Claims

1. A bispecific chimeric antigen receptor (CAR), comprising:

(i) an anti-CD20 antigen-binding region which comprises a light chain variable region (V_L1) and a heavy chain variable region (V_H1), wherein V_L1 comprises three complementarity determining regions (CDRs), CDR1, CDR2 and CDR3, having amino acid sequences about 80% to about 100% identical to the amino acid sequences set forth in SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, respectively, and wherein V_H1 comprises three CDRs, CDR1, CDR2 and CDR3, having amino acid sequences about 80% to about 100% identical to the amino acid sequences set forth in SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, respectively; and

(ii) an anti-CD19 antigen-binding region which comprises a light chain variable region (V_L2) and a heavy chain variable region (V_H2), wherein V_L2 comprises three complementarity determining regions (CDRs), CDR1, CDR2 and CDR3, having amino acid sequences about 80% to about 100% identical to the amino acid sequences set forth in SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 39, respectively, and wherein V_H2 comprises three CDRs, CDR1, CDR2 and CDR3, having amino acid sequences about 80% to about 100% identical to the amino acid sequences set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, respectively.

2. The bispecific CAR of claim 1, wherein V_L1 is located at the N-terminus of V_H1.

3. The bispecific CAR of claim 1, wherein V_H2 is located at the N-terminus of V_L2.

4. The bispecific CAR of claim 1, wherein V_L1 and V_H1 have amino acid sequences about 80% to about 100% identical to amino acid sequences set forth in SEQ ID NO: 4 and SEQ ID NO: 3, respectively.

5. The bispecific CAR of claim 1, wherein V_L2 and V_H2 have amino acid sequences about 80% to about 100% identical to amino acid sequences set forth in SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

6. The bispecific CAR of claim 1, wherein the anti-CD20 antigen-binding region is a single-chain variable fragment (scFv) that specifically binds CD20, and wherein the anti-CD19 antigen-binding region is a scFv that specifically binds CD19.

7. The bispecific CAR of claim 1, wherein the bispecific CAR further comprises one or more of the following:

(a) a signal peptide,

(b) a hinge region,

(c) a transmembrane domain,

(d) a co-stimulatory region, and

(e) a cytoplasmic signaling domain.

8. The bispecific CAR of claim 7, wherein the co-stimulatory region comprises a co-stimulatory region of 4-1BB (CD137), CD28, OX40, CD2, CD7, CD27, CD30, CD40, CD70, CD134, PD1, Dap10, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), NKG2D, GITR, TLR2, or combinations thereof.

9. The bispecific CAR of claim 7, wherein the cytoplasmic signaling domain comprises a cytoplasmic signaling domain of CD3ζ.

10. The bispecific CAR of claim 7, wherein the hinge region comprises a hinge region of Ig4, CD8, CD28, CD137, or combinations thereof.

11. The bispecific CAR of claim 7, wherein the transmembrane domain comprises a transmembrane domain of CD8, CD28, CD3ε, CD45, CD4, CD5, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or combinations thereof.

12. The bispecific CAR of claim 1, comprising an amino acid sequence about 80% to about 100% identical to the amino acid sequence set forth in SEQ ID NO. 16.

13. An immune cell expressing the bispecific CAR of claim 1.

14. The immune cell of claim 13, wherein the immune cell is a T cell or a natural killer (NK) cell.

15. A nucleic acid encoding the bispecific CAR of claim 1.

16. A vector comprising the nucleic acid of claim 15.

17. A method of treating cancer, the method comprising administering the immune cell of claim 13 to a subject in need thereof.

18. The method of claim 17, wherein the cancer is a hematologic cancer.

19. The method of claim 17, wherein the cancer is a B-cell malignancy.

20. The method of claim 17, wherein the cancer is Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia, and/or multiple myeloma.

21. The method of claim 17, wherein the cancer is acute myeloid leukemia (AML), multiple myeloma (MM), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia, acute lymphoblastic leukemia (ALL), diffuse large B cell lymphoma (DLBCL), or combinations thereof.

22. The method of claim 17, wherein the immune cell is allogeneic or autologous.

23. A method of treating cancer, the method comprising administering the immune cell of claim 13 to a subject in need of, wherein the bispecific CAR generates an area under the curve (AUC) ranging from about 1e+06 copies/μg genomic DNA (copies/gDNA) to about 3.2e+06 copies/gDNA in the blood of the subject in about 28 days after administration.

24. A method for treating cancer, the method comprising administering the immune cell of claim 13 to a subject in need of, wherein the bispecific CAR generates a maximum plasma concentration (C_{max}) ranging from about 1e+05 copies/μg genomic DNA (copies/gDNA) to about 3e+05 copies/gDNA in the blood of the subject.

25. The method of claim 24, wherein the bispecific CAR has a T_{max} ranging from about 8 days to about 15 days.

26. The method of claim 25, wherein the bispecific CAR has a T_{max} ranging from about 10 days to about 14 days.