

CLAIMS

1. A method of making a population of immune effector cells, e.g., T cells, NK cells, expressing a CAR, which population of immune effector cells is depleted of CD25+ T regulatory cells and CD25-expressing chronic lymphocytic leukemia (CLL) cells, the method comprising:
 - (i) providing a population of immune effector cells, e.g., T cells, from a subject having CLL, and
 - (ii) removing CD25+ T regulatory cells and CD25-expressing CLL cells from the population of immune effector cells,to thereby provide a population of immune effector cells that contains less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ T regulatory cells and less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25-expressing CLL cells.
2. A method of making a population of immune effector cells e.g., T cells, NK cells, expressing a CAR, which population of immune effector cells is depleted of CD25+T regulatory cells and CD25-expressing chronic lymphocytic leukemia (CLL) cells, comprising:
 - providing a population of immune effector cells from a subject having CLL, and
 - contacting the population of immune effector cells with an anti-CD25 antibody, or a CD25 binding ligandto thereby provide a population of immune effector cells which:
 - (a) has an increased lentiviral transduction efficiency compared to an anti-CD3 selected population of immune effector cells; andcontains less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ T regulatory cells and less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25-expressing CLL cells.
3. A method of making a population of immune effector cells, e.g., T cells, NK cells, expressing a CAR, which population of immune effector cells is depleted of CD25+T regulatory cells and CD25-expressing chronic lymphocytic leukemia (CLL) cells, the method comprising:
 - (i) providing a population of immune effector cells, e.g., T cells, from a subject having CLL;
 - (ii) removing CD25+ T regulatory cells and CD25-expressing CLL cells from the population of immune effector cells, to provide a population of immune effector cells that

contains less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ T regulatory cells and less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25-expressing CLL cells; and

(iii) transducing the population of immune effector cells depleted of CD25+ T regulatory cells and CD25-expressing CLL cells with a nucleic acid encoding a CAR, e.g., a CD19 CAR.

4. The method of claim 1 or claim 2, wherein the method comprises transducing the population of immune effector cells depleted of CD25+ T regulatory cells and CD25-expressing CLL cells with a nucleic acid encoding a CAR, e.g., a CD19 CAR.

5. The method of any one of claims 1-4, wherein the population of immune effector cells depleted of CD25+ T regulatory cells and CD25-expressing CLL cells contains less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ T regulatory cells.

6. The method of any one of claims 1 or 3-5, wherein CD25+ T regulatory cells and CD25-expressing CLL cells, are removed from the population using an anti-CD25 antibody, or fragment thereof, or a CD25 binding ligand.

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

8. The method of any of the preceding claims, wherein the population of immune effector cells depleted of CD25+ T regulatory cells and CD25-expressing CLL cells contains:

(i) less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25-expressing CLL cells;

(ii) less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ T regulatory cells and less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25-expressing CLL cells; or

(iii) less than 10%, 5%, 4%, 3%, 2%, 1% of CD25+ T regulatory cells and less than 10%, 5%, 4%, 3%, 2%, 1% of CD25-expressing CLL cells.

9. The method of claim 1 or claim 3, the method further comprising:

(a) removing cells from the population which express a tumor antigen, e.g., a tumor antigen other than CD25, e.g., CD19, CD30, CD38, CD123, CD20, CD14 or CD11b, to thereby provide a population of CD25+ T regulatory-depleted, and tumor antigen depleted cells; or

(b) removing cells from the population which express a check point inhibitor, e.g., one or more of PD1+ cells, LAG3+ cells, and TIM3+ cells, to thereby provide a population of immune effector cells depleted of CD25+ T regulatory cells that is also depleted of check point inhibitor cells, e.g., PD1+, LAG3+, and/or TIM3+ depleted cells.

10. The method of any of claims 1-9, wherein the population of immune effector cells depleted of CD25+ T regulatory cells and CD25-expressing CLL cells 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+.

11. The method of any of the preceding claims, further comprising:

(a) activating the population of immune effector cells depleted of CD25+ T regulatory cells and CD25-expressing CLL cells; and/or

(b) expanding the population of immune effector cells depleted of CD25+ T regulatory cells and CD25-expressing CLL cells, e.g., expressing a CD19 CAR.

12. The method of claim 11, wherein the population of immune effector cells depleted of CD25+ T regulatory cells and CD25-expressing CLL cells is expanded:

(i) for a period of 8 days or less, e.g., 7, 6, 5, 4, or 3 days,

(ii) for a period of 3 days or less than 3 days;

(iii) 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;

(iv) 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; or

(v) by culturing the cells in the presence of an agent that stimulates a CD3/TCR complex associated signal, e.g., a bead conjugated with anti-CD3 antibody, or a fragment thereof,

and a ligand that stimulates a costimulatory molecule on the surface of the cells, e.g., an anti-CD28 antibody, or a fragment thereof.

13. The method of claim 11 or 12, wherein the population of immune effector cells depleted of CD25+ T-regulatory cells and CD25-expressing CLL cells is:

(i) expanded in an appropriate media that includes one or more interleukin that results 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 flow cytometry,

(ii) expanded in the presence of any one of IL-15, IL-7, or IL-15 receptor alpha, or a combination thereof; and/or

(iii) cryopreserved after the appropriate expansion period.

14. The method of claim 13, wherein the population of immune effector cells depleted of CD25+ T-regulatory cells and CD25-expressing CLL cells expanded in the presence of IL-15 and IL-7 exhibit an increased cell proliferation capacity compared to the population expanded without removing T-regulatory cells, or compared to the population expanded in the presence of IL-15 alone or IL-17 alone.

15. The method of claim 1 or claim 2, wherein the population of immune effector cells depleted of CD25+ T regulatory cells and CD25-expressing CLL cells has an increased lentiviral transduction efficiency compared to an anti-CD3 selected population of immune effector cells.

16. The method of the preceding claims, wherein the CAR is CD19 CAR, e.g., a CD19 CAR that specifically binds to CD19.

17. The method of claim 16, wherein the CD19 CAR comprises an antigen binding domain comprising a light chain variable domain (VL) and a heavy chain variable domain (VH) having the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 51.

18. A reaction mixture comprising a population of CD25+ T regulatory-cell depleted and CD25-expressing chronic lymphocytic leukemia (CLL) cell-depleted immune effector cells, e.g., T cells, or NK cells from a subject having CLL, which:

(a) expresses a CAR; and

(b) contains less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ T regulatory cells and less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25-expressing CLL cells.

19. The reaction mixture of claim 18, wherein the population of immune effector cells depleted of CD25+ T regulatory cells and CD25-expressing CLL cells contains:

(a) less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ T regulatory cells and less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25-expressing CLL cells, or

(b) less than 10%, 5%, 4%, 3%, 2%, 1% of CD25+ T regulatory cells and less than 10%, 5%, 4%, 3%, 2%, 1% of CD25-expressing CLL cells.

20. The reaction mixture of claim 18, wherein the reaction mixture further comprises less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ T regulatory 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.

21. The reaction mixture of any of claims 18-20, wherein:

(a) 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, e.g., a bead conjugated with anti-CD3 antibody, or a fragment thereof, and/or a ligand that stimulates a costimulatory molecule on the surface of the cells, e.g., an anti-CD28 antibody, or a fragment thereof; or

(b) the reaction mixture further comprises 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, IL-7, or both IL-15 and IL-7.

22. The reaction mixture of any of claims 18-21, wherein a plurality of cells of the population in the reaction mixture comprises a nucleic acid molecule that comprises a CAR encoding sequence, e.g., a CD19 CAR encoding sequence.

23. The reaction mixture of any of claims 18-22, wherein a plurality of cells of the population in the reaction mixture comprises a vector comprising a nucleic acid sequence encoding a CAR, e.g., a CD19 CAR, wherein the vector is chosen from a DNA, a RNA, a plasmid, a lentivirus vector, adenoviral vector, or a retrovirus vector.

24. The reaction mixture of claim 22 or claim 23, wherein the CAR encoding sequence comprises an antigen binding domain comprising a light chain variable domain (VL) and a heavy chain variable domain (VH) having the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 51.
25. The reaction mixture of any of claims 18-24, further comprising a cryoprotectant or stabilizer, 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, optionally wherein the cryoprotectant is dextran.
26. A population of immune effector cells depleted of CD25⁺ T regulatory cells and CD25⁻ expressing CLL cells made according to the method of any of claims 1-25.