

WHAT IS CLAIMED IS:

1. A nucleic acid comprising a polynucleotide encoding a chimeric antigen receptor (CAR), wherein the CAR comprises:
 - a ligand binding domain capable of or configured to specifically bind to CD171;
 - a polypeptide spacer having a length greater than or equal to 120 and less than or equal to 230 consecutive amino acid residues;
 - a transmembrane domain; and
 - an intracellular signalling domain.
2. The nucleic acid of claim 1, wherein the polypeptide spacer comprises an IgG4 hinge-CH2-CH3 domain comprising an L235D substitution.
3. The nucleic acid of claim 1 or 2, wherein the polypeptide spacer comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:02.
4. The nucleic acid of any one of claims 1-3, wherein the ligand binding domain is derived from a CE7 monoclonal antibody.
5. The nucleic acid of any one of claims 1-4, wherein the ligand binding domain is comprises an scFv encoded by a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of SEQ ID NO:01.
6. The nucleic acid of any one of claims 1-5, wherein the transmembrane domain comprises a CD28 transmembrane domain.
7. The nucleic acid of claim 6, wherein the CD28 transmembrane domain comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:04.
8. The nucleic acid of any one of claims 1-7, wherein the intracellular signalling domain comprises 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, in combination with a CD3-zeta domain or functional portion thereof.
9. The nucleic acid of any one of claims 1-8, wherein the intracellular signalling domain comprises a CD28 cytoplasmic domain.

10. The nucleic acid of claim 9, wherein the CD28 cytoplasmic domain comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:18.
11. The nucleic acid of any one of claims 1-8, wherein the intracellular signalling domain lacks a CD28 cytoplasmic domain.
12. The nucleic acid of any one of claims 1-11, wherein the intracellular signalling domain comprises a 4-1BB costimulatory domain.
13. The nucleic acid of claim 12, wherein the 4-1BB costimulatory domain is encoded by a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of SEQ ID NO:07.
14. The nucleic acid of any one of claims 1-13, wherein the CD3-zeta domain or functional portion thereof is encoded by a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of SEQ ID NO:08.
15. The nucleic acid of any one of claims 1-14, further comprising a constitutive promoter operably linked to the polynucleotide encoding a chimeric antigen receptor.
16. The nucleic acid of claim 15, wherein the constitutive promoter comprises an EF1 α promoter.
17. The nucleic acid of any one of claims 1-14, further comprising an inducible promoter operably linked to the polynucleotide encoding a chimeric antigen receptor.
18. The nucleic acid of any one of claims 1-17, further comprising a polynucleotide encoding a cell-surface selectable marker.
19. The nucleic acid of claim 18, wherein the cell-surface selectable marker is selected from a truncated EGFR polypeptide (EGFRt) or a truncated Her2 polypeptide (Her2t).
20. The nucleic acid of claim 18 or 19, further comprising a ribosome skip sequence located between a polynucleotide encoding the intracellular signalling domain and the polynucleotide encoding a cell-surface selectable marker.
21. The nucleic acid of claim 20, wherein the ribosome skip sequence is selected from the group consisting of a P2A sequence, a T2A sequence, an E2A sequence, and an F2A sequence.
22. The nucleic acid of any one of claims 1-21, further comprising a polynucleotide encoding a suicide gene system.

23. The nucleic acid of claim 22, wherein the suicide gene system is selected from a herpes simplex virus thymidine kinase/ganciclovir (HSVTK/GCV) suicide gene system, or an inducible caspase suicide gene system.

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

the ligand binding domain is encoded by the nucleotide sequence of SEQ ID NO:01; the polypeptide spacer consists of the amino acid sequence of SEQ ID NO:02; the transmembrane domain comprises the amino acid sequence of SEQ ID NO:04; the intracellular signalling domain comprises: a 4-1BB costimulatory domain encoded by the nucleotide sequence of SEQ ID NO:07, and a CD3-zeta domain or functional portion thereof encoded by the nucleotide sequence of SEQ ID NO:08; and

further comprising: an EF1 promoter operably linked to a polynucleotide encoding the ligand binding domain, a polynucleotide comprising a T2A ribosome skip sequence, and a polynucleotide encoding an EGFRt polypeptide.

25. A polypeptide encoded by the nucleic acid of any one of claim 1-24.

26. A vector comprising the nucleic acid of any one of claims 1-24.

27. The vector of claim 26, wherein the vector is selected from the group consisting of a viral vector, a transposon vector, an integrase vector, and an mRNA vector.

28. The vector of claim 27, wherein the viral vector is selected from the group consisting of a lentiviral vector, a foamy viral vector, a retroviral vector, and a gamma retroviral vector.

29. The vector of claim 28, wherein the viral vector is a lentiviral vector.

30. A host cell comprising the nucleic acid of any one of claims 1-24.

31. The host cell of claim 30, wherein the host cell is a CD4⁺ T-cell or a CD8⁺ T-cell.

32. The host cell of claim 30, wherein the host cell is a precursor T-cell, or a hematopoietic stem cell.

33. The host cell of claim 30, wherein the host cell is a CD8⁺ cytotoxic T-cell selected from the group consisting of a naïve CD8⁺ T-cell, a CD8⁺ memory T-cell, a central memory CD8⁺ T-cell, a regulatory CD8⁺ T-cell, an IPS derived CD8⁺ T-cell, an effector memory CD8⁺ T-cell, and a bulk CD8⁺ T-cell.

34. The host cell of claim 30, wherein the host cell is a CD4+ T helper cell selected from the group consisting of a naïve CD4+ T-cell, a CD4+ memory T-cell, a central memory CD4+ T-cell, a regulatory CD4+ T-cell, an IPS derived CD4+ T-cell, an effector memory CD4+ T-cell, and a bulk CD4+ T-cell.

35. The host cell of any one of claims 30-34, wherein the cell is allogenic to a subject, preferably a human or autologous to a subject, preferably a human.

36. A pharmaceutical composition comprising the host cell of any one of claims 30-35 and a pharmaceutically acceptable excipient.

37. A method of treating, inhibiting or ameliorating a cancer in a subject, comprising:

administering the host cell of any one of claims 30-35 to the subject.

38. The method of claim 37, wherein the subject is administered a dose comprising a plurality of the host cell sufficient to treat, inhibit or ameliorate the cancer that is less than a dose sufficient to treat, inhibit or ameliorate the cancer of a plurality of cells comprising a CAR comprising a polypeptide spacer having a length less than 120 consecutive amino acid residues.

39. The method of claim 37 or 38, wherein a dose comprising a plurality of the host cell less than 5×10^6 cell/kg is administered to the subject.

40. The method of any one of claims 37-39, further comprising administering cetuximab to the subject.

41. The method of any one of claims 37-40, wherein the cancer comprises a CD171 expressing cell.

42. The method of any one of claims 37-41, wherein the cancer is selected from a neuroblastoma or a ganglioneuroblastoma.

43. A host cell of any one of claims 30-35 for use in treating, inhibiting or ameliorating a cancer in a subject.

44. Use of the host cell of any one of claims 30-35 in the manufacture of a medicament for treating, inhibiting or ameliorating a cancer in a subject.

45. The host cell of any one of claims 30-35 for use as a medicament.