

What is claimed is:

1. A method of making a population of Chimeric Antigen Receptor (CAR) expressing immune effector cells, comprising:

- a) providing a population of immune effector cells, *e.g.*, T cells;
- b) contacting the population of immune effector cells with a nucleic acid encoding a CAR polypeptide;
- c) contacting the population of immune effector cells with a Stat3 activator; and
- d) maintaining the cells under conditions that allow expression of the CAR polypeptide, thereby making a population of CAR expressing immune effector cells.

2. The method of claim 1, wherein the Stat3 activator is chosen from, one, two, three, four, five, six, seven, eight, or all of, or any combination of:

- i) a gp130 activator, *e.g.*, an antibody molecule that binds to gp130, *e.g.*, an anti-gp130 antibody as described herein, or an IL-6 molecule, an IL-11 molecule, an IL-27 molecule, a CNTF molecule, a CT-1 molecule, a CLC molecule, a LIF molecule, a NP molecule, an OSM molecule;
- ii) a soluble IL-6 receptor (sIL-6R), *e.g.*, as described herein;
- iii) an IL-6/IL-6R complex, *e.g.*, a dimer, *e.g.*, as described herein;
- iv) an IL-6 family cytokine (*e.g.*, an IL-6 molecule, an IL-11 molecule, an IL-27 molecule, an IL-31 molecule, a CNTF molecule, a CT-1 molecule, a CLC molecule, a LIF molecule, a NP molecule or an OSM molecule);
- v) a CCL20 molecule;
- vi) an IL-10R2 receptor (IL-10R2) activator, *e.g.*, an IL-10 molecule, an IL-22 molecule, an IL-26 molecule, an IL-28A molecule, an IL-28B molecule, an IL-29 molecule, or an antibody molecule that binds to IL-10R2, *e.g.*, as described herein;
- vii) an IL-10 family cytokine (*e.g.*, an IL-10 molecule, an IL-19 molecule, an IL-20 molecule, an IL-22 molecule, an IL-24 molecule, an IL-26 molecule, an IL-28A molecule, an IL-28B molecule or an IL-29 molecule);
- viii) an IL-17 family cytokine (*e.g.*, an IL17A molecule, an IL17B molecule, an IL17C molecule, an IL17D molecule, an IL17E molecule or an IL17F molecule); or

~~ix) an IL-23 molecule.~~

~~3. The method of claim 1 or 2, wherein the method further comprises introducing into at least one cell of the population of immune effector cells:~~

~~a gp130 molecule, e.g., by introducing into the at least one cell of the population of immune effector cells a nucleic acid encoding the gp130 molecule under conditions that allow for translation of the gp130 molecule; or~~

~~a Stat3 molecule (e.g., a constitutively active Stat3 molecule (STAT3C)), e.g., by introducing into the at least one cell of the population of immune effector cells a nucleic acid encoding the Stat3 molecule under conditions that allow for translation of the Stat3 molecule.~~

~~4. A method of making a population of Chimeric Antigen Receptor (CAR)-expressing immune effector cells, comprising:~~

~~a) providing a population of immune effector cells, e.g., T cells;~~

~~b) contacting the population of immune effector cells with a nucleic acid encoding a CAR polypeptide;~~

~~c) introducing into at least one cell of the population of immune effector cells:~~

~~a gp130 molecule, e.g., by introducing into the at least one cell of the population of immune effector cells a nucleic acid encoding the gp130 molecule under conditions that allow for translation of the gp130 molecule; or~~

~~a Stat3 molecule (e.g., a constitutively active Stat3 molecule (STAT3C)), e.g., by introducing into the at least one cell of the population of immune effector cells a nucleic acid encoding the Stat3 molecule under conditions that allow for translation of the Stat3 molecule; and~~

~~d) maintaining the cells under conditions that allow expression of the CAR polypeptide, gp130 molecule or Stat3 molecule,~~

~~thereby making a population of CAR-expressing immune effector cells.~~

~~5. The method of claim 4, wherein the method further comprises contacting the population of immune effector cells with a Stat3 activator chosen from, one, two, three, four, five, six, seven, eight, or all of, or any combination of:~~

- ~~i) a gp130 activator, e.g., an antibody molecule that binds to gp130, e.g., an anti-gp130 antibody as described herein, or an IL-6 molecule, an IL-11 molecule, an IL-27 molecule, a CNTF molecule, a CT-1 molecule, a CLC molecule, a LIF molecule, a NP molecule, an OSM molecule;~~
- ~~ii) a soluble IL-6 receptor (sIL-6R), e.g., as described herein;~~
- ~~iii) an IL-6/IL-6R complex, e.g., a dimer, e.g., as described herein;~~
- ~~iv) an IL-6 family cytokine (e.g., an IL-6 molecule, an IL-11 molecule, an IL-27 molecule, an IL-31 molecule, a CNTF molecule, a CT-1 molecule, a CLC molecule, a LIF molecule, a NP molecule or an OSM molecule);~~
- ~~v) a CCL20 molecule;~~
- ~~vi) an IL-10R2 receptor (IL-10R2) activator, e.g., an IL-10 molecule, an IL-22 molecule, an IL-26 molecule, an IL-28A molecule, an IL-28B molecule, an IL-29 molecule, or an antibody molecule that binds to IL-10R2, e.g., as described herein;~~
- ~~vii) an IL-10 family cytokine (e.g., an IL-10 molecule, an IL-19 molecule, an IL-20 molecule, an IL-22 molecule, an IL-24 molecule, an IL-26 molecule, an IL-28A molecule, an IL-28B molecule or an IL-29 molecule);~~
- ~~viii) an IL-17 family cytokine (e.g., an IL17A molecule, an IL17B molecule, an IL17C molecule, an IL17D molecule, an IL17E molecule or an IL17F molecule); or~~
- ~~ix) an IL-23 molecule.~~

~~6. The method of any of claims 3-5, wherein expression of the gp130 molecule or the Stat3 molecule is transient (e.g., inducible or non-inducible) or constitutive.~~

~~7. The method of any of claims, 3-6, wherein the gp130 molecule or the Stat3 molecule is introduced into the population of immune effector cells, prior to, concurrently, or after contacting the population of immune effector cells with:~~

- ~~a nucleic acid encoding a CAR polypeptide; or~~
- ~~a Stat3 activator, e.g., as described herein.~~

~~8. The method of claim 3 or 4, wherein the nucleic acid comprising a nucleotide encoding a Stat3 molecule (e.g., a constitutively active Stat3 (STAT3C)), further comprises a nucleotide sequence encoding a CAR, e.g., a CD19 CAR.~~

- ~~9. The method of any of claims 1-3, or 5-8, wherein the Stat3 activator is an antibody molecule that binds to gp130, e.g., an anti-gp130 antibody as described herein.~~
- ~~10. The method of claim 9, which results in a population of T cells, e.g., CD4⁺ or CD8⁺ T cells, that is enriched for, e.g., early memory T cells or non-exhausted early memory T cells.~~
- ~~11. The method of claim 10, wherein early memory T cells have one or both of the following characteristics: CD27⁺ and/or CD45RO^{dim/neg}.~~
- ~~12. The method of claim 10, wherein non-exhausted early memory T cells have one or more, e.g., all, of the following characteristics: (i) PD-1 negative; (ii) CD27^{hi}; (iii) CCR7^{hi}; or (iv) CD45RO^{dim/neg}.~~
- ~~13. The method of any of claims 10-12, wherein the enriched population of T cells, e.g., early memory T cells or non-exhausted early memory T cells, e.g., has an increased level or amount of, e.g., at least 5%, e.g., 5-90% more (e.g., at least 5-10, 10-20, 20-30, 30-50, 50-70, or 70-90% more, e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30% more), early memory T cells or non-exhausted early memory T cells compared to an otherwise similar population of T cells that was not contacted with the Stat3 activator.~~
- ~~14. The method of any of claims 1-3, or 5-8, wherein the Stat3 activator comprises one, two, three, or all of: an IL-6 molecule, an IL-17 molecule, an IL-22 molecule or a CCL20 molecule.~~
- ~~15. The method of any of claims 1-3, or 5-9, wherein the Stat3 activator is a naturally occurring molecule, a recombinant molecule, or a purified molecule.~~
- ~~16. The method of any of claims 1-3, or 5-10, wherein the Stat3 activator is not present in serum, e.g., not present in an amount sufficient to activate Stat3, e.g., phosphorylate Stat3, e.g., on tyrosine 705 (Y705), e.g., as measured by an assay of Example 2.~~

~~17. The method of any of claims 1-16, wherein the Stat3 activator is situated, e.g., immobilized, on a substrate, e.g., bead or cell.~~

~~18. The method of claim 17, wherein the Stat3 activator is situated on a Stat3 activator cell.~~

~~19. The method of claim 18, wherein the Stat3 activator cell is an artificial antigen-presenting cell.~~

~~20. The method of any of claims 17-19, wherein the Stat3 activator is expressed by the Stat3 activator cell or is conjugated to the surface of the Stat3 activator cell.~~

~~21. The method of any of claims 1-3, 5, or 9-20 wherein the Stat3 activator, e.g., as described herein, is provided in an amount sufficient to activate Stat3, e.g., phosphorylate Stat3, e.g., on tyrosine 705 (Y705), e.g., as measured by an assay of Example 2.~~

~~22. The method of any of claims 1-3, 5, or 9-21, wherein the Stat3 activator, e.g., as described herein, is provided in an amount sufficient to expand the population of immune effector cells, by at least 1.1, 1.2, 1.3, 1.4, 1.5, 2, 3, 4, 5, 6, 7, 8, 9 fold or more after a 12 day culture period, e.g., as measured by an assay of Example 2, compared to an otherwise similar population of cells cultured under similar conditions but not contacted with the Stat3 activator.~~

~~23. The method of any of claims 1-3, 5, or 9-22, wherein the Stat3 activator, e.g., as described herein, is provided in an amount sufficient to increase the percentage of cells in the immune effector cell population that are CD27⁺ PD-1⁻, e.g., by at least 1.1, 1.2, 1.3, 1.4, 1.5, 2, 3, 4, 5, fold or greater, compared to an otherwise similar population of cells cultured under similar conditions but not contacted with the Stat3 activator.~~

~~24. The method of any of claims 1-3, 5, or 9-23, wherein the Stat3 activator, e.g., as described herein, is provided in an amount sufficient to increase the expression level of gp130 by at least 1.5, 2, 3, 4, 5, 10 fold or more, in the immune effector cell population, e.g., as measured by an assay of Example 2, compared to an otherwise similar population of cells cultured under similar conditions but not contacted with the Stat3 activator.~~

- ~~25. The method of any of claims 1-3, 5 or 9-24, wherein the Stat3 activator, *e.g.*, as described herein, is chosen from one, two, three, four, or all (*e.g.*, five) of: an IL-6 molecule, an IL-17 molecule, an IL-22 molecule, an IL31 molecule, and a CCL20 molecule.~~
- ~~26. The method of any of claims 1-3, 5, or 9-25 wherein the Stat3 activator, *e.g.*, as described herein, comprises an IL-6 molecule, *e.g.*, recombinant IL-6.~~
- ~~27. The method of claim 21 wherein the IL-6 molecule, *e.g.*, recombinant IL-6 is provided at an amount of at least 1, 5, 10, 15, 20, or 30 ng/ml, or in a range of 1-20, 1-15, or 5-15 ng/ml, *e.g.*, at least 10 ng/ml.~~
- ~~28. The method of claims 2, 5, or 9-13, wherein the anti-gp130 antibody molecule is chosen from B-S12 or B-P8 or an antibody molecule having 1, 2, 3, 4, 5, or 6 CDRs from B-S12 or B-P8.~~
- ~~29. The method of claim 28 which comprises contacting the population of immune effector cells with both of B-S12 and B-P8.~~
- ~~30. The method of any of claims 28 or 29, wherein the total amount of anti-gp130 antibody molecule is about 0.1-1000, 0.5-500, or 1-100 ug/ml.~~
- ~~31. The method of any of claims 28-29, wherein the anti-gp130 antibody molecule is provided at an amount of at least 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2 ug/ml, *e.g.*, about 1 ug/ml.~~
- ~~32. The method of any of claims 28-31, wherein the anti-gp130 antibody:~~
~~induces gp130 mediated signaling, as measured by phosphorylation of STAT3; or~~
~~induces dimerization, *e.g.*, homodimerization of gp130, or heterodimerization of gp130, *e.g.*, with LIF, OSM or CNTF.~~

~~33. The method of any of the preceding claims, wherein the population of cells cultured in the presence of the Stat3 activator, *e.g.*, as described herein, exhibits:~~

~~activation of Stat3, *e.g.*, phosphorylation of Stat3, *e.g.*, on tyrosine 705 (Y705), *e.g.*, as measured by an assay of Example 2;~~

~~expansion of the population of immune effector cells, by at least 1.1, 1.2, 1.3, 1.4, 1.5, 2, 3, 4, 5, 6, 7, 8, 9 fold or more after a 12 day culture period, *e.g.*, as measured by an assay of Example 2;~~

~~increase in the percentage of cells in the immune effector cell population that are CD27+ PD-1, *e.g.*, by at least 1.1, 1.2, 1.3, 1.4, 1.5, 2, 3, 4, 5, fold or greater; and/or~~

~~increase in the expression level of gp130 by at least 1.5, 2, 3, 4, 5, or 10 fold or more, in the immune effector cell population, *e.g.*, as measured by an assay of Example 2,~~

~~compared to an otherwise similar population of cells cultured under similar conditions but not contacted with the Stat3 activator.~~

~~34. The method of any of the preceding claims, comprising expanding the population, *e.g.*, for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 days or for 1-7, 7-14, or 14-21 days.~~

~~35. The method of any of the preceding claims, further comprising assaying Stat3 pathway activation in the population of immune effector cells by measuring the level or activity of Stat3 transcriptional targets, *e.g.*, c-Myc, c-Fos, Sox2, Bcl-2, or RORC to determine a value for Stat3 pathway activation.~~

~~36. The method of claim 35, further comprising comparing the Stat3 pathway activation value with a reference value, wherein the reference value is obtained from an otherwise similar population of immune effector cells cultured under similar conditions but not contacted with the Stat3 activator, *e.g.*, as described herein.~~

~~37. The method of any of the preceding claims, further comprising, responsive to the comparison of the Stat3 pathway activation value with reference value, performing one or more of:~~

~~classifying the population as suitable or not suitable for use as a therapeutic;~~

~~formulating or packaging the population, or an aliquot thereof, for therapeutic use; or altering a culture parameter, e.g., i) altering the length of time in culture or ii) increasing or decreasing the concentration of the Stat3 activator, e.g., as described herein.~~

~~38. A method of making a population of Chimeric Antigen Receptor (CAR)-expressing immune effector cells, comprising:~~

- ~~a) providing a population of immune effector cells, e.g., T cells;~~
- ~~b) contacting the population of immune effector cells with a nucleic acid encoding a CAR polypeptide;~~
- ~~c) contacting the population of immune effector cells with an inhibitor of glycolysis, e.g., a small molecule inhibitor of glycolysis, e.g., a small molecule hexokinase inhibitor, e.g., a glucose analog, e.g., 2-deoxy D-glucose (2-DG), and~~
- ~~d) maintaining the cells under conditions that allow expression of the CAR polypeptide, thereby making a population of CAR-expressing immune effector cells.~~

~~39. The method of claim 38, wherein the inhibitor of glycolysis, e.g., a small molecule inhibitor of glycolysis, e.g., a small molecule hexokinase inhibitor, e.g., a glucose analog, e.g., 2-DG, is added in an amount sufficient to:~~

- ~~increase the population of immune effector cells at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100% or greater; or~~
- ~~increase the percentage of cells in the immune effector cell population that have a central memory phenotype, e.g., are CD45RO⁺CCR7⁺, e.g., by about at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100% or greater;~~
- ~~compared to an otherwise similar population of cells cultured under similar conditions but not treated with the inhibitor of glycolysis.~~

~~40. The method of claim 38 or 39, wherein the inhibitor of glycolysis, e.g., 2-DG, is added at a concentration of at least 0.5, 1, 1.5, 2, or 2.5mM, 0.5-2.5 mM, or 1-2 mM.~~

~~41. The method of any of claims 38-40, wherein the population of cells cultured in the presence of the glycolysis inhibitor exhibits:~~

~~an increase the population of immune effector cells at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100% or greater; or~~

~~an increase the percentage of cells in the immune effector cell population that have a central memory phenotype, *e.g.*, are CD45RO+CCR7+, *e.g.*, by about at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100% or greater;~~

~~compared to an otherwise similar population of cells cultured under similar conditions but not treated with the inhibitor of glycolysis.~~

~~42. The method of any of claims 38-41, comprising:~~

~~expanding the population, *e.g.*, for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 days or for 1-7, 7-14, or 14-21 days; or~~

~~expanding the population, *e.g.*, by at least a 1.5, 2, 2.5, 3, 4, 5, 5, 7, 8, 9, 10, 20, 30, 40, 50 fold change in cell number or more, *e.g.*, up to about 40 or 50 fold, *e.g.*, under growth conditions of Example 1.~~

~~43. The method of any of claims 38-42, further comprising assaying glucose metabolism in the population of immune effector cells to determine a glucose metabolism value, *e.g.*, using 2-NBDG uptake assay, *e.g.*, an assay of Example 1.~~

~~44. The method of any of claims 38-43, further comprising comparing the glucose metabolism value with a reference value.~~

~~45. The method of any of claims 38-44, further comprising, responsive to the comparison of the glucose metabolism value with reference value, performing one or more of:~~

~~classifying the population as suitable or not suitable for use as a therapeutic;~~

~~formulating or packaging the population, or an aliquot thereof, for therapeutic use; or~~

~~altering a culture parameter, *e.g.*, i) altering the length of time in culture or ii) increasing or decreasing the concentration of the inhibitor of glycolysis, *e.g.*, the small molecule inhibitor of glycolysis, *e.g.*, the small molecule hexokinase inhibitor, *e.g.*, the glucose analog, *e.g.*, 2-deoxy-D-glucose (2-DG).~~

~~46. The method of any of claims 38-45, further comprising contacting the population of immune effector cells with a Stat3 activator listed in claim 1, or a population of cells listed in claims 3 or 4.~~

~~47. The method of any of claims 1-37, further comprising contacting the population of immune effector cells with an inhibitor of glycolysis, e.g., the small molecule inhibitor of glycolysis, e.g., the small molecule hexokinase inhibitor, e.g., a glucose analog, e.g., 2-deoxy D-glucose (2-DG).~~

~~48. The method of any of the preceding claims, wherein (b) is performed before (c), (c) is performed before (b), or (b) and (c) are performed simultaneously.~~

~~49. The method of any of the preceding claims, wherein the nucleic acid is DNA or RNA.~~

~~50. The method of any of the preceding claims, wherein (b) comprises performing lentiviral transduction to deliver the nucleic acid to the immune effector cells.~~

~~51. The method of any of the preceding claims, further comprising contacting the population of immune effector cells with a population of cells that expresses an antigen (e.g., CD19) that binds the CAR.~~

~~52. The method of any of the preceding claims, further comprising contacting the population of immune effector 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, e.g., wherein the agent is a bead conjugated with an anti-CD3 antibody, or a fragment thereof, and/or an anti-CD28 antibody, or a fragment thereof.~~

~~53. The method of any of the preceding claims, wherein the CAR polypeptide is a CD19 CAR, a CD22 CAR, a CD123 CAR, a BCMA CAR, an EGFRvIII CAR, a CLL-1 CAR, a CD20 CAR, or a CD33 CAR.~~

~~54. The method of any of the preceding claims, wherein the CAR is a CD19 CAR, e.g., a CAR~~

~~comprising an scFv amino acid sequence of SEQ ID NO: 39-51 or a CAR comprising the amino acid sequence of SEQ ID NO: 77-89.~~

~~55. The method of any of the preceding claims, wherein the CAR comprises an antibody molecule which includes an anti-CD19 binding domain, a transmembrane domain, and an intracellular signaling domain comprising a stimulatory domain, and wherein said anti-CD19 binding domain comprises one or more of light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of any anti-CD19 light chain binding domain amino acid sequence listed in Table 3B, and one or more of heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of any anti-CD19 heavy chain binding domain amino acid sequence listed in Table 3A.~~

~~56. The method of claim 50, wherein the anti-CD19 binding domain comprises a sequence of SEQ ID NO: 40, or SEQ ID NO: 51.~~

~~57. The method of any of claims 54-56, wherein the CAR comprises a polypeptide having a sequence of SEQ ID NO: 78, or SEQ ID NO: 89.~~

~~58. A reaction mixture comprising:~~

- ~~a) (i) a population of CAR-expressing immune effector cells (e.g., a CAR-expressing cell described herein, e.g., a CD19-CAR-expressing cell) or (ii) an immune effector cell and a nucleic acid encoding a CAR (e.g., a CAR described herein, e.g., a CD19-CAR); and~~
- ~~b) an agent selected from:~~
 - ~~(i) a Stat3 activator;~~
 - ~~(ii) a cell or population of cells listed in claims 3 or 4; or~~
 - ~~(iii) a gp130 molecule or a Stat3 molecule, or nucleic acid encoding a gp130 molecule or a Stat3 molecule.~~

~~59. The reaction mixture of claim 58, wherein the Stat3 activator is chosen from:~~

~~b-i-i) a gp130 activator, e.g., an antibody molecule that binds to gp130, e.g., an anti-gp130 antibody as described herein, or an IL-6 molecule, an IL-11 molecule, an IL-27 molecule, a CNTF molecule, a CT-1 molecule, a CLC molecule, a LIF molecule, a NP molecule, an OSM molecule;~~

~~b-i-ii) a soluble IL-6 receptor (sIL-6R), e.g., as described herein;~~

~~b-i-iii) an IL-6/IL-6R complex, e.g., a dimer, e.g., as described herein;~~

~~b-i-iv) an IL-6 family cytokine (e.g., an IL-6 molecule, an IL-11 molecule, an IL-27 molecule, an IL-31 molecule, a CNTF molecule, a CT-1 molecule, a CLC molecule, a LIF molecule, a NP molecule or an OSM molecule);~~

~~b-i-v) a CCL20 molecule;~~

~~b-i-vi) an IL-10R2 receptor (IL-10R2) activator, e.g., an IL-10 molecule, an IL-22 molecule, an IL-26 molecule, an IL-28A molecule, an IL-28B molecule, an IL-29 molecule, or an antibody molecule that binds to IL-10R2, e.g., as described herein;~~

~~b-i-vii) an IL-10 family cytokine (e.g., an IL-10 molecule, an IL-19 molecule, an IL-20 molecule, an IL-22 molecule, an IL-24 molecule, an IL-26 molecule, an IL-28A molecule, an IL-28B molecule or an IL-29 molecule);~~

~~b-i-viii) an IL-17 family cytokine (e.g., an IL17A molecule, an IL17B molecule, an IL17C molecule, an IL17D molecule, an IL17E molecule or an IL17F molecule); or~~

~~b-i-ix) an IL-23 molecule.~~

~~60. The reaction mixture of claim 58 or 59, which comprises (a)(i) a population of CAR-expressing immune effector cells.~~

~~61. The reaction mixture of claim 58 or 59, which comprises (a)(ii) a nucleic acid encoding a CAR (e.g., a CAR described herein, e.g., a CD19 CAR).~~

~~62. The reaction mixture of any of claims 58-61, which comprises (b)(i) a Stat3 activator listed in claim 59.~~

~~63. The reaction mixture of any of claims 58-61, which comprises (b)(ii) the cell or population of cells listed in claims 3 or 4.~~

~~64. The reaction mixture of any of claims 58-61, which comprises (b)(iii) a gp130 molecule, or a Stat3 molecule, or nucleic acid encoding same.~~

~~65. The reaction mixture of claim 58, which comprises: (a)(i) a population of CAR-expressing immune effector cells; and (b)(i) a Stat3 activator listed in claim 59.~~

~~66. The reaction mixture of claim 58, which comprises: (a)(i) a population of CAR-expressing immune effector cells; and (b)(ii) the cell or population of cells listed in claims 3 or 4.~~

~~67. The reaction mixture of claim 58, which comprises: (a)(i) a population of CAR-expressing immune effector cells; and (b)(iii) a gp130 molecule, or a Stat3 molecule, or nucleic acid encoding same.~~

~~68. The reaction mixture of claim 58, which comprises: (a)(ii) a nucleic acid encoding a CAR; and (b)(i) a Stat3 activator listed in claim 59.~~

~~69. The reaction mixture of claim 58, which comprises: (a)(ii) a nucleic acid encoding a CAR; and (b)(ii) the cell or population of cells of claims 3 or 4.~~

~~70. The reaction mixture of claim 53, which comprises: (a)(ii) a nucleic acid encoding a CAR; and (b)(iii) a gp130 molecule, or a Stat3 molecule, or nucleic acid encoding a gp130 molecule or a Stat3 molecule.~~

~~71. The reaction mixture of any of claims 58-62, 65 or 68, which comprises one or more of: (a)(i) and b-i-i); (a)(i) and b-i-ii); (a)(i) and b-i-iii); (a)(i) and b-i-iv); (a)(i) and b-i-v); (a)(i) and b-i-vi); (a)(i) and b-i-vii); (a)(i) and b-i-viii); (a)(ii) and b-i-i); (a)(ii) and b-i-ii); (a)(ii) and b-i-iii); (a)(ii) and b-i-iv); (a)(ii) and b-i-v); (a)(ii) and b-i-vi); (a)(ii) and b-i-vii); and (a)(ii) and b-i-viii).~~

~~72. A reaction mixture comprising:~~

- ~~a) a population of CAR-expressing immune effector cells, e.g., a CAR-expressing cell described herein, e.g., a CD19 CAR-expressing cell, and~~
- ~~b) an inhibitor of glycolysis, e.g., a small molecule inhibitor of glycolysis, e.g., a small molecule hexokinase inhibitor, e.g., a glucose analog, e.g., 2-deoxy D-glucose (2-DG).~~

~~73. A reaction mixture comprising:~~

- ~~a) a population of immune effector cells,~~
- ~~b) a nucleic acid encoding a CAR, e.g., a CAR described herein, e.g., a CD19 CAR, and~~
- ~~c) an inhibitor of glycolysis, e.g., a small molecule inhibitor of glycolysis, e.g., a small molecule hexokinase inhibitor, e.g., a glucose analog, e.g., 2-deoxy D-glucose (2-DG).~~

~~74. The reaction mixture of claim 72 or 73, wherein the inhibitor of glycolysis, e.g., 2-DG, is present at a concentration of at least 0.5, 1, 1.5, 2, 2.5mM, 0.5-2.5 mM, or 1-2 mM.~~

~~75. The reaction mixture of any of claims 72-74, further comprising a Stat3 activator; a cell or population of cells; or a gp130 molecule, or a Stat3 molecule, or nucleic acid encoding a gp130 molecule or a Stat3 molecule, listed in claim 58 or 59.~~

~~76. The reaction mixture of any of claims 59-71, further comprising an inhibitor of glycolysis, e.g., a small molecule inhibitor of glycolysis, e.g., a small molecule hexokinase inhibitor, e.g., a glucose analog, e.g., 2-deoxy D-glucose (2-DG).~~

~~77. The reaction mixture of any of claims 59-76, further comprising a lentivirus, e.g., wherein the nucleic acid encoding a CAR is packaged in a lentivirus.~~

~~78. The reaction mixture of any of claims 58-77, wherein the nucleic acid is DNA or RNA.~~

~~79. The reaction mixture of any of claims 58-78, further comprising a population of cells that expresses an antigen (e.g., CD19) that binds the CAR.~~

~~80. A method of evaluating or predicting the responsiveness of a subject having a cancer (e.g., a cancer described herein), to a therapeutic treatment with a CAR-expressing cell, e.g., prior to~~

~~administration of the CAR-expressing cell, comprising evaluating in an immune effector cell from the subject:~~

~~i) a level of glucose metabolism, wherein:~~

~~a level of glucose metabolism that is lower than a glucose metabolism reference value is indicative that the subject is likely to respond to treatment with the CAR-expressing cell, e.g., to exhibit a complete response or a partial response, and~~

~~a level of glucose metabolism that is higher than a glucose metabolism reference value is indicative that the subject is less likely to respond to treatment with the CAR-expressing cell, e.g., does not exhibit a complete response or partial response; or~~

~~ii) a level of Stat3 activation as measured by, e.g., phosphorylation of Stat3 (e.g., on tyrosine 705 (Y705)) or level or activity of Stat3 transcriptional targets (e.g., c-Myc, c-Fos, Sox2 or Bel-2), wherein:~~

~~a level of Stat3 activation that is higher than a Stat3 activation reference value is indicative that the subject is likely to respond to treatment with the CAR-expressing cell, e.g., to exhibit a complete response or a partial response, and~~

~~a level of Stat3 activation that is lower than a Stat3 activation reference value is indicative that the subject is less likely to respond to treatment with the CAR-expressing cell, e.g., does not exhibit a complete response or partial response;~~

~~thereby evaluating the subject, or predicting the responsiveness of the subject to the CAR-expressing cell.~~

~~81. The method of claim 80, wherein the immune effector cell has not been contacted with a nucleic acid encoding a CAR.~~

~~82. The method of claim 80, wherein the immune effector cell has been contacted with a nucleic acid encoding a CAR, e.g., expresses a CAR polypeptide.~~

~~83. The method of any of claims 80-82, wherein the immune effector cell has been contacted with:~~

~~i) a Stat3 activator listed in claim 1;~~

~~ii) a cell or population of cells listed in claims 3 or 4;~~

~~iii) a gp130 molecule, or a Stat3 molecule, or nucleic acid encoding a gp130 molecule or a Stat3 molecule; or~~

~~iv) an inhibitor of glycolysis, e.g., a small molecule inhibitor of glycolysis, e.g., a small molecule hexokinase inhibitor, e.g., a glucose analog, e.g., 2-deoxy-D-glucose (2-DG) at a concentration of at least 0.5, 1, 1.5, 2, or 2.5mM.~~

~~84. The method of any of claims 80-83, wherein the method further comprises determining a fold change in cell number, e.g., number of CAR-expressing cells.~~

~~85. The method of any of claims 80-84, wherein the subject who is less likely to respond to treatment with the CAR-expressing cell is predicted, e.g., to not have a complete response (CR) or a partial response (PR), e.g., to be a non-responder (NR).~~

~~86. The method of any of claims 80-85, wherein, responsive to determination that:~~
~~i) the level of glucose metabolism is lower than the glucose metabolism reference value; or~~
~~ii) the level of Stat3 activation is higher than the Stat3 activation reference value,~~
~~the subject is selected for administration of, or is administered, a CAR-expressing therapy.~~

~~87. The method of any of claims 80-85, wherein, responsive to determination that:~~
~~i) the level of glucose metabolism is higher than the glucose metabolism reference value; or~~
~~ii) the level of Stat3 activation is lower than the Stat3 activation reference value,~~
~~the subject is selected for administration of, or is administered, a therapy other than a CAR-expressing therapy.~~

~~88. The method of any of claims 80-87, wherein the glucose metabolism reference value is the glucose metabolism value of a cell of a complete responder subject as described in Example 1, e.g., wherein the cell (e.g., a sample containing the cell) is contacted with mock stimulation, e.g., stimulation with an antigen other than the CAR antigen, e.g., as described in Example 1.~~

~~89. The method of any of claims 80-87, wherein the Stat3 activation reference value is the Stat3 activation value of a cell of a non-responder subject, e.g., as described in Example 2.~~

~~90. A method of evaluating or predicting the responsiveness of a subject having a cancer (e.g., a cancer described herein), wherein the subject has been treated with a CAR-expressing cell, comprising evaluating in a CAR-expressing cell from the subject:~~

~~i) a level of glucose metabolism, wherein:~~

~~a level of glucose metabolism that is lower than a glucose metabolism reference value is indicative that the subject is likely to respond to treatment with the CAR-expressing cell, e.g., to exhibit a complete response or a partial response, and~~

~~a level of glucose metabolism that is higher than a glucose metabolism reference value is indicative that the subject is less likely to respond to treatment with the CAR-expressing cell, e.g., does not exhibit a complete response or partial response; or~~

~~ii) a level of Stat3 activation as measured by, e.g., phosphorylation of Stat3 (e.g., on tyrosine 705 (Y705)) or level or activity of Stat3 transcriptional targets (e.g., c-Myc, c-Fos, Sox2 or Bcl-2), wherein:~~

~~a level of Stat3 activation that is higher than a Stat3 activation reference value is indicative that the subject is likely to respond to treatment with the CAR-expressing cell, e.g., to exhibit a complete response or a partial response, and~~

~~a level of Stat3 activation that is lower than a Stat3 activation reference value is indicative that the subject is less likely to respond to treatment with the CAR-expressing cell, e.g., does not exhibit a complete response or partial response,~~

~~thereby evaluating the subject, or predicting the responsiveness of the subject to the CAR-expressing cell.~~

~~91. The method of claim 90, further comprising obtaining the CAR-expressing cell from the subject prior to evaluating the level of glucose metabolism, or the level of Stat3 activation in the CAR-expressing cell.~~

~~92. The method of claim 90 or 91, wherein the subject who is less likely to respond to treatment with the CAR-expressing cell is predicted e.g., to not have a complete response (CR) or a partial response (PR), e.g., to be a non-responder (NR).~~

~~93. The method of any of claims 90-92, wherein, responsive to determination that:~~

~~i) the level of glucose metabolism is lower than the glucose metabolism reference value; or~~

ii) the level of Stat3 activation is higher than the Stat3 activation reference value, the subject is selected for administration of, or is administered, one or more additional doses of the CAR-expressing therapy.

94. The method of any of claims 90-93, wherein, responsive to determination that:

i) the level of glucose metabolism is higher than the glucose metabolism reference value; or
 ii) the level of Stat3 activation is lower than the Stat3 activation reference value, the subject is selected for administration of, or is administered, a therapy other than a CAR-expressing therapy.

95. The method of any of claims 90-94, wherein the glucose metabolism reference value is the glucose metabolism value of a cell of a complete responder subject as described in Example 1, *e.g.*, wherein the cell (*e.g.*, a sample containing the cell) is contacted with mock stimulation, *e.g.*, stimulation with an antigen other than the CAR antigen, *e.g.*, as described in Example 1.

96. The method of any of claims 90-95, wherein the Stat3 activation reference value is the Stat3 activation value of a cell of a non-responder subject, *e.g.*, as described in Example 2.

97. A method of evaluating a CAR-expressing cell, *e.g.*, CAR19-expressing cell, (*e.g.*, CTL019), said method comprising evaluating in the CAR-expressing cell in a sample from a subject:

i) a level of glucose metabolism, wherein:

a level of glucose metabolism that is lower than a glucose metabolism reference value is indicative that the sample is suitable for treatment, and

a level of glucose metabolism that is higher than a glucose metabolism reference value is indicative that the sample is less suitable for treatment; or

ii) a level of Stat3 activation, wherein:

a level of Stat3 activation that is higher than a Stat3 activation reference value is indicative that the sample is suitable for treatment, and

a level of Stat3 activation that is lower than a Stat3 activation reference value is indicative that the sample is less suitable for treatment,

thereby evaluating the CAR-expressing cell.

~~98. The method of claim 97, further comprising obtaining the CAR-expressing cell from the subject prior to evaluating the level of glucose metabolism or Stat3 activation in the CAR-expressing cell.~~

~~99. The method of claims 97 or 98, wherein, responsive to determination that:~~
~~i) the level of glucose metabolism is lower than the glucose metabolism reference value; or~~
~~ii) the level of Stat3 activation is higher than the Stat3 activation reference value,~~
~~the sample is selected for administration, or is administered, to the subject.~~

~~100. The method of claims 97 or 98, wherein, responsive to determination that:~~
~~i) the level of glucose metabolism is higher than the glucose metabolism reference value,~~
~~ii) the level of Stat3 activation is lower than the Stat3 activation reference value,~~
~~the sample is not selected for administration, or is not administered, to the subject.~~

~~101. The method of any of claims 97-100, wherein the glucose metabolism reference value is the glucose metabolism value of a cell of a complete responder subject as described in Example 1, e.g., wherein the cell (e.g., a sample containing the cell) is contacted with mock stimulation, e.g., stimulation with an antigen other than the CAR antigen, e.g., as described in Example 1.~~

~~102. The method of any of claims 97-100, wherein the Stat3 activation reference value is the Stat3 activation value of a cell of a non-responder subject, e.g., as described in Example 2.~~

CLAIMS

1. A method of predicting the responsiveness of a subject having a cancer described herein, to a therapeutic treatment with a CAR-expressing cell prior to administration of the CAR-expressing cell, comprising evaluating in a sample comprising an immune effector cell from the subject:

i) a level of glucose metabolism, wherein:

a level of glucose metabolism that is lower than a glucose metabolism reference value is indicative that the subject is likely to exhibit a complete response or a partial response to treatment with the CAR-expressing cell, and

a level of glucose metabolism that is higher than a glucose metabolism reference value is indicative that the subject is less likely to exhibit a complete response or partial response to treatment with the CAR-expressing cell,

wherein the glucose metabolism reference value is the glucose metabolism value of a cell of a complete responder subject as described in Example 1, and wherein the sample containing the cell is contacted with mock stimulation that is stimulation with an antigen other than the CAR antigen, as described in Example 1; or

ii) a level of Stat3 activation as measured by phosphorylation of Stat3 on tyrosine 705 (Y705) or level or activity of Stat3 transcriptional targets c-Myc, c-Fos, Sox2, or Bcl-2, wherein:

a level of Stat3 activation that is higher than a Stat3 activation reference value is indicative that the subject is likely to exhibit a complete response or a partial response to treatment with the CAR-expressing cell, and

a level of Stat3 activation that is lower than a Stat3 activation reference value is indicative that the subject is less likely to exhibit a complete response or partial response to treatment with the CAR-expressing cell,

thereby predicting the responsiveness of the subject to the CAR-expressing cell.