CLAIMS

What is claimed is:

1. An *in vitro* method of activating and expanding γδ T cells comprising

isolating γδ T cells from a blood sample of a human subject,

activating the isolated γδ T cells in the presence of an aminobisphosphonate, human recombinant interleukin 2 (IL-2), and human recombinant interleukin 15 (IL-15), and

expanding the activated γδ T cells in the absence of an aminobisphosphonate and in the presence of human recombinant interleukin 2 (IL-2) and human recombinant interleukin 15 (IL-15).

2. The method of claim 1, wherein the γδ T cells are isolated from a leukapheresis human sample.

3. The method of claim 1 or 2, wherein the aminobisphosphonate comprises pamidronic acid, alendronic acid, zoledronic acid, risedronic acid, ibandronic acid, incadronic acid, a salt thereof and/or a hydrate thereof.

4. The method of any one of claims 1-3, wherein the aminobisphosphonate is zoledronic acid.

5. The method of any one of claims 1-4, wherein activation is in the presence of zoledronic acid and a cytokine composition consisting of IL-2 and IL-15.

6. The method of any one of claims 1-5, wherein the activation is further in the presence of a Toll-like receptor 2 (TLR2) ligand.

7. The method of claim 6, wherein the TLR2 ligand is selected from the group consisting of Amphotericin B, L-theanine, tannin, and polyphenols.

8. The method of any one of claims 1-7, wherein the activation is further in the presence of N-acetyl cysteine (NAC).

9. The method of any one of claims 1-8, wherein the activation is further in the presence of a COX-2 inhibitor.

10. The method of claim 9, wherein the COX-2 inhibitor is Ibuprofen.

11. The method of any one of claims 1 – 10, wherein activation is in the presence of zoledronic acid at a concentration of about 1 µM to about 10 µM.

12. The method of any one of claims 1 – 11, wherein activation is in the presence of IL-2 at a concentration of about 10 IU/ml to about 100 IU/ml.

13. The method of any one of claims 1 – 10, wherein activation is in the presence of zoledronic acid at a concentration of about 1 µM to about 100 µM, IL-2 at a concentration from about 10 IU/ml to about 200 IU/ml, and IL-15 at a concentration of about 10 – 500 ng/ml.

14. The method of any one of claims 1 – 10 and 13, wherein expansion is in the presence of IL-2 at a concentration from about 10 IU/ml to about 100 IU/ml and/or IL-15 at a concentration of about 50 – 200 ng/ml.

15. A population of expanded γδ T cells prepared by the method of any one of claims 1-14, wherein the density of the expanded γδ T cells is at least about 1 x 10^5 cells/ml, at least about 1 x 10^6 cells/ml, at least about 1 x 10^7 cells/ml, at least about 1 x 10^8 cells/ml, or at least about 1 x 10^9 cells/ml.

16. A method of treating cancer, comprising administering to a patient in need thereof an effective amount of the engineered γδ T cell of claim 15.

17. The method of claim 16, wherein the cancer is selected from the group consisting of lung cancer, small cell lung cancer, melanoma, liver cancer, breast cancer, uterine cancer, Merkel cell carcinoma, pancreatic cancer, gallbladder cancer, bile duct cancer, colorectal cancer, urinary bladder cancer, non-small cell lung cancer, kidney cancer, leukemia, ovarian cancer, esophageal cancer, brain cancer, gastric cancer, and prostate cancer.

18. The method of claim 17, wherein the cancer is melanoma.

19. An *in vitro* method of activating and expanding γδ T cells comprising

isolating γδ T cells from peripheral blood mononuclear cells (PBMC) of a human subject,

wherein the isolating comprises

contacting the PBMC with anti-α and anti-β T cell receptor (TCR) antibodies,

depleting α- and/or β-TCR positive cells from the PBMC,

activating the isolated γδ T cells in the presence of an aminobisphosphonate, human recombinant interleukin 2 (IL-2), and human recombinant interleukin 15 (IL-15), and

expanding the activated γδ T cells in the absence of an aminobisphosphonate and in the presence of human recombinant interleukin 2 (IL-2) and human recombinant interleukin 15 (IL-15).

20. The method of any one of claims 1 – 19, further comprising transducing the activated γδ T cells with a recombinant viral vector.

21. The method of claim 20, wherein the viral vector is a retroviral vector, a lentiviral vector, an adeno-associated virus (AAV), or a transposon.

22. The method of claim 20 or 21, wherein the viral vector is a lentiviral vector.

23. The method of any one of claims 20 – 22, wherein the viral vector comprises a RD114TR gene having at least 95% identity to SEQ ID NO: 1 encoding an envelope protein.

24. The method of any one of claims 20 – 23, wherein the viral vector encodes CD8 and/or an αβ-TCR.

25. The method of any one of claims 20 – 24, wherein the transducing is in the presence of a transduction enhancer.

26. The method of claims 25, wherein the transduction enhancer is RetroNectin® or VectoFusin-1®.

27. An engineered γδ T cell prepared by the method of any one of claims 14-26.

28. A population of expanded and activated γδ T cells prepared by the method of any one of claims 1 – 14 and 19 - 26.

29. The method of any one of claims 1- 14 and 19 – 26, further comprising a phosphoantigen selected from the group consisting of (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), isoprenoid pyrophosphates (farnesyl pyrophosphate (FPP), geranylgeranyl pyrophosphate (GGPP), isopentenyl pyrophosphate (IPP), and dimethylallyl diphosphate (DMAPP).