

What is claimed is:

1. A method of making a population of immune effector cells (e.g., T cells, NK cells) that is depleted of T regulatory cells and can be engineered to express a CAR, the method comprising:

providing a population of immune effector cells (e.g., T cells), and

removing T regulatory cells, e.g., CD25+ T cells, from the population, to thereby provide a population of T regulatory-depleted cells, e.g., CD25+ depleted cells, that are suitable for expression of a CAR.

2. The method of claim 1, wherein the population of T regulatory-depleted cells contains less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells.

3. The method of claim 1, wherein the population of immune effector cells are cells of a subject having cancer, e.g., a subject having a CD25 expressing cancer such as, e.g., chronic lymphocytic leukemia (CLL).

4. The method of claim 3, wherein the population of T regulatory-depleted cells contains less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of tumor cells.

5. The method of claim 1, wherein the T regulatory cells, e.g., CD25+ T cells, are removed from the population using an anti-CD25 antibody, or fragment thereof.

6. The method of claim 5, wherein the anti-CD25 antibody, or fragment thereof, is conjugated to a substrate, e.g., a bead.

7. The method of claim 3, wherein the population of immune effector cells are obtained from a subject having a hematological cancer, e.g., a leukemia, e.g., chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), or a lymphoma, e.g., mantle cell lymphoma (MCL).

8. The method of claim 7, wherein the population of T regulatory-depleted cells contains less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of the leukemia cells, e.g., CLL cells, ALL cells, or lymphoma cells, e.g., MCL cells, HL cells, e.g., wherein the

population of T regulatory-depleted cells contains less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of tumor cells, e.g., CLL cells, e.g., wherein the population of T regulatory-depleted cells contains less than 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 10%, 5%, 4%, 3%, 2%, 1% of tumor cells, e.g., CLL cells.

9. The method of claim 1, the method further comprising:

removing cells from the population which express a tumor antigen, e.g., a tumor antigen that does not comprise CD25, e.g., CD19, CD30, CD38, CD123, CD20, CD14 or CD11b, to thereby provide a population of T regulatory-depleted, e.g., CD25+ depleted, and tumor antigen depleted cells that are suitable for expression of a CAR, e.g., a CAR described herein.

10. The method of claim 1, the method further comprising removing cells from the population which express a check point inhibitor, e.g., a check point inhibitor described herein, e.g., one or more of PD1+ cells, LAG3+ cells, and TIM3+ cells, to thereby provide a population of T regulatory-depleted, e.g., CD25+ depleted cells, and check point inhibitor depleted cells, e.g., PD1+, LAG3+ and/or TIM3+ depleted cells.

11. The method of claim 1, wherein the population of immune effector cells provided has been selected based upon the expression of one or more markers, e.g., CD3, CD28, CD4, CD8, CD45RA, and CD45RO, e.g., the provided population of immune effector cells (e.g., T cells) are CD3+ and/or CD28+.

12. The method of any of the preceding claims, further comprising: activating the population of T regulatory-depleted cells, e.g., CD25+ depleted cells, e.g., by a method described herein, and/or transducing a cell from the population of T regulatory-depleted cells, e.g., the population of CD25+ depleted cells, with a vector comprising a nucleic acid encoding a CAR, e.g., a CAR described herein, e.g., a CD19 CAR described herein.

13. The method of claim 12, further comprising expanding the population of T regulatory-depleted cells, e.g., engineered to express a CAR, a CAR described herein, e.g., a CD19 CAR described herein, e.g., by a method described herein.

14. The method of claim 13, wherein the population of cells is expanded for a period of 8 days or less, e.g., 7, 6, 5, 4, or 3 days, or wherein the population of cells is expanded

in culture for 5 days, and the resulting cells are more potent than the same cells expanded in culture for 9 days under the same culture conditions, e.g., wherein the cells expanded for 5 days show at least a one, two, three or four fold increase in cells doublings upon antigen stimulation as compared to the same cells expanded in culture for 9 days under the same culture conditions, or wherein the cells are expanded in culture for 5 days, and the resulting cells exhibit higher proinflammatory cytokine production, e.g., IFN- γ and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions.

15. The method of claim 13, wherein the population of cells is expanded by culturing the cells in the presence of an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a costimulatory molecule on the surface of the cells, e.g., as described herein. In one embodiment, the agent is a bead conjugated with anti-CD3 antibody, or a fragment thereof, and/or anti-CD28 antibody, or a fragment thereof.

16. The method of any of claims 13-15, wherein the population of cells is expanded in an appropriate media (e.g., media described herein) that includes one or more interleukin that result in at least a 200-fold (e.g., 200-fold, 250-fold, 300-fold, 350-fold) increase in cells over a 14 day expansion period, e.g., as measured by a method described herein such as flow cytometry, e.g., wherein the population of cells is expanded in the presence IL-15 and/or IL-7 (e.g., IL-15 and IL-7).

17. The method of any of claims 13-16, wherein the population of the cells is cryopreserved after the appropriate expansion period.

18. The method of any of the preceding claims further comprising contacting the population of immune effector cells with a nucleic acid encoding a telomerase subunit, e.g., hTERT, and wherein the nucleic acid is, e.g., DNA or RNA.

19. A reaction mixture comprising a population of T regulatory-depleted cells containing less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells.

20. A reaction mixture comprising a population of T regulatory-depleted cells containing less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25 expressing tumor cells, e.g., CLL cells.

21. The reaction mixture of claim 20, wherein the population of cells contains less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of tumor cells, e.g., CD25 expressing tumor cells, e.g., CLL cells, or wherein the population of cells contains less than 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 10%, 5%, 4%, 3%, 2%, 1% of tumor cells, e.g., CD25 expressing tumor cells, e.g., CLL cells.

22. The reaction mixture of any of claims 20-21, wherein the reaction mixture comprises a population of T regulatory-depleted cells containing less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of a checkpoint inhibitor expressing cells, e.g., a PD1+ cells, LAG3+ cells, or TIM3+ cells.

23. The reaction mixture of any of claims 19-22, wherein the reaction mixture further comprises an agent that activates and/or expands to cells of the population, e.g., an agent that stimulates a CD3/TCR complex associated signal and/or a ligand that stimulates a costimulatory molecule on the surface of the cells, e.g., wherein the agent is a bead conjugated with anti-CD3 antibody, or a fragment thereof, and/or anti-CD28 antibody, or a fragment thereof.

24. The reaction mixture of any of claims 19-23, further comprising one or more factor for proliferation and/or viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN- γ , IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGF β , and TNF- α or any other additives for the growth of cells, e.g., wherein the reaction mixture further comprises IL-15 and/or IL-7.

25. The reaction mixture of any of claims 19-24, wherein a plurality of the cells of the population in the reaction mixture comprise a nucleic acid molecule, e.g., a nucleic acid molecule described herein, that comprises a CAR encoding sequence, e.g., a CD19 CAR encoding sequence, e.g., as described herein, e.g., wherein a plurality of the cells of the population in the reaction mixture comprise a vector comprising a nucleic acid sequence encoding a CAR, e.g., a CAR described herein, e.g., a CD19 CAR described herein, e.g., wherein the vector is a vector described herein, e.g., a vector selected from the group consisting of a DNA, a RNA, a plasmid, a lentivirus vector, adenoviral vector, or a retrovirus vector.

26. The reaction mixture of any of claims 19-24, further comprising a cryoprotectant or stabilizer such as, e.g., a saccharide, an oligosaccharide, a polysaccharide and a polyol (e.g., trehalose, mannitol, sorbitol, lactose, sucrose, glucose and dextran), salts and crown ethers. In one embodiment, the cryoprotectant is dextran.

27. A method of making a population of immune effector cells (e.g., T cells, NK cells) engineered to express a CAR, the method comprising:

providing a population of immune effector cells (e.g., T cells), wherein a plurality of the immune effector cells comprise a nucleic acid encoding a CAR, e.g., a CAR described herein, e.g., a CD19 CAR described herein, and

expanding the cells of the population in the presence of one or more interleukin that result in at least a 200-fold (e.g., 200-fold, 250-fold, 300-fold, 350-fold) increase in cells over a 14 day expansion period, e.g., as measured by a method described herein such as flow cytometry.

28. The method of claim 27, wherein the population of cells is expanded in the presence of IL-15 and/or IL-7, e.g., IL-15 and IL-7.

29. The method of claim 27 or 28, wherein the population of cells is expanded for a period of less than 8 days, e.g., 7, 6, 5, 4 or 3 days.

30. The method of claim 27 or 28, wherein the cells are expanded in culture for 5 days, and the resulting cells are more potent than the same cells expanded in culture for 9 days under the same culture conditions.

31. The method of claim 30, wherein the cells expanded for 5 days show at least a one, two, three or four fold increase in cells doublings upon antigen stimulation as compared to the same cells expanded in culture for 9 days under the same culture conditions, or wherein the cells are expanded in culture for 5 days, and the resulting cells exhibit higher proinflammatory cytokine production, e.g., IFN- γ and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions.

32. The method of any of claims 27-31, wherein the population of cells is expanded by culturing the cells in the presence of an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a costimulatory molecule on the surface of the

cells, e.g., as described herein, e.g., wherein the agent is a bead conjugated with anti-CD3 antibody, or a fragment thereof, and/or anti-CD28 antibody, or a fragment thereof.

33. The method of any of claims 27-32, wherein the provided population of immune effector cells is a population of T regulatory-depleted cells containing less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells, or wherein the provided population of immune effector cells is a population of T regulatory-depleted cells containing less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25 expressing tumor cells, e.g., CLL cells.

34. The method of claim 33, wherein the provided population of cells contains less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of tumor cells, e.g., CD25 expressing tumor cells, e.g., CLL cells, or wherein the provided population of cells contains less than 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 10%, 5%, 4%, 3%, 2%, 1% of tumor cells, e.g., CD25 expressing tumor cells, e.g., CLL cells.

35. The method of any of claims 50-53, wherein the provided population of immune effector cells also contains less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of a checkpoint inhibitor expressing cells, e.g., a PD1+ cells, LAG3+ cells, or TIM3+ cells.

36. The method of any claims 33-35, further comprising contacting the population of immune effector cells with a nucleic acid encoding a telomerase subunit, e.g., hTERT, and wherein the nucleic acid is, e.g., DNA or RNA.

37. A reaction mixture comprising a population of immune effector cells, wherein a plurality of the cells of the population in the reaction mixture comprise a nucleic acid molecule, e.g., a nucleic acid molecule described herein, that comprises a CAR encoding sequence, e.g., a CD19 CAR encoding sequence, e.g., as described herein, and IL-7 and/or IL-15.

38. A reaction mixture comprising a population of immune effector cells, wherein a plurality of the cells of the population in the reaction mixture comprise a vector comprising a nucleic acid sequence encoding a CAR, e.g., a CAR described herein, e.g., a CD19 CAR described herein, and IL-7 and/or IL-15, and optionally wherein the vector is a vector described

herein, e.g., a vector selected from the group consisting of a DNA, a RNA, a plasmid, a lentivirus vector, adenoviral vector, or a retrovirus vector.

39. The reaction mixture of claim 37 or 38, further comprising an agent that activates and/or expands to cells of the population, e.g., an agent that stimulates a CD3/TCR complex associated signal and/or a ligand that stimulates a costimulatory molecule on the surface of the cells, e.g., wherein the agent is a bead conjugated with anti-CD3 antibody, or a fragment thereof, and/or anti-CD28 antibody, or a fragment thereof.

40. A method of making a population of immune effector cells (e.g., T cells, NK cells), the method comprising:

providing a population of immune effector cells (e.g., T cells), and

contacting the population of immune effector cells with a nucleic acid encoding a CAR and a RNA encoding a telomerase subunit, e.g., hTERT, under conditions suitable for expression of the CAR and the telomerase subunit.

41. The method of claim 40, wherein the nucleic acid encoding the CAR and the RNA encoding the telomerase subunit are part of the same nucleic acid molecule, or wherein the nucleic acid encoding the CAR and the RNA encoding the telomerase subunit are part of separate nucleic acid molecules.

42. The method of claim 40, which comprises contacting the population of immune effector cells with a nucleic acid encoding the CAR and the RNA encoding the telomerase subunit at substantially the same time, or which comprises contacting the population of immune effector cells with a nucleic acid encoding the CAR before contacting the population of immune effector cells with the RNA encoding the telomerase subunit, or which comprises contacting the population of immune effector cells with a nucleic acid encoding the CAR after contacting the population of immune effector cells with the RNA encoding the telomerase subunit.

43. The method of claim 40, wherein the RNA encoding the telomerase subunit is mRNA, or wherein the RNA encoding the telomerase subunit comprises a poly(A) tail or 5' cap structure.

44. The method of claim 40, which comprises transfecting, transducing, or electroporating the immune effector cells with the RNA encoding the telomerase subunit.

45. An immune effector cell (e.g., T cell or NK cell) comprising:
a nucleic acid encoding a CAR; and
an RNA (e.g., mRNA) encoding an exogenous telomerase subunit, e.g., hTERT.

46. An immune effector cell (e.g., T cell or NK cell) comprising:
a CAR; and
an exogenous telomerase subunit, e.g., hTERT,
wherein the cell does not comprise DNA encoding the exogenous telomerase
subunit.