

## **CLAIMS**

1. A method of optimizing acquisition of a population of immune effector cells suitable for engineering to express a chimeric antigen receptor (“CAR”) for use in a CAR therapy, comprising acquiring (e.g., obtaining or harvesting) the population of immune effector cells from a pediatric subject having a leukemia, according to two, three, or all of the following:

(i) the timing of immune effector cell acquisition from the subject;

(ii) the timing of the chemotherapy in relation to acquisition of the population of immune effector cells;

(iii) the type of chemotherapy;

(iv) the underlying malignancy;

wherein the acquired population of immune effector cells is suitable for use in a CAR therapy and

(a) includes at least 20% naïve T cells, at least 2% stem central memory T cells, and/or at least 4% central memory T cells;

(b) includes at least 50% central memory T cells;

(c) includes less than 55% effector memory and terminal effector T cells combined;

(d) shows an increased absolute T cell count (ATC), compared to a reference value (e.g., a sample from the subject at a later time point or after exposure to additional rounds of chemotherapy);

(e) comprises an absolute T cell count of at least 400 cells/microliter, an absolute naïve T cell count of at least 200 cells/microliter, an absolute stem central memory T cell count of at least 20 cells/microliter, and/or an absolute central memory T cell count of at least 40 cells/microliter; and/or

(f) is selected based upon the expression of one or more markers, e.g., CCR7, CD62L, CD45RO, and CD95, e.g., the population of immune effector cells (e.g., T cells) are CCR7+ and CD62L+,

wherein in any of (a)-(f) the naïve T cells are identified based upon an expression pattern of CCR7+, CD62L+, CD45RO–, CD95–, wherein the stem central memory T cells are identified based upon an expression pattern of CCR7+, CD62L+, CD45RO–, CD95+, and wherein the central memory T cells are identified based upon an expression pattern of CCR7+, CD62L+, CD45RO+, CD95.

2. A combination of a population of immune effector cells that express a CAR molecule and a chemotherapy for use in a combination therapy for treating, or in providing anti-tumor immunity to, a pediatric subject having a leukemia, wherein the population of immune effector cells are acquired (e.g., obtained or harvested) from the subject prior to introduction of the CAR molecule, by optimizing two, three, or all of the following:

(i) the timing of immune effector cell acquisition from the subject;

(ii) the timing of the chemotherapy in relation to acquisition of the population of immune effector cells;

(iii) the type of chemotherapy;

(iv) the underlying malignancy;

wherein the acquired population of immune effector cells

(a) includes at least 20% naïve T cells, at least 2% stem central memory T cells, and/or at least 4% central memory T cells;

(b) includes at least 50% central memory T cells;

(c) includes less than 55% effector memory and terminal effector T cells combined;

(d) shows an increased absolute T cell count (ATC), compared to a reference value (e.g., a sample from the subject at a later time point or after exposure to additional rounds of chemotherapy);

(e) comprises an absolute T cell count of at least 400 cells/microliter, an absolute naïve T cell count of at least 200 cells/microliter, an absolute stem central memory T cell count of at least 20 cells/microliter, and/or an absolute central memory T cell count of at least 40 cells/microliter; and/or

(f) is selected based upon the expression of one or more markers, e.g., CCR7, CD62L, CD45RO, and CD95, e.g., the population of immune effector cells (e.g., T cells) are CCR7+ and CD62L+,

wherein in any of (a)-(f) the naïve T cells are identified based upon an expression pattern of CCR7+, CD62L+, CD45RO-, CD95-, wherein the stem central memory T cells are identified based upon an expression pattern of CCR7+, CD62L+, CD45RO-, CD95+, and wherein the central memory T cells are identified based upon an expression pattern of CCR7+, CD62L+, CD45RO+, CD95+.

3. Use of a population of immune effector cells that express a CAR molecule in the manufacture of a medicament for the treatment of, or providing anti-tumor immunity to, a pediatric subject having a leukemia, wherein the subject is to be administered an effective amount of the population of immune effector cells that expresses a CAR molecule simultaneously, separately, or sequentially with a chemotherapy, wherein the population of immune effector cells are acquired (e.g., obtained or harvested) from the subject, by optimizing two, three, or all of the following:

(i) the timing of immune effector cell acquisition from the subject;

(ii) the timing of the chemotherapy in relation to the acquisition of the population of immune effector cells;

(iii) the type of chemotherapy;

(iv) the underlying malignancy;

wherein the population of immune effector cells is acquired from the subject prior to introduction of the CAR molecule; and

wherein the acquired population of immune effector cells

(a) includes at least 20% naïve T cells, at least 2% stem central memory T cells, and/or at least 4% central memory T cells;

(b) includes at least 50% central memory T cells;

(c) includes less than 55% effector memory and terminal effector T cells combined;

(d) shows an increased absolute T cell count (ATC), compared to a reference value (e.g., a sample from the subject at a later time point or after exposure to additional rounds of chemotherapy);

(e) comprises an absolute T cell count of at least 400 cells/microliter, an absolute naïve T cell count of at least 200 cells/microliter, an absolute stem central memory T cell count of at least 20 cells/microliter, and/or an absolute central memory T cell count of at least 40 cells/microliter; and/or

(f) is selected based upon the expression of one or more markers, e.g., CCR7, CD62L, CD45RO, and CD95, e.g., the population of immune effector cells (e.g., T cells) are CCR7+ and CD62L+, wherein in any of (a)-(f) the naïve T cells are identified based upon an expression pattern of CCR7+, CD62L+, CD45RO-, CD95-, wherein the stem central memory T cells are identified based upon an expression pattern of CCR7+, CD62L+, CD45RO-, CD95+, and wherein the central memory T cells are identified based upon an expression pattern of CCR7+, CD62L+, CD45RO+, CD95+, thereby treating, or providing anti-tumor immunity to, the leukemia.

4. The method of claim 1, wherein the acquisition (e.g., obtaining or harvesting) of the population of immune effector cells from the subject is further according to an immune effector cell (e.g., T cell) parameter, e.g., one or more of T cell number, T cell phenotype or T cell function.

5. The composition of claim 2 or the use of claim 3, wherein the acquisition of the population of immune effector cells (e.g., obtained or harvested) from the subject further comprises optimizing an immune effector cell (e.g., T cell) parameter, e.g., one or more of T cell number, T cell phenotype or T cell function.

6. The method of claim 1, the composition of claim 2, or the use of claim 3, wherein the population of immune effector cells shows an increase in one or more of: ex-vivo expansion of the population of immune effector cells, the efficacy of the population of immune effector cells for therapy, or the yield of the population of immune effector cells, when any of (i)-(iv) are optimized.

7. The composition or the use of any one of claims 2-6:

(i) wherein the population of immune effector cells that expresses a CAR molecule comprises a nucleic acid encoding a CAR, e.g., a CAR molecule described herein, e.g., a CD19 CAR described herein

(e.g., CTL019), optionally wherein the CAR molecule comprises a sequence according to Table 1 or Table 4; or

(ii) further comprising introducing into the population of immune effector cells a nucleic acid encoding a CAR, e.g., a CAR molecule described herein, e.g., a CD19 CAR described herein (e.g., CTL019), optionally wherein the CAR molecule comprises a sequence according to Table 1 or Table 4.

8. The method, the composition, or the use of any one of claims 1-7, wherein the subject is 18 years of age or younger (e.g., 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or younger).

9. The method, the composition, or the use of any one of claims 1-8, wherein:

(i) the leukemia is chosen from one or more of: a B-cell acute lymphocytic leukemia (B-ALL), T-cell acute lymphocytic leukemia (T-ALL), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), B cell promyelocytic leukemia, hairy cell leukemia, e.g., CLL or ALL;

(ii) the subject is classified as having standard-risk, high-risk, or very high-risk ALL; and/or

(iii) the subject does not have a relapsed cancer.

10. The method, the composition, or the use of any one of claims 1-9, wherein the acquisition of the population of immune effector cells occurs:

(i) prior to administration of the chemotherapy to the subject;

(ii) before the subject has undergone 2, 3, 4 or 5 cycles of chemotherapy; or

(iii) after the subject has undergone 1 cycle of chemotherapy, but before the subject has undergone more than 1 cycle (e.g., more than 1, 2, 3, 4, or 5 cycles) of chemotherapy.

11. The method, the composition, or the use of any one of claims 1-10, wherein the chemotherapy or cycle of chemotherapy comprises one or more of an induction, a consolidation, an interim maintenance, a delayed intensification, or a maintenance therapy cycle, optionally wherein:

(i) the population of immune effector cells is acquired from the subject before the subject has undergone a consolidation cycle of chemotherapy or an induction cycle of chemotherapy;

(ii) the population of immune effector cells is acquired from the subject before the subject has undergone a consolidation cycle of chemotherapy;

(iii) the subject is classified as having a high-risk or very high-risk cancer (e.g., ALL) and the cells are harvested before the subject has undergone a consolidation cycle;

(iv) the population of immune effector cells is acquired from the subject before the subject has undergone a delayed intensification cycle; or

(v) the subject is classified as having a high-risk or very high-risk cancer (e.g., ALL) and the cells are harvested before the subject has undergone a delayed intensification cycle.

12. The method, the composition, or the use of any one of claims 1-11, wherein one or more chemotherapy cycles or drugs are chosen from Table 8, optionally wherein:

- (i) the chemotherapy comprises a drug and dosing regimen described in Table 8;
- (ii) the chemotherapy comprises one or more of vincristine, dexamethasone, PEG-L-asparaginase, daunorubicin, 6-mercaptopurine, cyclophosphamide, cytarabine, methotrexate, doxorubicin, 6-thioguanine, and/or prednisone; or
- (iii) the population of immune effector cells is acquired from the subject before the subject has been administered cyclophosphamide and/or cytarabine.

13. The method, the composition, or the use of any one of claims 1-12, wherein the population of immune effector cells is acquired from the subject before the subject has been administered cyclophosphamide and/or cytarabine.

14. The method, the composition, or the use of any one of claims 1-13,

(i) further comprising removing T regulatory cells, e.g., CD25+ T cells, from the acquired population of immune effector cells, to thereby provide a population of T regulatory-depleted cells, e.g., CD25+ depleted cells;

optionally wherein the population of T regulatory-depleted cells contains

- (a) less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells;
- (b) 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;
- (c) 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;
- (d) 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; and/or
- (e) 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; and/or
- (ii) the acquired population of immune effector cells are cells of a subject having a CD25-expressing cancer such as, e.g., chronic lymphocytic leukemia (CLL).

15. The method, the composition, or the use of any one of claims 1-14:

(i) further comprising removing cells from the acquired population of immune effector cells 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;

(ii) further comprising removing cells from the population of acquired immune effector cells 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; and/or

(iii) wherein the acquired population of immune effector 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+.

16. The method, the composition, or the use of any one of the preceding claims, further comprising transducing a cell from the population of immune effector cells with a vector comprising a nucleic acid encoding a CAR, e.g., a CAR described herein, e.g., a CD19 CAR described herein, optionally further comprising expanding the population of immune effector cells, e.g., engineered to express a CAR, e.g., a CAR described herein, e.g., a CD19 CAR described herein, e.g., by a method described herein.

17. The method, the composition, or the use of claim 16, wherein:

(i) the population of immune effector cells is expanded for a period of 8 days or less, e.g., 7, 6 or 5 days; or

(ii) the population of immune effector 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, optionally wherein:

(a) the population of immune effector 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

(b) the population of immune effector cells are expanded in culture for 5 days, and the resulting cells exhibit higher proinflammatory cytokine production, e.g., IFN- $\gamma$  and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions, and/or

(iii) the population of immune effector 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 wherein the agent is a bead conjugated with anti-CD3 antibody, or a fragment thereof, and/or anti-CD28 antibody, or a fragment thereof.

18. The method, the composition, or the use of claim 16 or claim 17, wherein the population of immune effector cells is

(i) 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, optionally wherein the population of immune effector cells is expanded in the presence a cytokine, e.g., IL-15 and/or IL-7 (e.g., IL-15 and IL-7); and/or

(ii) cryopreserved after the expansion period.

19. An immune cell preparation or reaction mixture comprising a population of immune effector cells, made according to claim 1.

20. The immune cell preparation or reaction mixture of claim 19, wherein the population of immune effector cells:

(a) comprises at least 20% naïve T cells, at least 2% stem central memory T cells, and/or at least 4% central memory T cells;

(b) comprises an absolute T cell count of at least 400 cells/microliter, an absolute naïve T cell count of at least 200 cells/microliter, an absolute stem central memory T cell count of at least 20 cells/microliter, and/or an absolute central memory T cell count of at least 40 cells/microliter, optionally wherein the population of immune effector cells has been selected based upon the expression of one or more markers, e.g., CCR7, CD62L, CD45RO, and CD95, e.g., the population of immune effector cells (e.g., T cells) are CCR7<sup>+</sup> and CD62L<sup>+</sup>, further optionally wherein the naïve T cells are identified based upon an expression pattern of CCR7<sup>+</sup>, CD62L<sup>+</sup>, CD45RO<sup>-</sup>, CD95<sup>-</sup>, wherein the stem central memory T cells are identified based upon an expression pattern of CCR7<sup>+</sup>, CD62L<sup>+</sup>, CD45RO<sup>-</sup>, CD95<sup>+</sup>, and wherein the central memory T cells are identified based upon an expression pattern of CCR7<sup>+</sup>, CD62L<sup>+</sup>, CD45RO<sup>+</sup>, CD95<sup>+</sup>; and/or

(c) comprises a nucleic acid encoding a CAR, e.g., a CAR as described herein.