

CLAIMS

1. A method of expanding and isolating $\gamma\delta$ T cells from human peripheral blood mononuclear cells (PBMCs), the method comprising:
 - a. obtaining 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 of the pharmaceutical composition of claim 15.
17. The method of claim 16, wherein the disease or condition is cancer.

18. The method of claim 17, wherein the cancer is selected from a solid cancer or a liquid cancer.
19. The method 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 of claim 16, wherein the disease or condition is an autoimmune disease.
21. The method 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.