

## **DESCRIPTION**

### **UNIVERSAL ANTIGEN PRESENTING CELLS AND USES THEREOF**

**[0001]** This application claims the benefit of United States Provisional Patent Application No. 62/633,587, filed February 21, 2018, which is incorporated herein by reference in its entirety.

## **BACKGROUND**

**[0002]** The sequence listing that is contained in the file named “UTFCP1355WO\_ST25.txt”, which is KB (as measured in Microsoft Windows) and was created on February 21, 2019, is filed herewith by electronic submission and is incorporated by reference herein

### **1. Field**

**[0003]** The present invention relates generally to the fields of medicine and immunology. More particularly, it concerns antigen presenting cells and uses thereof, such as expanding natural killer (NK) cells.

### **2. Description of Related Art**

**[0004]** Natural killer (NK) cells do not need previous exposure to antigens for activation which is in contrast to T cells, making them highly effective in immuno-surveillance against tumors. While prior activation is not required, the immense killing powers of NK cells need stringent controls to forestall unintended cytotoxicity and autoimmunity. Generation of clinically sufficient quantities and optimally functional NK cells is another impediment. Thus, there is a need to focus on the molecular machinery governing NK cell biology to discriminate “resistant” from “sensitive” target cells to design helper cells to enhance NK-cell mediated cancer immunotherapy.

## **SUMMARY**

**[0005]** Accordingly, certain examples of the present disclosure provide methods and compositions concerning the manufacture, expansion, quality control, and functional characterization of clinical-grade NK cells intended for cell and immunotherapy.

**[0006]** In a first aspect, the present disclosure refers to a universal antigen presenting cell (UAPC) engineered to express (1) CD48 and/or CS1 (CD319), (2) membrane-bound interleukin-21 (mbIL-21), and (3) 41BB ligand (41BBL). In some examples, the UAPC expresses CD48. In other examples, the UAPC expresses CS1. In particular examples, the UAPC expresses CD48 and CS1.

**[0007]** In some examples, the UAPC has essentially no expression of endogenous HLA class I, II, or CD1d molecules. In certain examples, the UAPC expresses ICAM-1 (CD54) and LFA-3 (CD58).

**[0008]** In certain examples, the UAPC is further defined as a leukemia cell-derived aAPC. In some examples, the leukemia-cell derived UAPC is further defined as a K562 cell.

**[0009]** In some examples, engineered is further defined as retroviral transduction. In some examples, the retroviral transduction is further defined as transduction of a viral construct of SEQ ID NO:1 and/or SEQ ID NO:2. In certain examples, the UAPC is irradiated.

**[0010]** In a further example, there is provided a method for expanding immune cells comprising culturing the immune cells in the presence of an effective amount of UAPCs of the examples (*e.g.*, a universal antigen presenting cell (UAPC) engineered to express (1) CD48 and/or CS1 (CD319), (2) membrane-bound interleukin-21 (mbIL-21), and (3) 41BB ligand (41BBL)). In some examples, the immune cells and UAPCs are cultured at a ratio of 3:1 to 1:3, such as 3:1, 3:2, 1:1, 1:2, or 1:3. In particular examples, the immune cells and UAPCs are cultured at a ratio of 1:2.

**[0010a]** In another aspect, the present disclosure refers to a method for expanding immune cells comprising culturing the immune cells in the presence of an effective amount of UAPCs as disclosed herein.

**[0011]** In some examples, the expanding is in the presence of IL-2. In specific examples, the IL-2 is present at a concentration of 10-500 U/mL, such as 10-25, 25-50, 50-75, 75-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, or 400-500 U/mL. In certain examples, the IL-2 is present at a concentration of 100-300 U/mL. In particular examples, the IL-2 is present at a concentration of 200 U/mL. In some examples, the IL-2 is recombinant human IL-2. In specific examples, the IL-2 is replenished every 2-3 days, such as every 2 days or 3 days.

**[0012]** In certain examples, the UAPCs are added at least a second time. In some examples, the immune cells are NK cells or T cells. In particular examples, the immune cells are NK cells.

**[0013]** In particular examples, the immune cells are derived from cord blood (CB), peripheral blood (PB), stem cells, or bone marrow. In specific examples, the stem cells are induced pluripotent stem cells. In some examples, the immune cells are obtained from CB. In particular examples, the CB is pooled from 2 or more individual cord blood units. In specific examples, the CB is pooled from 3, 4, 5, 6, 7, or 8 individual cord blood units.

**[0014]** In some examples, the NK cells are CB mononuclear cells (CBMCs). In certain examples, the NK cells are further defined as CD56<sup>+</sup> NK cells.

**[0015]** In certain examples, the method is performed in serum-free media.

**[0016]** In additional examples, the immune cells are engineered to express a chimeric antigen receptor (CAR). In some examples, the CAR comprises a CD19, CD123, mesothelin, CD5, CD47, CLL-1, CD33, CD99, U5snRNP200, CD200, CS1, BAFF-R, ROR-1, or BCMA antigen-binding domain. In some examples, the CAR is a humanized CAR. In some examples, the CAR comprises IL-15. In certain examples, the CAR comprises a suicide gene. In some examples, the suicide gene is CD20, CD52, EGFRv3, or inducible caspase 9.

**[0017]** Further provided herein is a population of expanded immune cells produced according to the examples (*e.g.*, culturing the immune cells in the presence of an effective amount of UAPCs of the examples (*e.g.*, a universal antigen presenting cell (UAPC) engineered to express (1) CD48 and/or CS1 (CD319), (2) membrane-bound interleukin-21 (mbIL-21), and (3) 41BB ligand (41BBL)).

**[0017a]** In another aspect, the present disclosure refers to a population of expanded immune cells produced according to the methods as disclosed herein.

**[0018]** In another aspect, the present disclosure refers to a pharmaceutical composition comprising the population of expanded immune cells as disclosed herein and a pharmaceutically acceptable carrier. In another aspect, the present disclosure refers to a composition comprising an effective amount of the expanded immune cells as disclosed herein for use in the treatment of a disease or disorder in a subject.

**[0019]** A further example provides a method of treating a disease or disorder in a subject comprising administering a therapeutically effective amount of the expanded immune cells of the examples to the subject.

**[0019a]** In another aspect, the present disclosure refers to use of the expanded immune cells as disclosed herein in the manufacture of a medicament for treating a disease or disorder in a subject.

**[0020]** In some examples, the disease or disorder is cancer, inflammation, graft versus host disease, transplant rejection, an autoimmune disorder, an immunodeficiency disease, a B cell malignancy, or an infection. In particular examples, the cancer is a leukemia. In some examples, the leukemia is an acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), or a chronic myelogenous leukemia (CML). In particular examples, the disorder is graft versus host disease (GVHD). In some examples, the disorder is multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, type I diabetes, systemic lupus erythematosus, contact hypersensitivity, asthma or Sjogren's syndrome. In some examples, the subject is a human.

**[0021]** In certain examples, the immune cells are allogeneic. In some examples, the immune cells are autologous. In some examples, the immune cells are NK cells or T cells.

**[0022]** In additional examples, the method further comprises administering at least a second therapeutic agent. In some examples, the at least a second therapeutic agent is a therapeutically effective amount of an anti-cancer agent, immunomodulatory agent, or an immunosuppressive agent. In certain examples, the anti-cancer agent is chemotherapy, radiotherapy, gene therapy, surgery, hormonal therapy, anti-angiogenic therapy or immunotherapy.

**[0023]** In some examples, the immunosuppressive agent is a calcineurin inhibitor, an mTOR inhibitor, an antibody, a chemotherapeutic agent irradiation, a chemokine, an interleukins or an inhibitor of a chemokine or an interleukin.

**[0024]** In further examples, the immune cells and/or the at least a second therapeutic agent are administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion. In some examples, the second therapeutic agent is an antibody. In certain examples, the antibody is a monoclonal, bispecific, or trispecific antibody. In some examples, the antibody is rituximab.

**[0025]** Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred examples of the

disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

## **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0026]** The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The present disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

**[0027] FIG. 1:** Zipper model of human natural killer cell receptors and target cell ligand interactions. Human NK cells interact with target cells via many receptors, including killer immunoglobulin receptors (KIRs), natural cytotoxicity receptors (NCRs), NKG2 family of receptors, nectin binding receptors, SLAM family receptors and others. Receptors on NK cells are activating (KIR2DL4, KIR3DS1, NKp30, NKp44, NKp46, NKp80, CD94-NKG2C, DAP10, NKG2D, DAP10, CRTAM, DNAM, 2B4, NTB-A, CD3 zeta, CD100, CD160) or inhibitory (KIR2DL1/2/3/5A/5B, KIR3DL1/2/3, CD94-NKG2A, TIGIT, CD96, CEACAM-1, ILT2/LILRB1, KLRG1, LAIR1, CD161, Siglec-3/7/9)

**[0028] FIGS. 2A-2E:** (**FIG. 2A-2B**) Fold expansion of fresh or frozen NK cells with IL-2 or APCs. (**FIG. 2C**) Flow cytometry at Day 0, Day 7, or Day 14 for expression of CD3 and CD56 in NK cells. (**FIG. 2D**) Cell number of NT-NK or SG4-NK cells stimulated with APCs. (**FIG. 2E**) Growth kinetics of NK cells stimulated with Clone 9 APC or UAPC.

**[0029] FIGS. 3A-3D:** (**FIG. 3A**) Flow cytometry of parental K562 cells for indicated markers. No observed expression of mb-IL21, 41BBL, CD48, and SLAMF7 (CS1). (**FIG. 3B**) Flow cytometry analysis of APCs post-transduction of clone 46 (mbIL-21, 41BBL). (**FIG. 3C**) Flow cytometry analysis of APCs post-transduction of uAPC construct for expression of CD48 (nmIL-21, 41BBL, and CD48). (**FIG. 3D**) Flow cytometry analysis of APCs post-transduction of uAPC2 construct for expression of CS1 (mbIL-21, 41BBL, and CS1).

**[0030] FIGS. 4A-4D:** MMLV-retroviral transfer construct maps and annotations. (**FIG. 4A**) Retroviral transfer vectors for mb-IL21. (**FIG. 4B**) Retroviral transfer vectors for 41BBL.

## **WHAT IS CLAIMED IS:**

1. A universal antigen presenting cell (UAPC) engineered to express (1) CD48 and/or CS1 (CD319), (2) membrane-bound interleukin-21 (mbIL-21), and (3) 41BB ligand (41BBL).
2. The UAPC of claim 1, wherein the UAPC expresses CD48.
3. The UAPC of claim 1, wherein the UAPC expresses CS1.
4. The UAPC of claim 1, wherein the UAPC expresses CD48 and CS1.
5. The UAPC of claim 1, wherein the UAPC has essentially no expression of endogenous HLA class I, II, or CD1d molecules.
6. The UAPC of claim 1, wherein the UAPC expresses ICAM-1 (CD54) and LFA-3 (CD58).
7. The UAPC of claim 1, wherein the UAPC is a leukemia cell-derived UAPC.
8. The UAPC of claim 7, wherein the leukemia-cell derived UAPC is further defined as a K562 cell.
9. The UAPC of claim 1, wherein the UAPC has been engineered by retroviral transduction.
10. The UAPC of claim 9, wherein the retroviral transduction is further defined as transduction using a viral construct of SEQ ID NO:1 and/or SEQ ID NO:2.
11. The UAPC of any one of claims 1-10, wherein the UAPC is irradiated.
12. A method for expanding immune cells comprising culturing the immune cells in the presence of an effective amount of UAPCs of any one of claims 1-11.
13. The method of claim 12, wherein the immune cells and UAPCs are cultured at a ratio of 3:1 to 1:3.
14. The method of claim 12, wherein the immune cells and UAPCs are cultured at a ratio of 1:2.
15. The method of claim 12, wherein the expanding is in the presence of IL-2.

16. The method of claim 15, wherein the IL-2 is present at a concentration of 10-500 U/mL.
17. The method of claim 15, wherein the IL-2 is present at a concentration of 100-300 U/mL.
18. The method of claim 15, wherein the IL-2 is present at a concentration of 200 U/mL.
19. The method of claim 15, wherein the IL-2 is recombinant human IL-2.
20. The method of claim 15, wherein the IL-2 is replenished every 2-3 days.
21. The method of claim 12, wherein the UAPCs are added at least a second time.
22. The method of claim 12, wherein the immune cells are NK cells or T cells.
23. The method of claim 12, wherein the immune cells are NK cells.
24. The method of claim 12, wherein the immune cells are T cells.
25. The method of claim 12, wherein the immune cells are derived from cord blood (CB), peripheral blood (PB), stem cells, or bone marrow.
26. The method of claim 12, wherein the stem cells are induced pluripotent stem cells.
27. The method of claim 12, wherein the immune cells are obtained from CB.
28. The method of claim 27, wherein the CB is pooled from 2 or more individual cord blood units.
29. The method of claim 27, wherein the CB is pooled from 3, 4, 5, 6, 7, or 8 individual cord blood units.
30. The method of claim 23, wherein the NK cells are CB mononuclear cells (CBMCs).
31. The method of claim 23, wherein NK cells are further defined as CD56<sup>+</sup> NK cells.
32. The method of claim 12, wherein the method is performed in serum-free media.
33. The method of any one of claims 12-32, wherein the immune cells are engineered to express a chimeric antigen receptor (CAR).

34. The method of claim 33, wherein the CAR comprises a humanized antigen-binding domain.
35. The method of claim 33, wherein the CAR comprises a CD19, CD123, mesothelin, CD5, CD47, CLL-1, CD33, CD99, U5snRNP200, CD200, CS1, BAFF-R, ROR-1, or BCMA antigen-binding domain.
36. The method of claim 33, wherein the CAR comprises a CD19 or CD123 antigen-binding domain.
37. The method of claim 33, wherein the CAR comprises IL-15.
38. The method of claim 33, wherein the CAR comprises a suicide gene.
39. The method of claim 38, wherein the suicide gene is CD20, CD52, EGFRv3, or inducible caspase 9.
40. A population of expanded immune cells produced according to the methods of any one of claims 12-39.
41. A pharmaceutical composition comprising the population of expanded immune cells of claim 40 and a pharmaceutically acceptable carrier.
42. A composition comprising an effective amount of the expanded immune cells of claim 40 for use in the treatment of a disease or disorder in a subject.
43. Use of the expanded immune cells of claim 40 in the manufacture of a medicament for treating a disease or disorder in a subject.
44. The use of claim 43, wherein the disease or disorder is cancer, inflammation, graft versus host disease, transplant rejection, an autoimmune disorder, an immunodeficiency disease, a B cell malignancy, or an infection.
45. The use of claim 44, wherein the cancer is a leukemia.

46. The use of claim 45, wherein the leukemia is an acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), or a chronic myelogenous leukemia (CML).
47. The use of claim 43, wherein the immune cells are allogeneic.
48. The use of claim 43, wherein the immune cells are autologous.
49. The use of claim 43, wherein the immune cells are NK cells or T cells.
50. The use of claim 43, wherein the immune cells are NK cells.
51. The use of claim 43, wherein the disorder is graft versus host disease (GVHD).
52. The use of claim 43, wherein the disorder is multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, type I diabetes, systemic lupus erythematosus, contact hypersensitivity, asthma or Sjogren's syndrome.
53. The use of claim 43, wherein the subject is a human.
54. The use of claim 43, wherein at least a second therapeutic agent is to be further administered.
55. The use of claim 54, wherein the at least a second therapeutic agent is a therapeutically effective amount of an anti-cancer agent, immunomodulatory agent, or an immunosuppressive agent.
56. The use of claim 55, wherein the anti-cancer agent is chemotherapy, radiotherapy, gene therapy, surgery, hormonal therapy, anti-angiogenic therapy or immunotherapy.
57. The use of claim 55, wherein the immunosuppressive agent is a calcineurin inhibitor, an mTOR inhibitor, an antibody, a chemotherapeutic agent irradiation, a chemokine, an interleukins or an inhibitor of a chemokine or an interleukin.

58. The use of claim 54, wherein immune cells and/or the at least a second therapeutic agent are to be administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion.
59. The use of claim 54, wherein the second therapeutic agent is an antibody.
60. The use of claim 59, wherein the antibody is a monoclonal, bispecific, or trispecific antibody.
61. The use of claim 60, wherein the antibody is rituximab.