technologies for generating therapeutic doses of T cells, including engineered T cells, remain limited by cumbersome T cell manufacturing processes. Existing T cell manufacturing processes are not easily scalable, repeatable, reliable, or efficient and often produce an inferior T cell product that may be prone to exhaustion and loss of effector immune cell function.

To date, engineered T cell adoptive immunotherapies have met with only limited success and routinely show variable clinical activity. Therefore, such therapies are not suitable for widespread clinical use.

SUMMARY

The disclosure generally provides methods for adoptive cellular therapy.

In various examples, a method for manufacturing a T cell therapeutic is provided comprising: obtaining a population of cells that comprises T cells and antigen presenting cells (APCs); culturing the population of cells in a cell culture medium comprising i) one or more cytokines, ii) an anti-CD3 antibody or CD3-binding fragment thereof, and iii) 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, wherein the culture activates and stimulates the T cells; transducing the population of activated cells with a viral vector; and culturing the population of cells in a cell growth medium to expand the transduced T cells; thereby manufacturing the T cell therapeutic.

In one aspect, the present disclosure provides for a method for manufacturing a human T cell therapeutic comprising:

a) obtaining a population of peripheral blood mononuclear cells (PBMCs) that comprises T cells and antigen presenting cells (APCs);

b) culturing the population of PBMCs for about 16 hours to about 32 hours prior to transduction in a cell culture medium comprising i) interleukin-2 (IL-2), ii) an anti-CD3 antibody or CD3-binding fragment thereof, and iii) 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, wherein the culture activates and stimulates the T cells;

c) transducing the population of PBMCs activated in step b) with a lentiviral vector encoding an anti-BCMA chimeric antigen receptor (CAR); and

d) culturing the population of PBMCs from step c) in a cell growth medium to expand the transduced T cells;

thereby manufacturing the T cell therapeutic.

In another aspect, the present disclosure provides for a method for manufacturing a human T cell therapeutic comprising:

a) obtaining a population of peripheral blood mononuclear cells (PBMCs) that comprises T cells and antigen presenting cells (APCs);

b) culturing the population of PBMCs for about 16 hours to about 32 hours prior to transduction in a cell culture medium comprising:

i) interleukin-2 (IL-2);

ii) an anti-CD3 antibody or CD3-binding fragment thereof; and

iii) 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, wherein the culture activates and stimulates the T cells;

c) transducing the population of PBMCs activated in step b) with a lentiviral vector encoding an anti-BCMA CAR comprising a single chain variable fragment (scFv) that binds BCMA, a CD8α transmembrane domain, a CD137 intracellular co-stimulatory signaling domain, and a CD3ζ primary signaling domain; and

d) culturing the population of PBMCs from step c) in a cell growth medium to expand the transduced T cells;

thereby manufacturing the T cell therapeutic.

In particular examples, the population of cells is obtained from peripheral blood, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, or tumors.

In certain examples, the T cells are obtained from peripheral blood, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, tumors, or a T cell line.

In additional examples, the APCs are obtained from peripheral blood, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, or tumors.

In some examples, the population of cells comprises peripheral blood mononuclear cells (PBMCs).

In further examples, harvesting or obtaining the population of cells comprises leukapheresis.

In particular examples, isolating the population of cells comprises sedimentation.

In additional examples, the sedimentation comprises a FICOLL™ or a PERCOLL™ gradient.

In certain examples, the sedimentation is performed using a semiautomated flowthrough centrifuge.

In additional examples, the semiautomated flowthrough centrifuge is a Cobe 2991 cell processor, a Cell Saver 5+, or a Teruma Elutra.

In particular examples, the methods further comprise washing the population of cells in a buffer or cell culture medium.

In some examples, the population of cells are washed in T cell growth medium (TCGM) containing one or more cytokines.

In some examples, the one or more cytokines in the TCGM are selected from the group consisting of: IL-2, IL7, IL-15, IL-9, and IL-21.

In particular examples, the cytokine is IL-2.

In certain examples, the concentration of IL-2 is about 250 IU/mL.

In certain examples, the concentration of IL-2 is about from 100 IU/mL to about 300 IU/mL.

In some examples, the isolated population of cells comprises PBMCs.

In additional examples, the population of cells is cryopreserved in a controlled rate freezer.

In further examples, the cryopreserved population of cells is thawed.

In further examples, the population of cells is seeded for culturing in step (b) in TCGM at a density of about 1×10^8 cells/mL.

In further examples, the population of cells is seeded for culturing in step (b) in TCGM at a density of about 1×10^7 cells/mL.

In further examples, the population of cells is seeded for culturing in step (b) in TCGM at a density of about 1×10^6 cells/mL.

In further examples, about 1 x 10^8 cells are seeded for culturing in step (b) in TCGM at a density of about 1 x 10^6 cells/mL.

In particular examples, the population of cells is cultured in a cell culture bag or a bioreactor.

In certain examples, the TCGM comprises one or more cytokines selected from the group consisting of: IL-2, IL7, IL-15, IL-9, and IL-21.

In further examples, the one or more cytokines are selected from the group consisting of: IL-2, IL-7, and IL-15.

In additional examples, the one or more cytokines comprise IL-2.

In additional examples, the concentration of the one or more cytokines is about 250 IU/mL.

In additional examples, the concentration of the one or more cytokines is about from 25 IU/mL to about 500 IU/mL

In further examples, the population of cells is cultured with a soluble anti-CD3 antibody and a soluble anti-CD28 antibody.

In certain examples, the concentration of the anti-CD3 antibody is about 50 ng/mL.

In particular examples, the concentration of the anti-CD28 antibody is about 50 ng/mL.

In particular examples, the population of cells of step b) is cultured for about 12 hours to about 48 hours prior to transduction.

In further examples, the population of cells of step b) is cultured for about 16 hours to about 32 hours prior to transduction.

In additional examples, the population of cells of step b) is cultured for at least 18 hours prior to transduction.

In further examples, the population of cells of step b) is cultured for at least 24 hours prior to transduction.

In certain examples, the population of cells of step d) is transduced with a retroviral vector.

In certain examples, the population of cells of step d) is transduced with a lentiviral vector.

In further examples, about 1 x 10^9 TU to about 2 x 10^9 TU of viral vector are used to transduce the 1 x 10^8 seeded cells.

In further examples, about 1 x 10^9 TU to about 4 x 10^9 TU of viral vector are used to transduce the 1 x 10^8 seeded cells.

In particular examples, the viral vector is diluted to 20% v/v of the total culture volume.

In particular examples, the viral vector is diluted to about 20% to about 40% v/v of the total culture volume.

In additional examples, the population of cells is transduced for about 18 to about 48 hours.

In further examples, the population of cells is transduced for about 18 to about 36 hours.

In further examples, the population of cells is transduced for about 24 hours.

In additional examples, the viral vector comprises a polynucleotide encoding a chimeric antigen receptor.

In certain examples, the CAR comprises: an extracellular domain that binds an antigen selected from the group consisting of: alpha folate receptor, 5T4, αvβ6 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, FRα, 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-11Rα, IL-13Rα2, 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: CD8α; CD4, CD28, CD45, PD1, and CD152; one or more intracellular co-stimulatory 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 CD3ζ signaling domain.

In particular examples, the extracellular domain comprises an antibody or antigen binding fragment that binds the antigen.

In further examples, the transmembrane domain is derived from CD8α or CD28.

In certain examples, the one or more co-stimulatory signaling domains selected from the group consisting of: CD28, CD134, and CD137.

In further examples, comprising a hinge region polypeptide.

In additional examples, the hinge region polypeptide comprises a hinge region of IgG1 or CD8α.

In particular examples, the CAR further comprises a signal peptide.

In particular examples, the signal peptide comprises an IgG1 heavy chain signal polypeptide, a CD8α signal polypeptide, or a human GM-CSF receptor alpha signal polypeptide.

In additional examples, the population of cells in step d) is cultured for expansion for about 5 to about 8 days.

In certain examples, the population of cells in step d) is cultured for expansion for about 5 days to about 8 days in a cell culture bag.

In further examples, the population of cells in step d) is cultured for expansion for about 5 days in a cell culture bag and then cultured for about 3 days in a bioreactor.

In additional examples, the population of cells in step d) is cultured for expansion for about 5 days to about 8 days in a bioreactor.

In further examples, the bioreactor is a WAVE bioreactor or a GREX bioreactor.

In particular examples, the number of T cells is expanded at least 50 fold during the culture of step d).

In certain examples, the number of T cells is expanded at least 100 fold during the culture of step d).

In particular examples, the number of T cells is expanded at least 200 fold during the culture of step d).

In further examples, the number of T cells is expanded at least 300 fold during the culture of step d).

In additional examples, the number of T cells is expanded at least 400 fold during the culture of step d).

In additional examples, the number of T cells is expanded at least 500 fold during the culture of step d).

In further examples, the number of T cells is expanded at least 600 fold during the culture of step d).

In certain examples, the method further comprises recovering the manufactured T cell therapeutic.

In certain examples, recovering the T cell therapeutic comprises concentrating and washing the cells expanded in step d).

In particular examples, the T cell therapeutic is concentrated and washed using a semiautomated flowthrough centrifuge.

In further examples, the semiautomated flowthrough centrifuge is a Cell Saver 5+ or LOVO.

In particular examples, the method further comprises cryopreserving the T cell therapeutic.

In additional examples, the cryopreserved T cells are thawed for use in a method of adoptive cell therapy.

In various aspects, a composition comprising the manufactured T cells of any one of the preceding examples described *supra* and elsewhere herein and a physiologically acceptable excipient is provided.

In various other examples, a method of treating a malignancy in a subject in need thereof, comprising administering to the subject the T cell therapeutic of any one of the preceding examples described *supra* and elsewhere herein is provided.

In one aspect, the present disclosure provides for a use of the composition described *supra* and elsewhere herein in the manufacture of a medicament for treating a malignancy in a subject in need thereof.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1 shows transduction efficiency and VCN of transduced cells transduced at different times during the T cell manufacturing process. Cells were transduced with a GFP expressing lentivirus at 1-2 x 108 TU/106 PBMCs at D0, D1, and D2. FACS analysis of GFP expressing cells expressing CD3, CD8, or CD4 determined that transduction efficiency and VCN were highest in cells transduced 20 to 24 hours (D1) after activation.

Figure 2 shows the transduction efficiency of cells activated using different methods. PBMCs were activated using (i) plate bound anti-CD3 and anti-CD28 antibodies; (ii) soluble anti-CD3 and anti-CD28 antibodies; and (iii) bead bound anti-CD3 and anti-

CLAIMS

1. A method for manufacturing a human T cell therapeutic comprising:

a) obtaining a population of peripheral blood mononuclear cells (PBMCs) that comprises T cells and antigen presenting cells (APCs);

b) culturing the population of PBMCs for about 16 hours to about 32 hours prior to transduction in a cell culture medium comprising i) interleukin-2 (IL-2), ii) an anti-CD3 antibody or CD3-binding fragment thereof, and iii) 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, wherein the culture activates and stimulates the T cells;

c) transducing the population of PBMCs activated in step b) with a lentiviral vector encoding an anti-BCMA chimeric antigen receptor (CAR); and

d) culturing the population of PBMCs from step c) in a cell growth medium to expand the transduced T cells;

thereby manufacturing the T cell therapeutic.

2. The method of Claim 1, wherein the anti-BCMA CAR comprises: an extracellular domain comprising anti-BCMA antibody or antigen binding fragment thereof; a CD8α or CD28 transmembrane domain; one or more intracellular co-stimulatory signaling domains selected from the group consisting of: CD27, CD28, CD54 (ICAM), CD134 (OX40), CD137 (41BB), and CD278 (ICOS); and a CD3ζ primary signaling domain.

3. A method for manufacturing a human T cell therapeutic comprising:

a) obtaining a population of peripheral blood mononuclear cells (PBMCs) that comprises T cells and antigen presenting cells (APCs);

b) culturing the population of PBMCs for about 16 hours to about 32 hours prior to transduction in a cell culture medium comprising:

i) interleukin-2 (IL-2);

ii) an anti-CD3 antibody or CD3-binding fragment thereof; and

iii) 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, wherein the culture activates and stimulates the T cells;

c) transducing the population of PBMCs activated in step b) with a lentiviral vector encoding an anti-BCMA CAR comprising a single chain variable fragment (scFv) that binds BCMA, a CD8α transmembrane domain, a CD137 intracellular co-stimulatory signaling domain, and a CD3ζ primary signaling domain; and

d) culturing the population of PBMCs from step c) in a cell growth medium to expand the transduced T cells;

thereby manufacturing the T cell therapeutic.

4. The method of any one of Claims 1 to 3, wherein the population of PBMCs obtained in step a) is cryopreserved prior to culture in step b).

5. The method of any one of Claims 1 to 4, wherein the population of PBMCs obtained in step a) is cryopreserved in a controlled rate freezer prior to culture in step b).

6. The method of Claim 4 or Claim 5, wherein the cryopreserved population of PBMCs is thawed prior to culture in step b).

7. The method of any one of Claims 1 to 6, wherein obtaining the population of PBMCs comprises leukapheresis.

8. The method of any one of Claims 1 to 7, wherein obtaining the population of PBMCs comprises sedimentation.

9. The method of Claim 8, wherein the sedimentation comprises a FICOLL (trade mark) or a PERCOLL (trade mark) gradient.

10. The method of Claim 8 or Claim 9, wherein the sedimentation is performed using a semiautomated flowthrough centrifuge.

11. The method of Claim 10, wherein the semiautomated flowthrough centrifuge is a Cobe 2991 cell processor, a Cell Saver 5^+, or an Elutra.

12. The method of any one of Claims 1 to 11, further comprising washing the population of PBMCs obtained in step a) in a buffer or cell culture medium.

13. The method of Claim 12, wherein the population of PBMCs are washed in T cell growth medium (TCGM) containing one or more additional cytokines.

14. The method of Claim 12, wherein the one or more additional cytokines in the TCGM are selected from the group consisting of: IL7, IL-15, IL-9, and IL-21.

15. The method of any one of Claims 1 to 14, wherein the concentration of IL-2 is about 250 IU/mL.

16. The method of any one of Claims 1 to 15, wherein the population of PBMCs is seeded for culturing in step b) in TCGM at a density of about 1 x 10^6 cells/mL.

17. The method of Claim 16, wherein the population of PBMCs is cultured in a cell culture bag or a bioreactor.

18. The method of Claim 16 or Claim 17, wherein the TCGM further comprises one or more cytokines selected from the group consisting of: IL7, IL-15, IL-9, and IL-21.

19. The method of Claim 18, wherein the one or more cytokines are selected from the group consisting of: IL-7, and IL-15.

20. The method of Claim 18 or Claim 19, wherein the concentration of the one or more cytokines is about 250 IU/mL.

21. The method of any one of Claims 1 to 20, wherein the population of PBMCs is cultured with a soluble anti-CD3 antibody and a soluble anti-CD28 antibody.

22. The method of Claim 21, wherein the concentration of the anti-CD3 antibody is about 50 ng/mL.

23. The method of Claim 21, wherein the concentration of the anti-CD28 antibody is about 50 ng/mL.

24. The method of any one of Claims 1 to 23, wherein the population of PBMCs of step b) is cultured for at least 24 hours prior to transduction or for at least 24 hours prior to transduction.

25. The method of any one of Claims 1 to 23, wherein the population of PBMCs of step b) is cultured for about 20 to about 24 hours prior to transduction.

27. The method of any one of Claim 16 to 25, wherein about 1 x 10^9 TU to about 2 x 10^9 TU of lentiviral vector are used to transduce 1 x 10^8 seeded cells.

28. The method of any one of Claims 1 to 27, wherein the population of PBMCs is transduced for about 24 hours.

29. The method of any one of Claims 1 to 28, wherein the population of PBMCs in step d) is cultured for expansion for about 5 to about 8 days.

30. The method of any one of Claims 1 to 28, wherein the population of PBMCs in step d) is cultured for expansion for about 5 days to about 8 days in a cell culture bag.

31. The method of any one of Claims 1 to 28, wherein the population of PBMCs in step d) is cultured for expansion for about 5 days in a cell culture bag and then cultured for about 3 days in a bioreactor.

32. The method of any one of Claims 1 to 28, wherein the population of PBMCs in step d) is cultured for expansion for about 5 days to about 8 days in a bioreactor.

33. The method of Claim 31 or Claim 32, wherein the bioreactor is a WAVE bioreactor or a GREX bioreactor.

34. The method of any one of Claims 1 to 33, wherein the number of T cells is expanded at least 50 fold during the culture of step d).

35. The method of any one of Claims 1 to 33, wherein the number of T cells is expanded at least 100 fold during the culture of step d).

36. The method of any one of Claims 1 to 33, wherein the number of T cells is expanded at least 200 fold during the culture of step d).

37. The method of any one of Claims 1 to 33, wherein the number of T cells is expanded at least 300 fold during the culture of step d).

38. The method of any one of Claims 1 to 33, wherein the number of T cells is expanded at least 400 fold during the culture of step d).

39. The method of any one of Claims 1 to 33, wherein the number of T cells is expanded at least 500 fold during the culture of step d).

40. The method of any one of Claims 1 to 33, wherein the number of T cells is expanded at least 600 fold during the culture of step d).

41. The method of any one of Claims 1 to 40, wherein the method further comprises recovering the manufactured T cell therapeutic.

42. The method of Claim 41, wherein recovering the T cell therapeutic comprises concentrating and washing the cells expanded in step d).

43. The method of Claim 41, wherein the T cell therapeutic is concentrated and washed using a semiautomated flowthrough centrifuge.

44. The method of Claim 43, wherein the semiautomated flowthrough centrifuge is a Cell Saver 5^{+} or LOVO.

45. The method of any one of Claims 1 to 44, wherein the method further comprises cryopreserving the T cell therapeutic.

46. The method of Claim 45, wherein the cryopreserved T cells are thawed for use in a method of adoptive cell therapy.

47. A composition comprising the manufactured T cells of any one of Claims 1 to 46 and a physiologically acceptable excipient.

48. Use of the composition of Claim 47 in the manufacture of a medicament for treating a malignancy in a subject in need thereof.