CLAIMS

1. Use of a cell that expresses a chimeric antigen receptor (CAR) molecule that binds CD19, e.g., a CD19 CAR, in the manufacture of a medicament for use in combination therapy for the treatment of a subject having a disease associated with expression of CD19, and wherein said medicament is to be administered in combination with an effective number of one or more cells that express a CAR molecule that binds CD22, wherein the CD22 CAR molecule comprises a CD22 binding domain comprising:

(i) a light chain complementarity determining region 1 (LC CDR1) of SEQ ID NO: 647, a light chain complementarity determining region 2 (LC CDR2) of SEQ ID NO: 658, and a light chain complementarity determining region 3 (LC CDR3) of SEQ ID NO: 669, and

(ii) a heavy chain complementarity determining region 1 (HC CDR1) of SEQ ID NO: 497 or SEQ ID NO: 1344, a heavy chain complementarity determining region 2 (HC CDR2) of SEQ ID NO:508 or SEQ ID NO: 1353, and a heavy chain complementarity determining region 3 (HC CDR3) of SEQ ID NO: 519 or SEQ ID NO: 1362; or

(iii) a LC CDR1 of SEQ ID NO: 648, a LC CDR2 of SEQ ID NO:659, and a LC CDR3 of SEQ ID NO:670, and

(iv) a HC CDR1 of SEQ ID NO:498 or SEQ ID NO: 1345, a HC CDR2 or SEQ ID NO: 509 or SEQ ID NO: 1354, and a HC CDR3 of SEQ ID NO: 520 or SEQ ID NO: 1363,

wherein the cells comprise T cells.

2. Use of a cell that expresses a chimeric antigen receptor (CAR) molecule that binds CD19, e.g., a CD19 CAR, in combination with a CAR molecule that binds CD22, in the manufacture of a medicament for the treatment of a disease associated with expression of CD19, wherein the CAR molecule that binds CD22 comprises an effective number of one or more cells that express a CAR molecule that binds CD22, wherein the CD22 CAR molecule comprises a CD22 binding domain comprising:

(i) a light chain complementarity determining region 1 (LC CDR1) of SEQ ID NO: 647, a light chain complementarity determining region 2 (LC CDR2) of SEQ ID NO: 658, and a light chain complementarity determining region 3 (LC CDR3) of SEQ ID NO: 669, and

(ii) a heavy chain complementarity determining region 1 (HC CDR1) of SEQ ID NO: 497 or SEQ ID NO: 1344, a heavy chain complementarity determining region 2 (HC CDR2) of SEQ ID NO:508 or SEQ ID NO: 1353, and a heavy chain complementarity determining region 3 (HC CDR3) of SEQ ID NO: 519 or SEQ ID NO: 1362; or

(iii) a LC CDR1 of SEQ ID NO: 648, a LC CDR2 of SEQ ID NO:659, and a LC CDR3 of SEQ ID NO:670, and

(iv) a HC CDR1 of SEQ ID NO:498 or SEQ ID NO: 1345, a HC CDR2 or SEQ ID NO: 509 or SEQ ID NO: 1354, and a HC CDR3 of SEQ ID NO: 520 or SEQ ID NO: 1363,

wherein the cells comprise T cells.

3. The use of claim 2, wherein the CD19 CAR molecule and the CD22 CAR molecule are expressed in the same cell; or the CD19 CAR molecule and the CD22 CAR molecule are expressed in different cells.

4. The use of claims 1-3, wherein the one or more cells that express a CAR molecule that binds CD19 are to be administered concurrently with, before, or after the CAR molecule that binds CD22.

5. The use of any of the preceding claims, wherein the subject has relapsed or is identified as having relapsed after treatment with the one or more cells that express a CAR molecule that binds CD19, e.g., a CD19 CAR.

6. The use of claim 5, wherein the subject has relapsed or is identified as having relapsed based on one or more of:

(i) reappearance of blasts in the blood, bone marrow (e.g., reappearance of at least or more than 5% blasts in the bone marrow), or any extramedullary site, after a complete response;

(ii) detection of CD19- blasts above a predetermined threshold, e.g., over 1%, 2%, 3%, 4%, 5%, or 10%;

(iii) an increase in RNA levels of one or more of MIR199A1, MIR1203, ITM2C, or HLA-DQB1; or

(iv) a decrease in RNA levels of one or more of PPIAL4D, TTTY10, TXLNG2P,

MIR4650-1, KDM5D, USP9Y, PRKY, RPS4Y2, RPS4Y1, NCRNA00185, SULT1E1, and EIF1AY.

7. The use of any of the preceding claims, further comprising lymphodepletion to be performed on that subject, e.g., prior to the administration of the one or more cells that express a CAR molecule that binds CD19, wherein optionally the lymphodepletion comprises one or more of melphalan, cyclophosphamide, and fludarabine to be administered.

8. The use of any of the preceding claims, wherein the CD19 CAR molecule comprises an antibody or antibody fragment which includes a CD19 binding domain, a transmembrane domain, and an intracellular signaling domain comprising a stimulatory domain, and wherein said CD19 binding domain comprises 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 any CD19 scFv or light chain binding domain amino acid sequence listed in **Tables 2 or 3**, and 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 any CD19 scFv or heavy chain binding domain amino acid sequence listed in **Tables 2 or 3**.

9. The use of any of the preceding claims, wherein the CD19 CAR molecule comprises any light chain variable region of a scFv listed in **Tables 2 or 3** and any heavy chain variable region of a scFv listed **Tables 2 or 3**.

10. The use of any of the preceding claims, wherein the CD19 CAR molecule comprises a CD19 binding domain comprising a LC CDR1 of SEQ ID NO: 25, a LC CDR2 of SEQ ID NO: 26 and a LC CDR3 of SEQ ID NO: 27; and a HC CDR1 of SEQ ID NO: 19; a HC CDR2 of SEQ ID NO: 20 and a HC CDR3 of SEQ ID NO: 24.

11. The use of any of claims 1-9, wherein the CD19 CAR molecule comprises a CD19 binding domain comprising a LC CDR1 of SEQ ID NO: 25, a LC CDR2 of SEQ ID NO: 26 and a LC CDR3 of SEQ ID NO: 27; and a HC CDR1 of SEQ ID NO: 19; a HC CDR2 of any of SEQ ID NOs: 21-23 and a HC CDR3 of SEQ ID NO: 24.

12. The use of claim 10 or 11, wherein:

the CD19 CAR molecule comprises a CD19 binding domain which comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO: 59, or an amino acid sequence with at least 95% identity thereto, or

the CD19 CAR comprises a polypeptide selected from the group consisting of SEQ ID NOS: 31-42 or 58, or a sequence with at least 95% identity thereto.

13. The use of any of the preceding claims, wherein the CD22 CAR molecule comprises a CD22 binding domain comprising:

(i) a light chain variable region of SEQ ID NO: 690; or

(ii) a light chain variable region with 95-99% identity to the light chain variable region of SEQ ID NO: 690.

14. The use of any of the preceding claims, wherein the CD22 CAR molecule comprises a CD22 binding domain comprising:

(i) a heavy chain variable region of SEQ ID NO: 680, or

(ii) a heavy chain variable region with 95-99% identity to the heavy chain variable region of SEQ ID NO: 680.

15. The use of any of the preceding claims, wherein the CD22 binding domain comprises a (Gly4-Ser)n linker, wherein n=1, 2, 3 or 4 (SEQ ID NO: 53).

16. The use of any of the preceding claims, wherein the CD22 CAR molecule comprises a CD22 binding domain comprising:

(i) a light chain variable region of SEQ ID NO: 690; and

(ii) a heavy chain variable region of SEQ ID NO: 680.

17. The use of any of the preceding claims, wherein the CD22 CAR molecule comprises the amino acid sequence of SEQ ID NO: 140, or an amino acid sequence with 95-99% identity thereto.

18. The use of any of claims 1-12, wherein the CD22 CAR molecule comprises a CD22 binding domain comprising: a light chain variable region of SEQ ID NO: 689 or an amino acid sequence with 95-99% identity thereto.

19. The use of any of claims 1-12 or 18, wherein the CD22 CAR molecule comprises a CD22 binding domain comprising: a heavy chain variable region of SEQ ID NO: 679, or an amino acid sequence with 95-99% identity thereto.

20. The use of any of the preceding claims, wherein the CD19 CAR molecule or CD22 CAR molecule comprise 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.

21. The use of claim 20, wherein the CD19 CAR molecule or CD22 CAR molecule comprise an antigen binding domain which is connected to the transmembrane domain by a hinge region, wherein optionally, the hinge region comprises the amino acid sequence of SEQ ID NO:14, or an amino acid sequence with 95-99% identity thereto.

22. The use of any of the preceding claims, wherein the CD19 CAR molecule or CD22 CAR molecule comprise a costimulatory domain comprising a functional signaling domain obtained from a protein selected from the group consisting of OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137), wherein optionally, the costimulatory domain comprises the amino acid sequence of SEQ ID NO:16 or SEQ ID NO:51, or an amino acid sequence with 95-99% identity thereto.

23. The use of any of the preceding claims, wherein the CD19 CAR molecule or CD22 CAR molecule comprise an intracellular signaling domain that comprises a functional signaling domain of 4-1BB and/or a functional signaling domain of CD3 zeta; or

wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 16 and/or the amino acid sequence of SEQ ID NO:17 or SEQ ID NO:43, or an amino

acid sequence with 95-99% identity thereto.

24. The use of any of the preceding claims, wherein the CD19 CAR molecule or CD22 CAR molecule further comprises a leader sequence, wherein, optionally, the leader sequence comprises the amino acid sequence of SEQ ID NO: 13, or an amino acid sequence with 95-99% identity thereto.

25. The use of any of the preceding claims, wherein:
(a) the disease associated with CD19 expression is selected from:

(i) a proliferative disease such as a cancer, tumor, or malignancy;

(ii) a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome, or a preleukemia;

(iii) a non-cancer related indication; or

(iv) one or more of a hematologic cancer, acute leukemia, B-cell acute lymphoblastic leukemia (BALL), T-cell acute lymphoblastic leukemia (TALL), small lymphocytic lymphoma (SLL), acute lymphoblastic leukemia (ALL); chronic leukemia, chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), non-Hodgkin lymphoma, or myeloma; or
(b) the disease is a CD19-negative cancer, e.g., a CD19-negative relapsed cancer.

26. The use of any of the preceding claims, wherein the subject is to be administered:

an agent that increases the efficacy of a cell expressing a CAR molecule,

an agent that ameliorates one or more side effects associated with administration of a cell expressing a CAR molecule,

an agent that treats the disease associated with CD19; or

a checkpoint inhibitor.

27. The use of any of the preceding claims, wherein the subject received, has received, or is about to receive a pre-treatment, concurrent treatment, or post-CAR therapy treatment with an agent, e.g., a checkpoint inhibitor, prior to the initiation of a CAR therapy.

28. The use of any of the preceding claims, wherein the subject comprises a CD19-negative

cancer cell, and a cancer cell that is positive for one or more of:

(i) CD22; or

(ii) CD10, CD20, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, or CD79a.

29. The use of any of the preceding claims, wherein the subject is to be administered an inhibitor of one or more of the CD10, CD20, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, or CD79a for which the cancer cell is positive.

30. The use of any of the preceding claims, which further comprises a step of determining whether the subject comprises a CD19-negative cancer cell or whether the subject comprises a cancer cell that is positive for one or more of:

(i) CD22; or

(ii) CD10, CD20, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, or CD79a.

31. A composition comprising, separately or admixed:
(i) one or more cells that express a CAR molecule that binds CD19, e.g., a CD19 CAR, and
(ii) a B-cell inhibitor comprising one or more cells that express a CAR molecule that binds CD22, e.g., a CD22 CAR, wherein the CD22 CAR comprises a CD22 binding domain comprising:

(a) a light chain complementarity determining region 1 (LC CDR1) of SEQ ID NO: 647, a light chain complementarity determining region 2 (LC CDR2) of SEQ ID NO: 658, and a light chain complementarity determining region 3 (LC CDR3) of SEQ ID NO: 669, and

(b) a heavy chain complementarity determining region 1 (HC CDR1) of SEQ ID NO: 497 or SEQ ID NO: 1344, a heavy chain complementarity determining region 2 (HC CDR2) of SEQ ID NO:508 or SEQ ID NO: 1353, and a heavy chain complementarity determining region 3 (HC CDR3) of SEQ ID NO: 519 or SEQ ID NO: 1362; or

(c) a LC CDR1 of SEQ ID NO: 648, a LC CDR2 of SEQ ID NO:659, and a LC CDR3 of SEQ ID NO:670, and

(d) a HC CDR1 of SEQ ID NO:498 or SEQ ID NO: 1345, a HC CDR2 or SEQ ID NO: 509 or SEQ ID NO: 1354, and a HC CDR3 of SEQ ID NO: 520 or SEQ ID NO: 1363,

wherein the cells comprise T cells.

32. A composition comprising:
(i) a first nucleic acid encoding a CAR molecule that binds CD19, e.g., a CD19 CAR, and
(ii) a second nucleic acid encoding a CAR molecule that binds CD22, e.g., a CD22 CAR,
wherein the first nucleic acid and second nucleic acid are in the same or a separate nucleic acid molecule,

wherein the CD22 CAR comprises a CD22 binding domain comprising:

(a) a light chain complementarity determining region 1 (LC CDR1) of SEQ ID NO: 647, a light chain complementarity determining region 2 (LC CDR2) of SEQ ID NO: 658, and a light chain complementarity determining region 3 (LC CDR3) of SEQ ID NO: 669, and

(b) a heavy chain complementarity determining region 1 (HC CDR1) of SEQ ID NO: 497 or SEQ ID NO: 1344, a heavy chain complementarity determining region 2 (HC CDR2) of SEQ ID NO:508 or SEQ ID NO: 1353, and a heavy chain complementarity determining region 3 (HC CDR3) of SEQ ID NO: 519 or SEQ ID NO: 1362; or

(c) a LC CDR1 of SEQ ID NO: 648, a LC CDR2 of SEQ ID NO:659, and a LC CDR3 of SEQ ID NO:670, and

(d) a HC CDR1 of SEQ ID NO:498 or SEQ ID NO: 1345, a HC CDR2 or SEQ ID NO: 509 or SEQ ID NO: 1354, and a HC CDR3 of SEQ ID NO: 520 or SEQ ID NO: 1363.

33. A composition comprising one or more immune effector cells and:
(i) a first nucleic acid encoding, or a first polypeptide comprising a CAR molecule that binds CD19, e.g., a CD19 CAR, and
(ii) a second nucleic acid encoding, or a second polypeptide comprising, a CAR molecule that binds CD22, e.g., a CD22 CAR, wherein the CD22 CAR comprises a CD22 binding domain comprising:

(a) a light chain complementarity determining region 1 (LC CDR1) of SEQ ID NO: 647, a light chain complementarity determining region 2 (LC CDR2) of SEQ ID NO: 658, and a light chain complementarity determining region 3 (LC CDR3) of SEQ ID NO: 669, and

(b) a heavy chain complementarity determining region 1 (HC CDR1) of SEQ ID NO:

497 or SEQ ID NO: 1344, a heavy chain complementarity determining region 2 (HC CDR2) of SEQ ID NO:508 or SEQ ID NO: 1353, and a heavy chain complementarity determining region 3 (HC CDR3) of SEQ ID NO: 519 or SEQ ID NO: 1362; or

(c) a LC CDR1 of SEQ ID NO: 648, a LC CDR2 of SEQ ID NO:659, and a LC CDR3 of SEQ ID NO:670, and

(d) a HC CDR1 of SEQ ID NO:498 or SEQ ID NO: 1345, a HC CDR2 or SEQ ID NO: 509 or SEQ ID NO: 1354, and a HC CDR3 of SEQ ID NO: 520 or SEQ ID NO: 1363.

34. The composition of claim 33, wherein:

(i) the first nucleic acid or first polypeptide and the second nucleic acid or second polypeptide are each contained within, e.g., expressed by, a first immune effector cell;

(ii) the composition comprises a first immune effector cell containing e.g., expressing, the first nucleic acid or first polypeptide and a second immune effector cell containing e.g., expressing, the second nucleic acid or second polypeptide; or

(iii) the cells within the composition either comprise (i) the first nucleic acid encoding, or first polypeptide comprising the CAR molecule that binds CD19 or (ii) the second nucleic acid encoding, or polypeptide comprising the CAR molecule that binds CD22, but not both (i) and (ii).

35. A cell comprising a nucleic acid composition of claim 32, wherein the cell is a human T cell, e.g., a CD8+ T cell, or an NK cell.

36. A cell of claim 35, further expressing an inhibitory molecule that inhibits T cell function, wherein the molecule comprises:

a first polypeptide that 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;

a second polypeptide that 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.

37. A vector comprising a nucleic acid composition of claim 32, optionally wherein, the vector is a viral vector, e.g., a lentiviral vector, or an RNA CAR vector.

38. A method of making a cell, comprising introducing a nucleic acid composition of claim 32 into a T cell or NK cell.

39. A method of making a cell, comprising transducing a T cell or NK cell with a vector of claim 37.

40. The method of claim 38 or 39, wherein the method further comprises:

assaying a gene signature that indicates whether a subject treated with the cell is likely to relapse, or has relapsed;

assaying the gene signature in the cell prior to infusion into the subject; or

decreasing the T_{REG} signature of a population of cells comprising the transduced cell.

41. The method of claim 40, wherein decreasing the T_{REG} signature comprises performing CD25-depletion on the population of cells.

42. The use, composition, or method of any of claims 1-34 or 37-41, further comprising a cell, e.g., a hematopoietic stem cell, or a bone marrow cell, to be transplanted into the subject.

43. An isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antibody or antibody fragment which includes a CD22 binding domain, a transmembrane domain, and an intracellular signaling domain, and wherein said CD22 binding domain comprises:

(a) a light chain complementarity determining region 1 (LC CDR1) of SEQ ID NO: 647, a light chain complementarity determining region 2 (LC CDR2) of SEQ ID NO: 658, and a light chain complementarity determining region 3 (LC CDR3) of SEQ ID NO: 669, and

(b) a heavy chain complementarity determining region 1 (HC CDR1) of SEQ ID NO: 497 or SEQ ID NO: 1344, a heavy chain complementarity determining region 2 (HC CDR2) of

SEQ ID NO:508 or SEQ ID NO: 1353, and a heavy chain complementarity determining region 3 (HC CDR3) of SEQ ID NO: 519 or SEQ ID NO: 1362; or

(c) a LC CDR1 of SEQ ID NO: 648, a LC CDR2 of SEQ ID NO:659, and a LC CDR3 of SEQ ID NO:670, and

(d) a HC CDR1 of SEQ ID NO:498 or SEQ ID NO: 1345, a HC CDR2 or SEQ ID NO: 509 or SEQ ID NO: 1354, and a HC CDR3 of SEQ ID NO: 520 or SEQ ID NO: 1363.

44. An isolated CAR molecule comprising a CD22 binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the CD22 binding domain comprises:

(i) a light chain complementarity determining region 1 (LC CDR1) of SEQ ID NO: 647, a light chain complementarity determining region 2 (LC CDR2) of SEQ ID NO: 658, and a light chain complementarity determining region 3 (LC CDR3) of SEQ ID NO: 669, and

(ii) a heavy chain complementarity determining region 1 (HC CDR1) of SEQ ID NO: 497 or SEQ ID NO: 1344, a heavy chain complementarity determining region 2 (HC CDR2) of SEQ ID NO:508 or SEQ ID NO: 1353, and a heavy chain complementarity determining region 3 (HC CDR3) of SEQ ID NO: 519 or SEQ ID NO: 1362; or

(iii) a LC CDR1 of SEQ ID NO: 648, a LC CDR2 of SEQ ID NO:659, and a LC CDR3 of SEQ ID NO:670, and

(iv) a HC CDR1 of SEQ ID NO:498 or SEQ ID NO: 1345, a HC CDR2 or SEQ ID NO: 509 or SEQ ID NO: 1354, and a HC CDR3 of SEQ ID NO: 520 or SEQ ID NO: 1363.

45. Use of a cell comprising the CAR molecule of claim 44, in the manufacture of a medicament for the treatment of a mammal having a disease associated with expression of CD22, wherein the cell comprises a T cell.

46. A pharmaceutical composition, comprising the cell of claim 35; the nucleic acid of claim 43; or the CAR molecule of claim 44.