COMPOSITIONS AND METHODS FOR PREPARING T CELL COMPOSITIONS AND USES THEREOF

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/827,018, filed on March 30, 2019, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Adoptive immunotherapy or adoptive cellular therapy with lymphocytes (ACT) is the transfer of gene modified T lymphocytes to a subject for the therapy of disease. Adoptive immunotherapy has yet to realize its potential for treating a wide variety of diseases including cancer, infectious disease, autoimmune disease, inflammatory disease, and immunodeficiency. However, most, if not all adoptive immunotherapy strategies require T cell activation and expansion steps to generate a clinically effective, therapeutic dose of T cells. Existing strategies of obtaining patient cells, and ex vivo activation, expansion and recovery of effective number of cells for ACT is a prolonged, cumbersome and an inherently complex process - and poses a serious challenge. Accordingly, there remains a need for developing compositions and methods for expansion and induction of antigen specific T cells with a favorable phenotype and function and within a shorter time span.

SUMMARY

[0002a] In one aspect, provided herein is a cell population comprising isolated antigen-specific T cells for use in treating a cancer in a subject in need thereof, wherein the cell population is prepared by: contacting ~~comprising~~ T cells from the subject or allogeneic T cells ex vivo ~~that have been contacted~~ with antigen presenting cells (APCs) comprising one or more warehouse curated peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a prevalidated library of epitope sequences, wherein each epitope sequence known for cancer, by a method comprising, selecting an epitope sequence in the prevalidated library is matched to a protein encoded by an HLA allele, and wherein each of the at least one selected epitope sequence satisfies ~~at least two or three~~ each of the following criteria:

(i) binds to a protein encoded ~~by~~an the HLA allele expressed by the subject,

(ii) is immunogenic according to an immunogenicity assay,

(iii) is presented by APCs according to a mass spectrometry assay, and

(iv) stimulates T cells to be cytotoxic according to a cytotoxicity assay;

wherein the antigen-specific T cells comprise a T cell receptor (TCR) that binds to a peptide-MHC complex, the peptide-MHC complex comprising the at least one selected epitope sequence and the matched protein encoded by ~~an~~the HLA allele; and

wherein at least 0.01% of CD8+ T cells of the cell population are detected to be immunogenic to the at least one selected epitope sequence according to a multimer assay; and percentage of the detected CD8+ T cells is higher than percentage of CD8+ T cells detected in a control sample, wherein the control sample

comprises T cells that have been stimulated with APCs that (i) do not comprise a peptide containing the at least one selected epitope sequence, (ii) comprise a peptide derived from a different protein than the at least one selected epitope sequence, or (iii) comprise a peptide with a random sequence.

[0002b] In one aspect, provided herein is a pharmaceutical composition comprising a~~the~~ cell population comprising isolated antigen-specific T cells~~of as described herein~~ for use in treating cancer in a subject, wherein the cell population is prepared by: contacting T cells from the subject or allogeneic T cells ex vivo with antigen presenting cells (APCs) comprising one or more warehouse curated peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a prevalidated library of epitope sequences, wherein each epitope sequence known for cancer, by a method comprising, selecting an epitope sequence in the prevalidated library is matched to a protein encoded by an HLA allele, and wherein each of the at least one selected epitope sequence satisfies each of the following criteria:

(i) binds to a protein encoded by an HLA allele expressed by the subject,

(ii) is immunogenic according to an immunogenicity assay,

(iii) is presented by APCs according to a mass spectrometry assay, and

(iv) stimulates T cells to be cytotoxic according to a cytotoxicity assay;

wherein the antigen-specific T cells comprise a T cell receptor (TCR) that binds to a peptide-MHC complex, the peptide-MHC complex comprising the at least one selected epitope sequence and the matched protein encoded by the HLA allele; and

wherein at least 0.01% of CD8+ T cells of the cell population are detected to be immunogenic to the at least one selected epitope sequence according to a multimer assay; and percentage of the detected CD8+ T cells is higher than percentage of CD8+ T cells detected in a control sample, wherein the control sample comprises T cells that have been stimulated with APCs that (i) do not comprise a peptide containing the at least one selected epitope sequence, (ii) comprise a peptide derived from a different protein than the at least one selected epitope sequence, or (iii) comprise a peptide with a random sequence.

[0002c] In one aspect, provided herein is a use of a~~the~~ cell population comprising isolated antigen-specific T cells~~as described herein~~ in the manufacture of a medicament for treating cancer, wherein the cell population is prepared by: contacting T cells from the subject or allogeneic T cells ex vivo with antigen presenting cells (APCs) comprising one or more warehouse curated peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a prevalidated library of epitope sequences, wherein each epitope sequence known for cancer, by a method comprising, selecting an epitope sequence in the prevalidated library is matched to a protein encoded by an HLA allele, and wherein each of the at least one selected epitope sequence satisfies each of the following criteria:

(i) binds to a protein encoded by an HLA allele expressed by the subject,

(ii) is immunogenic according to an immunogenicity assay,

(iii) is presented by APCs according to a mass spectrometry assay, and

(iv) stimulates T cells to be cytotoxic according to a cytotoxicity assay;

wherein the antigen-specific T cells comprise a T cell receptor (TCR) that binds to a peptide-MHC complex, the peptide-MHC complex comprising the at least one selected epitope sequence and the matched protein encoded by the HLA allele; and

wherein at least 0.01% of CD8+ T cells of the cell population are detected to be immunogenic to the at least one selected epitope sequence according to a multimer assay; and percentage of the detected CD8+ T cells is higher than percentage of CD8+ T cells detected in a control sample, wherein the control sample comprises T cells that have been stimulated with APCs that (i) do not comprise a peptide containing the at least one selected epitope sequence, (ii) comprise a peptide derived from a different protein than the at least one selected epitope sequence, or (iii) comprise a peptide with a random sequence.

~~**[0002d]** In one aspect, provided herein is a cell population according as described herein for use in therapy.~~

[0002de]In one aspect, provided herein is a method of preparing a~~the~~ cell population comprising isolated antigen-specific T cells~~as described herein~~, comprising contacting T cells from a subject or allogeneic T cells ex vivo ~~a cell population comprising T cells~~ with antigen presenting cells (APCs) comprising one or more warehouse curated peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a prevalidated library of epitope sequences, wherein each epitope sequence in the prevalidated library is matched to a protein encoded by an HLA allele, and wherein each of the at least one selected epitope sequence satisfies ~~at least two or three or~~ each of the following criteria:

(i) binds to a protein encoded by the HLA allele,

(ii) is immunogenic according to an immunogenicity assay,

(iii) is presented by APCs according to a mass spectrometry assay, and

(iv) stimulates T cells to be cytotoxic according to a cytotoxicity assay;

thereby producing antigen-specific T cells comprising a T cell receptor (TCR) that binds to a peptide-MHC complex, the peptide-MHC complex comprising the at least one selected epitope sequence and the matched protein encoded by an HLA allele of the subject.

[0003] Provided herein is a method for treating cancer in a subject in need thereof comprising: selecting at least one epitope sequence from a library of epitope sequences, wherein each epitope sequence in the library is matched to a protein encoded by an HLA allele of the subject; and contacting a T cell from the subject or an allogeneic T cell with one or more peptides comprising the at least one selected epitope sequence, wherein each of the at least one selected epitope sequence is pre-validated to satisfy at least three of the following criteria: binds to a protein encoded by an HLA allele of the subject, is immunogenic according to an immunogenicity assay, is presented by antigen presenting cells (APCs) according to a mass spectrometry assay, and stimulates T cells to be cytotoxic according to a cytotoxicity assay.

[0004] In some examples, the at least one selected epitope sequence comprises a mutation and the method comprises identifying cancer cells of the subject to encode the epitope with the mutation; the at least one selected epitope sequence is within a protein overexpressed by cancer cells of the subject

and the method comprises identifying cancer cells of the subject to overexpress the protein containing the epitope; or the at least one epitope sequence comprises a protein expressed by a cell in a tumor microenvironment.

CLAIMS

1. A cell population comprising isolated antigen-specific T cells for use in treating a cancer in a subject in need thereof, wherein the cell population ~~comprising~~is prepared by: contacting T cells from the subject or allogeneic T cells ex vivo ~~that have been contacted~~ with antigen presenting cells (APCs) comprising one or more warehouse curated peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a prevalidated library of epitope sequences, wherein each epitope sequence known for cancer, by a method comprising, selecting an epitope sequence in the prevalidated library is matched to a protein encoded by an HLA allele, and wherein each of the at least one selected epitope sequence satisfies ~~at least two or three of~~ each of the following criteria:

   (i) binds to a protein encoded by ~~the~~ an HLA allele expressed by the subject,

   (ii) is immunogenic according to an immunogenicity assay,

   (iii) is presented by APCs according to a mass spectrometry assay, and

   (iv) stimulates T cells to be cytotoxic according to a cytotoxicity assay;

   wherein the antigen-specific T cells comprise a T cell receptor (TCR) that binds to a peptide-MHC complex, the peptide-MHC complex comprising the at least one selected epitope sequence and the matched protein encoded by ~~an~~ the HLA allele; and

   wherein at least 0.01% of CD8+ T cells of the cell population are detected to be immunogenic to the at least one selected epitope sequence according to a multimer assay; and percentage of the detected CD8+ T cells is higher than percentage of CD8+ T cells detected in a control sample, wherein the control sample comprises T cells that have been stimulated with APCs that (i) do not comprise a peptide containing the at least one selected epitope sequence, (ii) comprise a peptide derived from a different protein than the at least one selected epitope sequence, or (iii) comprise a peptide with a random sequence.

2. The cell population for use according to~~of~~ claim 1, wherein the at least one selected epitope sequence comprises a mutation expressed by cancer cells and not expressed by non-cancer cells.

3. The cell population for use according to~~of~~ claim 1, wherein the at least one selected epitope sequence is within a protein overexpressed by cancer cells; or is within a protein expressed by a cell in a tumor microenvironment.

4. The cell population for use according to~~of~~ claim 1 or 2, wherein the at least one selected epitope sequence is selected from one or more epitope sequences of Table 1A-1F, Table 2A-2C, Table 3, Table 4A-4M, Table 5, Table 6, Table 7, Table 8, Table 11, Table 12, Table 13 and Table 14.

5. The cell population for use according to ~~of~~ any one of claims 1-4, wherein one or more of the at least one selected epitope sequence is from a protein overexpressed by a cancer cell of the subject, is from a tissue-specific protein, is from a cancer testes protein, comprises a driver mutation, comprises a drug resistance mutation, comprises a tumor specific mutation, is a viral epitope, is a minor histocompatibility epitope, is from a RAS protein, is from a GATA3 protein, is from an EGFR protein, is from a BTK protein, is from a p53 protein, is from a TMPRSS2::ERG fusion polypeptide or is from a Myc protein.

6. The cell population for use according to~~of~~ any one of claims 1-4, wherein at least one of the at least one selected epitope sequence is from a protein encoded by a gene selected from the group consisting of *ANKRD30A*, *COL10A1*, *CTCFL*, *PPIAL4G*, *POTEE*, *DLL3*, *MMP13*, *SSX1*, *DCAF4L2*, *MAGEA4*, *MAGEA11*, *MAGEC2*, *MAGEA12*, *PRAME*, *CLDN6*, *EPYC*, *KLK3*, *KLK2*, *KLK4*, *TGM4*, *POTEG*, *RLN1*, *POTEH*, *SLC45A2*, *TSPAN10*, *PAGE5*, *CSAG1*, *PRDM7*, *TG*, *TSHR*, *RSPH6A*, *SCXB*, *HIST1H4K*, *ALPPL2*, *PRM2*, *PRM1*, *TNP1*, *LELP1*, *HMGB4*, *AKAP4*, *CETN1*, *UBQLN3*, *ACTL7A*, *ACTL9*, *ACTRT2*, *PGK2*, *C2orf53*, *KIF2B*, *ADAD1*, *SPATA8*, *CCDC70*, *TPD52L3*, *ACTL7B*, *DMRTB1*, *SYCN*, *CELA2A*, *CELA2B*, *PNLIPRP1*, *CTRC*, *AMY2A*, *SERPINI2*, *RBPJL*, *AQP12A*, *IAPP*, *KIRREL2*, *G6PC2*, *AQP12B*, *CYP11B1*, *CYP11B2*, *STAR*, *CYP11A1*, and *MC2R*.

7. The cell population for use according to~~of~~ any one of claims 1-6, wherein the protein encoded by an HLA allele is a protein encoded by an HLA allele selected from the group consisting of HLA-A01:01, HLA-A02:01, HLA-A03:01, HLA-A11:01, HLA-A24:01, HLA-A30:01, HLA-A31:01, HLA-A32:01, HLA-A33:01, HLA-A68:01, HLA-B07:02, HLA-B08:01, HLA-B15:01, HLA-B44:03, HLA-C07:01 and HLA-C07:02.

8. The cell population for use according to~~of~~ any one of the claims 1-7, wherein the at least one selected epitope sequence:

   (i) binds to the ~~matched~~ protein encoded by an HLA allele with an affinity of 500 nM or less according to a binding assay, or

   (ii) is predicted to bind to the matched protein encoded by the HLA allele with an affinity of 500 nM or less according to an MHC epitope prediction program implemented on a computer.

9. The cell population for use according to~~of~~ any one of the claims 1-8, wherein the at least one selected epitope sequence has been detected by mass spectrometry after elution from the APCs with a mass accuracy of the detected peptide to be less than 15 Da or less than 10,000 parts per million (ppm).

10. The cell population for use according to~~of~~ any one of the claims 1-9, wherein the immunogenicity assay is a multimer assay and the multimer assay comprises detecting T cells bound to a peptide-

MHC multimer by flow cytometry, wherein the comprising the peptide-MHC multimer comprises the at least one selected epitope sequence and the matched protein encoded by an HLA allele, and wherein the T cells have been stimulated with APCs comprising a peptide containing the at least one selected epitope sequence.

11. The cell population for use according to~~of~~ any one of the claims 1-10, wherein the at least one selected epitope sequence is immunogenic according to the multimer assay when (i) at least 10 T cells that have been stimulated with APCs comprising a peptide containing the at least one selected epitope sequence are detected in at least one out of six stimulations from the same starting sample, (ii) the detected T cells make up at least 0.005% of the $CD8^+$ cells analyzed, and (iii) the percentage of detected T cells of CD8+ T cells is higher than the percentage of detected T cells of CD8+ T cells detected in the~~a~~ control sample.

12. The cell population for use according to~~of~~ claim 11, wherein the control sample comprises T cells that have been stimulated with APCs that (i) do not comprise a peptide containing the at least one selected epitope sequence, (ii) comprise a peptide derived from a different protein than the at least one selected epitope sequence, or (iii) comprise a peptide with a random sequence.

13. The cell population for use according to~~of~~ any one of the claims 1-~~12~~9, wherein the immunogenicity assay is a functional assay, wherein the functional assay comprises detecting T cells with intracellular staining of IFNγ or TNFα by an immunoassay or detecting T cells with cell surface expression of CD107a and/or CD107b by an immunoassay, wherein the T cells have been stimulated with APCs comprising a peptide containing the at least one selected epitope sequence.

14. The cell population for use according to~~of~~ claim 13, wherein the at least one selected epitope sequence is immunogenic according to the functional assay when (i) at least 10 T cells that have been stimulated with APCs comprising a peptide containing the at least one selected epitope sequence are detected, (ii) the detected T cells make up at least 0.005% of the $CD8^+$ or the $CD4^+$ cells analyzed, and (iii) the percentage of detected T cells of CD8+ or $CD4^+$ T cells is higher than the percentage of detected T cells of CD8+ or $CD4^+$ T cells detected in a control sample.

15. The cell population for use according to~~of~~ any one of claims 1-14, wherein the T cells stimulated to be cytotoxic according to the cytotoxicity assay are T cells that have been stimulated with APCs comprising a peptide containing the at least one selected epitope sequence that kill cells presenting the at least one selected epitope sequence, wherein a number of cells presenting the at least one selected epitope sequence that are killed by the T cells is at least 2 fold higher than (a) a number of cells that do not present the at least one selected epitope sequence that are killed by the T cells or (b) a number of cells presenting the at least one selected epitope sequence killed by T cells that have been stimulated with APCs that (i) do not comprise a peptide containing the at

least one selected epitope sequence, (ii) comprise a peptide derived from a different protein than the at least one selected epitope sequence, or (iii) comprise a peptide with a random sequence.

16. The cell population for use according to~~of~~ any one of claims 1-15, wherein the T cells stimulated to be cytotoxic according to the cytotoxicity assay are T cells that produce a cytokine or IL2, wherein the cytokine is Interferon gamma (IFN-γ), Tumor Necrosis Factor (TNF) alpha (α) and/or TNF beta (β) or a combination thereof.

17. The cell population for use according to~~of~~ any one of claims 1-16, wherein at least 0.1% of the CD8+ T cells in the cell population are CD8+ tumor antigen-specific T cells derived from naïve CD8+ T cells.

18. The cell population for use according to~~of~~ any one of claims 1-16, wherein at least 0.1% of the CD4+ T cells in the cell population are CD4+ tumor antigen-specific T cells derived from naïve CD4+ T cells.

19. The cell population for use according to~~of~~ any one of claims 1-18, ~~wherein each of the at least one selected epitope sequence binds to a protein encoded by the HLA allele, is immunogenic according to an immunogenicity assay, is presented by APCs according to a mass spectrometry assay, and stimulates T cells to be cytotoxic according to a cytotoxicity assay~~ the at least one selected epitope sequence comprises a RAS epitope sequence comprising at least one mutation.

20. A pharmaceutical composition comprising ~~a~~the cell population comprising isolated antigen-specific T cells ~~of any one of claims 1-19~~ for use in treating cancer in a subject, wherein the cell population is prepared by: contacting T cells from the subject or allogeneic T cells ex vivo with antigen presenting cells (APCs) comprising one or more warehouse curated peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a prevalidated library of epitope sequences, wherein each epitope sequence known for cancer, by a method comprising, selecting an epitope sequence in the prevalidated library is matched to a protein encoded by an HLA allele, and wherein each of the at least one selected epitope sequence satisfies each of the following criteria:

(i) binds to a protein encoded by an HLA allele expressed by the subject,

(ii) is immunogenic according to an immunogenicity assay,

(iii) is presented by APCs according to a mass spectrometry assay, and

(iv) stimulates T cells to be cytotoxic according to a cytotoxicity assay;

wherein the antigen-specific T cells comprise a T cell receptor (TCR) that binds to a peptide-MHC complex, the peptide-MHC complex comprising the at least one selected epitope sequence and the matched protein encoded by the HLA allele; and

wherein at least 0.01% of CD8+ T cells of the cell population are detected to be immunogenic to the at least one selected epitope sequence according to a multimer assay; and percentage of the

detected CD8+ T cells is higher than percentage of CD8+ T cells detected in a control sample, wherein the control sample comprises T cells that have been stimulated with APCs that (i) do not comprise a peptide containing the at least one selected epitope sequence, (ii) comprise a peptide derived from a different protein than the at least one selected epitope sequence, or (iii) comprise a peptide with a random sequence.

21. Use of ~~the~~ a cell population comprising isolated antigen-specific T cells ~~of any one of claims 1-19~~ in the manufacture of a medicament for treating cancer, wherein the cell population is prepared by: contacting T cells from the subject or allogeneic T cells ex vivo with antigen presenting cells (APCs) comprising one or more warehouse curated peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a prevalidated library of epitope sequences, wherein each epitope sequence known for cancer, by a method comprising, selecting an epitope sequence in the prevalidated library is matched to a protein encoded by an HLA allele, and wherein each of the at least one selected epitope sequence satisfies each of the following criteria:

(i) binds to a protein encoded by an HLA allele expressed by the subject,

(ii) is immunogenic according to an immunogenicity assay,

(iii) is presented by APCs according to a mass spectrometry assay, and

(iv) stimulates T cells to be cytotoxic according to a cytotoxicity assay;

wherein the antigen-specific T cells comprise a T cell receptor (TCR) that binds to a peptide-MHC complex, the peptide-MHC complex comprising the at least one selected epitope sequence and the matched protein encoded by the HLA allele; and

wherein at least 0.01% of CD8+ T cells of the cell population are detected to be immunogenic to the at least one selected epitope sequence according to a multimer assay; and percentage of the detected CD8+ T cells is higher than percentage of CD8+ T cells detected in a control sample, wherein the control sample comprises T cells that have been stimulated with APCs that (i) do not comprise a peptide containing the at least one selected epitope sequence, (ii) comprise a peptide derived from a different protein than the at least one selected epitope sequence, or (iii) comprise a peptide with a random sequence.

~~22. A cell population according to any one of claims 1-19 for use in therapy.~~

2223. A method of preparing a~~the~~ cell population comprising isolated antigen-specific T cells ~~of any one of claims 1-19~~, comprising contacting T cells from a subject or allogeneic T cells ex vivo ~~a cell population comprising T cells~~ with antigen presenting cells (APCs) comprising one or more warehouse curated peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a prevalidated library of epitope sequences, wherein each epitope sequence in the prevalidated library is matched to a protein encoded by an

HLA allele, and wherein each of the at least one selected epitope sequence satisfies ~~at least two or three or~~ each of the following criteria:

(i) binds to a protein encoded by the HLA allele,

(ii) is immunogenic according to an immunogenicity assay,

(iii) is presented by APCs according to a mass spectrometry assay, and

(iv) stimulates T cells to be cytotoxic according to a cytotoxicity assay;

thereby producing antigen-specific T cells comprising a T cell receptor (TCR) that binds to a peptide-MHC complex, the peptide-MHC complex comprising the at least one selected epitope sequence and the matched protein encoded by an HLA allele of the subject.

2324. The method of claim 223, wherein the method further comprises depleting CD14+ cells and/or CD25+ cells from a population of immune cells comprising APCs and T cells prior to contacting the ~~cell population comprising~~ T cells ex vivo with the APCs comprising the one or more peptides containing the at least one selected epitope sequence.

2425. The method of claim 234, wherein the method further comprises, after depleting CD14+ cells and/or CD25+ cells from a population of immune cells comprising APCs and T cells, incubating the CD14+ and/or CD25+ depleted cell population in the presence of

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

(ii) (A) a polypeptide comprising the at least one selected epitope sequence, or (B) a polynucleotide encoding the polypeptide; to form a cell population of cells comprising stimulated T cells.