

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

CAGGTCAAACCTGGAAGAATCTGGCGGAGGGCTGGTGCAGGCAGGACGGAGCCTG
CGCCTGAGCTGCGCAGCATCCGAGCACACCTTCAGCTCCCACGTGATGGGCTGCT

TTCGGCAGGCCCCAGGCAAGGAGAGAGAGAGCGTGGCCGTGATCGGCTGGAGG
GACATCTCCACATCTTACGCCGATTCCGTGAAGGGCCGCTTCACCATCAGCCGGG
ACAACGCCAAGAAGACACTGTATCTGCAGATGAACAGCCTGAAGCCCGAGGACA
CCGCCGTGTACTATTGGCGAGCAAGGAGAATCGACGCAGCAGACTTTGATTCTG
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
YAESVKGRFTISRDNAKNTVVLQMNSLKPEDTALYYCAADRKSVMISIRPDYWGQG
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

GAGGTGCAGCTGCTGGAGAGCGGAGGCGGCCTGCTGCAGGCCGGAGGCTCTCTG
AGGCTGAGCTGTGCAGCATCCGGAAGAACCTTCACAATGGGCTGGTTTAGGCAG
GCACCAGGAAAGGAGAGGGAGTTCCGTGGCAGCAATCAGCCTGTCCCCTACCGTG
GCCTACTATGCCGAGAGCGTGAAGGGCAGGTTTACCATCTCCCGCGATAACGCC
AAGAATACAGTGCTGCTGCAGATGAACTCCCTGAAACCTGAGGACACAGCCCTG
TACTATTGTGCGCGCGATCGGAAGAGCGTGATGAGCATTAGACCAGACTATTGG
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

ATGGCTCTGCCCCGTCACCGCTCTGCTGCTGCCTCTGGCTCTGCTGCTGCACGCTGCTCGCCCT (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

ATCTACATCTGGGGGGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCCTTTACTGC (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 CD3 ζ .

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 EGLYNELQKDKMAEAYSEIGMKGERRRGKCHDGLYQGLSTATKDTYDALHMQAL 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

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAA
CCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGA
CAAGAGACGTGGCCGGGACCCTGAGATGGGGGAAAGCCGAGAAGGAAGAACC
CTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACA
GTGAGATTGGGATGAAAGGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCCTT
TACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGAGGCCCTTCACATGCAG
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
AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCA
GTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCAGAAGAAGAA
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 TTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 15).

62. The method of claim 60, wherein the hinge domain comprises a polypeptide encoded by the nucleic acid sequence of
ACCACGACGCCAGCGCCGGACCAACACCGGCGCCCACCATCGCGTCGCAG
CCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGGCGCAGTGCAG
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.~~

1. Use of CAR-T cells in the manufacture of a medicament for the treatment of cancer in a subject, wherein at least one dose of the CAR-T cells is to be administered to the subject, wherein the CAR-T cells comprise 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,

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.

2. The use of claim 1, wherein the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells in the dose of the CAR-T cells is:

a. less than 2

b. less than 1.8

c. less than 1.7

d. less than 1.6

e. less than 1.4

f. less than 1.2; or

g. less than 1.0.

3. The use of claim 1 or 2, wherein the ratio of the CD4+ CAR-T cells at C_{max} to the CD8+ CAR-T cells at C_{max} is:

a. less than 3.5

b. less than 2.0

c. less than 1.2

d. less than 0.8

e. less than 0.6

f. less than 0.4; or

g. less than 0.3.

4. The use of any one of claims 1 to 3, further comprising assaying the amount of CD4+ CAR-T cells and/or CD8+ CAR-T cells in the subject.
5. The use of any one of claims 1 to 4, wherein the cells are NKT cells, iPSC-T cells or gamma-delta T cells.
6. The use of any one of claims 1 to 5, wherein the first BCMA binding moiety and/or the second BCMA binding moiety is an anti-BCMA sdAb.
7. The use of claim 6, wherein the first BCMA binding moiety is a first anti-BCMA sdAb and the second BCMA binding moiety is a second anti-BCMA sdAb.
8. The use of any one of claims 1 to 7, wherein the first BCMA binding moiety comprises the amino acid sequence of
QVKLEESGGGLVQAGRSLRLSCAASEHTFSSHVMGWFRQAPGKERESVAVIGWRDI
STSYADSVKGRFTISRDNAAKKTLYLQMNSLKPEDTAVYYCAARRIDAADFDSWGQG
TQVTVSS (SEQ ID NO: 1).
9. The use of any one of claims 1 to 8, wherein the first BCMA binding moiety comprises a polypeptide encoded by the nucleic acid sequence of
CAGGTCAAACCTGGAAGAATCTGGCGGAGGCCTGGTGCAGGCAGGACGGAGCCTG
CGCCTGAGCTGCGCAGCATCCGAGCACACCTTCAGCTCCCACGTGATGGGCTGGT
TTCGGCAGGCCCCAGGCAAGGAGAGAGAGAGAGCGTGGCCGTGATCGGCTGGAGG
GACATCTCCACATCTTACGCCGATTCCGTGAAGGGCCGGTTCACCATCAGCCGGG
ACAACGCCAAGAAGACACTGTATCTGCAGATGAACAGCCTGAAGCCCGAGGACA
CCGCCGTGTACTATTGCGCAGCAAGGAGAATCGACGCAGCAGACTTTGATTCCTG
GGGCCAGGGCACCCAGGTGACAGTGTCTAGC (SEQ ID NO: 2).
10. The use of any one of claims 1 to 9, wherein the second BCMA binding moiety comprises the amino acid sequence of
EVQLVESGGGLVQAGGSLRLSCAASGRTFTMGWFRQAPGKEREFVAAISLSPTLAY
YAESVKGRFTISRDNAAKNTVVLQMNSLKPEDTALYYCAADRKSVMISIRPDYWGQG
TQVTVSS (SEQ ID NO: 3).

11. The use of any one of claims 1 to 10, wherein the second BCMA binding moiety comprises a polypeptide encoded by the nucleic acid sequence of
GAGGTGCAGCTGGTGGAGAGCGGAGGCGGCCTGGTGCAGGCCGGAGGCTCTCTG
AGGCTGAGCTGTGCAGCATCCGGAAGAACCTTCACAATGGGCTGGTTTAGGCAG
GCACCAGGAAAGGAGAGGGAGTTTCGTGGCAGCAATCAGCCTGTCCCCTACCCTG
GCCTACTATGCCGAGAGCGTGAAGGGCAGGTTTACCATCTCCCGCGATAACGCC
AAGAATACAGTGGTGCTGCAGATGAACTCCCTGAAACCTGAGGACACAGCCCTG
TACTATTGTGCCGCCGATCGGAAGAGCGTGATGAGCATTAGACCAGACTATTGG
GGGCAGGGAACACAGGTGACCGTGAGCAGC (SEQ ID NO: 4).

12. The use of any one of claims 1 to 11, wherein the first BCMA binding moiety and the second BCMA binding moiety are connected to each other via a peptide linker.

13. The use of any one of claims 1 to 12, wherein the CAR polypeptide further comprises a signal peptide located at the N-terminus of the polypeptide.

14. The use of any one of claims 1 to 13, wherein the transmembrane domain comprises the amino acid sequence of IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 8).

15. The use of any one of claims 1 to 14, wherein the transmembrane domain comprises a polypeptide encoded by the nucleic acid sequence of
ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGG
TTATCACCCCTTTACTGC (SEQ ID NO: 9).

16. The use of any one of claims 1 to 15, wherein the intracellular signaling domain comprises a primary intracellular signaling domain of an immune effector cell.

17. The use of any one of claims 1 to 16, wherein the intracellular signaling domain comprises one or more co-stimulatory signaling domains.

18. The use of any one of claims 1 to 17, 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.

19. The use of any one of claims 1 to 18, wherein the T cells are:

- a. autologous T cells; or
- b. allogeneic T cells.

20. The use of any one of claims 1 to 19, wherein the subject is human.

21. The use of any one of claims 1 to 20, wherein the dose of the CAR-T cells is to be administered only once during the course of the treatment.

22. The use of any one of claims 1 to 21, wherein the dose of the CAR-T cells is to be administered intravenously.

23. The use of any one of claims 1 to 22, wherein the cancer is multiple myeloma.

24. The use of claim 23, wherein the multiple myeloma is refractory multiple myeloma or relapsed multiple myeloma.