COMPOSITIONS AND METHODS FOR IMMUNOTHERAPY

CROSS-REFERENCE

[0001] This application claims priority to C.N. Patent Application No. 201811501797.8, filed December 07, 2018; PCT Application No. PCT/CN2019/073252, filed January 25, 2019; C.N. Patent Application No. 201910297171.8, filed April 12, 2019; and PCT Application No. PCT/CN2019/097996, filed July 26, 2019, each of which is entirely incorporated herein by reference.

BACKGROUND

[0002] Adoptive T cell therapy, as an example of immunotherapy, involves the isolation and ex vivo expansion of tumor specific T cells to achieve greater number of T cells than what could be obtained by vaccination alone. The tumor specific T cells are then infused into patients with cancer to provide their immune systems the ability to overwhelm remaining tumor via T cells which can attack and kill the cancer. While there are many forms of adoptive T cell therapy for cancer, they by and large suffer from various deficiencies. Amongst them are cellular exhaustion, long preparation time, and ineffective compositions of engineered cells.

SUMMARY

[0003] In view of the foregoing, there exists a considerable need for alternative compositions and methods to carry out immunotherapy. The compositions and methods of the present disclosure address this need, and provide additional advantages as well. In particular, the various aspects of the disclosure provide systems for immune cell regulation.

[0003a] In one aspect, provided herein is a population of cells comprising engineered immune cells expressing a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR), wherein said CAR or said engineered TCR comprises a ligand binding domain specific for a ligand,

wherein said engineered immune cells have not been subject to ex vivo expansion for 1 or more weeks, and

wherein said population is further characterized in that:

(i) central memory T cells (TCM) in said population are at least 1-fold more abundant than effector memory T cells (TEM) in said population; and/or

(ii) at least 15% of said population of cells are stem memory T cells (TSCM).

[0003b] In one aspect, provided herein is a use of the population as described herein, in the manufacture of a medicament for the treatment of cancer.

[0004] In an example, the present disclosure provides a method of administering a cell therapy comprising engineered immune cells expressing a chimeric antigen receptor (CAR) and/or an engineered T cell receptor (TCR), comprising: infusing a population of immune cells comprising the engineered immune cells into a subject in need thereof, wherein the engineered immune cells have not been subject to ex vivo expansion for no more than 2 weeks or 1 week, and wherein the population is further characterized in that: central memory T cells (TCM) in the population are more abundant than effector memory T cells (TEM). In some examples, the engineered immune cells have been subject to ex vivo expansion less than 13, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 days. In some examples, the engineered immune cells have been subject to ex vivo expansion less than 6, 5, 4, 3, 2, or 1 days. In some examples, the engineered immune cells have been subject to ex vivo expansion less than 5 days. In some examples, the engineered immune cells have been subject to ex vivo expansion less than 72, 48, 36, 32, or 24 hours. In some examples, the TCM are $CD45RO^{+}CD62L^{+}$. In some examples, the TEM are $CD45RO^{+}$CD62L-. In some examples, a population is further characterized in that it is less abundant in PD1 and LAG3. In some examples, reduced exhaustion of cells in a population is

the cytotoxicity of a comparable population that undergoes ex vivo expansion for no more than 2 weeks or 1 week. In an example, the cytotoxicity is at least 0.1 fold, 0.2 fold, 0.3 fold, 0.4 fold, 0.5 fold, 0.6 fold, 0.7 fold, 0.8 fold, 0.9 fold, 1.0 fold, 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold or 10 fold higher in the population as compared to the comparable population that undergoes an ex vivo expansion for no more than 2 weeks or 1 week when the population and the comparable population contact a target.

[0028] Provided herein is a method of administering a cell therapy comprising engineered immune cells expressing a chimeric antigen receptor (CAR) and/or an engineered T cell receptor (TCR), comprising: infusing a population of immune cells comprising the engineered immune cells into a subject in need thereof, wherein the engineered immune cells have not been subject to ex vivo expansion for no more than 2 weeks or 1 week, and wherein the population is further characterized in that a greater bone marrow migration is observed of the population as compared to the bone marrow migration of a comparable population that undergoes ex vivo expansion for no more than 2 weeks or 1 week. In some examples, the bone marrow migration is at least 1 fold higher in the population as compared to the comparable population that undergoes ex vivo expansion for no more than 2 weeks or 1 week when the population and the comparable population contact a target. In an example, the population is further characterized in that: central memory T cells (TCM) in the population are more abundant than effector memory T cells (TEM). In an example, TCM are CD45RO+CD62L+. In an example, TEM are CD45RO+CD62L-. In an example, engineered immune cells have been subject to ex vivo expansion less than 5 days. In an example, the engineered immune cells have been subject to ex vivo expansion less than 4 days. In an example, the engineered immune cells have been subject to ex vivo expansion less than 72 hours. In an example, the engineered immune cells have been subject to ex vivo expansion less than 48 hours. In an example, the engineered immune cells have been subject to ex vivo expansion less than 24 hours. In an example, the target is a cancer cell, a ligand of the TCR or the CAR, or a chemokine. In some examples, the chemokine is stromal cell-derived factor-1 (SDF-1), and wherein the SDF-1 is expressed in bone marrow of the subject. In some cases, the population has a greater percentage of CXCR4 positive cells as compared to the comparable population that undergoes the ex vivo expansion for no more than 2 weeks or 1 week. In an example, the population has a greater median percentage of CXCR4 positive cells that is at least 10% greater as compared to the median percentage of CXCR4 positive cells expressed by the comparable population that undergoes the ex vivo expansion for no more than 2 weeks or 1 week. In an example, the population has a greater density of CXCR4 on a

cell surface of the CXCR4 positive cells as compared to the density of CXCR4 on the cell surface of the comparable population that undergoes an ex vivo expansion for no more than 2 weeks or 1 week. In an example, the density is measured by evaluating a mean fluorescence intensity (MFI) of CXCR4 on the cell surface of the CXCR4 positive cells. In an example, the cytotoxicity is measured in an in vivo assay. In some cases, a reduced cancer burden is observed in the subject administered the population as compared to the cancer burden observed in a comparable subject administered a comparable population that undergoes an ex vivo expansion for no more than 2 weeks or 1 week. In an example, the cancer burden is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% in the subject treated with the population as compared to the comparable subject administered a comparable population that undergoes an ex vivo expansion for no more than 2 weeks or 1 week. In an example, the cancer burden is reduced by at least 0.5 fold, 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, or 10 fold in the subject treated with the population as compared to the comparable subject administered a comparable population that undergoes an ex vivo expansion for no more than 2 weeks or 1 week. In an example, complete remission (CR) is observed in the subject administered the population as compared to the cancer burden observed in a comparable subject administered a comparable population that undergoes an ex vivo expansion for no more than 2 weeks or 1 week. In some examples, a partial response (PR) is observed in the subject administered the population as compared to the cancer burden observed in a comparable subject administered a comparable population that undergoes an ex vivo expansion for no more than 2 weeks or 1 week. In an example, the population comprises at most $1x10^4$ cells per kg/body weight of engineered immune cells. In an example, the population comprises from about $1x10^4$ cells per kg/body weight of engineered immune cells to at most about $1x10^5$ cells per kg/body weight of engineered immune cells. In some cases, the engineered immune cells are T cells, NK cells, and/or NKT cells. In some cases, the TCR comprises (i) a ligand binding domain specific for a ligand and (ii) a transmembrane domain. In an example, the CAR comprises: (i) a ligand binding domain specific for a ligand, (ii) a transmembrane domain, and (iii) an intracellular signaling domain. In an example, the ligand of the TCR or CAR is VEGFR-2, CD19, CD20, CD30, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD80, CD86, CD81, CD123, cd171, CD276, B7H4, BCMA, CD133, EGFR, GPC3, PMSA, CD3, CEACAM6, c-Met, EGFRvIII, ErbB2, ErbB3, HER3, ErbB4 / HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, CD44V6, CEA, CA125, CD151, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A, mesothelin, NY-ESO-1, RANK, RORl, TNFRSF4, CD40, CD137,

TWEAK-R, LTPR, LIFRP, LRP5, MUC1, TCRα, TCRβ, TLR7, TLR9, PTCH1, WT-1, Robol, Frizzled, OX40, CD79, Notch-1-4, and/or Claudin18.2. In an example, the transmembrane domain is from CD8α, CD4, CD28, CD45, PD-1 and/or CD152. In an example, the intracellular signaling domain is from CD3ζ, CD28, CD54 (ICAM), CD134 (OX40), CD137 (4-1BB), GITR, CD152 (CTLA4), CD273 (PD-L2), CD274 (PD-L1), DAP10 and/or CD278 (ICOS). In some cases, the CAR comprises at least 2 intracellular signaling domains. In some cases, the CAR comprises at least 3 intracellular signaling domains. In some cases, the CAR further comprises a hinge. A hinge can be from CD28, IgG1 and/or CD8α. In an example, the engineered immune cells are from peripheral blood, cord blood, bone marrow, and/or induced pluripotent stem cells. In some cases, the engineered immune cells are from peripheral blood, and the peripheral blood cells are T cells.

INCORPORATION BY REFERENCE

[0029] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0031] **Figure 1** shows multiplicity of infection (MOI) vs. percent CAR-positive ratio in F-CART cells.

[0032] **Figure 2** shows a comparison between percent CAR-positive expression of conventional-CART (C-CART) and fast-CART (F-CART) generated cells.

[0033] **Figure 3** shows a phenotypic analysis using flow cytometry of control (starting material) and F-CART cells.

[0034] **Figure 4A** shows a linear graphical representation of cell proliferation of anti-CD19 F-CART vs. anti-CD19 C-CART cells. **Figure 4B** shows fold proliferation of anti-CD19 F-CART vs. anti-CD19 C-CART cells.

[0035] **Figure 5** depicts *in vitro* killing efficacy over 50 hours of anti-CD19 F-CART vs. anti-CD19 C-CART cells.

CLAIMS

WHAT IS CLAIMED IS:

1. A population of cells comprising engineered immune cells expressing a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR), wherein said CAR or said engineered TCR comprises a ligand binding domain specific for a ligand,

wherein said engineered immune cells have not been subject to ex vivo expansion for 1 or more weeks, and

wherein said population is further characterized in that:

(i) central memory T cells (TCM) in said population are at least 1-fold more abundant than effector memory T cells (TEM) in said population; and/or

(ii) at least 15% of said population of cells are stem memory T cells (TSCM).

2. The population of claim 1, wherein said population is characterized to exhibit (i) and/or (ii) after binding of the ligand to the ligand binding domain.

3. The population of any one of the preceding claims, wherein said engineered immune cells have been subject to ex vivo expansion less than 72 hours.

4. The population of any one of the preceding claims, wherein said engineered immune cells have been subject to ex vivo expansion less than 48 hours.

5. The population of any one of the preceding claims, wherein said engineered immune cells have been subject to ex vivo expansion less than 24 hours.

6. The population of any one of the preceding claims, wherein said TCM are CD45RO+CD62L+.

7. The population of any one of the preceding claims, wherein said TEM are CD45RO+CD62L-.

8. The population of any one of the preceding claims, wherein said population is further characterized in that reduced exhaustion of cells in said population is observed as compared to the exhaustion of cells in a control population that undergoes an ex vivo expansion for 1 or more weeks.

9. The population of claim 8, wherein said reduced exhaustion of said population is characterized in that said population comprises less cells expressing PD1 and LAG3.

10. The population of any one of the preceding claims, wherein said engineered immune cells are T cells.

11. The population of any one of the preceding claims, wherein said CAR further

comprises a transmembrane domain, and an intracellular signaling domain.

12. The population of any one of the preceding claims, wherein said ligand is VEGFR-2, CD19, CD20, CD30, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD80, CD86, CD81, CD123, cd171, CD276, B7H4, BCMA, CD133, EGFR, GPC3, PMSA, CD3, CEACAM6, c-Met, EGFRvIII, ErbB2, ErbB3, HER3, ErbB4 / HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, CD44V6, CEA, CA125, CD151, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A, mesothelin, NY-ESO-1, RANK, RORl, TNFRSF4, CD40, CD137, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, TCRα, TCRβ, TLR7, TLR9, PTCH1, WT-1, Robol, Frizzled, OX40, CD79, Notch-1-4, and/or Claudin18.2.

13. The population of claim 11 or 12, wherein said transmembrane domain is from CD8α, CD4, CD28, CD45, PD-1 and/or CD152.

14. The population of any one of claims 11-13, wherein said intracellular signaling domain is from CD3ζ, CD28, CD54 (ICAM), CD134 (OX40), CD137 (4-1BB), GITR, CD152 (CTLA4), CD273 (PD-L2), CD274 (PD-L1), DAP10 and/or CD278 (ICOS).

15. The population of any one of claims 11-14, wherein said CAR comprises at least 2 intracellular signaling domains.

16. The population of any one of claims 11-15, wherein said CAR comprises at least 3 intracellular signaling domains.

17. The population of any one of the preceding claims, wherein said CAR further comprises a hinge.

18. The population of claim 17, wherein said hinge is from CD28, IgG1 and/or CD8α.

19. The population of any one of the preceding claims, wherein said CAR further comprises a signal peptide, and wherein said signal peptide is derived from IgG1, GM-CSF and/or CD8α.

20. The population of any one of the preceding claims, wherein said engineered immune cells are from peripheral blood, cord blood, bone marrow, and/or induced pluripotent stem cells.

21. The population of claim 20, wherein said engineered immune cells are from peripheral blood, and wherein said peripheral blood cells are T cells.

22. The population of any one of the preceding claims, comprising at least 2 fold more TCM as compared to TEM.

23. The population of any one of the preceding claims, comprising at least 4 fold more

TCM as compared to TEM.

24. The population of claim 3, wherein said population of immune cells is allogeneic to said subject in need thereof.

25. The population of claim 3, wherein said population of immune cells is autologous to said subject in need thereof.

26. The population of any one of the preceding claims, wherein said population is further characterized in that a greater proliferation, cytotoxicity, and/or bone marrow migration is observed in said population as compared to the proliferation, cytotoxicity, and/or bone marrow migration of a control population that undergoes an ex vivo expansion for 1 or more weeks.

27. The population of claim 26, wherein said cytotoxicity is at least 0.1 fold higher in said population as compared to a control population that undergoes an ex vivo expansion for 1 or more weeks when said population and control population contact a target.

28. The population of claim 26, wherein said proliferation is at least 1 fold higher in said population as compared to a control population that undergoes an ex vivo expansion for 1 or more weeks when said population and control population contact a target.

29. The population of claim 26, wherein said bone marrow migration is at least 0.1 fold higher in said population as compared to a control population that undergoes an ex vivo expansion for 1 or more weeks when said population and control population contact a target.

30. The population of any one of the preceding claims, wherein said population has a greater percentage of CXCR4 positive cells as compared to a control population that undergoes an ex vivo expansion for 1 or more weeks.

31. The population of claim 30, wherein said population has a greater median percentage of CXCR4 positive cells that is at least 10% greater as compared to the median percentage of CXCR4 positive cells expressed by the control population that undergoes the ex vivo expansion for 1 or more weeks.

32. The population of claim 31, wherein said population has a greater density of CXCR4 on a cell surface of said CXCR4 positive cells as compared to the density of CXCR4 on the cell surface of the control population that undergoes the ex vivo expansion for 1 or more weeks.

33. The population of claim 1, wherein the population is prepared by subjecting a source

of immune cells to the steps of:

(a) activation by an activation moiety; and

(b) introduction of a polynucleotide encoding for at least said CAR and/or said TCR,;

wherein the step of (a) and (b) are carried out concurrently.

34. The population of claim 33, wherein said activation moiety comprises anti-CD3 antibody and/or anti-CD28 antibody.

35. The population of claim 34, wherein said activation moiety is conjugated to a solid phase.

36. The population of claim 35, wherein said solid phase is a bead.

37. The population of claim 33, wherein said introduction comprises transduction of said population of cells with a viral vector and/or a transposon vector.

38. The population of claim 33, wherein the steps of (a) and (b) are performed within 5 hours.

39. The population of claim 33, wherein the steps of (a) and (b) are performed within 3 hours.

40. The population of claim 33, wherein the steps of (a) and (b) are performed within 1 hour.

41. The population of claim 33, wherein the population is to be administered to a subject in need thereof without further ex vivo expansion following the steps of (a) and (b).

42. Use of the population of any one of the preceding claims, in the manufacture of a medicament for the treatment of cancer.

43. The use of claim 42, wherein said medicament is to be administered to a subject in need thereof.

44. The use of claim 43, wherein said medicament is to be intravenously administered to the subject.

45. The use of claim 42, wherein said cancer is hematological.

46. The use of claim 45, wherein said hematological cancer is leukemia, myeloma, and/or lymphoma.

47. The use of claim 46, wherein said leukemia is chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), B cell acute lymphoblastic leukemia (B-ALL), T-cell acute lymphoblastic leukemia (T-ALL), and/or acute lymphoblastic leukemia (ALL).

48. The use of claim 42, wherein said cancer is a solid cancer.

49. The use of claim 48, wherein said solid cancer is selected from the group comprising:

nephroblastoma, Ewing's sarcoma, neuroendocrine tumor, glioblastoma, neuroblastoma, melanoma, skin cancer, breast cancer, colon cancer, rectal cancer, prostate cancer, liver cancer, kidney cancer, pancreatic cancer, lung cancer, biliary tract cancer, cervical cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancer, medullary thyroid carcinoma, ovarian cancer, glioma, or bladder cancer.