

## CLAIMS

What is claimed is:

1. A method of preparing  $\gamma\delta$  T cells comprising  
isolating  $\gamma\delta$  T cells from a blood sample of a human subject,  
activating the isolated  $\gamma\delta$  T cells in the presence of a feeder cell and at least one  
cytokine optionally selected from the group consisting of interleukin (IL)-1, IL-  
2, IL-12, IL-15, IL-18, IL-21, interferon (IFN)- $\alpha$ , and IFN- $\beta$ ,  
introducing a vector comprising a nucleic acid encoding a T cell receptor (TCR) or a  
chimeric antigen receptor (CAR) into the activated  $\gamma\delta$  T cells, and  
expanding the introduced  $\gamma\delta$  T cells.
2. The method of claim 1, wherein the blood sample comprises leukapheresis product.
3. The method of claim 1 or 2, wherein the blood sample comprises peripheral blood  
mononuclear cells (PBMC).
4. The method of any one of claims 1-3, wherein the activating is further in the presence of  
an aminobisphosphonate.
5. The method of claim 4, wherein the aminobisphosphonate comprises pamidronic acid,  
alendronic acid, zoledronic acid, risedronic acid, ibandronic acid, incadronic acid, a salt  
thereof and/or a hydrate thereof.
6. The method of claim 4 or 5, wherein the aminobisphosphonate comprises zoledronic  
acid.
7. The method of any one of claims 1-6, wherein the at least one cytokine comprises IL-2  
and IL-15.
8. The method of any one of claims 1-7, wherein the isolating comprises contacting the  
blood sample with anti- $\alpha$  and anti- $\beta$  T cell receptor (TCR) antibodies and depleting  $\alpha$ -  
and/or  $\beta$ -TCR positive cells from the blood sample.

9. The method of any one of claims 1-8, wherein the feeder cell is a human cell, a non-human cell, a virus-infected cell, a non-virus infected cell, a cell extract, a particle, a bead, a filament, or a combination thereof.
10. The method of claim 9, wherein the human cell is a K562 cell.
11. The method of claim 9 or 10, wherein the human cell is an engineered tumor cell comprising at least one recombinant protein.
12. The method of claim 11, wherein the at least one recombinant protein is selected from the group consisting of CD86, 4-1BBL, IL-15, and any combination thereof.
13. The method of claim 12, wherein the IL-15 is membrane bound IL-15.
14. The method of any one of claims 1-13, wherein the feeder cell is irradiated.
15. The method of any one of claims 1-14, wherein the isolated  $\gamma\delta$  T cells and the feeder cell are mixed in a ratio of from about 1:1 to about 50:1 (feeder cell : isolated  $\gamma\delta$  T cells).
16. The method of any one of claims 1-15, wherein the vector is a viral vector or a non-viral vector.
17. The method of any one of claims 1-16, wherein the expanding is in the absence of an aminobisphosphonate and in the presence of at least one cytokine.
18. The method of any one of claims 1-17, further comprising restimulating the expanded  $\gamma\delta$  T cells.
19. The method of claim 18, wherein the restimulating comprises contacting the expanded  $\gamma\delta$  T cells with a further feeder cell.
20. The method of claim 19, wherein the further feeder cell can be the same or different from the feeder cell.
21. The method of claim 19 or 20, wherein the expanded  $\gamma\delta$  T cells and the further feeder cell are mixed in a ratio of from about 1:1 to about 50:1 (further feeder cell : expanded  $\gamma\delta$  T cells).

22. The method of any one of claims 19-21, wherein the further feeder cell is selected from the group consisting of monocytes, PBMCs, and combinations thereof.
23. The method of any one of claims 19-22, wherein the further feeder cell is autologous to the human subject.
24. The method of any one of claims 19-23, wherein the further feeder cell is allogenic to the human subject.
25. The method of any one of claims 19-24, wherein the further feeder cell is depleted of  $\alpha\beta$  T cells.
26. The method of any one of claims 19-25, wherein the further feeder cell is contacted with an aminobisphosphonate.
27. The method of claim 26, wherein the aminobisphosphonate comprises zoledronic acid.
28. The method of claim 19, wherein the further feeder cell is a human cell, a non-human cell, a virus-infected cell, a non-virus infected cell, a cell extract, a particle, a bead, a filament, or a combination thereof .
29. The method of claim 28, wherein the human cell is a K562 cell.
30. The method of claim 28 or 29, wherein the human cell is an engineered tumor cell comprising at least one recombinant protein.
31. The method of claim 30, wherein the at least one recombinant protein is selected from the group consisting of CD86, 4-1BBL, IL-15, and any combination thereof.
32. The method of claim 31, wherein the IL-15 is membrane bound IL-15.
33. The method of any one of claims 19-32, wherein the further feeder cell is irradiated.
34. An *in vitro* method of expanding  $\gamma\delta$  T cells comprising  
isolating  $\gamma\delta$  T cells from a blood sample of a human subject,  
activating the isolated  $\gamma\delta$  T cells in the presence of at least one cytokine optionally  
selected from the group consisting of interleukin (IL)-1, IL-2, IL-12, IL-15, IL-

18, IL-21, interferon (IFN)- $\alpha$ , and IFN- $\beta$  and one or more of an aminobisphosphonate or a feeder cell, expanding the activated  $\gamma\delta$  T cells, and restimulating the expanded  $\gamma\delta$  T cells.

35. The method of claim 34, wherein the blood sample comprises leukapheresis product.

36. The method of claim 34 or 35, wherein the blood sample comprises peripheral blood mononuclear cells (PBMC).

37. The method of any one of claims 34-36, wherein the aminobisphosphonate is present and comprises pamidronic acid, alendronic acid, zoledronic acid, risedronic acid, ibandronic acid, incadronic acid, a salt thereof and/or a hydrate thereof.

38. The method of any one of claims 34-37, wherein the aminobisphosphonate is present and comprises zoledronic acid.

39. The method of any one of claims 34-38, wherein the at least one cytokine comprises IL-2 and IL-15.

40. The method of any one of claims 34-39, wherein the isolating comprises contacting the blood sample with anti- $\alpha$  and anti- $\beta$  T cell receptor (TCR) antibodies and depleting  $\alpha$ - and/or  $\beta$ -TCR positive cells from the blood sample.

41. The method of any one of claims 34-40, wherein the feeder cell is present and is a human cell, a non-human cell, a virus-infected cell, a non-virus infected cell, a cell extract, a particle, a bead, a filament, or a combination thereof.

42. The method of claim 41, wherein the human cell is a K562 cell.

43. The method of claim 41 or 42, wherein the human cell is an engineered tumor cell comprising at least one recombinant protein.

44. The method of claim 43, wherein the at least one recombinant protein is selected from the group consisting of CD86, 4-1BBL, IL-15, and any combination thereof.

45. The method of claim 44, wherein the IL-15 is membrane bound IL-15.

46. The method of any one of claims 41-45, wherein the feeder cell is irradiated.
47. The method of any one of claims 41-46, wherein the isolated  $\gamma\delta$  T cells and the feeder cell are mixed in a ratio of from about 1:1 to about 50:1 (feeder cell : isolated  $\gamma\delta$  T cells).
48. The method of any one of claims 34-47, further comprising transducing the activated  $\gamma\delta$  T cells with a recombinant viral vector prior to the expanding.
49. The method of any one of claims 34-48, wherein the expanding is in the absence of an aminobisphosphonate and in the presence of at least one cytokine.
50. The method of any one of claims 34-49, wherein the restimulating comprises contacting the expanded  $\gamma\delta$  T cells with a further feeder cell.
51. The method of claim 50, wherein the further feeder cell can be the same or different from the feeder cell.
52. The method of claim 50 or 51, wherein the expanded  $\gamma\delta$  T cells and the further feeder cell are mixed in a ratio of from about 1:1 to about 50:1 (further feeder cell : expanded  $\gamma\delta$  T cells).
53. The method of any one of claims 50-52, wherein the further feeder cell is selected from the group consisting of monocytes, PBMCs, and combinations thereof.
54. The method of any one of claims 50-53, wherein the further feeder cell is autologous to the human subject.
55. The method of any one of claims 50-54, wherein the further feeder cell is allogenic to the human subject.
56. The method of any one of claims 50-55, wherein the further feeder cell is depleted of  $\alpha\beta$  T cells.
57. The method of any one of claims 50-56, wherein the further feeder cell is contacted with an aminobisphosphonate.
58. The method of claim 57, wherein the aminobisphosphonate comprises zoledronic acid.

59. The method of claim 50, wherein the further feeder cell is a human cell, a non-human cell, a virus-infected cell, a non-virus infected cell, a cell extract, a particle, a bead, a filament, or a combination thereof .

60. The method of claim 59, wherein the human cell is a K562 cell.

61. The method of claim 59 or 60, wherein the human cell is an engineered tumor cell comprising at least one recombinant protein.

62. The method of claim 61, wherein the at least one recombinant protein is selected from the group consisting of CD86, 4-1BBL, IL-15, and any combination thereof.

63. The method of claim 62, wherein the IL-15 is membrane bound IL-15.

64. The method of any one of claims 50-63, wherein the further feeder cell is irradiated.

65. A population of expanded  $\gamma\delta$  T cells prepared by the method of any one of claims 1-64, wherein the density of the expanded  $\gamma\delta$  T cells is at least about  $1 \times 10^5$  cells/ml, at least about  $1 \times 10^6$  cells/ml, at least about  $1 \times 10^7$  cells/ml, at least about  $1 \times 10^8$  cells/ml, or at least about  $1 \times 10^9$  cells/ml.

66. A method of treating cancer, comprising administering to a patient in need thereof an effective amount of the expanded  $\gamma\delta$  T cells prepared by the method of any one of claims 1-64 or the population of expanded  $\gamma\delta$  T cells of claim 65.

67. The method of claim 66, wherein the cancer is selected from the group consisting of acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytomas, neuroblastoma, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancers, brain tumors, such as cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas, Burkitt lymphoma, carcinoma of unknown primary origin, central nervous system lymphoma, cerebellar astrocytoma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-

cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, germ cell tumors, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gliomas, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, Hypopharyngeal cancer, intraocular melanoma, islet cell carcinoma, Kaposi sarcoma, kidney cancer, laryngeal cancer, lip and oral cavity cancer, liposarcoma, liver cancer, lung cancers, such as non-small cell and small cell lung cancer, lymphomas, leukemias, macroglobulinemia, malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma, melanomas, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, myelodysplastic syndromes, myeloid leukemia, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, pancreatic cancer, pancreatic cancer islet cell, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pituitary adenoma, pleuropulmonary blastoma, plasma cell neoplasia, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma, renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcomas, skin cancers, Merkel cell skin carcinoma, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, stomach cancer, T-cell lymphoma, throat cancer, thymoma, thymic carcinoma, thyroid cancer, trophoblastic tumor (gestational), cancers of unknown primary site, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom's macroglobulinemia, and Wilms tumor.

68. The method of claim 67, wherein the cancer is melanoma.

69. A method of treating an infectious disease, comprising administering to a patient in need thereof an effective amount of the expanded  $\gamma\delta$  T cells prepared by the method of any one of claims 1-64 or the population of expanded  $\gamma\delta$  T cells of claim 65.

70. The method of claim 69, wherein the infectious disease is selected from the group consisting of dengue fever, Ebola, Marburg virus, tuberculosis (TB), meningitis, and syphilis.

71. A method of treating an autoimmune disease, comprising administering to a patient in need thereof an effective amount of the expanded  $\gamma\delta$  T cells prepared by the method of any one of claims 1-64 or the population of expanded  $\gamma\delta$  T cells of claim 65.

72. The method of claim 71, wherein the autoimmune disease is selected from the group consisting of Arthritis, Chronic obstructive pulmonary disease, Ankylosing Spondylitis, Crohn's Disease (one of two types of idiopathic inflammatory bowel disease "IBD"), Dermatomyositis, Diabetes mellitus type 1, Endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome (GBS), Hashimoto's disease, Hidradenitis suppurativa, Kawasaki disease, IgA nephropathy, Idiopathic thrombocytopenic purpura, Interstitial cystitis, Lupus erythematosus, Mixed Connective Tissue Disease, Morphea, Myasthenia gravis, Narcolepsy, Neuromyotonia, Pemphigus vulgaris, Pernicious anaemia, Psoriasis, Psoriatic Arthritis, Polymyositis, Primary biliary cirrhosis, Relapsing polychondritis, Rheumatoid arthritis, Schizophrenia, Scleroderma, Sjogren's syndrome, Stiff person syndrome, Temporal arteritis (also known as "giant cell arteritis"), Ulcerative Colitis (one of two types of idiopathic inflammatory bowel disease "IBD"), Vasculitis, Vitiligo, and Wegener's granulomatosis.

73. A method of preparing  $\gamma\delta$  T cells comprising  
isolating  $\gamma\delta$  T cells from a blood sample of a human subject,  
activating the isolated  $\gamma\delta$  T cells in the absence of a feeder cell,  
introducing a vector comprising a nucleic acid encoding a T cell receptor (TCR) or a chimeric antigen receptor (CAR) into the activated  $\gamma\delta$  T cells, and  
expanding the transduced  $\gamma\delta$  T cells in the presence of a feeder cell.

74. The method of claim 73, wherein the activating, the transducing, and/or the expanding is performed in the presence of at least one cytokine selected from the group consisting of interleukin (IL)-1, IL-2, IL-12, IL-15, IL-18, IL-21, interferon (IFN)- $\alpha$ , and IFN- $\beta$ .

75. The method of claim 73 or 74, wherein the feeder cell is a human cell, a non-human cell, a virus-infected cell, a non-virus infected cell, a cell extract, a particle, a bead, a filament, or a combination thereof.

76. The method of any one of claims 73-75, wherein the feeder cell comprises peripheral blood mononuclear cells (PBMCs) and/or lymphoblastoid cells (LCLs).

77. The method of any one of claims 73-76, wherein the activating, the transducing, and/or the expanding is performed in the presence of OKT3.

78. The method of any one of claims 73-77, wherein the vector is a viral vector or a non-viral vector.

79. The method of any one of claims 1-64, wherein the expanded  $\gamma\delta$  T cells comprise  $\delta 1$  and/or  $\delta 2$  T cells.

80. The method of any one of claims 1-64 and 73-79, wherein the vector comprises a nucleic acid encoding a TCR and a nucleic acid encoding CD8 $\alpha\beta$  or CD8 $\alpha$ .