substitution located at positions 186-187 of SEQ ID NO:14.

23. The nucleic acid of any one of claims 1-22, wherein the CD3ζ signaling domain comprises the amino acid sequence encoded by SEQ ID NO:7.

24. The nucleic acid of any one of claims 1-23, wherein:

the ligand binding domain comprises an scFv that has a VL-linker-VH orientation;

the polypeptide spacer comprises a modified IgG4 hinge region;

the transmembrane domain comprises the amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:5; and

the intracellular signaling domain comprises the amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:6 and the amino acid sequence encoded by SEQ ID NO:7.

25. The nucleic acid of any one of claims 7, 9 and 24, wherein the modified IgG4 hinge region comprises the amino acid sequence set forth in SEQ ID NO: 51 or the amino acid set forth in SEQ ID NO:21.

26. The nucleic acid of any one of claims 1-25, wherein:

the ligand binding domain comprises an scFv comprising a VH domain having at least 90% sequence identity to the VH domain sequence present in the amino acid sequence encoded by SEQ ID NO:3 or the antibody FMC63, and a VL domain having at least 90% sequence identity to the VL domain sequence present in the amino acid sequence encoded by SEQ ID NO:3 or the antibody FMC63;

the polypeptide spacer the amino acid sequence set forth in SEQ ID NO:51;

the transmembrane domain comprises the amino acid sequence encoded by SEQ ID NO:5; and

the intracellular signaling domain comprises the amino acid sequence encoded by SEQ ID NO:6 and comprises the amino acid sequence encoded by of SEQ ID NO:7.

27. The nucleic acid of any one of claims 1-26, wherein:

the ligand binding domain comprises an scFv comprising a VH domain having at least 90% sequence identity to the VH domain sequence present in the amino acid sequence encoded by SEQ ID NO:3 or the antibody FMC63, and a VL domain having at least 90% sequence identity to the VL domain sequence present in the amino acid sequence encoded by SEQ ID NO:3 or the antibody FMC63;

the polypeptide spacer consists of the amino acid sequence encoded by SEQ ID NO:4;

the transmembrane domain comprises the amino acid sequence encoded by SEQ ID NO:5; and

the intracellular signaling domain comprises the amino acid sequence encoded by SEQ ID NO:6 and the amino acid sequence encoded by SEQ ID NO:7.

28. The nucleic acid of any one of claims 1-27, wherein the scFv is encoded by the sequence set forth in SEQ ID NO:3.

29. The nucleic acid of any one of claims 1-28, wherein the nucleic acid comprises:

a) the sequence set forth in SEQ ID NO:3 encoding the scFv;

b) the sequence set forth in SEQ ID NO:4 encoding the polypeptide spacer;

c) the sequence set forth in SEQ ID NO:5 encoding the transmembrane domain;

d) the sequence set forth in SEQ ID NO:6 encoding the costimulatory domain; and

e) the sequence set forth in SEQ ID NO:7 encoding the CD3ζ signaling domain.

30. The nucleic acid of any one of claims 1-29, wherein the nucleic acid encodes a chimeric receptor encoded by a nucleic acid construct comprising the sequence set forth in SEQ ID NO:10 or a sequence having at least 90% identity thereto.

31. A chimeric receptor polypeptide encoded by the nucleic acid of any one of claims 1-30.

32. A chimeric receptor polypeptide, the chimeric receptor comprising:

a) a ligand binding domain consisting of a single chain variable fragment (scFv) that binds to a CD19;

b) a transmembrane domain;

c) a polypeptide spacer located between the ligand binding domain and the transmembrane domain, wherein the polypeptide spacer is about 15 amino acids or less in length and comprises an amino acid sequence of X_1PPX_2P, wherein X_1 is a cysteine, glycine, or arginine and X_2 is a cysteine or a threonine (SEQ ID NO:1); and

d) an intracellular signaling domain that comprises a CD3ζ signaling domain and a costimulatory domain.

33. A chimeric receptor polypeptide, the chimeric receptor comprising:

a) a ligand binding domain that binds to a CD19, wherein the ligand binding domain comprises a single chain variable fragment (scFv) that comprises:

a variable light chain (VL) domain comprising a VL domain present in an amino acid sequence encoded by SEQ ID NO:3 or a sequence having at least 90% identity thereto, and a variable heavy chain (VH) domain comprising a VH domain present in an amino acid sequence encoded by SEQ ID NO:3 or a sequence having at least 90% identity thereto;

a VL domain and a VH domain comprising the VL domain and the VH domain amino acid sequences, respectively, present in the amino acid sequence encoded by SEQ ID NO:3; or

an amino acid sequence encoded by SEQ ID NO:3;

b) a transmembrane domain;

c) a polypeptide spacer located between the ligand binding domain and the transmembrane domain, wherein the polypeptide spacer is about 15 amino acids or less in length and comprises an amino acid sequence of X_1PPX_2P, wherein X_1 is a cysteine, glycine, or arginine and X_2 is a cysteine or a threonine (SEQ ID NO:1); and

d) an intracellular signaling domain that comprises a CD3ζ signaling domain and a costimulatory domain.

34. A chimeric receptor polypeptide encoded by a nucleic acid construct comprising the sequence set forth in SEQ ID NO:10 or a sequence having at least 90% identity thereto.

35. An expression vector comprising the nucleic acid of any one of claims 1-30 or a nucleic acid encoding the chimeric receptor polypeptide of any one of claims 31-34.

36. The expression vector of claim 35, further comprising a polynucleotide encoding a marker sequence, wherein the polynucleotide encoding the marker sequence is operably linked in frame with the nucleic acid encoding the chimeric receptor.

37. The expression vector of claim 36, wherein the nucleic acid encoding the chimeric receptor and the polynucleotide encoding the marker sequence are separated by a polynucleotide encoding a cleavable linker.

38. The expression vector of claim 36 or 37, wherein the marker sequence is a truncated EGFR sequence.

39. The expression vector of claim 38, wherein the truncated EGFR sequence is encoded by the polynucleotide of SEQ ID NO:9.

40. The expression vector of any one of claims 37-39, wherein the cleavable linker comprises a T2A peptide.

41. The expression vector of claim 40, wherein the T2A peptide is encoded by the polynucleotide of SEQ ID NO:8.

42. An expression vector encoding the amino acid sequence MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPS RFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQ SLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCA KHYYYGGSYAMDYWGQGTSVTVSSESKYGPPCPPCPMFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRLEGGGEGRGSLLTC GDVEENPGPRMLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHT PPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNK NLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGE PREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCH PNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM.

43. A host cell comprising the nucleic acid of any one of claims 1-30, the chimeric receptor polypeptide of any one of claims 31-34, or the expression vector of any one of claims 35-42.

44. The host cell of claim 43, wherein the host cell is an immune cell.

45. The host cell of claim 43 or 44, wherein the host cell is a T cell.

46. The host cell of any one of claims 43-45, wherein the host cell is a CD8+ T cell or a CD4+ T cell.

47. The host cell of any one of claims 43-46, wherein the host cell is a CD8+ T cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells.

48. The host cell of any one of claims 43-46, wherein the host cell is a CD4+ T cell selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells.

49. A composition comprising the host cell of any one of claims 43-48 in a pharmaceutically acceptable excipient.

50. The composition of claim 49, comprising a host cell of claims 47 and a host cell of claims 48.

51. The composition of claim 50, wherein the CD4+ T cells and CD8+ T cells are present at about a 1:1 ratio in the composition.

52. An in vitro method for preparing a host cell, comprising:

introducing the nucleic acid of any one of claims 1-30 or the expression vector of any one of claims 35-42 into a T cell; and

culturing the cells in the presence of anti-CD3 or anti CD28 or both, and at least one homeostatic cytokine.

53. The method of claim 52, wherein the T cell is a CD8+ T cell or a CD4+ T cell.

54. Use of a pharmaceutical composition, comprising the chimeric receptor polypeptide of any one of claims 31-34, the host cell of any one of claims 43-48, or the composition of any one of claims 49-51 in the manufacture of a medicament for the treatment of a tumor expressing CD19.

55. Use of a population of immune cells comprising the nucleic acid of any one of claims 1-30 or the chimeric receptor polypeptide of any one of claims 31-34 in the manufacture of a medicament for treatment of a tumor expressing CD19.

56. The use of claim 55, wherein the population of immune cells comprise CD8+ T cells.

57. The use of claim 56, wherein the population of immune cells further comprises CD4+ T cells.

58. Use of a population of CD8+ T cells comprising the nucleic acid of any one of claims 1-30 or the chimeric receptor polypeptide of any one of claims 31-34, in the manufacture of a medicament for treatment of a tumor expressing CD19.

59. The use of claim 58, wherein the population of CD8+ T cells is for use in combination with a population of CD4+ T cells comprising the nucleic acid of any one of claims 1-30 or the chimeric receptor polypeptide of any one of claims 31-34.

60. Use of a population of CD4+ T cells comprising the nucleic acid of any one of claims 1-30 or the chimeric receptor polypeptide of any one of claims 31-34, in the manufacture of a medicament for treatment of a tumor expressing CD19.

61. The use of claim 58, wherein the population of CD4+ T cells is for use in combination with a population of CD8+ T cells comprising the nucleic acid of any one of claims 1-30 or the chimeric receptor polypeptide of any one of claims 31-34.

62. Use of a combination comprising a population of CD8+ T cells comprising the nucleic acid of any one of claims 1-30 or the chimeric receptor polypeptide of any one of claims 31-34 and a population of CD4+ T cells comprising the nucleic acid of any one of claims 1-30 or the chimeric receptor polypeptide of any one of claims 31-34, in the manufacture of a medicament for treatment of a tumor expressing CD19.

63. The use of any one of claims 59, 61 and 62, wherein the population of CD8+ T cells and the population of CD4+ T cells is for use at a ratio of 1:1 CD8+ T cells to CD4+ T cells.

64. The use of any one of claims 59 and 61-63, wherein the population of CD8+ T cells and the population of CD4+ T cells express the same chimeric receptor.

65. The use of any one of claims 54-64, wherein the tumor is a solid tumor or hematologic malignancy.

66. The use of any one of claims 54-65, wherein the tumor is a lymphoma or a leukemia;

a B cell malignancy; or

a leukemia that is a chronic lymphocytic leukemia (CLL).