

To date, clinical efficacy of engineered T cell adoptive immunotherapies is limited by poor T cell expansion and persistence after infusion into patients. Therefore, such therapies are not suitable for widespread clinical use. Accordingly, there is a persistent, unmet need for improvements in T cell manufacturing and therapeutic T cell compositions that survive, expand, and persist *in vivo*.

SUMMARY

The disclosure generally provides adoptive T cell immunotherapies comprising persistent and potent anti-tumor T cell compositions and methods of making the same.

The present disclosure relates to improved T cell compositions and methods for manufacturing T cells. More particularly, the disclosure relates to methods of T cell manufacturing that result in with improved survival, expansion, and persistence *in vivo*.

In various embodiments, a method for manufacturing T cells comprising: (a) isolating a population of T cells, *e.g.*, tumor infiltrating cytotoxic T lymphocytes (TIL), from a subject; (b) activating the population of T cells and stimulating the population of T cells to proliferate, wherein the activation and stimulation steps are performed in the presence of an inhibitor of AKT/mTOR pathway; (c) culturing the T cells to proliferate; wherein the activating and stimulating steps performed in the presence of the inhibitor of the PI3K/AKT/mTOR pathway results in maintaining proliferation of the T cells compared to the proliferation of T cells that were activated and stimulated in the absence of the inhibitor of the PI3K/AKT/mTOR pathway is provided.

In various embodiments, a method for manufacturing T cells comprising: (a) activating a population of T cells and stimulating the population of T cells to proliferate, wherein the activation and stimulation steps are performed in the presence of an inhibitor of AKT/mTOR pathway; (b) transducing the T cells with a viral vector comprising an engineered T cell receptor (TCR) or a chimeric antigen receptor (CAR); (c) culturing the transduced T cells to proliferate; wherein the activating and stimulating steps performed in the presence of the inhibitor of the PI3K/AKT/mTOR pathway results in maintaining proliferation of the transduced T cells compared to the proliferation of transduced T cells that were activated and stimulated in the absence of the inhibitor of the PI3K/AKT/mTOR pathway is provided.

In a first aspect, there is provided a method for manufacturing T cells comprising: (a) activating a population of T cells and stimulating the population of T cells to proliferate, wherein the activation and stimulation steps are performed in the presence of a PI3K inhibitor; (b) transducing the T cells with a viral vector comprising an engineered T cell receptor (TCR) or a chimeric antigen receptor (CAR); (c) culturing the transduced T cells to proliferate; wherein the activating and stimulating steps performed in the presence of the PI3K inhibitor results in maintaining proliferation of the transduced T cells compared to the proliferation of transduced T cells that were activated and stimulated in the absence of the PI3K inhibitor.

the activated T cells to proliferate; wherein the restimulated T cells have maintained proliferation and decreased differentiation compared to the proliferation of T cells that were stimulated or restimulated in the absence of the inhibitor of the PI3K/AKT/mTOR pathway is provided. In a second aspect, there is provided a method for maintaining the proliferation and decreasing the differentiation of restimulated T cells expressing an engineered TCR or CAR comprising: (a) contacting all or a portion of a population of proliferated T cells comprising an engineered TCR or CAR with an anti-CD3 antibody or CD3-binding fragment thereof, and an anti-CD28 antibody or CD28-binding fragment thereof, which stimulates a CD28 accessory molecule on the surface of the T cells, in the presence of a PI3K inhibitor, thereby restimulating the activated T cells to proliferate; wherein the restimulated T cells have maintained proliferation and decreased differentiation compared to the proliferation of T cells that restimulated in the absence of the PI3K inhibitor.

In particular embodiments, the cells comprise an engineered TCR.

In certain embodiments, the cells comprise a CAR.

In other particular embodiments, the cells comprise a viral vector encoding an engineered TCR or CAR.

In additional embodiments, the vector is a retroviral vector.

In additional embodiments, the vector is a lentiviral vector.

In particular embodiments, the CAR comprises: an extracellular domain that binds an antigen selected from the group consisting of: alpha folate receptor, 5T4, $\alpha\text{v}\beta 6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD19, CD20, CD22, CD30, CD33, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD138, CD171, CEA, CSPG4, EGFR, EGFR family including ErbB2 (HER2), EGFRvIII, EGP2, EGP40, EPCAM, EphA2, EpCAM, FAP, fetal AchR, FR α , GD2, GD3, 'Glypican-3 (GPC3), HLA-A1+MAGE1, HLA-A2+MAGE1, HLA-A3+MAGE1, HLA-A1+NY-ESO-1, HLA-A2+NY-ESO-1, HLA-A3+NY-ESO-1, IL-11R α , IL-13R $\alpha 2$, Lambda, Lewis-Y, Kappa, Mesothelin, Muc1, Muc16, NCAM, NKG2D Ligands, NY-ESO-1, PRAME, PSCA, PSMA, ROR1, SSX, Survivin, TAG72, TEMs, and VEGFR2; a transmembrane domain derived from a polypeptide selected from the group consisting of: CD8 α ; CD4, CD28, CD45, PD-1, and CD152; one or more intracellular costimulatory signaling domains selected from the group consisting of: CD28, CD54 (ICAM), CD134 (OX40), CD137 (41BB), CD152 (CTLA4), CD273 (PD-L2), CD274 (PD-L1), and CD278 (ICOS); and a CD3 ζ signaling domain.

In some embodiments, the extracellular domain comprises an antibody or antigen binding fragment that binds the antigen.

In certain embodiments, the transmembrane domain is derived from CD8 α or CD28.

In further embodiments, the one or more costimulatory signaling domains are selected from the group consisting of: CD28, CD134, and CD137.

In particular embodiments, the CAR comprises a hinge region polypeptide.

In further embodiments, the hinge region polypeptide comprises a hinge region of IgG1 or CD8 α .

In further embodiments, the inhibitor of the PI3K/AKT/mTOR pathway is a pan-mTOR inhibitor selected from the group consisting of: AZD8055, INK128, and rapamycin.

In additional embodiments, the inhibitor of the PI3K/AKT/mTOR pathway is a selective mTORC1 inhibitor selected from the group consisting of: PF-04691502 and everolimus.

5 In some embodiments, the inhibitor of the PI3K/AKT/mTOR pathway is a s6 kinase inhibitor selected from the group consisting of: BI-D1870, H89, PF-4708671, FMK, and AT7867.

In other particular embodiments, the inhibitor of the PI3K/AKT/mTOR pathway is a DNA-PK inhibitor selected from the group consisting of: NU7441, PI-103, NU7026, PIK-75, and PP-121.

10 In particular embodiments, the population of activated T cells restimulated in the presence of an inhibitor of PI3K/AKT/mTOR pathway have an increased number of T cells expressing one or more markers selected from the group consisting of: CD62L, CCR7, CD28, CD27, CD122, and CD127 compared to a population of T cells activated and stimulated in the absence of the inhibitor of PI3K/AKT/mTOR pathway.

15 In additional embodiments, the population of activated T cells restimulated in the presence of an inhibitor of PI3K/AKT/mTOR pathway do not express CD57 or KLRG1 or express less CD57 or KLRG1 compared to a population of T cells activated and stimulated in the absence of the inhibitor of PI3K/AKT/mTOR pathway.

In various embodiments, a population of T cells comprising a vector comprising an
20 engineered TCR or CAR, wherein the cells have been activated and stimulated to proliferate in the presence of an inhibitor of PI3K/AKT/mTOR pathway is provided.

In various particular embodiments, a population of T cells comprising a vector comprising an engineered TCR or CAR, wherein the cells have been activated and stimulated to proliferate in the presence of an inhibitor of PI3K/AKT/mTOR pathway and
25 have been restimulated by contacting all or a portion of a population of proliferated immune effector cells with an anti-CD3 antibody or CD3-binding fragment thereof, and an anti-CD28 antibody or CD28-binding fragment thereof, which stimulates a CD28 accessory molecule on the surface of the immune effector cells is provided. In a third aspect, there is provided a population of immune effector cells comprising a vector encoding an
30 engineered TCR or CAR, wherein the cells have been activated and stimulated to proliferate in the presence of a PI3K inhibitor, wherein activation and stimulation performed in the presence of the PI3K inhibitor increases expression of one or more of CD62L, CCR7, CD28, CD27, CD122, or CD127 and decreases expression of CD57 or KLRG1 in the population of immune effector cells compared to a population of
35 immune effector cells activated and stimulated in the absence of the PI3K inhibitor.

In certain embodiments, the immune effector cells comprise T cells.

In one embodiment, the immune effector cells are TILs.

In various embodiments, a composition comprising a population of immune effector cells contemplated herein and a physiologically acceptable excipient is provided. In a fourth aspect, there is provided a composition comprising the population of immune effector cells of the third aspect and a physiologically acceptable excipient.

5 In various certain embodiments, a method of treating a cancer in a subject in need thereof, comprising administering to the subject a therapeutically effect amount of a T cell composition contemplated herein is provided. In a fifth aspect, there is provided use of the composition of the fourth aspect in the manufacture of a medicament for treating a cancer in a subject in need thereof.

10 In particular embodiments, the cancer is selected from the group consisting of Wilms' tumor, Ewing sarcoma, a neuroendocrine tumor, a glioblastoma, a neuroblastoma, a melanoma, skin cancer, breast cancer, colon cancer, rectal cancer, prostate cancer, liver cancer, renal cancer, pancreatic cancer, lung cancer, biliary cancer, cervical cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancer, medullary
15 thyroid carcinoma, ovarian cancer, glioma, lymphoma, leukemia, myeloma, acute lymphoblastic leukemia, acute myelogenous leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, and urinary bladder cancer.

 In one embodiment, the cancer is associated with or caused by a viral infection, *e.g.*,
20 infection by CMV, HPV, or EBV.

 In additional embodiments, the cancer is pancreatic cancer and the extracellular binding domain binds an epitope of PSCA or MUC1

 In some embodiments, the cancer is bladder cancer and the extracellular binding domain binds an epitope of PSCA or MUC1

25 In further embodiments, the cancer is glioblastoma multiforme and the extracellular binding domain binds an epitope of EPHA2, EGFRvIII, or CSPG4.

 In particular embodiments, the cancer is lung cancer and the extracellular binding domain binds an epitope of PSCA or GD2.

 In certain embodiments, the cancer is breast cancer and the extracellular binding
30 domain binds an epitope of CSPG4 or HER2.

 In additional embodiments, the cancer is melanoma and the extracellular binding domain binds an epitope of CSPG4 or GD2.

 In particular embodiments, the cancer is a B-cell malignancy and the binding domain binds an epitope of BCMA.

35 In various embodiments, a method of treating a hematological malignancy in a subject in need thereof, comprising administering to the subject a therapeutically effect amount of a T cell composition contemplated herein is provided.

In a sixth aspect, there is provided use of the composition of the fourth aspect in the manufacture of a medicament for treating a hematological malignancy in a subject in need thereof.

In certain embodiments, the hematological malignancy is a B-cell malignancy selected from the group consisting of: multiple myeloma (MM), chronic lymphocytic leukemia (CLL), or non-Hodgkin's lymphoma (NHL).

In particular embodiments, the MM is selected from the group consisting of: overt multiple myeloma, smoldering multiple myeloma, plasma cell leukemia, non-secretory myeloma, IgD myeloma, osteosclerotic myeloma, solitary plasmacytoma of bone, and extramedullary plasmacytoma.

In certain embodiments, the NHL is selected from the group consisting of: Burkitt lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B-cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1 shows a representative example of the maintenance of T cell proliferation in T cells treated with an AKT inhibitor. T cells were cultured with various concentrations of the AKT inhibitor, MK-22067 for up to seven days. The right-most panels show the percent divided T cells from T cell cultures initiated from six normal donor PBMC samples. Each symbol in the right-most panel represents a unique culture done in parallel with a titrated MK-2206 dose. The left-most panels show a representative example from these experiments. A) After three days of culture with MK-2206, T cell proliferation was only slightly decreased when compared to the no treatment control. B) After 7 days of culture with MK-2206, T cell proliferation was not substantially different compared to the no treatment control.

Figure 2 shows a representative example of CD62L expression of T cells treated with an AKT inhibitor. T cells were cultured with various concentrations of the AKT inhibitor, MK-22067 for up to seven days. The right-most panels show the percent CD62L expressing T cells from T cell cultures initiated from six normal donor PBMC samples. Each symbol in the right-most panel represents a unique culture done in parallel with a titrated MK-2206 dose. The left-most panels show a representative example from these experiments. A) After three days of culture with MK-2206, CD62L expression on the MK-2206 treated T cells was not substantially different when compared to the no treatment

CLAIMS

1. A method for manufacturing T cells comprising:
 - (a) activating a population of T cells and stimulating the population of T cells to proliferate, wherein the activation and stimulation steps are performed in the presence of a PI3K inhibitor;
 - (b) transducing the T cells with a viral vector comprising an engineered T cell receptor (TCR) or a chimeric antigen receptor (CAR);
 - (c) culturing the transduced T cells to proliferate;wherein the activating and stimulating steps performed in the presence of the PI3K inhibitor results in maintaining proliferation of the transduced T cells compared to the proliferation of transduced T cells that were activated and stimulated in the absence of the PI3K inhibitor.
2. The method of claim 1, wherein the method comprises isolating peripheral blood mononuclear cells as the source of T cells.
3. The method of claim 1, wherein activation of the T cells comprises contacting the T cells with an anti-CD3 antibody or CD3-binding fragment thereof.
4. The method of claim 1, wherein stimulation of the T cells comprises contacting the T cells with an anti-CD28 antibody or a CD28-binding fragment thereof, B7-1 or a CD28-binding fragment thereof, or B7-2 or a CD28-binding fragment thereof.
5. The method of claim 1, wherein the cells are transduced with the viral vector prior to T cell proliferation.

6. The method of claim 1, wherein the cells are transduced with the viral vector after T cell proliferation.

7. The method of any one of claims 1 to 6, wherein the vector is a retroviral vector.

8. The method of any one of claims 1 to 7, wherein the vector is a lentiviral vector.

9. The method of any one of claims 1 to 8, wherein the cells comprise a chimeric antigen receptor (CAR).

10. The method of claim 9, wherein the CAR comprises

a) an extracellular domain that binds an antigen selected from the group consisting of: alpha folate receptor, 5T4, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD19, CD20, CD22, CD30, CD33, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD138, CD171, CEA, CSPG4, EGFR, EGFR family including ErbB2 (HER2), EGFRvIII, EGP2, EGP40, EPCAM, EphA2, EpCAM, FAP, fetal AchR, FR α , GD2, GD3, Glypican-3 (GPC3), HLA-A1+MAGE1, HLA-A2+MAGE1, HLA-A3+MAGE1, HLA-A1+NY-ESO-1, HLA-A2+NY-ESO-1, HLA-A3+NY-ESO-1, IL-11R α , IL-13R α 2, Lambda, Lewis-Y, Kappa, Mesothelin, Muc1, Muc16, NCAM, NKG2D Ligands, NY-ESO-1, PRAME, PSCA, PSMA, ROR1, SSX, Survivin, TAG72, TEMs, and VEGFR2;

b) a transmembrane domain derived from a polypeptide selected from the group consisting of: CD8 α ; CD4, CD28, CD45, PD-1, and CD152;

c) one or more intracellular costimulatory signaling domains selected from the group consisting of: CD28, CD54 (ICAM), CD134 (OX40), CD137 (41BB), CD152 (CTLA4), CD273 (PD-L2), CD274 (PD-L1), and CD278 (ICOS); and

d) a CD3 ζ signaling domain.

11. The method of claim 10, wherein the extracellular domain comprises an antibody or antigen binding fragment that binds the antigen.

12. The method of claim 10, wherein the transmembrane domain is derived from CD8 α or CD28.
13. The method of claim 10, wherein the one or more costimulatory signaling domains selected from the group consisting of: CD28, CD134, and CD137.
14. The method of claim 10, further comprising a hinge region polypeptide.
15. The method of claim 14, wherein the hinge region polypeptide comprises a hinge region of IgG1 or CD8 α .
16. The method of claim 10, further comprising a signal peptide.
17. The method of claim 16, wherein the signal peptide comprises an IgG1 heavy chain signal polypeptide or a CD8 α signal polypeptide.
18. The method of any one of claims 1 to 17, wherein the PI3K inhibitor is a pan-PI3K inhibitor selected from the group consisting of: BEZ235, LY294002, and GDC-0941.
19. The method of any one of claims 1 to 17, wherein the PI3K inhibitor is a selective PI3K inhibitor selected from the group consisting of: BYL719, GSK2636771, TGX-221, AS25242, CAL-101, and IPI-145.
20. The method of any one of claims 1 to 17, wherein the PI3K inhibitor is the PI3-K inhibitor ZSTK474.
21. The method of any one of claims 1 to 20, wherein the population of T cells activated and stimulated in the presence of a PI3K inhibitor have an increased number of T cells expressing one or more markers selected from the group consisting of: CD62L, CCR7, CD28,

CD27, CD122, and CD127 compared to a population of T cells activated and stimulated in the absence of the PI3K inhibitor.

22. The method of claim 21, wherein the population of T cells activated and stimulated in the presence of a PI3K inhibitor do not express CD57 or KLRG1 or express less CD57 or KLRG1 compared to a population of T cells activated and stimulated in the absence of the PI3K inhibitor.

23. A method for maintaining the proliferation and decreasing the differentiation of restimulated T cells expressing an engineered TCR or CAR comprising:

(a) contacting all or a portion of a population of proliferated T cells comprising an engineered TCR or CAR with an anti-CD3 antibody or CD3-binding fragment thereof, and an anti-CD28 antibody or CD28-binding fragment thereof, which stimulates a CD28 accessory molecule on the surface of the T cells, in the presence of a PI3K inhibitor, thereby restimulating the activated T cells to proliferate;

wherein the restimulated T cells have maintained proliferation and decreased differentiation compared to the proliferation of T cells that restimulated in the absence of the PI3K inhibitor.

24. The method of claim 23, wherein the cells comprise an engineered TCR.

25. The method of claim 23, wherein the cells comprise a CAR.

26. The method of any one of claims 23 to 25, wherein the cells comprise a viral vector encoding an engineered TCR or CAR.

27. The method of claim 26, wherein the vector is a retroviral vector.

28. The method of claim 27, wherein the vector is a lentiviral vector.

29. The method of any one of claims 25 to 28, wherein the CAR comprises
- a) an extracellular domain that binds an antigen selected from the group consisting of: alpha folate receptor, 5T4, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD19, CD20, CD22, CD30, CD33, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD138, CD171, CEA, CSPG4, EGFR, EGFR family including ErbB2 (HER2), EGFRvIII, EGP2, EGP40, EPCAM, EphA2, EpCAM, FAP, fetal AchR, FR α , GD2, GD3, 'Glypican-3 (GPC3), HLA-A1+MAGE1, HLA-A2+MAGE1, HLA-A3+MAGE1, HLA-A1+NY-ESO-1, HLA-A2+NY-ESO-1, HLA-A3+NY-ESO-1, IL-11R α , IL-13R α 2, Lambda, Lewis-Y, Kappa, Mesothelin, Muc1, Muc16, NCAM, NKG2D Ligands, NY-ESO-1, PRAME, PSCA, PSMA, ROR1, SSX, Survivin, TAG72, TEMs, and VEGFR2;
 - b) a transmembrane domain derived from a polypeptide selected from the group consisting of: CD8 α ; CD4, CD28, CD45, PD-1, and CD152;
 - c) one or more intracellular costimulatory signaling domains selected from the group consisting of: CD28, CD54 (ICAM), CD134 (OX40), CD137 (41BB), CD152 (CTLA4), CD273 (PD-L2), CD274 (PD-L1), and CD278 (ICOS); and
 - d) a CD3 ζ signaling domain.
30. The method of claim 29, wherein the extracellular domain comprises an antibody or antigen binding fragment that binds the antigen.
31. The method of claim 29, wherein the transmembrane domain is derived from CD8 α or CD28.
32. The method of claim 29, wherein the one or more costimulatory signaling domains selected from the group consisting of: CD28, CD134, and CD137.
33. The method of claim 29, further comprising a hinge region polypeptide.

34. The method of claim 33, wherein the hinge region polypeptide comprises a hinge region of IgG1 or CD8 α .

35. The method of claim 29, further comprising a signal peptide.

36. The method of claim 35, wherein the signal peptide comprises an IgG1 heavy chain signal polypeptide or a CD8 α signal polypeptide.

37. The method of any one of claims 23 to 36, wherein the PI3K inhibitor is selected from the group consisting of: BEZ235, LY294002, GDC-0941, BYL719, GSK2636771, TGX-221, AS25242, CAL-101, IPI-145, and ZSTK474.

38. The method of claim 37, wherein the PI3K inhibitor is a pan-PI3K inhibitor selected from the group consisting of: BEZ235, LY294002, and GDC-0941.

39. The method of claim 37, wherein the PI3K inhibitor is a selective PI3K inhibitor selected from the group consisting of: BYL719, GSK2636771, TGX-221, AS25242, CAL-101, and IPI-145.

40. The method of claim 37, wherein the PI3K inhibitor is the PI3-K inhibitor ZSTK474.

41. The method of any one of claims 23 to 40, wherein the population of activated T cells restimulated in the presence of the PI3K inhibitor have an increased number of T cells expressing one or more markers selected from the group consisting of: CD62L, CCR7, CD28, CD27, CD122, and CD127 compared to a population of T cells activated and stimulated in the absence of the PI3K inhibitor.

42. The method of any one of claim 41, wherein the population of activated T cells restimulated in the presence of the PI3K inhibitor do not express CD57 or KLRG1 or express less CD57 or KLRG1 compared to a population of T cells activated and stimulated in the absence of the PI3K inhibitor.

43. A population of immune effector cells comprising a vector encoding an engineered TCR or CAR, wherein the cells have been activated and stimulated to proliferate in the presence of a PI3K inhibitor, wherein activation and stimulation performed in the presence of the PI3K inhibitor increases expression of one or more of CD62L, CCR7, CD28, CD27, CD122, or CD127 and decreases expression of CD57 or KLRG1 in the population of immune effector cells compared to a population of immune effector cells activated and stimulated in the absence of the PI3K inhibitor.

44. The population of immune effector cells of claim 43, wherein the immune effector cells comprise T cells.

45. A composition comprising the population of immune effector cells of claim 43 and a physiologically acceptable excipient.

46. Use of the composition of claim 45 in the manufacture of a medicament for treating a cancer in a subject in need thereof.

47. The use of claim 46, wherein the cancer is selected from the group consisting of Wilms' tumor, Ewing sarcoma, a neuroendocrine tumor, a glioblastoma, a neuroblastoma, a melanoma, skin cancer, breast cancer, colon cancer, rectal cancer, prostate cancer, liver cancer, renal cancer, pancreatic cancer, lung cancer, biliary cancer, cervical cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancer, medullary thyroid carcinoma, ovarian cancer, glioma, lymphoma, leukemia, myeloma, acute lymphoblastic leukemia, acute

myelogenous leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, and urinary bladder cancer.

48. The use of claim 46, wherein the cancer is pancreatic cancer and the CAR comprises an extracellular binding domain that binds an epitope of PSCA or MUC1

49. The use of claim 46, wherein the cancer is bladder cancer and the CAR comprises an extracellular binding domain that binds an epitope of PSCA or MUC1

50. The use of claim 46, wherein the cancer is glioblastoma multiforme and the CAR comprises an extracellular binding domain that binds an epitope of EPHA2, EGFRvIII, or CSPG4.

51. The use of claim 46, wherein the cancer is lung cancer and the CAR comprises an extracellular binding domain that binds an epitope of PSCA or GD2.

52. The use of claim 46, wherein the cancer is breast cancer and the CAR comprises an extracellular binding domain that binds an epitope of CSPG4 or HER2.

53. The use of claim 46, wherein the cancer is melanoma and the CAR comprises an extracellular binding domain that binds an epitope of CSPG4 or GD2.

54. The use of claim 46, wherein the cancer is a B-cell malignancy and the CAR comprises an extracellular binding domain that binds an epitope of BCMA.

55. Use of the composition of claim 45 in the manufacture of a medicament for treating a hematological malignancy in a subject in need thereof.

56. The use of claim 55, wherein the hematological malignancy is a B-cell malignancy selected from the group consisting of: multiple myeloma (MM), chronic lymphocytic leukemia (CLL), or non-Hodgkin's lymphoma (NHL).

57. The use of claim 56, wherein the MM is selected from the group consisting of: overt multiple myeloma, smoldering multiple myeloma, plasma cell leukemia, non-secretory myeloma, IgD myeloma, osteosclerotic myeloma, solitary plasmacytoma of bone, and extramedullary plasmacytoma.

58. The use of claim 56, wherein the NHL is selected from the group consisting of: Burkitt lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), diffuse large B-cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma.