

WHAT IS CLAIMED IS:

1. A chimeric receptor nucleic acid comprising:
 - a) a polynucleotide coding for a ligand binding domain, wherein the ligand binding domain binds to and/or interacts with CD171;
 - b) a polynucleotide coding for a polypeptide spacer of a length, wherein the spacer is optimized;
 - c) a polynucleotide coding for a transmembrane domain; and
 - d) a polynucleotide coding for an intracellular signaling domain.
2. The chimeric receptor nucleic acid of claim 1, wherein the ligand binding domain is an antibody fragment.
3. The chimeric receptor nucleic acid of claim 2, wherein the ligand binding domain is single chain variable fragment.
4. The chimeric receptor nucleic acid of any one of claims 1-4, wherein the spacer is 15 amino acids or less but not less than 1 or 2 amino acids.
5. The chimeric receptor nucleic acid of any one of claims 1-4, wherein the spacer comprises an amino acid sequence of X_1PPX_2P .
6. The chimeric receptor nucleic acid of claim 5, wherein the spacer region comprises a portion of a hinge region of a human antibody.
7. The chimeric receptor nucleic acid of any one of claims 1 to 6, wherein the intracellular signaling domain comprises all of a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, and B7-H3 or combinations thereof.
8. The chimeric receptor nucleic acid of claim 7, wherein the intracellular signaling domain comprises a portion of CD3 zeta and a portion of 4-1BB.
9. The chimeric receptor nucleic acid of any one of claims 1-8, further comprising a nucleic acid that encodes a marker sequence.
10. A chimeric receptor polypeptide coded for by a chimeric receptor nucleic acid of any one of claims 1 to 9.
11. An expression vector comprising an isolated chimeric receptor nucleic acid of any one of claims 1-9.

12. A host cell comprising an expression vector of claim 11.

13. The host cell of claim 12, wherein the host cell is a CD8+ T cytotoxic lymphocyte 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.

14. The host cell of claim 13, wherein the CD8+ cytotoxic T lymphocyte cell is a central memory T cell and, wherein the central memory T cell is positive for CD45RO+, CD62L+, and CD8+.

15. The host cell according to claim 12, wherein the host cell is a CD4+ T helper lymphocyte 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.

16. The host cell of claim 15, wherein the CD4+ helper lymphocyte cell is a naïve CD4+ T cell and, wherein the naïve CD4+ T cell is positive for CD45RA+, CD62L+ and CD4+ and negative for CD45RO.

17. A composition comprising a host cell of any one of claims 12-16 or 31-31 in a pharmaceutically acceptable excipient.

18. A composition of comprising a host cell of claims 13 or 14 and a host cell of claims 15 or 16.

19. A method for preparing a host cell of any one of claims 12-16 or 30-31 comprising:

a) providing a library of nucleic acids coding for a chimeric receptor of any one of claims 1-9 or 11, wherein each of the plurality of nucleic acids code for a chimeric receptor that differs in length;

b) introducing each of the plurality of the nucleic acids into a separate isolated T lymphocyte population and expanding each T lymphocyte population *in vitro*;

c) administering each genetically modified T lymphocyte population into an animal model bearing a tumor and determining whether a genetically modified T lymphocyte population has an anti-tumor response; and

d) selecting a nucleic acid coding for the chimeric receptor that provides for the anti-tumor response.

20. The method of claim 19, further comprising introducing the selected nucleic acid coding for the chimeric receptor into a host cell.

21. A method for preparing a host cell of any one of claims 12-16 or 30-31 comprising:
- a) introducing a nucleic acid of any one of claims 1-9 or an expression vector of claim 11 into a lymphocyte population that has a CD45RA-, CD45RO+, and CD62L+ phenotype; and
 - b) culturing the cells in the presence of anti-CD3 and/or anti CD28, and at least one homeostatic cytokine until the cells expand sufficiently for use as a cell infusion.
22. The method of any one of claims 19-21, wherein the lymphocyte is CD8+ or CD4+.
23. A use of the host cell of any one of claims 12-16 or 30-31 or the composition of claims 17 or 18 in the treatment or inhibition of cancer or a solid tumor expressing CD171.
24. The use of claim 23, wherein the cancer is a neuroblastoma.
25. The use of claim 23, wherein the solid tumor is selected from the group consisting of a breast cancer, brain cancer, colon cancer, renal cancer, pancreatic cancer, and ovarian cancer.
26. A method of performing cellular immunotherapy in a subject having cancer or a tumor comprising: administering the composition of any one of claims 17 to 18 or a host cell of claims 12-16 to the subject.
27. The method of claim 26, wherein the cancer is neuroblastoma.
28. The method of claim 26, wherein the tumor is selected from the group consisting of a breast cancer, brain cancer, colon cancer, renal cancer, pancreatic cancer, and ovarian cancer.
29. The method of claim 1, wherein the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand, as compared to a reference chimeric receptor.
30. The host cell of claim 12, wherein the host cell is a precursor T cell.
31. The host cell of claim 12, wherein the host cell is a hematopoietic stem cell.

CLAIMS

1. A nucleic acid encoding a chimeric receptor comprising:
 - a) a polynucleotide encoding an extracellular ligand binding domain, wherein the ligand binding domain binds specifically to CD171;
 - b) a polynucleotide encoding an extracellular polypeptide spacer comprising the amino acid sequence set forth in SEQ ID NO:01, wherein the polypeptide spacer has a length of 119 consecutive amino acids or less;
 - c) a polynucleotide encoding a transmembrane domain; and
 - d) a polynucleotide encoding an intracellular signaling domain;wherein the polypeptide spacer is located between the ligand binding domain and the transmembrane domain.
2. The nucleic acid of claim 1, wherein the chimeric receptor comprises an extracellular domain consisting of the ligand binding domain and the extracellular polypeptide spacer.
3. The nucleic acid of claim 1 or 2, wherein the polypeptide spacer is selected from the group consisting of:
 - a polypeptide spacer having a length less than or equal to 15 amino acids;
 - a polypeptide spacer comprising the amino acid sequence set forth in SEQ ID NO: 59; and
 - a polypeptide spacer comprising the amino acid sequence set forth in SEQ ID NO: 21.
4. The nucleic acid of claim 1 or 2, wherein the polypeptide spacer is selected from the group consisting of:
 - a polypeptide spacer comprising the amino acid sequence set forth in SEQ ID NO: 59 and having a length of 119 consecutive amino acids; and
 - a polypeptide spacer comprising the amino acid sequence set forth in SEQ ID NO: 21 and having a length of 12 consecutive amino acids.
5. The nucleic acid of any one of claims 1-4, wherein the polypeptide spacer comprises a portion of a hinge region of a human antibody.

6. The nucleic acid of any one of claims 1-5, wherein the ligand binding domain comprises an antibody fragment.

7. The nucleic acid of any one of claims 1-6, wherein the ligand binding domain is a single chain variable fragment.

8. The nucleic acid of any one of claims 1-7, wherein the polynucleotide encoding a ligand binding domain comprises the nucleotide sequence of residues 2150 to 2875 set forth in SEQ ID NO:54.

9. The nucleic acid of any one of claims 1-8, wherein the intracellular signaling domain comprises all of or a portion of CD3 zeta in combination with a costimulatory domain selected from the group consisting of CD27, CD28, 4-1BB, OX-40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, NKG2C, B7-H3, and a combination thereof.

10. The nucleic acid of claim 9, wherein the intracellular signaling domain comprises a portion of CD3 zeta and a portion of 4-1BB.

11. The nucleic acid of any one of claims 1-10, further comprising a polynucleotide encoding a selectable marker.

12. The nucleic acid of any one of claims 1-11, wherein the nucleic acid comprises at least 95% sequence identity to the nucleotide sequence of residues 2084 to 3778 of SEQ ID NO:55, residues 2084 to 3457 of SEQ ID NO:56, or residues 2084 to 3580 of SEQ ID NO:57.

13. The nucleic acid of any one of claims 1-12, wherein the nucleic acid comprises the nucleotide sequence of residues 2084 to 3778 of SEQ ID NO:55, residues 2084 to 3457 of SEQ ID NO:56, or residues 2084 to 3580 of SEQ ID NO:57.

14. A chimeric receptor polypeptide encoded by the nucleic acid of any one of claims 1-13.

15. An expression vector comprising the nucleic acid of any one of claims 1-13.

16. A host cell comprising the nucleic acid of any one of claims 1-13 or the expression vector of claim 15.

17. The host cell of claim 16, wherein the host cell is a CD8⁺ cytotoxic T lymphocyte cell selected from the group consisting of a naïve CD8⁺ T cell, a central memory CD8⁺ T cell, an effector memory CD8⁺ T cell, and a bulk CD8⁺ T cell; or wherein the host cell is a CD4⁺ helper T lymphocyte cell selected from the group consisting of a naïve CD4⁺ T cell, a central memory CD4⁺ T cell, an effector memory CD4⁺ T cell, and a bulk CD4⁺ T cell.

18. The host cell of claim 15, wherein the host cell is a precursor T cell, or a hematopoietic stem cell.

19. A pharmaceutical composition comprising the host cell of any one of claims 16-18 and a pharmaceutically acceptable excipient.

20. A method for preparing the host cell of any one of claims 16-18 comprising:

a) introducing the nucleic acid of any one of claims 1-13 into a lymphocyte population that has a CD45RA-, CD45RO+, and CD62L+ phenotype; and

b) culturing the cells in the presence of anti-CD3 and/or anti CD28, and at least one homeostatic cytokine until the cells expand sufficiently for use as a cell infusion.

21. The method of claim 20, wherein the lymphocyte population is CD8+ or CD4+.

22. Use of the host cell of any one of claims 16-18 in the manufacture of a medicament for treating or inhibiting a cancer or a solid tumor expressing CD171.

23. The use of claim 22, wherein the cancer is a neuroblastoma.

24. The use of claim 22, wherein the cancer is selected from the group consisting of a breast cancer, a brain cancer, a colon cancer, a renal cancer, a pancreatic cancer, and an ovarian cancer.

25. Use of the host cell of any one of claims 16-18 in the manufacture of a medicament for performing cellular immunotherapy in a subject having a cancer or a tumor.

26. The use of claim 25, wherein the cancer is neuroblastoma.

27. The use of claim 25, wherein the cancer is selected from the group consisting of a breast cancer, brain cancer, colon cancer, renal cancer, pancreatic cancer, and ovarian cancer.