CLAIMS

What is claimed is:

1. A method of producing an *ex vivo* 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) enriching CD137 (4-1BB)-expressing T cells from the 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 *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.
2. The method of claim 1, wherein enriching further comprises enriching CD69 expressing T cells from the second population of T cells to produce 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.
4. A method for producing an *ex vivo* population of expanded antigen specific therapeutic 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), 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
    (c) expanding the second population of T cells in a 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.
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.