

CLAIMS

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, from an apheresis sample,

and

removing T regulatory cells, e.g., CD25+ T cells, from the population of immune effector cells,

to thereby provide a population of T regulatory-depleted cells, e.g., CD25+ depleted cells, that are suitable for expression of a CAR, wherein the population of T-regulatory depleted cells contains less than 50% of CD25+ cells and less than 50% of tumor cells compared to the population of immune effector cells from the apheresis sample.
2. A method of making a population of cells comprising immune effector cells, comprising:

providing a population of cells comprising immune effector cells from an apheresis sample from a human subject having cancer, and

contacting the population of cells with an anti-CD25 antibody, or a CD25 binding ligand to thereby provide a population of CD25-depleted cells, wherein the population of CD25-depleted cells is:
 - (a) suitable for expression of a CAR;
 - (b) has an increased lentiviral transduction efficiency compared to an anti-CD3 selected population of immune effector cells; and
 - (c) contains less than 50% of CD25+ T regulatory cells and less than 50% of CD25+ tumor cells compared to the population of immune effector cells from the apheresis sample.
3. A method of making a population of cells comprising immune effector cells, comprising:

providing a population of cells comprising immune effector cells from an apheresis sample from a human subject having cancer; and contacting the populations of cells with an anti-CD25 antibody or a CD25 binding ligand, thereby providing a population of CD25-depleted cells,

contacting the population of CD25-depleted cells with an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a costimulatory molecule on the surface of the cells; and

culturing the population of CD25-depleted cells in the presence of IL-15 alone, or both IL-15 and IL-7, wherein the cultured cells have an increased proliferation capacity compared to an otherwise similar population of cells which has not been depleted of CD25 cells, or is cultured in the presence of IL-7 alone,

wherein the population of CD25-depleted cells contains less than 50% of CD25+ T regulatory cells and less than 50% of CD25+ tumor cells compared to the population of immune effector cells from the apheresis sample.

4. The method of any one of claims 1-3, 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).
5. The method of any one of claims 1-4, wherein the population of T regulatory-depleted cells contains less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells compared to the population of immune effector cells from the apheresis sample.
6. The method of any one of claims 1-5, 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 compared to the population of immune effector cells from the apheresis sample.
7. The method of any one of claims 1 or 4-6, wherein the T regulatory cells, e.g., CD25+ T cells, are removed from the population using an anti-CD25 antibody, or fragment thereof.
8. The method of claim 7, wherein the anti-CD25 antibody, or fragment thereof, is conjugated to a substrate, e.g., a bead.
9. The method of any of one claims 1-8, 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).

10. The method of claim 9, wherein the population of T regulatory-depleted cells contains:
- (i) less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of the leukemia cells;
 - (ii) less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 15%, 10%, 5%, 4%, 3%, 2%, 1% of leukemia or lymphoma cells; or
 - (iii) less than 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells and less than 10%, 5%, 4%, 3%, 2%, 1% of leukemia or lymphoma cells
- compared to the population of immune effector cells from the apheresis sample.
11. The method of claim 1, the method further comprising:
- (a) 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; 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 T regulatory-depleted, e.g., CD25+ depleted cells, and check point inhibitor depleted cells, e.g., PD1+, LAG3+ and/or TIM3+ depleted cells.
12. The method of any of claims 1-11, 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+.
13. The method of any of the preceding claims, further comprising:
- (a) activating the population of T regulatory-depleted cells, e.g., CD25+ depleted cells, 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 CD19 CAR; and/or
 - (b) expanding the population of T regulatory-depleted cells, e.g., engineered to express a CAR, e.g., a CD19 CAR described herein.

14. The method of claim 13, wherein the population of 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.
15. The method of claim 13 or 14, wherein the population of 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.
16. The method of claim 15, wherein the population of T-regulatory depleted 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.
17. The method of claim 1, wherein the population of T regulatory depleted cells has an increased lentiviral transduction efficiency compared to an anti-CD3 selected population of immune effector cells.

18. A reaction mixture comprising immune effector cells from an apheresis sample comprising a population of T regulatory-depleted cells, e.g., T cells or NK cells, that can be engineered with a CAR, wherein the population of T regulatory-depleted cells contains less than 50% of CD25+ cells and less than 50% of tumor cells compared to the population of immune effector cells from the apheresis sample.

19. The reaction mixture of claim 18, 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 compared to the population of immune effector cells from the apheresis sample.

20. The reaction mixture of claim 18 or 19, wherein the population of T regulatory-depleted cells contains:

(i) 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;

(ii) 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, and optionally wherein the population of cells contains:

(a) 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

(b) 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,

compared to the population of immune effector cells from the apheresis sample.

21. The reaction mixture of claim 18, 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.

22. The reaction mixture of any of claims 18-21, 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.
23. The reaction mixture of any of claims 18-22, 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.
24. The reaction mixture of any of claims 18-23, 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 a vector selected from the group consisting of a DNA, a RNA, a plasmid, a lentivirus vector, adenoviral vector, or a retrovirus vector.
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 made according to the method of any of claims 1-25.