

CLAIMS

1. Use of cells in the manufacture of a medicament for the treatment of cancer in a subject, wherein a dose of the cells is to be administered to the subject, wherein the cells comprise a chimeric antigen receptor (CAR) polypeptide comprising:

a) an extracellular antigen binding domain comprising a first -BCMA binding moiety and a second BCMA binding moiety;

b) a transmembrane domain; and

c) an intracellular signaling domain,

wherein the cells are T cells,

wherein the first BCMA binding moiety comprises the amino acid sequence of QVKLEESGGGLVQAGRSLRLSCAASEHTFSSHVMGWFRQAPGKERESVAVIGWRDISTS YADSVKGRFTISRDNAAKKTLYLQMNSLKPEDTAVYYCAARRIDAADFDSWGQGTQVT VSS (SEQ ID NO: 1),

wherein the second BCMA binding moiety comprises the amino acid sequence of EVQLVESGGGLVQAGGSLRLSCAASGRTFTMGWFRQAPGKEREFVAAISLSPTLAYYYAE SVKGRFTISRDNAAKNTVVLQMNSLKPEDTALYYCAADRKSVMISIRPDYWGQGTQVTVS S (SEQ ID NO: 3),

wherein the cancer is refractory or relapsed multiple myeloma,

wherein the dose comprises 5.0×10^5 to 1.0×10^6 of the T cells per kilogram of the mass of the subject,

wherein a single dose of the T cells is to be administered to the subject by infusion during the course of the treatment, and

wherein the subject has received multiple prior lines of therapy comprising a proteasome inhibitor, an immunomodulatory agent, and an anti-CD38 monoclonal antibody.

2. The use of claim 1, wherein the cells are expanded *in vitro* prior to infusion.

3. The use of claim 1 or 2, wherein the T cells are heterologous T cells.

4. The use of any one of claims 1 to 3, wherein the dose comprises 5.5×10^5 to 8×10^5 of the T cells per kilogram of the mass of the subject.
5. The use of any one of claims 1 to 4, wherein the dose comprises about 7.5×10^5 of the T cells per kilogram of the mass of the subject.
6. The use of any one of claims 1 to 5, wherein the dose comprises 1×10^6 to 1×10^8 of the total amount of the T cells.
7. The use of any one of claims 1 to 6, wherein the dose comprises 2×10^7 to 8×10^7 of the total amount of the T cells.
8. The use of any one of claims 1 to 7, wherein the dose comprises about 5.25×10^7 of the total amount of the T cells.
9. The use of any one of claims 1 to 3, wherein the dose of the T cells is to be administered, resulting in 5.0×10^5 to 1.0×10^6 of the T cells per kilogram of the mass of the subject.
10. The use of any one of claims 1 to 3 and 9, wherein the dose of the T cells is to be administered, resulting in 5.5×10^5 to 8×10^5 of the T cells per kilogram of the mass of the subject.
11. The use of any one of claims 1 to 3 and 9 to 10, wherein the dose of the T cells is to be administered, resulting in about 7.5×10^5 of the T cells per kilogram of the mass of the subject.
12. The use of any one of claims 1 to 3 and 9 to 11, wherein the dose of the T cells is to be administered, resulting in 1×10^6 to 1×10^8 of the T cells in the subject.
13. The use of any one of claims 1 to 3 and 9 to 12, wherein the dose of the T cells is to be administered, resulting in 2×10^7 to 8×10^7 of the T cells in the subject.

14. The use of any one of claims 1 to 3 and 9 to 13, wherein the dose of the T cells is to be administered, resulting in about 5.25×10^7 of the T cells in the subject.

15. The use of any one of claims 1 to 14, wherein the T cells comprise CD4⁺ T cells and CD8⁺ T cells and a ratio of the CD4⁺ T cells to the CD8⁺ T cells in the dose of the T cells is:

- a. less than 4
- b. less than 2
- c. less than 1.8
- d. less than 1.7
- e. less than 1.6
- f. less than 1.4
- g. less than 1.2; or
- h. less than 1.0.

16. The use of any one of claims 1 to 15, wherein the first BCMA binding moiety and/or the second BCMA binding moiety is an anti-BCMA single-domain antibody (sdAb).

17. The use of claim 16, wherein the first BCMA binding moiety is a first anti-BCMA sdAb and the second BCMA binding moiety is a second anti-BCMA sdAb.

18. The use of any one of claims 1 to 17, wherein the first BCMA binding moiety comprises a polypeptide encoded by the nucleic acid sequence of

CAGGTCAAACCTGGAAGAATCTGGCGGAGGCCTGGTGCAGGCAGGACGGAGCCTGCG
CCTGAGCTGCGCAGCATCCGAGCACACCTTCAGCTCCCACGTGATGGGCTGGTTTCG
GCAGGCCCCAGGCAAGGAGAGAGAGAGCGTGGCCGTGATCGGCTGGAGGGACATC
TCCACATCTTACGCCGATTCCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACGCC
AAGAAGACACTGTATCTGCAGATGAACAGCCTGAAGCCCGAGGACACCGCCGTGTA

CTATTGCGCAGCAAGGAGAATCGACGCAGCAGACTTTGATTCTGGGGCCAGGGCA
CCCAGGTGACAGTGTCTAGC (SEQ ID NO: 2).

19. The use of any one of claims 1 to 18, wherein the second BCMA binding moiety comprises a polypeptide encoded by the nucleic acid sequence of

GAGGTGCAGCTGGTGGAGAGCGGAGGCGGCCTGGTGCAGGCCGGAGGCTCTCTGAG
GCTGAGCTGTGCAGCATCCGGAAGAACCTTCACAATGGGCTGGTTTAGGCAGGCAC
CAGGAAAGGAGAGGGAGTTCGTGGCAGCAATCAGCCTGTCCCCTACCCTGGCCTAC
TATGCCGAGAGCGTGAAGGGCAGGTTTACCATCTCCCGCGATAACGCCAAGAATAC
AGTGGTGCTGCAGATGAACTCCCTGAAACCTGAGGACACAGCCCTGTACTATTGTGC
CGCCGATCGGAAGAGCGTGATGAGCATTAGACCAGACTATTGGGGGCAGGGAACAC
AGGTGACCGTGAGCAGC (SEQ ID NO: 4).

20. The use of any one of claims 1 to 19, wherein the first BCMA binding moiety and the second BCMA binding moiety are connected to each other via a peptide linker.

21. The use of claim 20, wherein the peptide linker comprises the amino acid sequence of GGGGS (SEQ ID NO: 5).

22. The use of any one of claims 1 to 21, wherein the CAR polypeptide further comprises a signal peptide located at the N-terminus of the polypeptide.

23. The use of claim 22, wherein the signal peptide is derived from CD8 α .

24. The use of claim 23, wherein the signal peptide comprises the amino acid sequence of MALPVTALLLPLALLLHAARP (SEQ ID NO: 6).

25. The use of claim 23, wherein the signal peptide comprises a polypeptide encoded by the nucleic acid sequence of

ATGGCTCTGCCCCGTCACCGCTCTGCTGCTGCCTCTGGCTCTGCTGCTGCACGCTGCTC
GCCCT (SEQ ID NO: 7).

26. The use of any one of claims 1 to 23, wherein the transmembrane domain comprises the amino acid sequence of IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 8).

27. The use of any one of claims 1 to 23, wherein the transmembrane domain comprises a polypeptide encoded by the nucleic acid sequence of
ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACCTGGTTA
TCACCCTTTACTGC (SEQ ID NO: 9).

28. The use of any one of claims 1 to 27, wherein the intracellular signaling domain comprises a primary intracellular signaling domain of an immune effector cell.

29. The use of any one of claims 1 to 27, wherein the intracellular signaling domain is derived from CD3 ζ .

30. The use of any one of claims 1 to 29, wherein the intracellular signaling domain comprises one or more co-stimulatory signaling domains.

31. The use of claim 30, wherein the intracellular signaling domain comprises the amino acid sequence of
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO: 10).

32. The use of claim 30, wherein the intracellular signaling domain comprises a polypeptide encoded by the nucleic acid sequence of
AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACC
AGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG

AGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGG
AAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATT
GGGATGAAAGGCGAGCGCCGGAGGGGGCAAGGGGACGATGGCCTTTACCAGGGTCT
CAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCG
CTAA (SEQ ID NO: 11).

33. The use of any of claims 30 to 32, wherein the intracellular signaling domain comprises the amino acid sequence of KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 12).

34. The use of any of claims 30 to 32, wherein the intracellular signaling domain comprises a polypeptide encoded by the nucleic acid sequence of
AAACGGGGCAGAAAGAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGT
ACAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAG
GAGGATGTGAACTG (SEQ ID NO: 13).

35. The use of any one of claims 1 to 34, 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.

36. The use of claim 35, wherein the hinge domain comprises the amino acid sequence of TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 15).

37. The use of claim 35, wherein the hinge domain comprises a polypeptide encoded by the nucleic acid sequence of
ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCC
CCTGTCCCTGCGCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGACACGA
GGGGGCTGGACTTCGCCTGTGAT (SEQ ID NO: 14).

38. The use of any one of claims 1 to 37, wherein the T cells are:

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

39. The use of any one of claims 1 to 38, wherein the subject is human.

40. The use of any one of claims 1 to 39, wherein the dose of the T cells is to be administered intravenously.

41. The use of claim 1, wherein the T cells comprise CD4⁺ T cells and CD8⁺ T cells and a ratio of the CD4⁺ T cells to the CD8⁺ T cells in the dose of the T cells is at a median of about 1.5.

42. The use of claim 1, wherein the T cells comprise CD4⁺ T cells and CD8⁺ T cells and a central memory T cell (T_{cm}) phenotype of the CD8⁺ T cells is predominant in the CD8⁺ T cells comprising the CAR polypeptide in the subject after the T cells are to be administered to the subject.

43. The use of claim 42, wherein a ratio of the T_{cm} CD8⁺ T cells in the CD8⁺ T cells comprising the CAR polypeptide in the subject ranges from 29% to 99%.

44. The use of claim 43, wherein the ratio of the T_{cm} CD8⁺ T cells in the CD8⁺ T cells comprising the CAR polypeptide in the subject is at a median of about 90%.

45. The use of claim 42, further wherein an effector memory T cell (T_{em}) phenotype of the CD4⁺ T cells in the CD4⁺ T cells comprising the CAR polypeptide in the subject is predominant.

46. The use of claim 45, wherein a ratio of the T_{em} CD4⁺ T cells in the CD4⁺ T cells comprising the CAR polypeptide in the subject ranges from 70% to 98%.

47. The use of claim 46, wherein the ratio of the Tem CD4⁺ T cells in the CD4⁺ T cells comprising the CAR polypeptide in the subject is at a median of about 87%.