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.

BRIEF SUMMARY

The disclosure generally provides methods for adoptive cellular therapy.

In various embodiments, 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, there is provided a method for manufacturing a T cell therapeutic comprising:

a) obtaining an isolated 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) exogenous 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 engineered T cell receptor (TCR) or chimeric antigen receptor (CAR); and

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

thereby manufacturing the T cell therapeutic.

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

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

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

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

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

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

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

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

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

In various other embodiments, 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 embodiments described *supra* and elsewhere herein is provided.

In yet another aspect, use of the composition as described herein in the manufacture of a medicament for treating a malignancy in a subject in need thereof is provided.

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 T cell therapeutic comprising:

a) obtaining an isolated 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) exogenous 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 engineered T cell receptor (TCR) or chimeric antigen receptor (CAR); and

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

thereby manufacturing the T cell therapeutic.

2. The method of Claim 1, wherein harvesting or obtaining the population of PBMCs comprises leukapheresis.

3. The method of any one of Claim 1 or Claim 2, wherein isolating the population of PBMCs comprises sedimentation.

4. The method of Claim 3, wherein the sedimentation comprises a FICOLL (trade mark) or a PERCOLL (trade mark) gradient.

5. The method of Claim 3 or Claim 4, wherein the sedimentation is performed using a semiautomated flowthrough centrifuge.

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

7. The method of any one of Claims 1 to 6, further comprising washing the population of PBMCs in a buffer or cell culture medium.

8. The method of Claim 7, wherein the population of PBMCs are washed in T cell growth medium (TCGM) containing one or more additional cytokines.

9. The method of Claim 8, wherein the one or more additional cytokines in the TCGM are selected from the group consisting of: IL7, IL-15, IL-9, and IL-2 1.

10. The method of Claim 1, wherein the concentration of IL-2 is about 250 IU/mL.

11. The method of Claim 1, wherein the population of PBMCs is cryopreserved in a controlled rate freezer.

12. The method of Claim 12, wherein the cryopreserved population of PBMCs is thawed.

13. The method of Claim 1 or Claim 12, 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.

14. The method of Claim 13, wherein the population of PBMCs is cultured in a cell culture bag or a bioreactor.

15. The method of Claim 13 or Claim 14, wherein the TCGM further comprises one or more cytokines selected from the group consisting of: IL7, IL-15, IL-9, and IL-21.

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

17. The method of Claim 15 or Claim 16, wherein the concentration of the one or more cytokines is about 250 IU/mL.

18. The method of any one of Claims 1 to 17, wherein the population of PBMCs is cultured with a soluble anti-CD3 antibody and a soluble anti-CD28 antibody.

19. The method of Claim 18, wherein the concentration of the anti-CD3 antibody is about 50 ng/mL.

20. The method of Claim 19, wherein the concentration of the anti-CD28 antibody is about 50 ng/mL.

21. The method of any one of Claims 1 to 20, wherein the population of PBMCs of step b) is cultured for at least 24 hours prior to transduction or for at least 18 hours prior to transduction.

22. The method of any one of Claims 1 to 21, wherein the population of PBMCs of step b) is cultured for about 20 to about 24 hours prior to transduction.

23. The method of any one of Claim 13 to 22, wherein about 1 x 10^9 TU to about 2 x 10^9 TU of lentiviral vector are used to transduce the 1 x 10^8 seeded cells.

24. The method of any one of Claims 1 to 23, wherein the lentiviral vector is diluted to 20% v/v of the total culture volume.

25. The method of any one of Claims 1 to 23, wherein the lentiviral vector is diluted to about 40% to about 50% v/v of the total culture volume.

26. The method of any one of Claims 1 to 25, wherein the population of PBMCs is transduced for about 24 hours.

27. The method of any one of Claims 1-26, 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, 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;

b) a transmembrane domain derived from a polypeptide selected from the group consisting of: CD8α, CD4, CD28, CD45, PD1, and CD152;

c) 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

d) a CD3ζ signaling domain.

28. The method of Claim 27, wherein the extracellular domain comprises an antibody or antigen binding fragment that binds the antigen.

29. The method of Claim 27, wherein the transmembrane domain is derived from CD8α or CD28.

30. The method of Claim 27, wherein the one or more co-stimulatory signaling domains selected from the group consisting of: CD28, CD134, and CD137.

31. The method of Claim 27, further comprising a hinge region polypeptide.

32. The method of Claim 31, wherein the hinge region polypeptide comprises a hinge region of IgG1 or CD8α.

33. The method of Claim 27, further comprising a signal peptide.

34. The method of Claim 33, wherein the signal peptide comprises an IgG1 heavy chain signal polypeptide, a CD8α signal polypeptide, or a human GM-CSF receptor alpha signal polypeptide.

35. The method of any one of Claims 1 to 34, wherein the population of PBMCs in step d) is cultured for expansion for about 5 to about 8 days.

36. The method of any one of Claims 1 to 34, 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.

37. The method of any one of Claims 1 to 34, 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.

38. The method of any one of Claims 1 to 34, wherein the population of PBMCs in step d) is cultured for expansion for about 5 days to about 8 days in a bioreactor.

39. The method of Claim 37 or Claim 38 wherein the bioreactor is a WAVE bioreactor or a GREX bioreactor.

40. The method of any one of Claims 1 to 39, wherein the number of T cells is expanded at least 50 fold during the culture of step d).

41. The method of any one of Claims 1 to 39, wherein the number of T cells is expanded at least 100 fold during the culture of step d).

42. The method of any one of Claims 1 to 39, wherein the number of T cells is expanded at least 200 fold during the culture of step d).

43. The method of any one of Claims 1 to 39, wherein the number of T cells is expanded at least 300 fold during the culture of step d).

44. The method of any one of Claims 1 to 39, wherein the number of T cells is expanded at least 400 fold during the culture of step d).

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

46. The method of any one of Claims 1 to 39, wherein the number of T cells is expanded at least 600 fold during the culture of step d).

47. The method of any one of Claims 1 to 46, wherein the method further comprises recovering the manufactured T cell therapeutic.

48. The method of Claim 47, wherein recovering the T cell therapeutic comprises concentrating and washing the cells expanded in step d).

49. The method of Claim 48, wherein the T cell therapeutic is concentrated and washed using a semiautomated flowthrough centrifuge.

50. The method of Claim 49, wherein the semiautomated flowthrough centrifuge is a Cell Saver 5^+ or LOVO.

51. The method of any one of Claims 47 to 50, wherein the method further comprises cryopreserving the T cell therapeutic.

52. The method of Claim 51, wherein the cryopreserved T cells are thawed for use in a method of adoptive cell therapy.

53. A composition comprising the manufactured T cells of any one of Claims 1 to 52 and a physiologically acceptable excipient.

54. Use of the composition of Claim 53 in the manufacture of a medicament for treating a malignancy in a subject in need thereof.