

CLAIMS

1. A method of expanding and isolating $\gamma\delta$ T cells from human peripheral blood mononuclear cells (PBMCs), the method comprising:
 - a. ~~obtaining being provided with~~ human PBMCs;
 - b. culturing the human PBMCs in a culture media comprising interleukin-2 (IL-2) and an anti-TCR V γ 9 monoclonal antibody or antigen binding fragment thereof to expand the $\gamma\delta$ T cells; and
 - c. isolating the $\gamma\delta$ T cells.
2. The method of claim 1, wherein the concentration of the IL-2 is about 50 IU/mL to about 5000 IU/mL.
3. The method of claim 2, wherein the concentration of IL-2 is about 100 IU/mL to about 1000 IU/mL.
4. The method of claim 1, wherein the IL-2 is recombinant human IL-2 (rhIL-2).
5. The method of claim 1, wherein the anti-TCR V γ 9 monoclonal antibody or antigen binding fragment thereof is chimeric.
6. The method of claim 1, wherein the anti-TCR V γ 9 monoclonal antibody or antigen binding fragment thereof is human or humanized.
7. The method of claim 1, wherein the $\gamma\delta$ T cell is a V γ 9⁺ $\gamma\delta$ T cell of a V γ 9⁻ $\gamma\delta$ T cell.
8. The method of claim 1, wherein the $\gamma\delta$ T cells are isolated by flow cytometry, magnetic separation, and negative selection.
9. An isolated $\gamma\delta$ T cell produced by the method of claim 8.
10. A method of generating a chimeric antigen receptor (CAR)- $\gamma\delta$ T cell, the method comprising:
 - a. obtaining an isolated $\gamma\delta$ T cell of claim 9;
 - b. contacting the $\gamma\delta$ T cell with a nucleic acid encoding a chimeric antigen receptor (CAR), the CAR comprising:
 - i. an extracellular domain;
 - ii. a transmembrane domain; and
 - iii. an intracellular signaling domain,

wherein the CAR optionally further comprises a signal peptide at the amino terminus and a hinge region connecting the extracellular domain and the transmembrane domain, and wherein contacting the $\gamma\delta$ T cell with the nucleic acid encoding the CAR generates a CAR $\gamma\delta$ T cell.

11. The method of claim 10, wherein the CAR comprises:
 - i. an extracellular domain comprising an antigen binding domain and/or an antigen binding fragment;
 - ii. a transmembrane domain comprising a CD8 α transmembrane domain;
 - iii. an intracellular signaling domain comprising a CD3 ζ or 4-1BB intracellular domain;
 - iv. a signal peptide comprising a CD8 α signal peptide; and
 - v. a hinge region comprising a CD8 α hinge region.
12. The method of claim 11, wherein the CAR comprises:
 - i. the transmembrane domain having an amino acid sequence at least 90% identical to SEQ ID NO:1;
 - ii. the intracellular domain having an amino acid sequence at least 90% identical to SEQ ID NO:2 or SEQ ID NO:3;
 - iii. the signal peptide having an amino acid sequence at least 90% identical to SEQ ID NO:4; and
 - iv. the hinge region having an amino acid sequence at least 90% identical to SEQ ID NO:5.
13. The method of claim 10, wherein the extracellular domain comprises an antigen binding domain and/or an antigen binding fragment that specifically binds a tumor antigen.
14. A CAR- $\gamma\delta$ T cell produced by the method of claim 10.
15. A pharmaceutical composition comprising the CAR- $\gamma\delta$ T cell of claim 14 and a pharmaceutically acceptable carrier.
16. ~~A method of treating or preventing a disease or condition in a subject in need thereof, the method comprising administering a therapeutically effective amount~~ Use of the pharmaceutical composition of claim 15 in the manufacture of a medicament for the

treatment or prevention of a disease or condition in a subject, wherein a therapeutically effective amount is to be administered to the subject in need thereof.

17. The ~~method-use~~ of claim 16, wherein the disease or condition is cancer.

18. The ~~method-use~~ of claim 17, wherein the cancer is selected from a solid cancer or a liquid cancer.

19. The ~~method-use~~ of claim 18, wherein the cancer is selected from the group consisting of a lung cancer, a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, an endometrial cancer, a prostate cancer, a thyroid cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), a Hodgkin's lymphoma/disease (HD), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

20. The ~~method-use~~ of claim 16, wherein the disease or condition is an autoimmune disease.

21. The ~~method-use~~ of claim 20, wherein the autoimmune disease is selected from the group consisting of alopecia, amyloidosis, ankylosing spondylitis, Castleman disease (CD), celiac disease, crohn's disease, endometriosis, fibromyalgia, glomerulonephritis, Graves' disease, Guillain-Barre syndrome, IgA nephropathy, lupus, lyme disease, Meniere's disease, multiple sclerosis, narcolepsy, neutropenia, psoriasis, psoriatic arthritis, rheumatoid arthritis, sarcoidosis, scleroderma, type 1 diabetes, ulcerative colitis, and vitiligo.

22. A method of producing a pharmaceutical composition comprising a CAR- $\gamma\delta$ T cell, wherein the methods comprises combining the CAR- $\gamma\delta$ T cell of claim 14 with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

23. The pharmaceutical composition of claim 15 for use in therapy, wherein a therapeutically effective amount is to be administered to the subject in need thereof.