

CLAIMS

1. A method for preparing a mixed population of modified cells, the method comprising: contacting cells with (i) a first vector comprising a polynucleotide encoding a first antigen binding molecule that binds a first antigen and (ii) a second vector comprising a polynucleotide encoding a second antigen binding molecule that binds a second antigen to obtain a mixed population of modified cells, wherein the first antigen is different from the second antigen, the first antigen binding molecule binding a cell surface molecule of a B cell, the second antigen binding molecule binding a solid tumor antigen, and wherein the cells are T cells, natural killer (NK) cells, or dendritic cells.

2. The method of claim 1, wherein the cells are T cells.

3. The method of claim 1 or 2, wherein the mixed population of modified cells comprises modified cells comprising the first antigen binding molecule, modified cells comprising the second antigen binding molecule, and modified cells comprising the first and second antigen binding molecules.

4. The method of any one of claims 1-3, wherein the first and second antigen binding molecules are chimeric antigen receptors (CARs).

5. The method of claim 4, wherein the CAR comprises an extracellular domain, a transmembrane domain, and an intracellular domain.

6. The method of claim 5, wherein the intracellular domain comprises a co-stimulatory domain comprising an intracellular domain of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, or a combination thereof.

7. The method of claim 5 or 6, wherein the intracellular domain comprises a CD3 zeta signaling domain.

8. The method of any one of claims 1-7, wherein the first antigen comprises CD19, CD22, CD20, BCMA, CD5, CD7, CD2, CD16, CD56, CD30, CD14, CD68, CD11b, CD18, CD169, CD1c, CD33, CD38, CD138, or CD13.

9. The method of any one of claims 1-8, wherein the first antigen comprises CD19, CD22, CD20, or BCMA.

10. The method of any one of claims 1-9, wherein the first antigen binding molecule comprises amino acid of SEQ ID NO: 5.

11. The method of any one of claims 1-10, wherein the second antigen comprises tMUC1, PRLR, CLCA1, MUC12, GUCY2C, GPR35, CR1L, MUC 17, TMPRSS11B, MUC21, TMPRSS11E, CD207, SLC30A8, CFC1, SLC12A3, SSTR1, GPR27, FZD10, TSHR, SIGLEC15, SLC6A3, KISS1R, QRFPR, GPR119, CLDN6, UPK2, ADAM12, SLC45A3, ACP, MUC21, MUC16, MS4A12, ALPP, CEA, EphA2, FAP, GPC3, IL13-R α 2, Mesothelin, PSMA, ROR1, VEGFR-II, GD2, FR- α , ErbB2, EpCAM, EGFRvIII, CLDN18.2, or EGFR.

12. The method of any one of claims 1-11, wherein the second antigen binding molecule comprises amino acid of SEQ ID NO: 70, 8, or 11.

13. The method of any one of claims 1-12, wherein the mixed population of modified cells comprises a dominant negative variant of a receptor of programmed death 1 (PD-1), cytotoxic T lymphocyte antigen-4 (CTLA- 4), B- and T-lymphocyte attenuator (BTLA), T cell immunoglobulin mucin-3 (TIM-3), lymphocyte-activation protein 3 (LAG-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), leukocyte-associated immunoglobulin-like receptor 1 (LAIR1), natural killer cell receptor 2B4 (2B4), or CD 160.

14. The method of any one of claims 1-13, wherein the first binding molecule is a CAR, and the second binding molecule is a modified TCR.

15. The method of any one of claims 1-14, wherein the mixed population of modified cells comprises a polynucleotide encoding a therapeutic agent comprising IL12, IL6, and/or INF γ .

16. The method of any one of claims 1-15, wherein the first and second vectors comprise lentiviral vectors.

17. A composition comprising a mixed population of modified cells, wherein the mixed population of modified cells comprises (i) modified cells comprising a first vector comprising a polynucleotide encoding a first antigenbinding molecule that binds a first antigen; (ii) modified cells comprising a second vector comprising a polynucleotide encoding a second antigen binding molecule that binds a second antigen; (iii) modified cells comprising the first and second vector; wherein the first antigen binding molecule binds a cell surface molecule of a B cell and the second antigen binding molecule binds a solid tumor antigen, and wherein the modified cells are T cells, natural killer (NK) cells, or dendritic cells.

18. The composition of claim 17, wherein the composition comprises a mixed population of modified cells, wherein the mixed population of modified cells comprises modified cells comprising the first antigen binding molecule, modified cells comprising the second antigen binding molecule binding, and modified cells comprising the first and second antigen binding molecules.

19. The composition of claim 17 or 18, wherein the first and second antigen binding molecules comprise CARs.

20. The composition of claim 19, wherein the CARs comprise an extracellular domain, a transmembrane domain, and an intracellular domain.

21. The composition of claim 20, wherein the intracellular domain comprises a co-stimulatory domain comprising an intracellular domain of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, or a combination thereof.

22. The composition of claim 20 or 21, wherein the intracellular domain comprises a CD3 zeta signaling domain.

23. The composition of any one of claims 17-22, wherein the first antigen comprises CD19, CD22, CD20, BCMA, CD5, CD7, CD2, CD16, CD56, CD30, CD14, CD68, CD11b, CD18, CD169, CD1c, CD33, CD38, CD138, or CD13.

24. The composition of any one of claim 17-23, wherein the first antigen comprises CD19, CD20, CD22, or BCMA.

25. The composition of any one of claims 17-24, wherein the first antigen binding molecule comprises amino acid of SEQ ID NO: 5.

26. The composition of any one of claims 17-25, wherein the second antigen comprises tMUC1, PRLR, CLCA1, MUC12, GUCY2C, GPR35, CR1L, MUC 17, TMPRSS11B, MUC21, TMPRSS11E, CD207, SLC30A8, CFC1, SLC12A3, SSTR1, GPR27, FZD10, TSHR, SIGLEC15, SLC6A3, KISS1R, QRFPR, GPR119, CLDN6, UPK2, ADAM12, SLC45A3, ACPP, MUC21, MUC16, MS4A12, ALPP, CEA, EphA2, FAP, GPC3, IL13-R α 2, Mesothelin, PSMA, ROR1, VEGFR-II, GD2, FR- α , ErbB2, EpCAM, EGFRvIII, CLDN18.2 or EGFR.

27. The composition of any one of claims 17-26, wherein the second antigen binding molecule comprises amino acid of SEQ ID NO: 70, 8, or 11.

28. The composition of any one of claims 17-27, wherein the mixed population of modified cells comprises a dominant negative variant of a receptor of programmed death 1 (PD-1), cytotoxic T lymphocyte antigen-4 (CTLA- 4), B- and T-lymphocyte attenuator (BTLA), T cell immunoglobulin mucin-3 (TIM-3), lymphocyte-activation protein 3 (LAG-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), leukocyte-associated immunoglobulin-like receptor 1 (LAIR1), natural killer cell receptor 2B4 (2B4), or CD 160.

29. The composition of any one of claims 17-28, wherein the first binding molecule is a CAR, and the second binding molecule is a modified TCR.

30. The composition of any one of claims 17-29, wherein the mixed population of modified cells comprises a polynucleotide encoding a therapeutic agent comprising IL2, IL6 and/or INF γ .

31. The composition of any one of claims 17-30, wherein the first and second vectors comprise lentiviral vectors.

32. The composition of any one of claims 17-31, wherein the modified cells are T cells.

33. The composition of any one of claims 17-32, wherein the composition is a pharmaceutical composition.

34. The composition of any one of claims 17-33, wherein the composition further comprises a carrier or a pharmaceutically acceptable carrier.

35. Use of an effective amount of the composition of any one of claims 17-34 in the manufacture of a medicament for enhancing expansion of cells in a subject.