

CLAIMS

1. A method of enriching for, or making, an immune effector cell (e.g., cell population) suitable for use in a CAR therapy, comprising acquiring (e.g., obtaining or harvesting) the immune effector cell, according to one, two, three, four, five or more (all) of the following:

- (i) the timing of immune effector cell acquisition from a subject (e.g., a hematological cancer patient);
 - (ii) the timing of the chemotherapy in relation to the immune effector cell acquisition;
 - (iii) the type of chemotherapy;
 - (iv) the underlying malignancy;
 - (v) 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,
- or a combination of two, three, four, or five of (i)-(v),
thereby enriching for, or making, the immune effector cell suitable for use in a CAR therapy.

2. A composition comprising an immune effector cell (e.g., cell population) that expresses a CAR molecule (a “CAR-expressing cell”) for use, in combination with a chemotherapy, in treating, or in providing anti-tumor immunity to, a subject having a hematological cancer, wherein the immune effector cell is acquired (e.g., obtained or harvested) from the subject, by optimizing one, two, three, four, or more (all) of the following:

- (i) the timing of immune effector cell acquisition from a subject (e.g., a cancer patient);
 - (ii) the timing of the chemotherapy in relation to the immune effector cell acquisition;
 - (iii) the type of chemotherapy;
 - (iv) the underlying malignancy;
 - (v) 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,
- or a combination of two, three, four, or five of (i)-(v),
(optionally) wherein the immune effector cell is acquired from the subject prior to introduction of the CAR molecule.

3. Use of an immune effector cell (e.g., a cell population) that expresses a CAR molecule (a “CAR-expressing cell” or a “CAR therapy”) in the manufacture of a medicament for the treatment of, or providing anti-tumor immunity to, a subject having a hematological cancer, wherein the subject is to be administered an effective amount of an immune effector cell (e.g., a cell population) that expresses a CAR molecule (a “CAR-expressing cell” or a “CAR therapy”), in combination with a

chemotherapy, wherein the immune effector cell is acquired (e.g., obtained or harvested) from the subject, by optimizing one, two, three, four, or more (all) of the following:

- (i) the timing of immune effector cell acquisition from a subject (e.g., a cancer patient);
- (ii) the timing of the chemotherapy in relation to the immune effector cell acquisition;
- (iii) the type of chemotherapy;
- (iv) the underlying malignancy;
- (v) 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,

or a combination of two, three, four, or five of (i)-(v),

(optionally) wherein the immune effector cell is acquired from the subject prior to introduction of the CAR molecule,

thereby treating, or providing anti-tumor immunity to, the hematological cancer.

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

5. The composition or the use of any of claims 2-4, wherein the CAR-expressing cell 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.

6. The composition or the use of any of claims 2-5, further comprising introducing into the immune effector cell population 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.

7. The method, the composition, or the use of any of claims 1-6, wherein the subject from which immune cells are acquired and/or the subject to be treated, is a human cancer patient, optionally 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), or the subject is older than 18 years of age.

8. The method, the composition, or the use of any of claims 1-7, wherein the subject has a disease associated with expression of a tumor- or cancer associated-antigen.

9. The method, the composition, or the use of any of claims 1-8, wherein the hematological cancer 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, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma (MCL), marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma (HL), plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, and Waldenstrom macroglobulinemia.

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

- (i) the hematological cancer is a leukemia, e.g., CLL, ALL, or a lymphoma, e.g., MCL, NHL, or HL;
- (ii) the subject has a leukemia, and is a paediatric patient, e.g., is 18 years of age or younger;
- (iii) the subject is classified as having standard-risk, high-risk, or very high-risk ALL;
- (iv) the subject does not have a lymphoma, e.g., the subject does not have Non-Hodgkin lymphoma (NHL); or
- (v) the subject does not have a relapsed cancer.

11. The method, the composition, or the use of any of claims 1-10, wherein the immune effector cell population acquisition 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.

12. The method, the composition, or the use of any of claims 1-11, 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 immune effector cell population is acquired from the subject before the subject has undergone a consolidation cycle of chemotherapy or an induction cycle of chemotherapy;
- (ii) wherein the immune effector cell population 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 immune effector cell population 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.

13. The method, the composition, or the use of any of claims 1-12, wherein:

(i) one or more chemotherapy cycles or drugs are chosen from Table 8; and/or;

(ii) the chemotherapy comprises a drug and dosing regimen described in Table 8.

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

(i) 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

(ii) the immune effector cell population is acquired from the subject before the subject has been administered cyclophosphamide and/or cytarabine.

15. The method, the composition, or the use of any of claims 1-14, wherein the acquired immune effector cell population comprises a higher number of less differentiated T cells, e.g., a higher number of one or more of naïve T cells, stem central memory T cells, and/or central memory T cells, e.g., 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).

16. The method, the composition, or the use of claim 15, wherein the acquired immune effector cell population includes one or more of:

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

(ii) at least 50% central memory T cells; or

(iii) less than 55% effector memory and terminal effector T cells combined.

17. The method, the composition, or the use of claim 15, wherein:

(i) the acquired immune effector cell population 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); or

(ii) the acquired immune effector cell population 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.

18. The method, the composition, or the use of any of claims 1-17, wherein the acquired immune effector cell population 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+.

19. The method, the composition, or the use of claim 16, wherein 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+.

20. The method, the composition, or the use of any of claims 1-19, wherein the immune cell population is acquired, from a subject having a haematological cancer, e.g., a leukemia, e.g., CLL, ALL, or a lymphoma, e.g., MCL, NHL, or HL.

21. The method, the composition, or the use of any of claims 1-20, further comprising removing T regulatory cells, e.g., CD25+ T cells, from the acquired immune cell population, to thereby provide a population of T regulatory-depleted cells, e.g., CD25+ depleted cells.

22. The method, the composition, or the use of claim 21, wherein the population of T regulatory-depleted cells contains less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells.

23. The method, the composition, or the use of any of claims 1-22, wherein the acquired immune effector cell population are cells of a subject having cancer, e.g., a subject having a CD25-expressing cancer such as, e.g., chronic lymphocytic leukemia (CLL).

24. The method, the composition, or the use of claim 22, 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.

25. The method, the composition, or the use of claim 21, wherein the T regulatory cells, e.g., CD25+ T cells, are removed from the acquired immune effector cell population using an anti-CD25 antibody, or fragment thereof.

26. The method, the composition, or the use of claim 25, wherein the anti-CD25 antibody, or fragment thereof, is conjugated to a substrate, e.g., a bead.

27. The method, the composition, or the use of claim 24, wherein:

(i) 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;

(ii) 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; or

(iii) 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.

28. The method, the composition, or the use of any of claims 1-27, further comprising:

removing cells from the acquired immune effector cell 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.

29. The method, the composition, or the use of any of claims 1-27, further comprising removing cells from the acquired immune effector cell 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.

30. The method, the composition, or the use of any of claims 1-27, wherein the acquired immune effector cell population 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+.

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

32. The method, the composition, or the use of claim 31, further comprising expanding the immune effector cell population, 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.

33. The method, the composition, or the use of any of the preceding claims, further comprising: 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.

34. The method, the composition, or the use of claim 33, further comprising expanding the population of T regulatory-depleted 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.

35. The method, the composition, or the use of claim 32 or 34, wherein:

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

36. The method, the composition, or the use of claim 35, wherein:

- (i) 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
- (ii) 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.

37. The method, the composition, or the use of claim 32 or 34, 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.

38. The method, the composition, or the use of any of claims 32-37-33, 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.

39. The method, the composition, or the use of claim 38, wherein the population of cells is expanded in the presence a cytokine, e.g., IL-15 and/or IL-7 (e.g., IL-15 and IL-7).

40. The method, the composition, or the use of any of claims 32-39, wherein the population of the cells is cryopreserved after the expansion period.

41. The method, the composition, or the use 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.

42. An immune cell preparation or reaction mixture, e.g., comprising a population of immune effector cells (e.g., comprising a CAR molecule or a nucleic acid encoding a CAR molecule), made according to any of the methods described herein.

43. The immune effector cell preparation (e.g., reaction mixture) of claim 42, which is chosen from:

(i) the population of immune effector cells comprises at least 20% naïve T cells, at least 2% stem central memory T cells, and/or at least 4% central memory T cells, and/or

(ii) the population of immune effector cells 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.

44. The immune effector cell preparation (e.g., reaction mixture) of claim 42, which 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+ and CD62L+.

45. The immune effector cell preparation (e.g., reaction mixture) of claim 43, wherein 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+.

46. The immune effector cell preparation (e.g., reaction mixture) of any of claims 43-45, which comprises a nucleic acid encoding a CAR, e.g., a CAR as described herein.