SUMMARY

[0004] This disclosure provides novel and improved T cell therapeutics for clinical development and use. Although autologous T cell therapeutic is safe to use, several drastic improvements are necessary to meet therapeutic standards and development in the field has been both rapid and fraught with difficulties. Applicant's previously disclosed application provides hallmark developments in the composition and methods for T cell therapy in cancer, (WO2019/094642). The instant application results from a surprising discovery that depletion of certain cells expressing specific markers at different stages of the ex vivo immune cell preparation provides highly immunogenic cell composition. The present disclosure is derived also in part from the discovery of new and improved methods for antigenic stimulation thereby resulting in improved cell composition for the therapeutics development. Provided herein are new methods and compositions wherein, at least in part, selective depletion of certain immune cells from the ex vivo stimulation and cell expansion milieu provides new therapeutic compositions and improved methods.

[0004a] In one aspect, provided herein is a use of an expanded population of cells comprising tumor antigen-specific T cells and $1x10^8$ to $1x10^{11}$ total cells in the manufacture of a medicament for treating a cancer in a human subject in need thereof, wherein the expanded population of cells is produced according to a method comprising: (a) depleting CD14+ cells and/or CD25+ cells from peripheral blood mononuclear cells (PBMCs) comprising antigen presenting cells (APCs) and T cells, thereby forming a CD14 and/or CD25 depleted PBMCscomprising a first population of APCs and T cells, wherein the PBMCs are from a biological sample from the human subject with cancer; (b) directly incubating the first population of APCs and T cells from step (a) for a first time period in the presence of: (i) FMS-like tyrosine kinase 3 receptor ligand (FLT3L), and (ii) (A) a polypeptide comprising at least one tumor antigen epitope sequence expressed by cancer cells of the human subject with cancer, or (B) a polynucleotide encoding the polypeptide; thereby forming a population of cells comprising stimulated T cells; (c) expanding the stimulated T cells from step (b), thereby forming an expanded population of cells comprising tumor antigen-specific T cells, wherein the tumor antigen-specific T cells comprise T cells that are specific to a complex comprising (i) the at least one tumor antigen epitope sequence from step (b)(ii), and, (ii) an MHC protein expressed by the cancer cells, or APCs of the human subject of (b)(ii); wherein at least 0.1% of CD8+ T cells in the expanded population of cells are CD8+ tumor antigen-specific T cells derived from naïve CD8+ T cells.

[0004b] In one aspect, provided herein is an improved ex vivo method for preparing tumor antigen-specific T cells, the method comprising: (a) depleting CD14+ cells and/or CD25+ cells from peripheral blood mononuclear cells (PBMCs) comprising antigen presenting cells (APCs) and T cells, thereby forming CD14 and/or CD25 depleted PBMCs comprising a first population of APCs and T cells, wherein the PBMCs are from a human subject with cancer; (b) directly incubating the CD14 and/or CD25 depleted PBMCs from step (a) for a first time period in the presence of: (i) FMS-like tyrosine kinase 3 receptor ligand (FLT3L), and (ii) (A) a polypeptide comprising at least one tumor antigen

epitope sequence expressed by cancer cells of the human subject with cancer, or (B) a polynucleotide encoding the polypeptide; thereby forming a population of cells comprising stimulated T cells; (c) expanding the stimulated T cells from step (b), thereby forming an expanded population of cells comprising tumor antigen-specific T cells, wherein the tumor antigen-specific T cells comprise T cells that are specific to a complex comprising (i) the at least one tumor antigen epitope sequence from step (b)(ii), and, (ii) an MHC protein expressed by the cancer cells or APCs of the human subject: of (b)(ii); and (d) preparing a pharmaceutical composition comprising the expanded population of cells comprising tumor antigen-specific T cells from (c) for use in treating cancer in the human subject, (i) wherein the cancer is unresectable melanoma, or (ii) wherein the human subject has previously received a PD-1 inhibitor or PD-L1 inhibitor and a CTLA-4 inhibitor containing regimen and has disease progression, or (iii) wherein the human subject has received or is currently receiving a PD-1 inhibitor or PD-L1 inhibitor for at least 3 months and has stable disease or asymptomatic progressive disease.

[0004b] In one aspect, provided herein is a pharmaceutical composition comprising the expanded population as described herein, comprising $1x10^8$ to $1x10^{11}$ total cells comprising tumor antigen-specific T cells, wherein the tumor antigen-specific T cells comprise T cells specific to at least two different tumor antigen epitope sequences expressed by cancer cells of a human subject with cancer and not expressed by non-cancer cells of the human subject; wherein each tumor antigen specific T cell is specific to a complex comprising a tumor antigen epitope sequence of the at least two different tumor antigen epitope sequences and an MHC protein expressed by the cancer cells or APCs of the human subject; and a pharmaceutically acceptable carrier; and wherein

(a) the fraction of CD8+ tumor antigen-specific T cells of the total number of CD8+ T cells in the expanded population of cells comprising tumor antigen specific T cells is at least two-fold higher than the fraction of CD8+ tumor antigen-specific T cells of the total number of CD8+ T cells in the PBMCs;

(b) the fraction of CD4+ tumor antigen-specific T cells of the total number of CD4+ T cells in the expanded population of cells comprising tumor antigen specific T cells is at least two-fold higher than the fraction of CD4+ tumor antigen-specific T cells of the total number of CD4+ T cells in the PBMCs;

(c) at least 0.1% of the CD8+ T cells in the expanded population of cells comprising tumor antigen specific T cells are CD8+ tumor antigen-specific T cells derived from naïve CD8+ T cells; and/or (d) at least 0.1% of the CD4+ T cells in the expanded population of cells comprising tumor antigen specific T cells are CD4+ tumor antigen-specific T cells derived from naïve CD4+ T cells.

[0004c] In another aspect, provided herein is use of the pharmaceutical composition as described herein in the manufacture of a medicament for treating a cancer in a human subject, wherein

(i) the cancer is unresectable melanoma, or

(ii) wherein the human subject has previously received a PD-1 inhibitor or PD-L1 inhibitor and a CTLA-4 inhibitor containing regimen and has disease progression, or

(iii) wherein the human subject has received or is currently receiving a PD-1 inhibitor or PD-L1 inhibitor for at least 3 months and has stable disease or asymptomatic progressive disease.

[0004d] In another aspect, provided herein is the pharmaceutical composition as described herein for use in therapy.

[0005] Provided herein is an improved ex vivo method for preparing tumor antigen-specific T cells, the method comprising: depleting CD14+ cells and/or CD25+ cells from a population of immune cells comprising antigen presenting cells (APCs) and T cells, thereby forming a CD 14 and/or CD25 depleted population of immune cells comprising a first population of APCs and T cells, wherein the population of immune cells is from a biological sample from a human subject; and incubating the CD14 and/or CD25 depleted population of immune cells comprising a first population of APCs and T cells for a first time period in the presence of: FMS-like tyrosine kinase 3 receptor ligand (FLT3L), and (A) a polypeptide comprising at least one tumor antigen epitope sequence expressed by cancer cells of a human subject with cancer, or (B) a polynucleotide encoding the polypeptide; thereby forming a population of cells comprising stimulated T cells; expanding the population of cells comprising stimulated T cells, thereby forming an expanded population of cells comprising tumor antigen-specific T cells, wherein the tumor antigen-specific T cells comprise T cells that are specific to a complex comprising (i) the at least one tumor antigen epitope sequence and (ii) an MHC protein expressed by the cancer cells or APCs of the human subject of (b)(ii); and administering the expanded population of cells comprising tumor antigen-specific T cells to the human subject, wherein the expanded population of cells comprising tumor antigen-specific T cells comprises from $1xl0^{8}$ to $1x10^{11}$ total cells.

[0006] Provided herein is an improved ex vivo method for preparing tumor antigen-specific T cells, the method comprising: depleting CD14+ cells and/or CD25+ cells from a population of immune cells comprising antigen presenting cells (APCs) and T cells, thereby forming a CD 14 and/or CD25 depleted population of immune cells comprising a first population of APCs and T cells, wherein the population of immune cells is from a biological sample from a human subject; and incubating the CD14 and/or CD25 depleted population of immune cells comprising a first population of APCs and T cells for a first time

CLAIMS

What is claimed is:

1. Use of an expanded population of cells comprising tumor antigen-specific T cells and $1x10^8$ to $1x10^{11}$ total cells in the manufacture of a medicament for treating a cancer in a human subject in need thereof, wherein the expanded population of cells is produced according to a method comprising:

(a) depleting CD14+ cells and/or CD25+ cells from peripheral blood mononuclear cells (PBMCs) comprising antigen presenting cells (APCs) and T cells, thereby forming a CD14 and/or CD25 depleted PBMCs comprising a first population of APCs and T cells, wherein the PBMCs are from the human subject with cancer;

(b) directly incubating the first population of APCs and T cells from step (a) for a first time period in the presence of:

(i) FMS-like tyrosine kinase 3 receptor ligand (FLT3L), and

(ii) (A) a polypeptide comprising at least one tumor antigen epitope sequence expressed by cancer cells of the human subject with cancer, or (B) a polynucleotide encoding the polypeptide;

thereby forming a population of cells comprising stimulated T cells;

(c) expanding the stimulated T cells from step (b), thereby forming an expanded population of cells comprising tumor antigen-specific T cells, wherein the tumor antigen-specific T cells comprise T cells that are specific to a complex comprising (i) the at least one tumor antigen epitope sequence from step (b)(ii), and, (ii) an MHC protein expressed by the cancer cells, or APCs of the human subject of (b)(ii); wherein at least 0.1% of CD8+ T cells in the expanded population of cells are CD8+ tumor antigen-specific T cells derived from naïve CD8+ T cells.

2. An improved ex vivo method for preparing tumor antigen-specific T cells, the method comprising:

(a) depleting CD14+ cells and/or CD25+ cells from peripheral blood mononuclear cells (PBMCs) comprising antigen presenting cells (APCs) and T cells, thereby forming CD14 and/or CD25 depleted PBMCs comprising a first population of APCs and T cells, wherein the PBMCs are from a human subject with cancer;

(b) directly incubating the CD14 and/or CD25 depleted PBMCs from step (a) for a first time period in the presence of:

(i) FMS-like tyrosine kinase 3 receptor ligand (FLT3L), and

(ii) (A) a polypeptide comprising at least one tumor antigen epitope sequence expressed by cancer cells of the human subject with cancer, or (B) a polynucleotide encoding the polypeptide;

thereby forming a population of cells comprising stimulated T cells;

(c) expanding the stimulated T cells from step (b), thereby forming an expanded population of cells comprising tumor antigen-specific T cells, wherein the tumor antigen-specific T cells comprise T cells that are specific to a complex comprising (i) the at least one tumor antigen epitope sequence from step (b)(ii), and, (ii) an MHC protein expressed by the cancer cells or APCs of the human subject of (b)(ii); and

(d) preparing a pharmaceutical composition comprising the expanded population of cells comprising tumor antigen-specific T cells from (c) for use in treating cancer in the human subject with cancer,

(i) wherein the cancer is unresectable melanoma, or

(ii) wherein the human subject has previously received a PD-1 inhibitor or PD-L1 inhibitor and a CTLA-4 inhibitor containing regimen and has disease progression, or

(iii) wherein the human subject has received or is currently receiving a PD-1 inhibitor or PD-L1 inhibitor for at least 3 months and has stable disease or asymptomatic progressive disease.

3. The improved ex vivo method of claim 2, wherein the polynucleotide encoding the polypeptide is an mRNA.

4. The improved ex vivo method of claim 2, wherein depleting comprises depleting CD14+ cells and/or CD25+ cells directly from a washed and/or cryopreserved PBMC sample from the human subject; thereby forming a CD14 and/or CD25 depleted population of PBMCs comprising a first population of APCs and T cells; and wherein incubating comprises incubating the first population of APCs and T cells for a first time period in the presence of:

(i) FMS-like tyrosine kinase 3 receptor ligand (FLT3L), and

(ii) (A) a polypeptide comprising at least two different tumor antigen epitope sequences expressed by cancer cells of the human subject with cancer, or (B) a polynucleotide encoding the polypeptide;

thereby forming a population of cells comprising stimulated T cells.

5. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein incubating comprises introducing the polynucleotide encoding the polypeptide or

the mRNA into the CD14 and/or CD25 depleted PBMCs comprising the first population of APCs and T cells.

6. The use according to claim 5 or the improved ex vivo method of claim 5, wherein introducing comprises electroporating or nucleofecting.

7. The use according to claim 6 or the improved ex vivo method of claim 6, wherein the electroporating or nucleofecting is carried out without separating the T cells from the APCs of the first population of APCs and T cells.

8. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein the polypeptide comprising at least one tumor antigen epitope sequence comprises at least two different tumor antigen epitope sequences expressed by cancer cells of the human subject.

9. The use or the improved ex vivo method of claim 5, wherein incubating comprises incubating the CD14 and/or CD25 depleted PBMCs for the first time period in the presence of an mRNA encoding a polypeptide comprising at least two different tumor antigen epitope sequences expressed by cancer cells of the human subject with cancer.

10. The use or the improved ex vivo method of claim 9 wherein the mRNA comprises a 5’ CAP.

11. The use or the improved ex vivo method of claim 10, wherein the 5’ CAP is CAP-1.

12. The use or the improved ex vivo method of claim 10, wherein the mRNA comprises a 3’ polyA tail, wherein the polyA tail is from 120 to 135 nucleotides in length.

13. The use or the improved ex vivo method of claim 8, wherein a first tumor antigen epitope sequence of the at least two different tumor antigen epitope sequences is connected to a second tumor antigen epitope sequence of the at least two different tumor antigen epitope sequences via a linker sequence.

14. The use or the improved ex vivo method of claim 10, wherein the 5’ CAP is operably linked to a tumor antigen epitope sequence of the at least two different tumor antigen epitope sequences via a linker sequence.

15. The use or the improved ex vivo method of claim 8, wherein the at least two different tumor antigen epitope sequences are expressed as a single polypeptide chain.

16. The use or the improved ex vivo method of claim 8, wherein the at least two different tumor antigen epitope sequences are each 8 to 12 amino acids in length or 15 to 25 amino acids in length.

17. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein the polypeptide comprises at least 3, 4, 5, 6, 7, 8, 9, 10 or more different tumor antigen epitope sequences expressed by cancer cells of the human subject with cancer.

18. The use according to claim 1 or the improved ex vivo method of any one of claims 2-17, wherein

(a) the percentage of CD3+ cells in the expanded population of cells comprising tumor antigen-specific T cells is at least 40%, at least 50% or at least 60% of the total cell population;

(b) the percentage of CD107a+ cells in the expanded population of cells comprising tumor antigen-specific T cells is at least 10% of the tumor antigen-specific T cell population;

(c) the percentage of TNFα+ cells in the expanded population of cells comprising tumor antigen-specific T cells is at least 5% of the tumor antigen-specific T cell population;

(d) the percentage of IFNγ+ cells in the expanded population of cells comprising tumor antigen-specific T cells is at least 15% of the tumor antigen-specific T cell population;

(e) the percentage of TNFα+ and IFNγ+ cells in the expanded population of cells comprising tumor antigen-specific T cells is at least 2% of the tumor antigen-specific T cell population;

(f) the percentage of TNFα+ and CD107a+ cells in the expanded population of cells comprising tumor antigen-specific T cells is at least 0.5% of the tumor antigen-specific T cell population;

(g) the percentage of IFNγ+ and CD107a+ cells in the expanded population of cells comprising tumor antigen-specific T cells is at least 5% of the tumor antigen-specific T cell population;

(h) the percentage of TNFα+ and IFNγ+ and CD107a+ cells in the expanded population of cells comprising tumor antigen-specific T cells is at least 0.1% of the tumor antigen-specific T cell population;

(i) the percentage of CD4+ T cells in the expanded population of cells comprising tumor antigen-specific T cells that are effector memory T cells (CD62L- and CD45RA-) is at least 60%.;

(j) the percentage of CD4+ T cells in the expanded population of cells comprising tumor antigen-specific T cells that are effector T cells (CD62L- and CD45RA+) is at most 5%.;

(k) the percentage of CD4+ T cells in the expanded population of cells comprising tumor antigen-specific T cells that are central memory T cells (CD62L+ and CD45RA-) is at least 10%.;

(l) the percentage of CD8+ T cells in the expanded population of cells comprising tumor antigen-specific T cells that are naive T cells (CD62L+ and CD45RA+) is at most 25%;

(m) the percentage of CD8+ T cells in the expanded population of cells comprising tumor antigen-specific T cells that are effector memory T cells (CD62L- and CD45RA-) is at least 60%.;

(n) the percentage of CD8+ T cells in the expanded population of cells comprising tumor antigen-specific T cells that are effector T cells (CD62L- and CD45RA+) is at most 10%; and/or

(o) the percentage of CD8+ T cells in the expanded population of cells comprising tumor antigen-specific T cells that are central memory T cells (CD62L+ and CD45RA-) is at least 15%.

19. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein the human subject is refractory to an anti-checkpoint inhibitor therapy or has a mutation in a BRAF gene and has previously received a B-raf inhibitor or a B-raf/MEK combination therapy.

20. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein depleting further comprises depleting CD11b+ cells from the PBMCs from the human subject that has not been subject to a step of monocyte maturation into mature dendritic cells (DCs).

21. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein steps (b) and (c) are performed in less than 28 days.

22. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein

(a) fraction of CD8+ tumor antigen-specific T cells of total number of CD8+ T cells in the expanded population of cells comprising tumor antigen specific T cells is at least two-fold higher than fraction of CD8+ tumor antigen-specific T cells of total number of CD8+ T cells in the PBMCs from the human subject;

(b) fraction of CD4+ tumor antigen-specific T cells of total number of CD4+ T cells in the expanded population of cells comprising tumor antigen specific T cells is at least two-fold higher than fraction of CD4+ tumor antigen-specific T cells of total number of CD4+ T cells in the PBMCs from the human subject;

(c) at least 0.1% of the CD8+ T cells in the expanded population of cells comprising tumor antigen specific T cells are CD8+ tumor antigen-specific T cells derived from naïve CD8+ T cells; and/or

(d) at least 0.1% of the CD4+ T cells in the expanded population of cells comprising tumor antigen specific T cells are CD4+ tumor antigen-specific T cells derived from naïve CD4+ T cells.

23. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein expanding comprises (A) contacting the population of cells comprising stimulated T cells with a second population of mature APCs, wherein the second population of mature APCs (i) have been incubated with FLT3L and (ii) present the at least one tumor antigen epitope sequence; and (B) expanding the population of cells comprising stimulated T cells for a second time period, thereby forming an expanded population of T cells.

24. The use or the improved ex vivo method of claim 23, wherein the second population of mature APCs have been incubated with FLT3L for at least 1 day prior to contacting the population of cells comprising stimulated T cells with the second population of mature APCs.

25. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein the improved ex vivo method further comprises harvesting the expanded population of cells comprising tumor antigen-specific T cells, cryopreserving the expanded population of cells comprising tumor antigen-specific T cells or preparing a pharmaceutical composition containing the expanded population of cells comprising tumor antigen-specific T cells.

26. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein the polypeptide is from 8 to 50 amino acids in length.

27. The use according to claim 1 or the improved ex vivo method of any one of claims 2-4, wherein depleting further comprising depleting CD19+ cells from the PBMCs comprising a first population of APCs and T cells.

28. A pharmaceutical composition comprising the expanded population of claim 1, comprising $1x10^8$ to $1x10^{11}$ total cells comprising tumor antigen-specific T cells, wherein the tumor antigen-specific T cells comprise T cells specific to at least two different tumor antigen epitope sequences expressed by cancer cells of a human subject with cancer and not expressed by non-cancer cells of the human subject; wherein each tumor antigen specific T cell is specific to a complex comprising a tumor antigen epitope sequence of the at least two different tumor antigen epitope sequences and an MHC protein expressed by the cancer cells or APCs of the human subject; and a pharmaceutically acceptable carrier; and

wherein

(a) the fraction of CD8+ tumor antigen-specific T cells of the total number of CD8+ T cells in the expanded population of cells comprising tumor antigen specific T cells is at least two-fold

higher than the fraction of CD8+ tumor antigen-specific T cells of the total number of CD8+ T cells in the PBMCs;

(b) the fraction of CD4+ tumor antigen-specific T cells of the total number of CD4+ T cells in the expanded population of cells comprising tumor antigen specific T cells is at least two-fold higher than the fraction of CD4+ tumor antigen-specific T cells of the total number of CD4+ T cells in the PBMCs;

(c) at least 0.1% of the CD8+ T cells in the expanded population of cells comprising tumor antigen specific T cells are CD8+ tumor antigen-specific T cells derived from naïve CD8+ T cells; and/or

(d) at least 0.1% of the CD4+ T cells in the expanded population of cells comprising tumor antigen specific T cells are CD4+ tumor antigen-specific T cells derived from naïve CD4+ T cells.

29. Use of the pharmaceutical composition of claim 28 in the manufacture of a medicament for treating a cancer in a human subject, wherein

(i) the cancer is unresectable melanoma, or

(ii) wherein the human subject has previously received a PD-1 inhibitor or PD-L1 inhibitor and a CTLA-4 inhibitor containing regimen and has disease progression, or

(iii) wherein the human subject has received or is currently receiving a PD-1 inhibitor or PD-L1 inhibitor for at least 3 months and has stable disease or asymptomatic progressive disease.

30. The pharmaceutical composition of claim 28 for use in therapy.