

CLAIMS

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
CGCCTGAGCTGCGCAGCATCCGAGCACACCTTCAGCTCCACGTGATGGGCTGGT
TTCGGCAGGCCCCAGGCAAGGAGAGAGAGAGCGTGCCGTGATCGGCTGGAGG
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.