

CLAIMS

1. A method of treating a subject who has a cancer, the method comprising administering to the subject at least one dose of cells comprising a chimeric antigen receptor (CAR) polypeptide comprising:
 - a) an extracellular antigen binding domain comprising a first anti-BCMA binding moiety and a second BCMA binding moiety;
 - b) a transmembrane domain; and
 - c) an intracellular signaling domain,optionally wherein the cells are CAR-T cells.
2. The method of claim 1, wherein the cells are expanded in vitro prior to infusion.
3. The method of claim 1 or claim 2, wherein the cells are T cells, NK cells, iPSC-NK cells.
4. The method of claim 3, wherein the cells are CAR-T cells.
5. The method of claim 4, wherein the cells are NKT cells, iPSC-T cells or gamma-delta T cells.
6. The method of any one of claims 1 to 5, wherein the cells are heterologous or autologous.
7. The method of any one of claims 1 to 6, wherein the dose comprises 4.0×10^5 to 1.0×10^6 of the CAR-T cells per kilogram of the mass of the subject.
8. The method of any one of claims 1 to 7, wherein the dose comprises 5.5×10^5 to 8×10^5 of the CAR-T cells per kilogram of the mass of the subject.
9. The method of any one of claims 1 to 8, wherein the dose comprises about 7.5×10^5 of the CAR-T cells per kilogram of the mass of the subject.
10. The method of any one of claims 1 to 9, wherein the dose comprises 1×10^6 to 1×10^8 of the CAR-T cells.

11. The method of any one of claims 1 to 10, wherein the dose comprises 2×10^7 to 8×10^7 of the CAR-T cells.
12. The method of any one of claims 1 to 11, wherein the dose comprises about 5.25×10^7 of the CAR-T cells.
13. The method of any one of claims 1 to 6, wherein the cells are administered at a dose resulting in 4.0×10^5 to 1.0×10^6 of the CAR-T cells per kilogram of the mass of the subject.
14. The method of any one of claims 1 to 6 and 13, wherein the cells are administered at a dose resulting in 5.5×10^5 to 8×10^5 of the CAR-T cells per kilogram of the mass of the subject.
15. The method of any one of claims 1 to 6, 13 and 14, wherein the cells are administered at a dose resulting in about 7.5×10^5 of the CAR-T cells per kilogram of the mass of the subject.
16. The method of any one of claims 1 to 7 and 13-15, wherein the cells are administered at a dose resulting in 1×10^6 to 1×10^8 of the CAR-T cells in the subject.
17. The method of any one of claims 1 to 7 and 13-16, wherein the cells are administered at a dose resulting in 2×10^7 to 8×10^7 of the CAR-T cells in the subject.
18. The method of any one of claims 1 to 7 and 13-17, wherein the cells are administered at a dose resulting in about 5.25×10^7 of the CAR-T cells in the subject.
19. The method of any one of claims 1 to 18, wherein the dose of the CAR-T cells is administered only once during the course of the treatment.
20. The method of any one of claims 1 to 19, wherein the dose of the CAR-T cells is administered intravenously.
21. The method of any one of claims 1 to 20, wherein the cancer is multiple myeloma.

22. The method of claim 21, wherein the multiple myeloma is refractory multiple myeloma or relapsed multiple myeloma.
23. The method of any one of claims 1 to 22, wherein the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells in the dose of the CAR-T cells is less than 4.
24. The method of claim 23, wherein the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells in the dose of the CAR-T cells is less than 2.
25. The method of claim 24, wherein the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells in the dose of the CAR-T cells is less than 1.8.
26. The method of claim 25, wherein the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells in the dose of the CAR-T cells is less than 1.7.
27. The method of claim 26, wherein the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells in the dose of the CAR-T cells is less than 1.6.
28. The method of claim 27, wherein the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells in the dose of the CAR-T cells is less than 1.4.
29. The method of claim 28, wherein the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells in the dose of the CAR-T cells is less than 1.2.
30. The method of claim 29, wherein the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells in the dose of the CAR-T cells is less than 1.0.
31. The method of any one of claims 1 to 30, wherein the ratio of the CD4+ CAR-T cells at C_{max} to the CD8+ CAR-T cells at C_{max} is less than 3.5.
32. The method of any one of claims 1 to 31, wherein the ratio of the CD4+ CAR-T cells at C_{max} to the CD8+ CAR-T cells at C_{max} is less than 2.0.

33. The method of claim 32, wherein the ratio of the CD4+ CAR-T cells at C_{max} to the CD8+ CAR-T cells at C_{max} is less than 1.2.
34. The method of claim 33, wherein the ratio of the CD4+ CAR-T cells at C_{max} to the CD8+ CAR-T cells at C_{max} is less than 0.8.
35. The method of claim 34, wherein the ratio of the CD4+ CAR-T cells at C_{max} to the CD8+ CAR-T cells at C_{max} is less than 0.6.
36. The method of claim 35, wherein the ratio of the CD4+ CAR-T cells at C_{max} to the CD8+ CAR-T cells at C_{max} is less than 0.4.
37. The method of claim 36, wherein the ratio of the CD4+ CAR-T cells at C_{max} to the CD8+ CAR-T cells at C_{max} is less than 0.3.
38. The method of any one of claims 1 to 37, further comprising assaying the amount of CD4+ CAR-T cells and/or CD8+ CAR-T cells in the subject.
39. The method of any one of claims 1 to 38, wherein the first BCMA binding moiety and/or the second BCMA binding moiety is an anti-BCMA sdAb.
40. The method of claim 39, wherein the first BCMA binding moiety is a first anti-BCMA sdAb and the second BCMA binding moiety is a second anti-BCMA sdAb.
41. The method of any one of claims 1 to 40, wherein the first BCMA binding moiety comprises the amino acid sequence of
QVKLEESGGGLVQAGRSLRLSCAASEHTFSSHVMGWFRQAPGKERESVAVIGWRDI
STSYADSVKGRFTISRDNAKKTLYLQMNSLKPEDTAVYYCAARRIDAADFDSWGQG
TQVTVSS (SEQ ID NO: 1).
42. The method of any one of claims 1 to 40, wherein the first BCMA binding moiety comprises a polypeptide encoded by the nucleic acid sequence of
CAGGTCAAACCTGGAAGAATCTGGCGGAGGCCTGGTGCAGGCAGGACGGAGCCTG
CGCCTGAGCTGCGCAGCATCCGAGCACACCTTCAGCTCCACGTGATGGGCTGGT

TTCGGCAGGCCCCAGGCAAGGAGAGAGAGAGCGTGGCCGTGATCGGCTGGAGG
GACATCTCCACATCTTACGCCGATTCCGTGAAGGGCCGGTTCACCATCAGCCGGG
ACAACGCCAAGAAGACACTGTATCTGCAGATGAACAGCCTGAAGCCCCGAGGACA
CCGCCGTGTACTATTGCGCAGCAAGGAGAATCGACGCAGCAGACTTTGATTCTG
GGGCCAGGGCACCCAGGTGACAGTGTCTAGC (SEQ ID NO: 2).

43. The method of any one of claims 1 to 42, wherein the second BCMA binding moiety comprises the amino acid sequence of

EVQLVESGGGLVQAGGSLRLSCAASGRTFTMGWFRQAPGKEREFVAAISLSPTLAY
YAESVKGRFTISRDNKNTVVLQMNSLKPEDTALYYCAADRKSVMISIRPDYWGQG
TQVTVSS (SEQ ID NO: 3).

44. The method of any one of claims 1 to 43, wherein the second BCMA binding moiety comprises a polypeptide encoded by the nucleic acid sequence of

GAGGTGCAGCTGGTGGAGAGCGGAGGCGGCCTGGTGCAGGCCGGAGGCTCTCTG
AGGCTGAGCTGTGCAGCATCCGGAAGAACCTTCACAATGGGCTGGTTTAGGCAG
GCACCAGGAAAGGAGAGGGAGTTCGTGGCAGCAATCAGCCTGTCCCCTACCCTG
GCCTACTATGCCGAGAGCGTGAAGGGCAGGTTTACCATCTCCCGCGATAACGCC
AAGAATACAGTGGTGCTGCAGATGAACTCCCTGAAACCTGAGGACACAGCCCTG
TACTATTGTGCCGCCGATCGGAAGAGCGTGATGAGCATTAGACCAGACTATTGG
GGGCAGGGAACACAGGTGACCGTGAGCAGC (SEQ ID NO: 4).

45. The method of any one of claims 1 to 44, wherein the first BCMA binding moiety and the second BCMA binding moiety are connected to each other via a peptide linker.

46. The method of claim 45, wherein the peptide linker comprises the amino acid sequence of GGGGS (SEQ ID NO: 5).

47. The method of any one of claims 1 to 46, wherein the CAR polypeptide further comprises a signal peptide located at the N-terminus of the polypeptide.

48. The method of claim 47, wherein the signal peptide is derived from CD8 α .

49. The method of claim 48, wherein the signal peptide comprises the amino acid sequence of

MALPVTALLLPLALLLHAARP (SEQ ID NO: 6).

50. The method of claim 48, wherein the signal peptide comprises a polypeptide encoded by the nucleic acid sequence of

ATGGCTCTGCCCCGTCACCGCTCTGCTGCTGCCTCTGGCTCTGCTGCTGCACGCTGC
TCGCCCT (SEQ ID NO: 7).

51. The method of any one of claims 1 to 48, wherein the transmembrane domain comprises the amino acid sequence of IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 8).

52. The method of any one of claims 1 to 48, wherein the transmembrane domain comprises a polypeptide encoded by the nucleic acid sequence of

ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGG
TTATCACCCCTTTACTGC (SEQ ID NO: 9).

53. The method of any one of claims 1 to 52, wherein the intracellular signaling domain comprises a primary intracellular signaling domain of an immune effector cell.

54. The method of any one of claims 1 to 52, wherein the intracellular signaling domain is derived from CD3ζ.

55. The method of any one of claims 1 to 54, wherein the intracellular signaling domain comprises one or more co-stimulatory signaling domains.

56. The method of claim 55, wherein the intracellular signaling domain comprises the amino acid sequence of

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
PPR (SEQ ID NO: 10).

57. The method of claim 55, wherein the intracellular signaling domain comprises a polypeptide encoded by the nucleic acid sequence of

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGGTACCAGCAGGGCCAGAA
CCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGA
CAAGAGACGTGGCCGGGACCCTGAGATGGGGGAAAGCCGAGAAGGAAGAACC
CTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACA
GTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTT
TACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG
GCCCTGCCCCCTCGCTAA (SEQ ID NO: 11).

58. The method of any one of claims 55 to 57, wherein the intracellular signaling domain comprises the amino acid sequence of
KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 12).

59. The method of any one of claims 55 to 57, wherein the intracellular signaling domain comprises a polypeptide encoded by the nucleic acid sequence of
AAACGGGGCAGAAAGAACTCCTGTATATATTCAAACAACCATTTATGAGACCA
GTACAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAA
GAAGGAGGATGTGAACTG (SEQ ID NO: 13).

60. The method of any one of claims 1 to 59, wherein the CAR polypeptide further comprises a hinge domain located between the C-terminus of the extracellular antigen binding domain and the N-terminus of the transmembrane domain.

61. The method of claim 60, wherein the hinge domain comprises the amino acid sequence of TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 15).

62. The method of claim 60, wherein the hinge domain comprises a polypeptide encoded by the nucleic acid sequence of
ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAG
CCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCAC
ACGAGGGGGCTGGACTTCGCCTGTGAT (SEQ ID NO: 14).

63. The method of any one of claims 1 to 62, wherein the T cells are autologous T cells.

64. The method of any one of claims 1 to 62, wherein the T cells are allogeneic T cells.

65. The method of any one of claims 1 to 64, wherein the subject is human.