CLAIMS

What is claimed is:

~~1. A method for producing a therapeutic population of T cells comprising:~~

~~(a) culturing T cells from a biological sample from a subject in a first cell culture medium comprising antigen presenting cells (APCs) to produce a first population of T cells, wherein the APCs present an epitope of a peptide antigen in complex with an MHC protein;~~

~~(b) optionally, culturing the first population of T cells in a second cell culture medium to produce a second population of T cells;~~

~~(c) enriching CD137 (4-1BB) expressing T cells and/or CD69 expressing T cells from the first or second population of T cells to produce a third population of T cells; and~~

~~(d) expanding the third population of T cells in a third cell culture medium to obtain a therapeutic population of T cells comprising antigen-specific T cells.~~

~~2. The method of claim 1, wherein the method comprises culturing the first population of T cells in a second cell culture medium to produce the second population of T cells, and enriching CD137 (4-1BB)-expressing T cells and/or CD69 expressing T cells from the second population of T cells to produce the third population of T cells.~~

~~3.~~1. A method ~~for~~ of producing an *ex vivo* ~~therapeutic~~ population of expanded antigen specific T cells, the method comprising:

(a) culturing T cells from a biological sample from a subject in a first cell culture medium comprising antigen presenting cells (APCs) to produce a first population of T cells, wherein the APCs present an epitope of a peptide antigen in complex with an MHC protein;

(b) culturing the first population of T cells in a second cell culture medium to produce a second population of T cells;

(c) ~~optionally,~~ enriching CD137 (4-1BB)-expressing T cells ~~and/or CD69 expressing T cells~~ from the second population of T cells to produce a third population of T cells; and

(d) expanding the ~~second or~~ third population of T cells in a third cell culture medium to obtain ~~a therapeutic~~*ex vivo* population of expanded antigen specific T cells, wherein antigen specific T cells in the third population of T cells is at least ten-fold compared to antigen specific T cells in the first or the second population of T cells ~~comprising antigen-specific T cells; wherein the concentration of the peptide antigen in the third culture medium is at least 2-fold lower than the concentration of the peptide antigen in the first culture medium and/or second culture medium~~.

~~4.~~2. The method of claim ~~3~~1, wherein ~~the method comprises~~ enriching further comprises enriching ~~CD137 (4-1BB) expressing T cells and/or~~ CD69 expressing T cells from the second population of T cells to produce ~~a~~ the third population of T cells.

3. The method of claim 2, wherein enriching comprises contacting with an enriching reagent comprising an anti-CD137 reagent and anti-CD69 reagent respectively.

~~5. The method of any one of claims 1-4, wherein enriching CD137 (4-1BB) expressing T cells and/or CD69 expressing T cells from the second population of T cells begins 10, 11, 12, 13, 14, 15, 16, 17 or 18 days after beginning the culturing of the T cells from a biological sample from a subject in a first cell culture medium.~~

~~6. The method of any one of claims 1-5, wherein the APCs (i) comprise a polynucleotide sequence encoding the peptide antigen, or (ii) are loaded with the epitope of a peptide antigen.~~

~~7. The method of any one of claims 1-6, wherein the peptide antigen is directly added to the first cell culture medium.~~

~~8. The method of any one of claims 1-7, wherein the first cell culture medium comprises a first concentration of the peptide antigen~~

~~9. The method of any one of claims 1-7, wherein the method further comprises supplementing the first cell culture medium with an amount of the peptide antigen such that the first cell culture medium comprises a first concentration of the peptide antigen.~~

~~10. The method of claim 8 or 9, wherein the first concentration of the peptide antigen is from 1 nM to 100 μM or from 100 nM to 10 μM.~~

~~11. The method of any one of claims 8-10, wherein the first concentration of the peptide antigen is about 1 μM, 2 μM, 3 μM, 4 μM or 5 μM.~~

~~12. The method of any one of claims 1-11, wherein the second cell culture medium comprises a second concentration of the peptide antigen~~

~~13. The method of any one of claims 1-11, wherein the method further comprises supplementing the second cell culture medium with an amount of the peptide antigen such that the second cell culture medium comprises a second concentration of the peptide antigen.~~

~~14. The method of claim 12 or 13, wherein the second concentration of the peptide antigen is higher, lower or about the same as the first concentration of the peptide antigen.~~

~~15. The method of any one of claims 12-14, wherein the second concentration of the peptide antigen is from 1 nM to 100 μM or from 100 nM to 10 μM.~~

~~16. The method of any one of claims 12-15, wherein second concentration of the peptide antigen is about 1 μM, 2 μM, 3 μM, 4 μM or 5 μM.~~

~~17. The method of any one of claims 12-16, wherein culturing the first population of T cells in the second cell culture medium begins 9, 10, 11, 12, 13, 14, 15, 16 or 17 days after beginning the culturing of the T cells from a biological sample from a subject in a first cell culture medium.~~

~~18. The method of any one of claims 1-17, wherein the third cell culture medium comprises a third concentration of the peptide antigen~~

19. The method of any one of claims 1-17, wherein the method further comprises supplementing the third cell culture medium with an amount of the peptide antigen such that the third cell culture medium comprises a third concentration of the peptide antigen.

20. The method of claim 18 or 19, wherein the third concentration of the peptide antigen is at least 2-fold lower than the first concentration of the peptide antigen.

21. The method of any one of claims 18-20, wherein the third concentration of the peptide antigen is at least 2-fold lower than the second concentration of the peptide antigen.

22. The method of any one of claims 18-21, wherein the third concentration of the peptide antigen is at least 3, 4, 5, 6, 7, 8, 9 or 10-fold lower than the first concentration of the peptide antigen.

23. The method of any one of claims 18-22, wherein the third concentration of the peptide antigen is at least 3, 4, 5, 6, 7, 8, 9 or 10-fold lower than the second concentration of the peptide antigen.

24. The method of any one of claims 18-23, wherein the third concentration of the peptide antigen is from 0.1 nM to 10 μM.

25. The method of any one of claims 18-24, wherein the third concentration of the peptide antigen is about 0.1 nM, 0.5, nM, 1 nM, 10 nM, 25 nM, 50 nM, 100 nM, 150 nM, 200 nM, 300 nM, 400 nM, 500 nM, 1 μM or 10 μM.

26. The method of any one of claims 18-25, wherein expanding the second or third population of T cells in the third cell culture medium begins 11, 12, 13, 14, 15, 16 , 17, 18 or 19 days after beginning the culturing of the T cells from a biological sample from a subject in a first cell culture medium

27. The method of any one of claims 18-26, wherein expanding the second or third population of T cells in the third cell culture medium begins 1, 2, 3 4 or 5 days after enriching CD137 (4-1BB) expressing T cells and/or CD69 expressing T cells from the second population of T cells.

28. The method of any one of claims 1-27, wherein expanding the second or third population of T cells in a third cell culture medium comprises expanding the second or third population of T cells in the presence of an increasing concentration of the peptide antigen.

29. The method of claim 28, wherein expanding the second or third population of T cells in the presence of an increasing concentration of the peptide antigen comprises expanding the second or third population of T cells in a fourth cell culture medium comprising a fourth concentration of the peptide antigen

30. The method of claim 28, wherein expanding the second or third population of T cells in the presence of an increasing concentration of the peptide antigen comprises supplementing the third cell culture medium with an amount of the peptide antigen such that the third cell culture medium comprises a fourth concentration of the peptide antigen, wherein the fourth concentration of the peptide antigen is at least 1.1-fold higher than the third concentration of the peptide antigen.

31. The method of claim 29 or 30, wherein the fourth concentration of the peptide antigen is at least 2, 3, 4, 5, 6, 7, 8, 9 or 10-fold higher than the third concentration of the peptide antigen.

32. The method of any one of claims 29-31, wherein the fourth concentration of the peptide antigen is from 1 nM to 50 μM.

33. The method of any one of claims 29-32, wherein the fourth concentration of the peptide antigen is about 1 nM, 10 nM, 25 nM, 50 nM, 100 nM, 150 nM, 200 nM, 300 nM, 400 nM, 500 nM, 600 nM, 700 nM, 800 nM, 900 nM, 1 μM, 10 μM, 25 μM or 50 μM.

34. The method of any one of claims 29-33, wherein expanding the second or third population of T cells in a fourth cell culture medium comprising a fourth concentration of the peptide antigen or supplementing the third cell culture medium with an amount of the peptide antigen such that the third cell culture medium comprises a fourth concentration of the peptide antigen begins 12, 13, 14, 15, 16, 17, 18, 19 or 20 days after beginning the culturing of the T cells from a biological sample from a subject in a first cell culture medium.

35. The method of any one of claims 29-34, wherein expanding the second or third population of T cells in a fourth cell culture medium comprising a fourth concentration of the peptide antigen or supplementing the third cell culture medium with an amount of the peptide antigen such that the third cell culture medium comprises a fourth concentration of the peptide antigen begins 1, 2, 3, 4 or 5 days after beginning expanding the second or third population of T cells in a third cell culture medium comprising a third concentration of the peptide antigen or 1, 2, 3, 4 or 5 days after supplementing the third cell culture medium with an amount of the peptide antigen such that the third cell culture medium comprises a third concentration of the peptide antigen.

36. The method of any one of claims 29-35, wherein expanding the second or third population of T cells in the presence of an increasing concentration of the peptide antigen comprises expanding the second or third population of T cells in a fifth cell culture medium comprising a fifth concentration of the peptide antigen

37. The method of any one of claims 29-35, wherein expanding the second or third population of T cells in the presence of an increasing concentration of the peptide antigen comprises supplementing the fourth cell culture medium with an amount of the peptide antigen such that the fourth cell culture medium comprises a fifth concentration of the peptide antigen, wherein the fifth concentration of the peptide antigen is at least 1.1-fold higher than the fourth concentration of the peptide antigen.

38. The method of claim 36 or 37, wherein the fifth concentration of the peptide antigen is at least 2, 3, 4, 5, 6, 7, 8, 9 or 10-fold higher than the third concentration of the peptide antigen and/or the fourth concentration of the peptide antigen .

39. The method of any one of claims 36-38, wherein the fifth concentration of the peptide antigen is from 10 nM to 100 μM.

40. The method of any one of claims 36-39, wherein the fifth concentration of the peptide antigen is about 10 nM, 25 nM, 50 nM, 100 nM, 150 nM, 200 nM, 300 nM, 400 nM, 500 nM, 600 nM, 700 nM, 800 nM, 900 nM, 1 μM, 10 μM, 25 μM, 50 μM, 75 μM or 100 μM.

41. The method of any one of claims 36-40, wherein expanding the second or third population of T cells in a fifth cell culture medium comprising a fifth concentration of the peptide antigen or supplementing the fourth cell culture medium with an amount of the peptide antigen such that the fourth cell culture

medium comprises a fifth concentration of the peptide antigen begins 13, 14, 15, 16, 17, 18, 19, 20 or 21 days after beginning the culturing of the T cells from a biological sample from a subject in a first cell culture medium.

42. The method of any one of claims 36-41, wherein expanding the second or third population of T cells in a fifth cell culture medium comprising a fifth concentration of the peptide antigen or supplementing the fourth cell culture medium with an amount of the peptide antigen such that the fourth cell culture medium comprises a fifth concentration of the peptide antigen begins 1, 2, 3, 4 or 5 days after expanding the second or third population of T cells in a fourth cell culture medium comprising a fourth concentration of the peptide antigen or 1, 2, 3 4 or 5 days after supplementing the fourth cell culture medium with an amount of the peptide antigen such that the fourth cell culture medium comprises a fourth concentration of the peptide antigen.

43. The method of any one of claims 36-42, wherein expanding the second or third population of T cells in a fifth cell culture medium comprising a fifth concentration of the peptide antigen or supplementing the fourth cell culture medium with an amount of the peptide antigen such that the fourth cell culture medium comprises a fifth concentration of the peptide antigen begins 2, 3, 4, 5 or 6 days after expanding the second or third population of T cells in a third cell culture medium comprising a third concentration of the peptide antigen or 2, 3, 4, 5 or 6 days after supplementing the third cell culture medium with an amount of the peptide antigen such that the third cell culture medium comprises a third concentration of the peptide antigen.

44. The method of any one of claims 1-43, wherein the number of antigen-specific T cells in the second or third population of T cells is greater than the number of antigen-specific T cells in the first population of T cells.

45. The method of any one of claims 1-44, wherein the frequency of antigen-specific T cells in the second or third population of T cells is greater than the frequency of antigen-specific T cells in the first population of T cells, wherein the frequency of antigen-specific T cells in a population of T cells is the [number of antigen-specific T cells in the population]/[total number of T cells in the population] x 100.

46. The method of any one of claims 1-45, wherein the frequency of antigen-specific T cells in the therapeutic population of T cells is greater than the frequency of antigen-specific T cells in the first population of T cells, wherein the frequency of antigen-specific T cells in a population of T cells is the [number of antigen-specific T cells in the population]/[total number of T cells in the population] x 100.

47. The method of any one of claims 1-46, wherein the frequency of antigen-specific T cells in the therapeutic population of T cells is greater than the frequency of antigen-specific T cells in the second population of T cells, wherein the frequency of antigen-specific T cells in a population of T cells is the [number of antigen-specific T cells in the population]/[total number of T cells in the population] x 100.

48. The method of any one of claims 1-47, wherein the frequency of antigen-specific T cells in the therapeutic population of T cells is greater than the frequency of antigen-specific T cells in the third

population of T cells, wherein the frequency of antigen-specific T cells in a population of T cells is the [number of antigen-specific T cells in the population]/[total number of T cells in the population] x 100.

49. The method of any one of claims 1-48, culturing of the first population of T cells is performed for a period of from 5 to 25 days, from 7 to 16 days, from 13 to 15 days or about 13 or 14 days.

50. The method of any one of claims 1-49, wherein culturing of the second population of T cells is performed for a period of **1**, 2, 3, or 4 days.

51. The method of any one of claims 1-50, wherein culturing of the second population of T cells is performed for a period of from 5 to 25 days, from 7 to 14 days, from 11 to 13 days, 21 days or less, or about 12 days.

52. The method of any one of claims 1-51, wherein expanding the second or third population of T cells is performed for a period of from 5 to 25 days, from 7 to 14 days, from 11 to 13 days, 21 days or less, or about 12 days.

53. The method of any one of claims 1-52, wherein expanding the second or third population of T cells is performed for a period of from 4 to 24 days, from 6 to 13 days, from 10 to 12 days, 20 days or less, or about 11 days.

54. The method of any one of claims 1-53, wherein the method expands antigen-specific T cells.

55. The method of any one of claims 1-54, wherein the method expands naive T cells from the first population of T cells.

56. The method of any one of claims 1-55, wherein the method expands naive T cells from the first population of T cells that have become antigen-specific T cells.

57. The method of any one of claims 1-56, wherein the method comprises expanding antigen-specific T cells.

58. The method of any one of claims 1-57, wherein culturing T cells from a biological sample from a subject in a first cell culture medium expands antigen-specific T cells.

59. The method of any one of claims 1-58, wherein culturing the first population of T cells in a second cell culture medium expands antigen-specific T cells.

60. The method of any one of claims 1-59, wherein expanding the second or third population of T cells in a third cell culture medium expands antigen-specific T cells.

61. The method of any one of claims 1-60, wherein the first population of T cells is not obtained from a tumor infiltrating lymphocyte (TIL) sample.

62. The method of any one of claims 1-61, wherein the first and second culture mediums are the same.

63. The method of any one of claims 1-62, wherein the first and second culture mediums are different.

64. The method of any one of claims 1-63, wherein the first culture medium comprises GM-CSF, IL-4, FLT3L, TNF-α, IL-1β, PGE1, IL-6, IL-7, IL-12, IFN-α, R848, LPS, ss-rna40, poly I:C, or any combination thereof.

65. The method of any one of claims 1-64, wherein the second culture medium comprises a soluble anti-CD3 antibody, an anti-CD3 antibody conjugated to a bead, soluble anti-CD28 antibody, an anti-CD28

~~antibody conjugated to a bead, insulin, one or more non-essential amino acids, glucose, glutamine, IL-2, IL-7, IL-15, IL-12, a CD137 agonist, an AKT inhibitor, a MEM vitamin solution, sodium pyruvate or any combination thereof.~~

~~66. The method of any one of claims 1-65, wherein the first culture medium comprises FMS-like tyrosine kinase 3 receptor ligand (FLT3L).~~

~~67. The method of any one of claims 1-66, wherein the second culture medium comprises FLT3L.~~

~~68. The method of any one of claims 1-67, wherein the second culture medium does not comprise additional APCs.~~

~~69. The method of any one of claims 1-67, wherein a number of APCs present in the second or the third culture medium is less than the number of APCs present in the first cell culture medium.~~

~~70. The method of any one of claims 1-67, wherein supplementing does not comprise supplementing with APCs.~~

~~71. The method of any one of claims 1-70, wherein the method comprises enriching CD137-expressing T cells from the second population of T cells after (a) and before (b).~~

~~72. The method of any one of claims 1-71, wherein enriching comprises enriching with an enriching reagent comprising an anti-CD137 reagent.~~

~~73. The method of claim 72, wherein the enriching reagent is an antibody or binding fragment thereof.~~

~~74. The method of claim 72 or 73, wherein the enriching reagent is coupled to a solid surface.~~

~~75. The method of any one of claims 1-74, wherein enriching comprises immunoprecipitating.~~

~~76. The method of any one of claims 1-75, wherein the second and/or the third culture media is supplemented with a T cell activator.~~

~~77. The method of any one of claims 1-76, wherein the T cell activator comprises soluble CD3 and or CD28 coated beads.~~

~~78. The method of any one of claims 1-77, wherein the method further comprises harvesting the therapeutic population of T cells comprising antigen-specific T cells.~~

~~79. The method of claim 78, wherein the method further comprises transferring the harvested therapeutic population of T cells comprising antigen-specific T cells to an infusion bag.~~

~~80. The method of any one of claims 1-79, wherein the method further comprises administering the therapeutic population of T cells comprising antigen-specific T cells to the subject.~~

~~81. The method of any one of claims 1-80, wherein the subject has a disease or condition.~~

~~82. The method of claim 81, wherein the disease or condition is cancer.~~

~~83. The method of claim 82, wherein the cancer is a solid cancer.~~

~~84. The method of claim 82, wherein the cancer is melanoma, pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC) or non-small cell lung cancer (NSCLC).~~

~~85. The method of claim 82, wherein the cancer is unresectable melanoma or RAS mutant PDAC.~~

~~86. The method of any one of claims 1-85, wherein the subject is human.~~

87. The method of any one of claims 1-86, wherein the subject has previously received a PD-1 inhibitor, PD-L1 inhibitor, CTLA-4 inhibitor or any combination thereof.

88. The method of any one of claims 1-87, wherein the subject has disease progression.

89. The method of any one of claims 1-88, wherein the subject has received or is currently receiving a PD-1 inhibitor or PD-L1 inhibitor for at least 3 months.

90. The method of any one of claims 1-89, wherein the subject has stable disease or asymptomatic progressive disease.

91. The method of any one of claims 1-90, wherein the method further comprises depleting CD14+ cells from the biological sample prior to (a).

92. The method of any one of claims 1-91, wherein the method further comprises depleting CD25+ cells from the biological sample prior to (a).

93. The method of any one of claims 1-92, wherein the method further comprises depleting CD56+ cells from the biological sample prior to (a).

94. The method of any one of claims 1-93, wherein the biological sample is peripheral blood mononuclear cell (PBMC) sample.

95. The method of any one of claims 1-94, wherein the biological sample is a washed and/or cryopreserved peripheral blood mononuclear cell (PBMC) sample.

96. The method of any one of claims 1-95, wherein the expanded population of T cells or the third population of T cells comprises from $1x10^7$ to $1x10^{11}$ total cells.

97. The method of any one of claims 1-96, wherein the APCs comprise a polynucleotide encoding the epitope of the peptide antigen.

98. The method of claim 97, wherein the polynucleotide is mRNA.

99. The method of any one of claims 1-96, wherein the APCs have been contacted with a polypeptide comprising the peptide antigen.

100. The method of any one of claims 1-99, wherein the peptide antigen is a RAS peptide antigen.

101. The method of any one of claims 1-99, wherein the method comprises selecting the epitope by a method comprising:

(a) generating cancer cell nucleic acids from a first biological sample comprising cancer cells obtained from the subject and generating non-cancer cell nucleic acids from a second biological sample comprising non-cancer cells obtained from the same subject;

(b) sequencing the cancer cell nucleic acids by whole genome sequencing or whole exome sequencing, thereby obtaining a first plurality of nucleic acid sequences comprising cancer cell nucleic acid sequences; and sequencing the non-cancer cell nucleic acids by whole genome sequencing or whole exome sequencing, thereby obtaining a second plurality of nucleic acid sequences comprising non-cancer cell nucleic acid sequences;

(c) identifying cancer specific nucleic acid sequences from the first plurality of nucleic acid sequences that (i) encode epitopes containing a cancer specific mutation, (ii) that are specific to the cancer

~~cells and (iii) that do not include a nucleic acid sequence from the second plurality of nucleic acid sequences;~~

~~(d) predicting or calculating or measuring which epitopes form a complex with a protein encoded by an HLA allele of the same subject by an HLA peptide binding analysis; and~~

~~(e) selecting an epitope predicted or calculated or measured in (d), to bind to the protein encoded by an HLA allele of the same subject with an IC_{50} of less than 500 nM.~~

~~102. The method of any one of claims 1-101, wherein culturing a first population of T cells comprises adding a pulse amount of the peptide antigen prior to expanding the second population of T cells, prior to enriching CD137 (4-1BB) expressing T cells.~~

~~103. The method of claim 102, wherein the pulse amount of the peptide is added at most about 2 days prior to expanding the second population of T cells, prior to enriching CD137 (4-1BB) expressing T cells.~~

~~104. The method of claim 102 or 103, wherein the pulse amount of the peptide is higher than the first amount of the peptide antigen.~~

~~105. The method of any one of claims 100-104, wherein the RAS peptide antigen is a RAS peptide neoantigen or an antigen derived from a RAS mutation.~~

~~106.~~4. A method for producing an *ex vivo* ~~therapeutic~~ population of expanded antigen specific therapeutic T cells, the method comprising:

(a) culturing ~~a first population of~~ T cells from a biological sample from a subject in a first cell culture medium comprising antigen presenting cells (APCs) ~~to produce a second population of T cells~~, wherein the APC's present an epitope of a peptide antigen in complex with an MHC protein;

(b) culturing the first population of T cells in a second cell culture medium to produce a second population of T cells; and

~~(b)~~(c) expanding the second population of T cells in a ~~second~~ third cell culture medium in presence of an increasing concentration of a plurality of peptide antigens comprising the peptide antigen, thereby obtaining the *ex vivo* population of expanded antigen specific therapeutic T cells. ~~comprising a first amount of the peptide antigen to produce a third population of T cells;~~

~~(c) supplementing the second cell culture medium with a second amount of the peptide antigen, wherein the second amount of the peptide antigen is higher than the first amount of the peptide antigen; and~~

~~(d) expanding the third population of T cells to obtain a therapeutic population of T cells comprising antigen specific T cells.~~

~~107. A method for producing a therapeutic population of T cells comprising:~~

~~(a) culturing a first population of T cells from biological sample from a subject in a first cell culture medium comprising antigen presenting cells (APCs) to produce a second population of T cells, wherein the APCs present an epitope of a peptide antigen in complex with an MHC protein;~~

~~(b) enriching CD137 (4-1BB) expressing T cells and/or CD69 expressing T cells to produce an enriched second population of T cells;~~

~~(c) culturing the enriched population of T cells in a second culture medium that is supplemented with pulses of an increasing concentration of the peptide antigen, starting with a dose that is lower than that present in the first culture medium; and~~

~~(d) expanding the enriched second population of T cells in a second cell culture medium to obtain a therapeutic population of T cells comprising antigen specific T cells.~~

~~108. A pharmaceutical composition comprising a therapeutic population of T cells comprising antigen specific T cells produced according to the method of any one of claims 1-107.~~

5. The method of any one of claims 1-4, wherein the biological sample is a sample of peripheral blood mononuclear cells (PBMCs) from the subject; and wherein the method further comprises depleting CD14+ cells from the PBMC prior to culturing.
6. The method of claim 5, wherein the method further comprises depleting CD25+ cells from the PBMC prior to culturing.
7. The method of claim 1 or 4, wherein the first cell culture medium comprises one or more peptide antigens comprising the peptide antigen; or wherein the APCs comprise a polynucleotide encoding the peptide antigen.
8. The method of any one of the claims 1-3, wherein the second culture medium is spiked with an amount of the peptide antigen such that a concentration of the peptide antigen in the first culture medium is that is at least the same as, or higher than a second concentration of the peptide antigen comprised in the second culture medium, wherein the first concentration of the peptide antigen or the second concentration of the peptide antigen ranges from 1 nM to 100 µM or 100 nM to 10 µM.
9. The method of claim 8, wherein the first concentration of the peptide antigen or the second concentration of the peptide antigen is 1 µM, 2 µM, 3 µM, 4 µM or 5 µM.
10. The method of claim 2, wherein the enriching CD137 (4-1BB)-expressing T cells and CD69 expressing T cells from the second population of T cells begins at least 10 days after beginning the culturing of the T cells from the biological sample from the subject in the first cell culture medium.
11. The method of claim 2, wherein expanding the third population of T cells in the third cell culture medium begins at least 1 day after enriching CD137 (4-1BB)-expressing T cells and CD69 expressing T cells.
12. The method of any one of the claims 1-11, wherein expanding in the third cell culture medium comprises expanding the population of T cells in the presence of an increasing concentration of the peptide antigen.
13. The method of claim 12, wherein expanding in the third cell culture medium comprises expanding the population of T cells in the presence of a third concentration of the peptide antigen, followed by further expanding the population of T cells in the presence of a fourth concentration of the peptide antigen, and additionally followed by further expanding at least once the population of T cells in the presence of at least a fifth concentration of the peptide antigen, wherein the third concentration of the peptide antigen

is at least 2-fold lower than the first concentration of the peptide antigen, and wherein the third concentration of the peptide antigen ranges from 0.1 nM to 10 µM.

14. The method of claim 13, wherein the further expanding the third population of T cells in the presence of the fourth concentration of the peptide antigen is in a fourth cell culture medium, and the further expanding at least once the third population in the presence of at least a fifth concentration of the peptide antigen is in a fifth cell culture medium.

15. The method of claim 13, wherein the fourth concentration of the peptide antigen is at least 1.1-fold higher than the third concentration of the peptide antigen and wherein the fifth concentration of the peptide antigen is at least 1.1-fold higher than the fourth concentration of the peptide antigen.

16. The method of claim 13, wherein the fifth concentration of the peptide antigen is at least 2-fold relative to the third concentration of the peptide antigen or the fourth concentration of the peptide antigen.

17. The method of claim 13, wherein the fifth concentration of the peptide antigen is at least 10 nM.

18. The method of any one of claims 1-17, wherein expanding the third population of T cells in the presence of the third cell culture medium begins at least 11 days after beginning culturing the T cells from the biological sample in the first cell culture medium.

19. The method of claim 13, wherein the further expanding the third population of T cells in the presence of the fourth concentration of the peptide antigen begins at least 1, day after expanding the third population of T cells in the third cell culture medium, and wherein the further expanding the third population of T cells in the presence of the at least fifth concentration of the peptide antigen begins at least 2 days after expanding the third population of T cells in the third cell culture medium.

20. The method of claim 1 or 4, wherein frequency of antigen-specific T cells in the *ex vivo* population of expanded antigen specific T cells is greater than frequency of antigen-specific T cells in the first population of T cells, and wherein the frequency of antigen-specific T cells in the *ex vivo* population of expanded antigen specific T cells is greater than frequency of antigen-specific T cells in the third population of T cells;

wherein the frequency of antigen-specific T cells is: [number of antigen-specific T cells in the population]/[total number of T cells in the population] x 100.

21. The method of claim 18, wherein frequency of antigen-specific T cells in the *ex vivo* expanded population of antigen specific T cells is greater than the frequency of antigen-specific T cells in the first population of T cells by at least 1.2 fold for the peptide antigen.

22. The method of claim 1, wherein

(i) the *ex vivo* population of expanded antigen specific T cells or the third population of T cells comprises from $1x10^7$ to $1x10^{11}$ total cells,

(ii) the *ex vivo* population of expanded antigen specific T cells comprises activated CD8+ T cells and CD4+ T cells; or

(iii) obtaining the *ex vivo* population of expanded antigen specific T cells occurs at or less than 26 days from culturing T cells from the biological sample from the subject in the first cell culture medium.

23. A pharmaceutical composition comprising a population of T cells comprising the *ex vivo* population of expended antigen-specific T cells of any one of claims 1-4 and a therapeutically acceptable excipient.

24. The pharmaceutical composition of claim 23, wherein the *ex vivo* population of expanded antigen specific T cells or the third population of T cells comprises from $1x10^7$ to $1x10^{11}$ total cells and comprises activated CD8+ T cells and CD4+ T cells.

25. Use of a composition comprising the *ex vivo* population of expanded antigen specific T cells of any one of claims 1-4 in the manufacture of a medicament for treating cancer in a human subject.

26. The use of claim 25, wherein obtaining the *ex vivo* population of expanded antigen specific T cells occurs at or less than 26 days from culturing T cells from the biological sample from the subject.

27. A composition comprising the *ex vivo* population of expanded antigen specific T cells of any one of claims 1-4 for use in therapy.