CLAIMS

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~~).~~), wherein the fold-expansion of the expanded γδ T cells is from 3000-fold to 35,000-fold.

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.~~15. 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~~).~~), wherein the fold-expansion of the expanded γδ T cells is from 3000-fold to 35,000-fold.

16. ~~17.~~The method of any one of claims 1~~ – 16~~-15, further comprising transducing the activated γδ T cells with a recombinant viral vector.

~~18.~~17. The method of claim ~~17~~16, wherein the viral vector is a retroviral vector, a lentiviral vector, an adeno-associated virus (AAV), or a transposon.

18. ~~19.~~The method of claim ~~17~~16 or ~~18~~17, wherein the viral vector is a lentiviral vector.

~~20.~~19. The method of any one of claims ~~17 – 19~~16-18, wherein the viral vector comprises a RD114TR gene having at least 95% identity to SEQ ID NO: 1 encoding an envelope protein.

20. ~~21.~~The method of any one of claims ~~17 – 20~~16-19, wherein the viral vector encodes CD8 and/or an αβ-TCR.

21. ~~22.~~The method of any one of claims ~~17 – 21~~16-20, wherein the transducing is in the presence of a transduction enhancer.

22. ~~23.~~The method of ~~claims 22~~claim 21, wherein the transduction enhancer is ~~RetroNectin®~~a fibronectin fragment coated onto plates or ~~VectoFusin-1®.~~a soluble cationic peptide.

~~24. An engineered γδ T cell prepared by the method of any one of claims 14-17.~~

~~25. A population of expanded and activated γδ T cells prepared by the method of any one of claims 1 – 14 and 16 - 23.~~

~~26.~~ 23. The method of any one of claims 1- ~~14 and 16 – 23,~~22, wherein the activation is further ~~comprising~~in the presence of 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).

24. The method of claim 4, wherein the fold-expansion of the expanded γδ T cells is from 10,000-fold to 35,000-fold.

25. The method any one of claims 1-24, wherein the activated γδ T cells are expanded in the absence of a phosphoantigen.

26. The method of claim 25, wherein the phosphoantigen is 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).