

## **CLAIMS**

1. A method of evaluating a subject, e.g., evaluating or monitoring the effectiveness of a CAR-expressing cell therapy in a subject, having a hematological cancer, comprising  
acquiring a value for the level of CD27+ CD45RO- immune effector cells, in a sample  
wherein said level is predictive of the subject's response to the CAR-expressing cell therapy, thereby evaluating the subject.
2. The method of claim 1, wherein a greater level of CD27+ CD45RO- immune effector cells in the immune effector cell population, compared to a non-responder number of CD27+ CD45RO- immune effector cells indicates that the subject has an increased likelihood of being responsive to the CAR-expressing cell therapy.
3. The method of claim 2, wherein the greater level of CD27+ CD45RO- immune effector cells comprises a greater percentage (e.g., 7% or greater number).
4. The method of any one of claims 1-3, further comprising identifying the subject as having an increased likelihood of being responsive to the CAR-expressing cell therapy, e.g., identifying the subject as a responder, e.g., a complete responder, partial responder or non-relapser.
5. The method of any one of claims 1-4, wherein the sample comprises an apheresis sample.
6. The method of any one of claims 1-5, wherein the sample comprises a manufactured CAR-expressing cell product sample.
7. The method of any one of claims 1-6, further comprising acquiring a measure of one, two, three, four, five, six or more (e.g., all) of the following:
  - (i) the level or activity of one, two, three, or more (e.g., all) of resting T<sub>EFF</sub> cells, resting T<sub>REG</sub> cells, younger T cells (e.g., younger CD4 or CD8 cells, or gamma/delta T cells), or early memory T cells, or a combination thereof, in a sample;

(ii) the level or activity of one, two, three, or more (*e.g.*, all) of activated T<sub>EFF</sub> cells, activated T<sub>REG</sub> cells, older T cells (*e.g.*, older CD4 or CD8 cells), or late memory T cells, or a combination thereof, in a sample;

(iii) the level or activity of an immune cell exhaustion marker, *e.g.*, one, two or more immune checkpoint inhibitors (*e.g.*, PD-1, TIM-3 and/or LAG-3) in a sample;

(iv) the level or activity of one, two, three, four, five, ten, twenty or more of the biomarkers listed in Table 1A, Table 1B, Table 7A, Table 7B, Table 8, Table 9, Table 10, Table 14 (*e.g.*, CCL20, IL-17a and/or IL-6), Table 16, Table 17, Table 18, Table 20, FIG. 2B, PD-1, LAG-3, TIM-3, CD57, CD27, CD122, CD62L, KLRG1, or a CD19 CAR-expressing cell gene set signature;

(v) a cytokine level or activity (*e.g.*, quality of cytokine repertoire) in a CAR-expressing cell product sample wherein the cytokine is chosen from one, two, three, four, five or more (or all) of the cytokines listed in Table 16;

(vi) a transduction efficiency of a CAR-expressing cell in a manufactured CAR-expressing cell product sample; or

(vii) a quantity of CD27<sup>+</sup> PD-1<sup>-</sup> cells in a sample, *e.g.*, a quantity greater than or equal to  $1 \times 10^7$  cells.

8. The method of any one of claims 1-7, further comprising arranging for one, two, three, four, five, six, seven, or more (*e.g.*, all) of the following to be carried out:

the administration of a CAR-expressing cell therapy;

the administration of an altered dosing of a CAR-expressing cell therapy;

the administration of a CAR-expressing cell therapy according to an altered schedule or time course;

the administration of, *e.g.*, to a non-responder or a partial responder, an additional agent in combination with a CAR-expressing cell therapy, *e.g.*, a checkpoint inhibitor, *e.g.*, a checkpoint inhibitor described herein;

the administration of to a non-responder or partial responder a therapy that increases the number of younger T cells in the subject prior to treatment with a CAR-expressing cell therapy;

the modification of a manufacturing process of a CAR-expressing cell therapy, e.g., enriching for younger T cells prior to introducing a nucleic acid encoding CAR, or increasing the transduction efficiency, e.g., for a subject identified as a non-responder or a partial responder;

the administration of an altered infusion dose of the CAR-expressing cell to achieve clinical efficacy;

the administration of an alternative therapy, e.g., for a non-responder or partial responder or relapser;

the administration of an alternative therapy, e.g., for a non-responder or partial responder, e.g., a standard of care for a particular cancer type; or

if the subject is, or is identified as, a non-responder or a relapser, the decrease of the T<sub>REG</sub> cell population and/or T<sub>REG</sub> gene signature, e.g., by CD25 depletion, administration of cyclophosphamide, anti-GITR antibody, mTOR inhibitor, or a combination thereof.

9. Use of a CAR-expressing cell therapy in the manufacture of a medicament for the treatment of a hematological cancer in a subject wherein the therapeutically effective dose of a CAR-expressing cell therapy in the form of the medicament is to be administered to the subject, if the subject is identified, or has been identified as being responsive to a therapy comprising a CAR-expressing cell population,

wherein said identifying comprises measuring, in a sample from the subject or population of subjects a value for the level of CD27+ CD45RO- immune effector cells,

wherein the subject identified as being responsive to the CAR-expressing cell therapy has an increased level of CD27+ CD45RO- immune effector cells compared to a non-responder number of CD27+ CD45RO- immune effector cells

thereby treating the subject.

10. The use of claim 9, wherein the increased level of CD27+ CD45RO- immune effector cells comprises a greater percentage (e.g., 7% or greater number) of CD27+ CD45RO- immune effector cells.

11. Use of a CAR-expressing cell therapy in the manufacture of a medicament for the treatment of a hematological cancer in a subject, comprising acquiring a value for the level of CD27+ CD45RO- immune effector cells,

wherein an increased level of CD27+ CD45RO- immune effector cells in the immune effector cell population, compared to a non-responder number of CD27+ CD45RO- immune effector cells indicates that the subject has an increased likelihood of being responsive to the CAR-expressing cell therapy,

responsive to said value, arranging for one, two, three, four, five, six, seven, or all of the following to be carried out:

the administration of a CAR-expressing cell therapy to a responder or a non-relapser;

the administration of an altered dosing of a CAR-expressing cell therapy;

the administration of a CAR-expressing cell therapy according to an altered schedule or time course;

the administration of an additional agent in combination with a CAR-expressing cell therapy to a non-responder or a partial responder;

the administration of to a non-responder or partial responder a therapy that increases the number of younger T cells or naïve T cells in the subject prior to treatment with a CAR-expressing cell therapy;

the modification of a manufacturing process of a CAR-expressing cell therapy, by enriching for younger T cells or naïve T cells prior to introducing a nucleic acid encoding a CAR, or increasing the transduction efficiency for a subject identified as a non-responder or a partial responder;

the administration of an alternative therapy such as a standard of care for a particular cancer type for a non-responder or partial responder or relapser; or

if the subject is, or is identified as, a non-responder or a relapser, decreasing the T<sub>REG</sub> cell population and/or T<sub>REG</sub> gene signature.

12. The use of claim 11, wherein the increased level of CD27+ CD45RO- immune effector cells comprises a greater percentage (e.g., 7% or greater number) of CD27+ CD45RO- immune effector cells.

13. The use of any one of claims 9-12, further comprising acquiring a measure of one, two, three, four, five, six or more (e.g., all) of the following:

(i) the level or activity of one, two, three, or more (e.g., all) of resting T<sub>EFF</sub> cells, resting T<sub>REG</sub> cells, younger T cells (e.g., younger CD4 or CD8 cells, or gamma/delta T cells), or early memory T cells, or a combination thereof, in a sample;

(ii) the level or activity of one, two, three, or more (e.g., all) of activated T<sub>EFF</sub> cells, activated T<sub>REG</sub> cells, older T cells (e.g., older CD4 or CD8 cells), or late memory T cells, or a combination thereof, in a sample;

(iii) the level or activity of an immune cell exhaustion marker, e.g., one, two or more immune checkpoint inhibitors (e.g., PD-1, TIM-3 and/or LAG-3) in a sample;

(iv) the level or activity of one, two, three, four, five, ten, twenty or more of the biomarkers listed in Table 1A, Table 1B, Table 7A, Table 7B, Table 8, Table 9, Table 10, Table 14 (e.g., CCL20, IL-17a and/or IL-6), Table 16, Table 17, Table 18, Table 20, FIG. 2B, PD-1, LAG-3, TIM-3, CD57, CD27, CD122, CD62L, KLRG1, or a CD19 CAR-expressing cell gene set signature;

(v) a cytokine level or activity (e.g., quality of cytokine repertoire) in a CAR-expressing cell product sample (e.g., CTL019), wherein the cytokine is chosen from one, two, three, four, five or more (or all) of the cytokines listed in Table 16;

(vi) a transduction efficiency of a CAR-expressing cell in a manufactured CAR-expressing cell product sample; or

(vii) a quantity of CD27+ PD-1- cells in a sample, e.g., a quantity greater than or equal to  $1 \times 10^7$  cells.

14. The use of any of claims 9-13, wherein the sample comprises an apheresis sample.

15. The use of any of claims 9-13, wherein the sample comprises a CAR19-expressing cell product sample.

16. The method of any one of claims 1-8, or the use of any one of claims 9-16, wherein the CAR-expressing cell therapy comprises a CAR having an antigen binding domain that binds to CD19.

17. The method or use of claim 16, wherein the CAR-expressing cell therapy is a CAR19 therapy (e.g., CTL019 therapy).

18. The method of any one of claims 1-8, or the use of any one of claims 9-16, wherein the CAR-expressing cell therapy comprises a CAR having an antigen binding domain that binds to BCMA, e.g., a CAR BCMA-expressing cell therapy.

19. The method of any one of claims 1-8 or 16-18, or the use of any of claims 9-18, wherein

(a) the CAR-expressing cell therapy comprises a plurality of CAR-expressing immune effector cells;

(b) the measure of the level of CD27 + CD45RO- immune effector cells, or one or more of (i)-(vii) is obtained from:

an apheresis sample acquired from the subject, wherein optionally the apheresis sample is evaluated prior to infusion or re-infusion; or

from a manufactured CAR- expressing cell product sample (e.g., CTL019), wherein optionally the manufactured CAR-expressing cell product is evaluated prior to infusion or re-infusion; and/or

(c) the subject is evaluated prior to, during, or after receiving the CAR-expressing cell therapy.

20. The method of any one of claims 1-8 or 16-19, or the use of any of claims 9-19, wherein the hematological cancer is an ALL or a CLL; and/or the subject is a human patient.

21. The method of any one of claims 1-8 or 16-20, or the use of any of claims 9-20, further comprises identifying the subject as:

(a) having an increased likelihood of being responsive to the CAR-expressing cell therapy, e.g., identifying the subject as a responder, e.g., a complete responder, partial responder or non-relapser; or

(b) a non-responder or relapser,

based on a measure of the level of CD27 + CD45RO- immune effector cells, or one or more of (i)-(vii).

22. The method of any one of claims 1-8 or 16-21, or the use of any of claims 9-21, wherein

(a) the measure of the level of CD27 + CD45RO- immune effector cells, or one or more of (i)-(vii) evaluates a profile for one or more of gene expression, flow cytometry or protein expression;

(b) the level or activity of a CD8+ T cell is evaluated using a profile or signature indicative of the percentage of CD8+ T cell in the sample;

(c) the level or activity of CD27+ CD45RO- immune effector cells is evaluated using a profile or signature indicative of the percentage of CD27+ CD45RO- immune effector cells in the sample;

(d) the level or activity is evaluated using a profile or gene signature according to one, two, three, four, five, ten, twenty, fifty, sixty, seventy, one hundred or more of a biomarker or gene set listed in Tables 1A, 1B, 3, 4, 5, 6, 7A, 7B, or FIG. 2B; and/or

(e) the biomarker is a secreted or a cell surface biomarker listed in Table 8.

23. The method or the use of claim 22, wherein

(a) a non-responder has, or is identified as having, a greater level or activity of one, two, three, four, five, six, seven, or more (e.g., all) of IL22, IL-2RA, IL-21, IRF8, IL8, CCL17, CCL22, effector T cells, or regulatory T cells, as compared to a responder;

(b) a responder (e.g., a complete responder) has, or is identified as having, a greater level or activity of one, two, or more (all) of GZMK, PPF1BP2, or naïve T cells as compared to a non-responder;

(c) a complete responder has, or is identified as having, a greater, e.g., a statistically significant greater, percentage of CD8+ T cells compared to a reference value, e.g., a non-responder percentage of CD8+ T cells;

(d) a complete responder or a partial responder has, or is identified as having, a greater, e.g., a statistically significant greater, percentage of CD4+ T cells compared to a reference value, e.g., a non-responder percentage of CD4+ T cells;

(e) a complete responder has, or is identified as having, a greater percentage of one, two, three, or more (*e.g.*, all) of resting T<sub>EFF</sub> cells, resting T<sub>REG</sub> cells, younger T cells (*e.g.*, younger CD4 or CD8 cells, or gamma/delta cells), or early memory T cells, or a combination thereof, compared to a reference value, *e.g.*, a non-responder number of resting T<sub>EFF</sub> cells, resting T<sub>REG</sub> cells, younger T cells (*e.g.*, younger CD4 or CD8 cells), early memory T cells;

(f) a non-responder has, or is identified as having, a greater percentage of one, two, three, or more (*e.g.*, all) of activated T<sub>EFF</sub> cells, activated T<sub>REG</sub> cells, older T cells (*e.g.*, older CD4 or CD8 cells), or late memory T cells, or a combination thereof, compared to a reference value, *e.g.*, a responder number of activated T<sub>EFF</sub> cells, activated T<sub>REG</sub> cells, older T cells (*e.g.*, older CD4 or CD8 cells), or late memory T cells;

(g) a non-responder has, or is identified as having:

a greater percentage of PD-1 or LAG-3 expressing immune effector cells (*e.g.*, CD4+ T cells and/or CD8+ T cells) (*e.g.*, CAR-expressing CD4+ cells and/or CD8+ T cells) compared to the percentage of PD-1 or LAG-3 expressing immune effector cells from a responder, or a greater percentage of PD-1+/LAG-3+ cells in the CAR-expressing cell population (*e.g.*, a CAR19+ cell population) compared to a responder (*e.g.*, a complete responder) to the CAR-expressing cell therapy; an exhausted phenotype of PD1+ CAR+ and co-expression of LAG3 in the CAR-expressing cell population (*e.g.*, a CAR19+ cell population); or a greater percentage of PD-1+/TIM-3+ cells in the CAR-expressing cell population (*e.g.*, a CAR19+ cell population) compared to the responder (*e.g.*, complete responder);

(h) a partial responder has, or is identified as having:

a higher percentage of PD-1+/LAG-3+ cells than responders in the CAR-expressing cell population (*e.g.*, a CAR19+ cell population); or a higher percentage of PD-1+/TIM-3+ cells than responders in the CAR-expressing cell population (*e.g.*, a CAR19+ cell population);

(i) the presence of CD8+ CD27+ CD45RO- T cells in an apheresis sample is a positive predictor of the subject response to a CAR-expressing cell therapy (*e.g.*, a CAR19 therapy (*e.g.*, CTL019 therapy));

(j) a high percentage of PD1+ CAR+ and LAG3+ or TIM3+ T cells in an apheresis sample is a poor prognostic predictor of the subject response to a CAR-expressing cell therapy (e.g., a CAR19 therapy (e.g., CTL019 therapy));

(k) a responder (e.g., a complete responder) to a CAR19 therapy has, or is identified as having, the biomarker profile of Table 9;

(l) a non-responder to a CAR19 therapy has, or is identified as having, the biomarker comprising one or more of PD-1+ immune effector cells, TIM-3+ immune effector cells, LAG-3+ immune effector cells, KLRG1+ immune effector cells, CD27-immune effector cells, activated T<sub>EFF</sub> cells, activated T<sub>REG</sub> cells, activated TH1, activated TH2 cells, stimulated memory cells, or late T memory cells, or a combination thereof;

(m) a non-responder to a CAR19 therapy has, or is identified as having, the biomarker profile of Table 10;

(n) gene expression of one, two, three, four or more (all) of KLRG1, CD57, CD27, CD122, or CD62L is predictive of patient response to CTL019 therapy;

(o) a relapser has, or is identified as having, elevated levels of one, two, three, four, five, six, seven, eight, nine, ten or more of C5orf32, CCL17, CSF1, CTSL1, EMP1, EPAS1, GCLM, GK, GPR56, HMOX1, IKBIP, IL10, IL13, IL1RN, IL4, IL5, IL9, MIR155, PANX2, PGAM4, PRKAR1B, TNFRSF11A, TNFRSF1B, TNFRSF8, VTRNA1-3, or ZNF282; and/or

(p) the responder (e.g., the complete or partial responder) has one, two, three or more (or all) of the following profile:

(i) has a greater number of CD27+ immune effector cells compared to a reference value, e.g., a non-responder number of CD27+ immune effector cells;

(ii) has a greater number of CD8+ T cells compared to a reference value, e.g., a non-responder number of CD8+ T cells;

(iii) has a lower number of cells expressing one or more checkpoint inhibitors, e.g., a checkpoint inhibitor chosen from PD-1, LAG-3, TIM-3, or KLRG-1, or a combination, compared to a reference value, e.g., a non-responder number of cells expressing one or more checkpoint inhibitors; or

(iv) has a greater number of one, two, three, or more (all) of resting T<sub>EFF</sub> cells, resting T<sub>REG</sub> cells, younger cells, or early memory T cells, or a combination thereof, compared to a reference value, e.g., a non-responder number of resting

T<sub>EFF</sub> cells, resting T<sub>REG</sub> cells, naïve CD4 cells, unstimulated memory cells or early memory T cells.

24. The method of any one of claims 1-8 or 16-23, or the use of any of claims 9-23, wherein:

the cytokine level or activity of (v) is chosen from one, two, three, four, five, six, seven, eight, or more (or all) of cytokine CCL20/MIP3a, IL17A, IL6, GM-CSF, IFN $\gamma$ , IL10, IL13, IL2, IL21, IL4, IL5, IL9 or TNF $\alpha$ , or a combination thereof; or

a transduction efficiency of 15% or higher in (vi) is indicative of increased responsiveness or decreased relapse.

25. The method of any one of claims 1-8 or 16-24, or the use of any of claims 9-24, wherein the method or use comprises a measure of the level or activity of one, two, three, four, five, ten, twenty or more (e.g., all) of the biomarkers having a FDR p-value of below 0.1 or 0.01 listed in Table 1A, 1B, 17, 18, or 20.

26. A method of evaluating the potency of a CAR-expressing cell product, said method comprising acquiring a value for the level of CD27<sup>+</sup> CD45RO<sup>-</sup> immune effector cells, in the CAR-expressing cell product; wherein an increase in the level of CD27<sup>+</sup> CD45RO<sup>-</sup>, is indicative of increased potency of the CAR-expressing cell product.

27. The method of claim 26, wherein the method comprises acquiring a value for one, two, three, four, five, six, seven, or more (e.g., all) of:

(i) the level or activity of one, two, three, or more (e.g., all) of resting T<sub>EFF</sub> cells, resting T<sub>REG</sub> cells, younger T cells (e.g., younger CD4 or CD8 cells, or gamma/delta T cells), or early memory T cells, or a combination thereof, in the CAR-expressing cell product;

(ii) the level or activity of one, two, three, or more (e.g., all) of activated T<sub>EFF</sub> cells, activated T<sub>REG</sub> cells, older T cells (e.g., older CD4 or CD8 cells), or late memory T cells, or a combination thereof, in the CAR-expressing cell product;

(iii) the level or activity of an immune cell exhaustion marker, e.g., one, two or more immune checkpoint inhibitors (e.g., PD-1, TIM-3 and/or LAG-3) in the CAR-expressing cell product;

(iv) the level or activity of one, two, three, four, five, ten, twenty or more of the biomarkers listed in Table 1A, Table 1B, Table 7A, Table 7B, Table 8, Table 9, Table 10, Table 14 (e.g., CCL20, IL-17a and/or IL-6), Table 16, Table 17, Table 18, Table 20, FIG. 2B, PD-1, LAG-3, TIM-3, CD57, CD27, CD122, CD62L, KLRG1, or a CD19 CAR-expressing cell gene set signature;

(v) a cytokine level or activity (e.g., a cytokine repertoire) in the CAR- expressing cell product sample, wherein the cytokine is chosen from one, two, three, four, five or more (or all) of the cytokines listed in Table 16;

(vi) a transduction efficiency of CAR-expressing cells in the product;

(vii) a quantity of CD27<sup>+</sup> PD-1<sup>-</sup> cells in a sample, e.g., a quantity greater than or equal to  $1 \times 10^7$  cells; or

(viii) the level or activity of a T<sub>REG</sub> cell or cell population;  
wherein an increase in (i), (v), (vi), (vii), or any combination thereof, is indicative of increased potency of the CAR-expressing cell product, and  
wherein an increase in (ii), (iii), (viii), or any combination thereof, is indicative of decreased potency of the CAR-expressing cell product.

28. The method of claim 27, wherein:

the cytokine is chosen from one, two, three, four, five, six, seven, eight, or more (or all) of CCL20/MIP3a, IL17A, IL6, GM-CSF, IFN $\gamma$ , IL10, IL13, IL2, IL21, IL4, IL5, IL9 or TNF $\alpha$ , or a combination thereof; or

a transduction efficiency of 15% or higher is indicative of increased potency.

29. A method for optimizing manufacturing of a CAR-expressing cell product, comprising:

(1) acquiring a sample comprising a population of CAR-expressing immune effector cells;

(2) activating the population of CAR-expressing cells *in vitro*; and

(3) evaluating the potency of the activated CAR-expressing cell population by determining the level of CD27<sup>+</sup> CD45RO<sup>-</sup> immune effector cells in the CAR-expressing cell product, wherein an increase in the level of CD27<sup>+</sup> CD45RO<sup>-</sup> immune effector cells, is

indicative of increased potency of the CAR-expressing cell product, thereby optimizing manufacturing of the product.

30. The method of claim 29, comprising determining one, two, three, four, five, six, seven, or more (e.g., all) of:

(i) the level or activity of one, two, three, or more (e.g., all) of resting T<sub>EFF</sub> cells, resting T<sub>REG</sub> cells, younger T cells (e.g., younger CD4 or CD8 cells, or gamma/delta T cells), or early memory T cells, or a combination thereof, in the CAR-expressing cell product;

(ii) the level or activity of one, two, three, or more (e.g., all) of activated T<sub>EFF</sub> cells, activated T<sub>REG</sub> cells, older T cells (e.g., older CD4 or CD8 cells), or late memory T cells, or a combination thereof, in the CAR-expressing cell product;

(iii) the level or activity of an immune cell exhaustion marker, e.g., one, two or more immune checkpoint inhibitors (e.g., PD-1, TIM-3 and/or LAG-3) in the CAR-expressing cell product;

(iv) the level or activity of one, two, three, four, five, ten, twenty or more of the biomarkers listed in Table 1A, Table 1B, Table 7A, Table 7B, Table 8, Table 9, Table 10, Table 14 (e.g., CCL20, IL-17a and/or IL-6), Table 16, Table 17, Table 18, Table 20, FIG. 2B, PD-1, LAG-3, TIM-3, CD57, CD27, CD122, CD62L, KLRG1, or a CD19 CAR-expressing cell gene set signature;

(v) a cytokine level or activity in a CAR-expressing cell product sample, wherein the cytokine is chosen from one, two, three, four, five or more (or all) of the cytokines listed in Table 16;

(vi) a transduction efficiency of CAR-expressing cells in the product;

(vii) a quantity of CD27<sup>+</sup> PD-1<sup>-</sup> cells in a sample, e.g., a quantity greater than or equal to  $1 \times 10^7$  cells; or

(viii) the level or activity of a T<sub>REG</sub> cell or cell population, wherein an increase in (i), (v), (vi), (vii), or any combination thereof, is indicative of increased potency of the CAR-expressing cell product, and wherein an increase in (ii), (iii), (viii), or any combination thereof, is indicative of decreased potency of the CAR-expressing cell product.

31. The method of any one of claims 1-8 or 16-30, or the use of any one of claims 9-25, wherein the CAR-expressing immune effector cells comprise CD4<sup>+</sup> T cells or CD8<sup>+</sup> T cells.

32. The method of any one of claims 26-31, wherein the CAR-expressing cell product comprises a CAR having an antigen binding domain that binds to CD19, optionally wherein the CAR-expressing cell product is a CAR19-expressing cell product, e.g., CTL019.

33. The method of any one of claims 26-32, wherein the CAR-expressing cell product comprises a CAR having an antigen binding domain that binds to BCMA.

34. The method of any one of claims 26-33, further comprising a step of enriching for, e.g., isolating, cells having:

an increase in the level of CD27<sup>+</sup> CD45RO<sup>-</sup> immune effector cells, or any of (i), (v), (vi), (vii), or any combination thereof, or

a decrease in any of (ii), (iii), (viii), or any combination thereof; or

wherein the cytokine level or activity is chosen from one or more of cytokine CCL20/MIP3a, IL17A, IL6, GM-CSF, IFN $\gamma$ , IL10, IL13, IL2, IL21, IL4, IL5, IL9 or TNF $\alpha$ , or a combination thereof.

35. The method of any one of claims 26-34, wherein the level of CD27<sup>+</sup> CD45RO<sup>-</sup> immune effector cells, or (i), (ii), (iii), (iv), (v), (vi), (vii), (viii), or any combination thereof (e.g., all) are evaluated following activation *in vitro*.

36. The method of any one of claims 1-8 or 16-35, or the use of any one of claims 9-25, wherein

(i) the subject has received or is to receive a treatment, e.g., a pre-treatment or concurrent treatment with an agent, e.g., an mTOR inhibitor, and/or a checkpoint inhibitor, e.g., a pre-treatment prior to the initiation of a CAR-expressing cell therapy or a treatment post- CAR-expressing cell therapy;

(ii) the hematological cancer is associated with CD19 expression or BCMA expression;

(iii) the hematological cancer is selected from the group consisting of 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, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin lymphoma, Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, and Waldenstrom macroglobulinemia; or

(iv) the CD19 CAR-expressing cell gene signature comprises a value for expression of at least 5, 6, 7, 8, 9 or 10 genes comprising a CD19 CAR-expressing cell gene signature.

37. A system for evaluating a hematological cancer in a subject, comprising:

at least one processor operatively connected to a memory, the at least one processor when executing is configured to:

acquire a value for the level of CD27+ CD45RO- immune effector cells, wherein an increased level of CD27+ CD45RO- immune effector cells is indicative of the subject's increased likelihood of being responsive to a CAR-expressing cell therapy,

responsive to a determination of the level of CD27+ CD45RO- immune effector cells, perform one, two, three, four, five, six, seven, or all of:

identify the subject as a complete responder, partial responder, non-responder, relapser or non-relapser;

recommend administering a CAR-expressing cell therapy;

recommend a selection or alteration of a dosing of a CAR-expressing cell therapy;

recommend a selection or alteration of a schedule or time course of a CAR-expressing cell therapy;

recommend administering to a non-responder or a partial responder an additional agent in combination with a CAR-expressing cell therapy;

recommend administering to a non-responder or partial responder a therapy that increases the number of naïve T cells in the subject prior to treatment with a CAR-expressing cell therapy;

recommend modifying a manufacturing process of a CAR-expressing cell therapy by enriching for naïve T cells prior to introducing a nucleic acid encoding a CAR for a subject identified as a non-responder or a partial responder;

recommend administering an adjusted CAR-expressing cell infusion dose to achieve clinical efficacy;

recommend administering an alternative therapy for a non-responder or partial responder or relapser;

recommend a selection of an alternative therapy such as a standard of care for a particular cancer type for a non-responder or partial responder; or

if the subject is, or is identified as, a non-responder or a relapser, recommend decreasing the T<sub>REG</sub> cell population and/or T<sub>REG</sub> gene signature.

38. The system of claim 37, further comprising acquiring a value for one, two, three, four, five, six, or more (all) of the following:

(i) the level or activity of one, two, three, or more (*e.g.*, all) of resting T<sub>EFF</sub> cells, resting T<sub>REG</sub> cells, younger T cells (*e.g.*, younger CD4 or CD8 cells, or gamma/delta T cells), or early memory T cells, or a combination thereof, in a sample;

(ii) the level or activity of one, two, three, or more (*e.g.*, all) of activated T<sub>EFF</sub> cells, activated T<sub>REG</sub> cells, older T cells (*e.g.*, older CD4 or CD8 cells), or late memory T cells, or a combination thereof, in a sample;

(iii) the level or activity of an immune cell exhaustion marker, *e.g.*, one, two or more immune checkpoint inhibitors (*e.g.*, PD-1, TIM-3 and/or LAG-3) in a sample;

(iv) the level or activity of one, two, three, four, five, ten, twenty or more of the biomarkers listed in Table 1A, Table 1B, Table 7A, Table 7B, Table 8, Table 9, Table 10, Table 14 (*e.g.*, CCL20, IL-17a and/or IL-6), Table 16, Table 17, Table 18, Table 20, FIG. 2B, PD-1, LAG-3, TIM-3, CD57, CD27, CD122, CD62L, KLRG1, or a CD19 CAR-expressing cell gene set signature;

(v) a cytokine level or activity (*e.g.*, quality of cytokine repertoire) in a CAR19-expressing cell product sample (*e.g.*, CTL019), wherein the cytokine is chosen from one, two, three, four, five or more (or all) of the cytokines listed in Table 16;

(vi) a transduction efficiency of a CAR-expressing cell in a manufactured CAR-expressing cell product sample; or

(vii) a quantity of CD27<sup>+</sup> PD-1<sup>-</sup> cells in a sample, *e.g.*, a quantity greater than or equal to  $1 \times 10^7$  cells.

39. The system of claim 37 or 38, wherein the sample comprises an apheresis sample.
40. The system of claim 37 or 38, wherein the sample comprises a manufactured CAR-expressing cell product sample.
41. The system of claim 37 or 38, wherein the CAR-expressing cell therapy comprises a CAR having an antigen binding domain that binds to CD19, optionally wherein the CAR-expressing cell therapy is a CAR19-expressing cell therapy, e.g., CTL019.
42. The system of claim 37 or 38, wherein the CAR-expressing cell therapy comprises a CAR having an antigen binding domain that binds to BCMA.