

CLAIMS

1. An isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), that specifically binds to CLL-1 chosen from CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12, wherein the CAR comprises a human anti-CLL-1 binding domain, a transmembrane domain, and an intracellular signaling domain, and wherein said anti-CLL-1 binding domain comprises:

(i) a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of an amino acid sequence of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Tables 5, 7, or 3; and

(ii). a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of an amino acid sequence of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Tables 6, 8, or 4.

2. The isolated nucleic acid molecule of claim 1, which encodes a CAR comprising:

(i) the amino acid sequence of a light chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Table 2;

(ii) an amino acid sequence having at least one, two, or three modifications of the amino acid sequence of the light chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of the light chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2; and/or

(iv) the amino acid sequence of a heavy chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Table 2;

(v) an amino acid sequence having at least one, two, or three modifications of the amino acid sequence of the heavy chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2; or

(vi) an amino acid sequence with 95-99% identity to the amino acid sequence of the heavy chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2.

3. The isolated nucleic acid molecule of claim 1 or 2, wherein:

(i) the encoded CLL-1 binding domain comprises the amino acid sequence selected from a group consisting of SEQ ID NO:47, 44, 48, 49, 50, 73, 70, 74, 75, 76, 86, 83, 87, 88, or 89;

(ii) the encoded CLL-1 binding domain comprises an amino acid sequence having at least one, two, or three modifications to any of SEQ ID NO: 47, 44, 48, 49, 50, 73, 70, 74, 75, 76, 86, 83, 87, 88, or 89; or

(iii) the encoded CLL-1 binding domain comprises an amino acid sequence with 95-99% identity to any of SEQ ID NO: 47, 44, 48, 49, 50, 73, 70, 74, 75, 76, 86, 83, 87, 88, or 89; or

(iv) the CLL-1 binding domain is encoded by a nucleotide sequence selected from a group consisting of SEQ ID NO: 60, 57, 61, 62, or 63, or a sequence with 95-99% identity thereof.

4. The isolated nucleic acid molecule of any of claims 1, 2, or 3, wherein the encoded CAR includes a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta, or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, and CD154.

5. The isolated nucleic acid molecule of any of claims 1, 2, 3, or 4, wherein:

(i) the encoded transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6, an amino acid sequence comprising at least one, two, or three modifications but not more than 20, 10, or 5 modifications of the amino acid sequence of SEQ ID NO:6, or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:6;

(ii) the nucleic acid sequence encoding the transmembrane domain comprises a sequence of SEQ ID NO:17, or a sequence with 95-99% identity thereof; or

(iii) the nucleic acid sequence encoding the transmembrane domain comprises a sequence encoding a transmembrane domain provided in any of SEQ ID NOs: 112, 109, 113, 114, or 115.

6. The isolated nucleic acid molecule of any of claims 1, 2, 3, or 4-5, wherein the encoded CLL-1 binding domain is connected to the transmembrane domain by a hinge region.

7. The nucleic acid molecule of claim 6, wherein:

(i) the encoded hinge region comprises the amino acid sequence of SEQ ID NO:2, or a sequence with 95-99% identity thereof;

(ii) the nucleic acid sequence encoding the hinge region comprises the nucleotide sequence of SEQ ID NO: 13, or a sequence with 95-99% identity thereof; or

(iii) the nucleic acid sequence encoding the hinge region comprises a sequence encoding a hinge region provided in any of SEQ ID NOs: 112, 109, 113, 114, or 115.

8. The isolated nucleic acid molecule of any of claims 1, 2, 3, or 4-7, wherein the encoded intracellular signaling domain comprises a costimulatory intracellular signaling domain, wherein the costimulatory intracellular signaling domain comprises a functional signaling domain obtained from a costimulatory molecule selected from the group consisting of a MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

9. The isolated nucleic acid molecule of claim 8, wherein the encoded costimulatory domain comprises the amino acid sequence of SEQ ID NO:7, or an amino acid sequence having at least one, two, or three modifications but not more than 20, 10, or 5 modifications of the amino acid sequence of SEQ ID NO:7, or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7.

10. The isolated nucleic acid molecule of claim 8, wherein the nucleic acid sequence encoding the costimulatory domain comprises the nucleotide sequence of SEQ ID NO:18, or a sequence with 95-99% identity thereof, or the nucleic acid sequence encoding the costimulatory domain comprises a sequence encoding a 4-1BB domain provided in any of SEQ ID NOs: 112, 109, 113, 114 or 115, or a sequence with 95-99% identity thereof.

11. The isolated nucleic acid molecule of any of claims 1, 2, 3, or 4-10, wherein the encoded intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta.

12. The isolated nucleic acid molecule of any of claims 1, 2, 3, or 4-11, wherein the encoded intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and/or the sequence of SEQ ID NO:9 or SEQ ID NO:10; or an amino acid sequence having at least one, two, or three modifications but not more than 20, 10, or 5 modifications of the amino acid sequence of SEQ ID NO:7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10; or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10.

13. The isolated nucleic acid molecule of any of claims 1, 2, 3, or 4-12, wherein the encoded intracellular signaling domain comprises the sequence of SEQ ID NO:7 and the sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

14. The isolated nucleic acid molecule of any of claims 1, 2, 3, or 4-13, wherein the nucleic acid sequence encoding the intracellular signaling domain comprises the sequence of SEQ ID NO:18, or a sequence with 95-99% identity thereof, and/or

(i) the sequence of SEQ ID NO:20 or SEQ ID NO:21, or a sequence with 95-99% identity thereof, or

(ii) the sequence encoding a CD3 zeta domain provided in any of SEQ ID NOs: 112, 109, 113, 114, or 115, or a sequence with 95-99% identity thereof.

15. The isolated nucleic acid molecule of any of claims 1, 2, 3, or 4-14, further comprising a leader sequence which encodes the amino acid sequence of SEQ ID NO:1.

16. The isolated nucleic acid molecule of any of claims 1, 2, 3, or 4-15, which encodes a CAR comprising:

(i) the amino acid sequence of any of SEQ ID NOs:99, 96, 100, 101, or 102;

(ii) an amino acid sequence having at least one, two, or three modifications but not more than 30, 20, or 10 modifications to any of SEQ ID NOs: 99, 96, 100, 101, or 102; or

(iii) an amino acid sequence with 95-99% identity to any of SEQ ID NOs: 99, 96, 100, 101, or 102.

17. The isolated nucleic acid molecule of any of claims 1, 2, 3, or 4-16, comprising the nucleotide sequence of any of SEQ ID NOs: 112, 109, 113, 114, or 115, or a nucleotide sequence with 95-99% identity to any of SEQ ID NOs: 112, 109, 113, 114, or 115.

18. The isolated nucleic acid molecule of any of claims 1-17, wherein the anti-CLL-1 binding domain comprises:

the heavy chain complementarity determining region 1 (HC CDR1),

the heavy chain complementarity determining region 2 (HC CDR2),

the heavy chain complementarity determining region 3 (HC CDR3),

the light chain complementarity determining region 1 (LC CDR1),

the light chain complementarity determining region 2 (LC CDR2), and

the light chain complementarity determining region 3 (LC CDR 3)

of a CLL-1 CAR amino acid sequence selected from CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11 and CLL-1-CAR12, wherein

(a) the CDR sequences according to the Kabat numbering are:

(i) LC CDR1 of SEQ ID NO:364, LC CDR2 of SEQ ID NO:378, and LC CDR3 of SEQ ID NO:392; and HC CDR1 of SEQ ID NO:322, HC CDR2 of SEQ ID NO:336, and HC CDR3 of SEQ ID NO:350 for CLL-1 CAR9;

(ii) LC CDR1 of SEQ ID NO:361, LC CDR2 of SEQ ID NO:375, and LC CDR3 of SEQ ID NO:389; and HC CDR1 of SEQ ID NO:319; HC CDR2 of SEQ ID NO:333 and HC CDR3 of SEQ ID NO:347 for CLL-1 CAR6;

(iii) LC CDR1 of SEQ ID NO:365, LC CDR2 of SEQ ID NO:379, and LC CDR3 of SEQ ID NO:393; and HC CDR1 of SEQ ID NO:323; HC CDR2 of SEQ ID NO:337, and HC CDR3 of SEQ ID NO:351 for CLL-1 CAR10;

(iv) LC CDR1 of SEQ ID NO:366, LC CDR2 of SEQ ID NO:380, and LC CDR3 of SEQ ID NO:394; and HC CDR1 of SEQ ID NO:324; HC CDR2 of SEQ ID NO:338, and HC CDR3 of SEQ ID NO:352 for CLL-1 CAR11; and

(v) LC CDR1 of SEQ ID NO:367, LC CDR2 of SEQ ID NO:381, and LC CDR3 of SEQ ID NO:395; and HC CDR1 of SEQ ID NO:325; HC CDR2 of SEQ ID NO:339, and HC CDR3 of SEQ ID NO:353 for CLL-1 CAR12;

(b) the CDR sequences according to the Chothia numbering are:

(i) LC CDR1 of SEQ ID NO: 448, LC CDR2 of SEQ ID NO: 462, and LC CDR3 of SEQ ID NO:476; and HCL CDR1 of SEQ ID NO:406, HC CDR2 of SEQ ID NO:420, and HC CDR3 of SEQ ID NO:434 for CLL-1 CAR9;

(ii) LC CDR1 of SEQ ID NO:445, LC CDR2 of SEQ ID NO:459, and LC CDR3 of SEQ ID NO:473; and HC CDR1 of SEQ ID NO:403; HC CDR2 of SEQ ID NO:417, and HC CDR3 of SEQ ID NO:431 for CLL-1 CAR6;

(iii) LC CDR1 of SEQ ID NO:449, LC CDR2 of SEQ ID NO:463, and LC CDR3 of SEQ ID NO:477; and HC CDR1 of SEQ ID NO:407; HC CDR2 of SEQ ID NO:421, and HC CDR3 of SEQ ID NO:435 for CLL-1 CAR10;

(iv) LC CDR1 of SEQ ID NO:450, LC CDR2 of SEQ ID NO:464, and LC CDR3 of SEQ ID NO:478; and HC CDR1 of SEQ ID NO:408; HC CDR2 of SEQ ID NO:422, and HC CDR3 of SEQ ID NO:436 for CLL-1 CAR11; and

(v) LC CDR1 of SEQ ID NO:451, LC CDR2 of SEQ ID NO:465, and LC CDR3 of SEQ ID NO:479; and HC CDR1 of SEQ ID NO:409; HC CDR2 of SEQ ID NO:423, and HC CDR3 of SEQ ID NO:437 for CLL-1 CAR12;

(c) the CDR sequences according to the combined numbering are:

(i) LC CDR1 of SEQ ID NO:164, LC CDR2 of SEQ ID NO:177, and LC CDR3 of SEQ ID NO:190; and HC CDR1 of SEQ ID NO:125, HC CDR2 of SEQ ID NO:138, and HC CDR3 of SEQ ID NO:151 for CLL-1 CAR9;

(ii) LC CDR1 of SEQ ID NO:161, LC CDR2 of SEQ ID NO:174, and LC CDR3 of SEQ ID NO:187; and HC CDR1 of SEQ ID NO:122; HC CDR2 of SEQ ID NO:135, and HC CDR3 of SEQ ID NO:148 for CLL-1 CAR6;

(iii) LC CDR1 of SEQ ID NO:165, LC CDR2 of SEQ ID NO:178, and LC CDR3 of SEQ ID NO:191; and HC CDR1 of SEQ ID NO:126; HC CDR2 of SEQ ID NO:139, and HC CDR3 of SEQ ID NO:152 for CLL-1 CAR10;

(iv) LC CDR1 of SEQ ID NO:166, LC CDR2 of SEQ ID NO:179, and LC CDR3 of SEQ ID NO:192; and HC CDR1 of SEQ ID NO:127; HC CDR2 of SEQ ID NO:140, and HC CDR3 of SEQ ID NO:153 for CLL-1 CAR11; and

(v) LC CDR1 of SEQ ID NO:167, LC CDR2 of SEQ ID NO:180, and LC CDR3 of SEQ ID NO:193; and HC CDR1 of SEQ ID NO:128; HC CDR2 of SEQ ID NO:141, and HC CDR3 of SEQ ID NO:154 for CLL-1 CAR12.

19. The isolated nucleic acid molecule of claim 18, wherein the anti-CLL-1 binding domain comprises a light chain variable region and a heavy chain variable region comprising:

(i) the amino acid sequence of SEQ ID NOs: 86 and 73, respectively; SEQ ID NOs: 83 and/or 70, respectively, SEQ ID NOs: 87 and/or 74, respectively; SEQ ID NOs: 88 and/or 75, respectively; or SEQ ID NO: 89 and/or 76, respectively;

(ii) an amino acid sequence having at least one, two, or three modifications but not more than 30, 20, or 10 modifications of the amino acid sequence of SEQ ID NOs: 86 and/or 73, respectively; SEQ ID NOs: 83 and/or 70, respectively, SEQ ID NOs: 87 and/or 74, respectively; SEQ ID NOs: 88 and/or 75, respectively; or SEQ ID NO: 89 and/or 76, respectively; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NOs: 86 and/or 73, respectively; SEQ ID NOs: 83 and/or 70, respectively, SEQ ID NOs: 87 and/or 74, respectively; SEQ ID NOs: 88 and/or 75, respectively; or SEQ ID NO: 89 and/or 76, respectively.

20. The isolated nucleic acid molecule of claim 19, wherein the light chain variable region and heavy chain variable region comprise the amino acid sequence of SEQ ID NOs: 86 and 73, respectively; SEQ ID NOs: 83 and 70, respectively; SEQ ID NOs: 87 and 74, respectively; SEQ ID NOs: 88 and 75, respectively; or SEQ ID NO: 89 and 76, respectively.

21. An isolated polypeptide molecule encoded by the nucleic acid molecule of any one of claims 1-20.

22. An isolated chimeric antigen receptor (CAR) polypeptide, that specifically binds to CLL-1, wherein the CAR comprises an antibody or antibody fragment which includes a human anti-CLL-1 binding domain, a transmembrane domain, and an intracellular signaling domain comprising a costimulatory domain and/or a primary signaling domain, and wherein said anti-CLL-1 binding domain comprises:

(i) a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of an amino acid sequence of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Tables 5, 7, or 3; and

(ii) a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of an amino acid sequence of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Tables 6, 8, or 4.

23. The isolated CAR polypeptide of claim 22, comprising:

(i) the amino acid sequence of a light chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Table 2;

(ii) an amino acid sequence having at least one, two, or three modifications of the amino acid sequence of the light chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of the light chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2; and/or

(iv) the amino acid sequence of a heavy chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Table 2;

(v) an amino acid sequence having at least one, two, or three modifications of the amino acid sequence of the heavy chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2;

(vi) an amino acid sequence with 95-99% identity to the amino acid sequence of the heavy chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2; or

(vii) any one of (i)-(iii) and any one of (iv)-(vi).

24. The isolated CAR polypeptide of claim 22 or 23, comprising:

(i) the amino acid sequence selected from a group consisting of SEQ ID NO: 47, 44, 48, 49, 50, 73, 70, 74, 75, 76, 86, 83, 87, 88, or 89;

(ii) an amino acid sequence having at least one, two, or three modifications to any of SEQ ID NO: 47, 44, 48, 49, 50, 73, 70, 74, 75, 76, 86, 83, 87, 88, or 89; or

(iii) an amino acid sequence with 95-99% identity to any of SEQ ID NO: 47, 44, 48, 49, 50, 73, 70, 74, 75, 76, 86, 83, 87, 88, or 89.

25. The isolated CAR polypeptide of any of claims 22-24, wherein the transmembrane domain comprises a transmembrane domain from a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, and CD154.

26. The isolated CAR polypeptide of any of claims 22-25, wherein:

(i) the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6,

- (ii) an amino acid sequence comprises at least one, two, or three modifications but not more than 20, 10, or 5 modifications of the amino acid sequence of SEQ ID NO:6, or
- (iii) a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:6.

27. The isolated CAR polypeptide of any of claims 22-26, wherein the CLL-1 binding domain is connected to the transmembrane domain by a hinge region.

28. The isolated CAR polypeptide of claim 27, wherein the hinge region comprises SEQ ID NO:2, or a sequence with 95-99% identity thereof.

29. The isolated CAR polypeptide of any of claims 22-28, wherein the intracellular signaling domain comprises a costimulatory intracellular signaling domain, wherein the costimulatory intracellular signaling domain comprises a functional signaling domain obtained from a costimulatory molecule selected from the group consisting of a MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFRR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

30. The isolated CAR polypeptide of any of claims 22-29, wherein the costimulatory domain comprises the amino acid sequence of SEQ ID NO:7, or an amino acid sequence having at least one, two, or three modifications but not more than 20, 10, or 5 modifications of the

amino acid sequence of SEQ ID NO:7, or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7.

31. The isolated CAR polypeptide of any of claims 22-30, wherein the intracellular signaling domain comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta.

32. The isolated CAR polypeptide of any of claims 22-31, wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and/or the sequence of SEQ ID NO:9 or SEQ ID NO:10; or an amino acid sequence having at least one, two, or three modifications but not more than 20, 10, or 5 modifications of the amino acid sequence of SEQ ID NO:7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10; or a sequence with 95-99% identity to the amino acid sequence of SEQ ID NO:7 and/or the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10.

33. The isolated CAR polypeptide of any of claims 22-32, wherein the intracellular signaling domain comprises the sequence of SEQ ID NO:7 and the sequence of SEQ ID NO:9 or SEQ ID NO:10, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

34. The isolated CAR polypeptide of any of claims 22-33, further comprising a leader sequence which comprises the amino acid sequence of SEQ ID NO:1.

35. The isolated CAR polypeptide of any of claims 22-34, comprising:

- (i) the amino acid sequence of any of SEQ ID NOs: 99, 96, 100, 101, or 102;
- (ii) an amino acid sequence having at least one, two, or three modifications but not more than 30, 20, or 10 modifications to any of SEQ ID NOs: 99, 96, 100, 101, or 102; or
- (iii) an amino acid sequence with 95-99% identity to any of SEQ ID NOs: 99, 96, 100, 101, or 102.

36. The isolated CAR polypeptide of any one of claims 22-35, wherein the anti-CLL-1 binding domain comprises the heavy chain complementarity determining region 1 (HC CDR1), the heavy chain complementarity determining region 2 (HC CDR2), the heavy chain complementarity determining region 3 (HC CDR3), the light chain complementarity determining region 1 (LC CDR1), the light chain complementarity determining region 2 (LC CDR2), and the light chain complementarity determining region 3 (LC CDR 3) of a CLL-1 CAR amino acid sequence selected from CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11 and CLL-1-CAR12, wherein

(a) the CDR sequences according to the Kabat numbering are:

(i) LC CDR1 of SEQ ID NO:364, LC CDR2 of SEQ ID NO:378, and LC CDR3 of SEQ ID NO:392; and HC CDR1 of SEQ ID NO:322, HC CDR2 of SEQ ID NO:336, and HC CDR3 of SEQ ID NO:350 for CLL-1 CAR9;

(ii) LC CDR1 of SEQ ID NO:361, LC CDR2 of SEQ ID NO:375, and LC CDR3 of SEQ ID NO:389; and HC CDR1 of SEQ ID NO:319; HC CDR2 of SEQ ID NO:333, and HC CDR3 of SEQ ID NO:347 for CLL-1 CAR6;

(iii) LC CDR1 of SEQ ID NO:365, LC CDR2 of SEQ ID NO:379, and LC CDR3 of SEQ ID NO:393; and HC CDR1 of SEQ ID NO:323; HC CDR2 of SEQ ID NO:337, and HC CDR3 of SEQ ID NO:351 for CLL-1 CAR10;

(iv) LC CDR1 of SEQ ID NO:366, LC CDR2 of SEQ ID NO:380, and LC CDR3 of SEQ ID NO:394; and HC CDR1 of SEQ ID NO:324; HC CDR2 of SEQ ID NO:338, and HC CDR3 of SEQ ID NO:352 for CLL-1 CAR11; and

(v) LC CDR1 of SEQ ID NO:367, LC CDR2 of SEQ ID NO:381, and LC CDR3 of SEQ ID NO:395; and HC CDR1 of SEQ ID NO:325; HC CDR2 of SEQ ID NO:339, and HC CDR3 of SEQ ID NO:353 for CLL-1 CAR12;

(b) the CDR sequences according to the Chothia numbering are:

(i) LC CDR1 of SEQ ID NO: 448, LC CDR2 of SEQ ID NO: 462, and LC CDR3 of SEQ ID NO:476; and HCL CDR1 of SEQ ID NO:406, HC CDR2 of SEQ ID NO:420, and HC CDR3 of SEQ ID NO:434 for CLL-1 CAR9;

(ii) LC CDR1 of SEQ ID NO:445, LC CDR2 of SEQ ID NO:459, and LC CDR3 of SEQ ID NO:473; and HC CDR1 of SEQ ID NO:403; HC CDR2 of SEQ ID NO:417, and HC CDR3 of SEQ ID NO:431 for CLL-1 CAR6;

(iii) LC CDR1 of SEQ ID NO:449, LC CDR2 of SEQ ID NO:463, and LC CDR3 of SEQ ID NO:477; and HC CDR1 of SEQ ID NO:407; HC CDR2 of SEQ ID NO:421, and HC CDR3 of SEQ ID NO:435 for CLL-1 CAR10;

(iv) LC CDR1 of SEQ ID NO:450, LC CDR2 of SEQ ID NO:464, and LC CDR3 of SEQ ID NO:478; and HC CDR1 of SEQ ID NO:408; HC CDR2 of SEQ ID NO:422, and HC CDR3 of SEQ ID NO:436 for CLL-1 CAR11; and

(v) LC CDR1 of SEQ ID NO:451, LC CDR2 of SEQ ID NO:465, and LC CDR3 of SEQ ID NO:479; and HC CDR1 of SEQ ID NO:409; HC CDR2 of SEQ ID NO:423, and HC CDR3 of SEQ ID NO:437 for CLL-1 CAR12;

(c) the CDR sequences according to the combined numbering are:

(i) LC CDR1 of SEQ ID NO:164, LC CDR2 of SEQ ID NO:177, and LC CDR3 of SEQ ID NO:190; and HC CDR1 of SEQ ID NO:125, HC CDR2 of SEQ ID NO:138, and HC CDR3 of SEQ ID NO:151 for CLL-1 CAR9;

(ii) LC CDR1 of SEQ ID NO:161, LC CDR2 of SEQ ID NO:174, and LC CDR3 of SEQ ID NO:187; and HC CDR1 of SEQ ID NO:122; HC CDR2 of SEQ ID NO:135, and HC CDR3 of SEQ ID NO:148 for CLL-1 CAR6;

(iii) LC CDR1 of SEQ ID NO:165, LC CDR2 of SEQ ID NO:178, and LC CDR3 of SEQ ID NO:191; and HC CDR1 of SEQ ID NO:126; HC CDR2 of SEQ ID NO:139, and HC CDR3 of SEQ ID NO:152 for CLL-1 CAR10;

(iv) LC CDR1 of SEQ ID NO:166, LC CDR2 of SEQ ID NO:179, and LC CDR3 of SEQ ID NO:192; and HC CDR1 of SEQ ID NO:127; HC CDR2 of SEQ ID NO:140, and HC CDR3 of SEQ ID NO:153 for CLL-1 CAR11; and

(v) LC CDR1 of SEQ ID NO:167, LC CDR2 of SEQ ID NO:180, and LC CDR3 of SEQ ID NO:193; and HC CDR1 of SEQ ID NO:128; HC CDR2 of SEQ ID NO:141, and HC CDR3 of SEQ ID NO:154 for CLL-1 CAR12.

37. The isolated CAR polypeptide of claim 36, wherein

the anti-CLL-1 binding domain comprises a light chain variable region and/or a heavy chain variable region comprising:

(i) the amino acid sequence of SEQ ID NOs: 86 and/or 73, respectively; SEQ ID NOs: 83 and/or 70, respectively, SEQ ID NOs: 87 and/or 74, respectively; SEQ ID NOs: 88 and/or 75, respectively; or SEQ ID NO: 89 and/or 76, respectively;

(ii) an amino acid sequence having at least one, two, or three modifications but not more than 30, 20, or 10 modifications of the amino acid sequence of SEQ ID NOs: 86 and/or 73, respectively; SEQ ID NOs: 83 and/or 70, respectively, SEQ ID NOs: 87 and/or 74, respectively; SEQ ID NOs: 88 and/or 75, respectively; or SEQ ID NO: 89 and/or 76, respectively ; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of SEQ ID NOs: 86 and/or 73, respectively; SEQ ID NOs: 83 and/or 70, respectively, SEQ ID NOs: 87 and/or 74, respectively; SEQ ID NOs: 88 and/or 75, respectively; or SEQ ID NO: 89 and/or 76, respectively.

38. The isolated CAR polypeptide of claim 37, wherein

the light chain variable region and heavy chain variable region comprise the amino acid sequence of SEQ ID NOs: 86 and 73, respectively; SEQ ID NOs: 83 and 70, respectively; SEQ ID NOs: 87 and 74, respectively; SEQ ID NOs: 88 and 75, respectively; or SEQ ID NO: 89 and 76, respectively.

39. An anti-CLL-1 binding domain which specifically binds to CLL-1, comprising:

(i) a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of an amino acid sequence of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Tables 5, 7, or 3; and

(ii). a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of an amino acid sequence of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Tables 6, 8, or 4.

40. The anti-CLL-1 binding domain of claim 39, comprising:

(i) the amino acid sequence of a light chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Table 2;

(ii) an amino acid sequence having at least one, two, or three modifications of the amino acid sequence of the light chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of the light chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2; and/or

(iv) the amino acid sequence of a heavy chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 listed in Table 2;

(v) an amino acid sequence having at least one, two, or three modifications of the amino acid sequence of the heavy chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2; or

(vi) an amino acid sequence with 95-99% identity to the amino acid sequence of the heavy chain variable region of CLL-1-CAR9, CLL-1-CAR6, CLL-1-CAR10, CLL-1-CAR11, or CLL-1-CAR12 provided in Table 2.

41. The anti-CLL-1 binding domain of claim 39 or 40, comprising:

(i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 47, 44, 48, 49, and 50;

(ii) an amino acid sequence having at least one, two, or three modifications to any of SEQ ID NOs: 47, 44, 48, 49, or 50; or

(iii) an amino acid sequence with 95-99% identity to any of SEQ ID NOs: 47, 44, 48, 49, or 50.

42. An isolated anti-CLL-1 binding domain or a chimeric antigen receptor (CAR) polypeptide comprising an anti-CLL-1 binding domain which specifically binds to CLL-1, wherein the anti-CLL1 binding domain comprises:

(i) a heavy chain complementarity determining region 1 (HC CDR1), a heavy chain complementarity determining region 2 (HC CDR2), and a heavy chain complementarity

determining region 3 (HC CDR3) of an amino acid sequence of a CLL-1 CAR amino acid sequence, wherein

(a) the CDR sequences according to the Kabat numbering are:

(i) HC CDR1 of SEQ ID NO:322, HC CDR2 of SEQ ID NO:336 and HC CDR3 of SEQ ID NO:350;

(b) the CDR sequences according to the Chothia numbering are:

(i) HC CDR1 of SEQ ID NO:406, HC CDR2 of SEQ ID NO:420 and HC CDR3 of SEQ ID NO:434;

(c) the CDR sequences according to the combined numbering are:

(i) HC CDR1 of SEQ ID NO:125, HC CDR2 of SEQ ID NO:138 and HC CDR3 of SEQ ID NO:151; and

(ii) a light chain complementarity determining region 1 (LC CDR1), a light chain complementarity determining region 2 (LC CDR2), and a light chain complementarity determining region 3 (LC CDR3) of a CLL-1 CAR amino acid sequence, wherein

(a) the CDR sequences according to the Kabat numbering are:

(i) LC CDR1 of SEQ ID NO:364, LC CDR2 of SEQ ID NO:378 and LC CDR3 of SEQ ID NO:392;

(b) the CDR sequences according to the Chothia numbering are:

(i) LC CDR1 of SEQ ID NO: 448, LC CDR2 of SEQ ID NO: 462 and LC CDR3 of SEQ ID NO:476;

(c) the CDR sequences according to the combined numbering are:

(i) LC CDR1 of SEQ ID NO:164, LC CDR2 of SEQ ID NO:177 and LC CDR3 of SEQ ID NO:190.

43. The isolated anti-CLL-1 binding domain or CAR polypeptide of claim 42, wherein the anti-CLL-1 binding domain comprises a heavy chain variable region of SEQ ID NO: 73; and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 86.

44. The isolated anti-CLL-1 binding domain or CAR polypeptide of claim 42, wherein the anti-CLL-1 binding domain comprises the amino acid sequence of SEQ ID NOs: 47.

45. The isolated anti-CLL-1 binding domain or CAR polypeptide of claim 42, wherein the anti-CLL-1 binding domain is encoded by a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOs: 60.

46. A vector comprising a nucleic acid molecule encoding a CAR of any of claims 22-38 or 42-45, wherein the vector is selected from the group consisting of a DNA vector, an RNA vector, a plasmid, a lentivirus vector, adenoviral vector, or a retrovirus vector.

47. The vector of claim 46, further comprising an EF-1 promoter comprising the sequence of SEQ ID NO: 11.

48. A cell, e.g., an immune effector cell, comprising the nucleic acid of any of claims 1-20, the CAR polypeptide of any of claims 21-38 or 42-45, the anti-CLL-1 binding domain of any of claims 39-45, or the vector of claim 46 or 47.

49. An *in vitro* or *ex vivo* method of making a cell, e.g., an immune effector cell, comprising transducing an immune effector cell with a vector of either of claim 46 or 47.

50. An *in vitro* or *ex vivo* method of generating a population of RNA-engineered cells, comprising introducing an *in vitro* transcribed RNA or synthetic RNA into a cell, where the RNA comprises a nucleic acid encoding a CAR polypeptide of any of the preceding claims.

51. An immune effector cell, e.g., a population of immune effector cells, comprising the CAR nucleic acid of any of claims 1-20, the CAR polypeptide of any of claims 21-38 or 42-45, or the anti-CLL-1 binding domain of any of claims 39-45 for use in a method of providing an anti-tumor immunity in a mammal, wherein the immune effector cell is a T cell or an NK cell.

52. The immune effector cell for use according to claim 51, wherein the immune effector cell is an autologous T cell or an allogeneic T cell.

53. An immune effector cell, e.g., a population of immune effector cells, comprising the CAR nucleic acid of any of claims 1-20, the CAR polypeptide of any of claims 21-38 or 42-45, or the anti-CLL-1 binding domain of any of claims 39-45 for use in a method of treating a mammal having a hematologic cancer associated with expression of CLL-1, wherein the immune effector cell is a T cell or an NK cell.

54. The immune effector cell for use according to claim 53, wherein the hematologic cancer associated with CLL-1 expression is a cancer or malignancy, or a precancerous condition chosen from one or more of a myelodysplasia, a myelodysplastic syndrome, or a preleukemia.

55. The immune effector cell for use according to claim 53 or 54, wherein the hematological cancer is an acute leukemia chosen from one or more of acute myeloid leukemia (AML); acute lymphoblastic B-cell leukemia (B-cell acute lymphoid leukemia, BALL), acute lymphoblastic T-cell leukemia (T-cell acute lymphoid leukemia (TALL), B-cell prolymphocytic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia (CML), myelodysplastic syndrome, plasma cell myeloma, or a combination thereof.

56. The immune effector cell for use according to any of claims 53-55, wherein the immune effector cell, e.g., the population of immune effector cells, are to be administered in combination with one or more of:

- (i) an agent that increases the efficacy of the immune effector cell comprising the CAR nucleic acid or CAR polypeptide;
- (ii) an agent that ameliorates one or more side effects associated with administration of the cell comprising the CAR nucleic acid or CAR polypeptide; or
- (iii) an agent that treats the hematologic cancer associated with the expression of CLL-1.

57. The isolated nucleic acid molecule of any of claims 1-20, the isolated CAR polypeptide molecule of any of claims 21-38 or 42-45, the anti-CLL-1 binding domain of any of

claims 39-45, the vector of either of claim 46 or 47, or the immune effector cell of claim 48 for use as a medicament.

58. The isolated nucleic acid molecule of any of claims 1-20, the isolated CAR polypeptide molecule of any of claims 21-38 or 42-45, the anti-CLL-1 binding domain of any of claims 39-45, the vector of either of claim 46 or 47, or the immune effector cell of claim 48 for use in the treatment of a hematologic cancer associated with expression of CLL-1.

59. The immune effector cell, e.g., a population of immune effector cells, of claim 41, further comprising a fusion protein comprising a first domain and a second domain, wherein:
the first domain comprises an inhibitory molecule, wherein the inhibitory molecule is chosen from PD1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, or TGFR beta, or a fragment thereof; and;

the second domain comprises an intracellular signaling domain, wherein the intracellular signaling domain comprises a co-stimulatory intracellular signaling domain derived from 4-1BB, CD27 or CD28, or a primary signaling domain derived from CD3 zeta, or both.

60. The immune effector cell for use according to claim 56, wherein the agent is an mTOR inhibitor and the subject is to be administered a low, immune enhancing dose of an mTOR inhibitor, e.g., RAD001 or rapamycin.

61. The immune effector cell for use according to claim 60, wherein the mTOR inhibitor is to be administered for an amount of time sufficient to decrease the proportion of PD-1 positive T cells, increase the proportion of PD-1 negative T cells, or increase the ratio of PD-1 negative T cells/ PD-1 positive T cells, in the peripheral blood of the subject, or in a preparation of T cells isolated from the subject.

62. An immune effector cell comprising the CAR nucleic acid molecule of any of claims 1-20, the CAR polypeptide of any of claims 21-38 or 42-45, or the anti-CLL-1 binding domain of any of claims 39-45 for use in a method of conditioning a subject prior to cell transplantation,

wherein the immune effector cell is a T cell or an NK cell, and wherein conditioning a subject prior to cell transplantation comprises reducing the number of CLL-1 -expressing cells in a subject, e.g., CLL-1 -expressing normal cells or CLL-1 -expressing cancer cells.

63. The immune effector cell for use according to claim 62, wherein the cell transplantation is a stem cell transplantation, e.g., a hematopoietic stem cell transplantation, or a bone marrow transplantation.

64. An immune effector cell, *e.g.*, a population of immune effector cells, comprising the nucleic acid of any of claims 1-20, the CAR polypeptide of any of claims 21-38 or 42-45, or the anti-CLL-1 binding domain of any of claims 39-45, or the vector of claim 46 or 47, for use in a method of treating a mammal having a hematologic cancer associated with expression of CLL-1, wherein an effective amount of the immune effector cell or population of immune effector cells is to be administered to the mammal, in combination with a chemotherapeutic agent, wherein the chemotherapeutic agent is to be administered prior to administration of the immune effector cell, *e.g.*, the population of immune effector cells, wherein the immune effector cell is a T cell or an NK cell; and optionally, wherein the chemotherapeutic agent increases CLL-1 expression on the cancer cell or wherein the chemotherapeutic agent is cytarabine.

65. The immune effector cell for use of claim 64, wherein:

(i) the chemotherapeutic agent, *e.g.*, cytarabine, is to be administered at least 5 days, 10 days, 15 days, or 30 days prior to administration of the immune effector cell, *e.g.*, population of immune effector cells, expressing the CAR molecule; or

(ii) the hematologic cancer associated with expression of CLL-1 is a leukemia, *e.g.*, AML, or a minimal residual disease (MRD) of a hematological cancer.