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 antigen-specific T cells, the cell population comprising T cells that have been contacted with antigen presenting cells (APCs) comprising one or more peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a library of epitope sequences, wherein each epitope sequence in the 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 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;

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 HLA allele.

[0002b] In one aspect, provided herein is a pharmaceutical composition comprising the cell population of as described herein for use in treating cancer in a subject.

[0002c] In one aspect, provided herein is a use of the cell population as described herein in the manufacture of a medicament for treating cancer.

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

[0002e] In one aspect, provided herein is a method of preparing the cell population as described herein, comprising contacting a cell population comprising T cells with antigen presenting cells (APCs) comprising one or more peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a library of epitope sequences, wherein each epitope sequence in the 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.

[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.

[0005] In some examples, one or more of the least one selected epitope sequence comprises an epitope that is not expressed by cancer cells of the subject.

[0006] In some examples, the epitope that is not expressed by cancer cells of the subject is expressed by cells in a tumor microenvironment of the subject.

[0007] In some examples, an epitope that binds to a protein encoded by an HLA allele of the subject binds to an MHC molecule encoded by the HLA allele with an affinity of 500 nM or less according to a binding assay.

[0008] In some examples, an epitope that binds to a protein encoded by an HLA allele of the subject is predicted to bind to an MHC molecule encoded by the HLA allele with an affinity of 500 nM or less using an MHC epitope prediction program implemented on a computer.

[0009] In some examples, the MHC epitope prediction program implemented on a computer is NetMHCpan In some examples, the MHC epitope prediction program implemented on a computer is NetMHCpan version 4.0.

[0010] In some examples, the epitope that is presented by antigen presenting cells (APCs) according to a mass spectrometry assay are detected by mass spectrometry after elution from the APCs with a mass accuracy of the detected peptide to be less than 15 Da, 10 Da or 5 Da, or less than 10,000 or 5,000 parts per million (ppm).

[0011] In some examples, the epitope that is immunogenic according to an immunogenicity assay is immunogenic according to a multimer assay or a functional assay.

[0012] In some examples, the multimer assay comprises flow cytometry analysis.

[0013] In some examples, the multimer assay comprises detecting T cells bound to a peptide-MHC multimer comprising the at least one selected epitope sequence and the matched HLA allele, wherein the T cells have been stimulated with APCs comprising a peptide containing the at least one selected epitope sequence.

[0014] In some examples, epitope 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, (ii) the detected T cells make up at least 0.1% or 0.01% or 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 a control sample.

[0015] In some examples, the epitope is immunogenic according to the multimer assay when 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.

[0016] In some examples, 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.

[0017] In some examples, the T cells have been stimulated with APCs comprising a peptide containing the at least one selected epitope sequence for at least 3, 4, 5, 6, 7, 8,9, 10, 11, 12, 13, 14, 15, 16, 7, 18, 19, 20 or more days.

[0018] In some examples, antigen-specific T cells have been expanded at least 5-fold, 10-fold, 20, fold, 50-fold, 100-fold, 500-fold or 1,000-fold or more in the presence of APCs comprising a peptide containing the at least one selected epitope sequence.

[0019] In some examples, the functional assay comprises an immunoassay.

[0020] In some examples, the functional assay comprises detecting T cells with intracellular staining of IFNγ or TNFα or cell surface expression of CD107a and/or CD107b, wherein the T cells have been stimulated with APCs comprising a peptide containing the at least one selected epitope sequence

[0021] In some examples, the epitope 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 0.1% or 0.01% or 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.

[0022] In some examples, 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 epitope.

[0023] In some examples, a number of cells presenting the epitope that are killed by the T cells is at least 1.1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 50, 100, 500, or 1,000 fold higher than a number of cells that do not present the epitope that are killed by the T cells.

[0024] In some examples, a number of cells presenting the epitope that are killed by the T cells is at least 1.1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 50, 100, 500, or 1,000 fold higher than a number of cells presenting the epitope 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.

[0025] In some examples, a number of cells presenting a mutant epitope that are killed by the T cells is at least 1.1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 50, 100, 500, or 1,000 fold higher than a number of cells presenting a corresponding wild-type epitope that are killed by the T cells.

[0026] In some examples, the T cells stimulated to be cytotoxic according to the cytotoxicity assay are T cells stimulated to be specifically cytotoxic according to the cytotoxicity assay.

[0027] In some examples, the method comprises selecting the subject using a circulating tumor DNA assay.

[0028] In some examples, the method comprises selecting the subject using a gene panel.

[0029] In some examples, the T cell is from a biological sample from the subject.

[0030] In some examples, the T cell is from an apheresis or a leukopheresis sample from the subject.

[0031] In some examples, the T cell is an allogeneic T cell.

[0032] In some examples, each of the at least one selected epitope sequence is pre-validated to satisfy each 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.

[0033] In some examples, at least one of the one or more peptides is a synthesized peptide or a peptide expressed from a nucleic acid sequence.

[0034] In some examples, the method comprises identifying a protein encoded by an HLA allele of the subject or identifying an HLA allele in the genome of the subject.

[0035] In some examples, 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.

[0036] In some examples, the method comprises expanding the T cell contacted with the one or more peptides in vitro or ex vivo to obtain a population of T cells specific to the at least one selected epitope sequence in complex with an MHC protein.

[0037] In some examples, the method further comprises administering the population of T cells to the subject.

[0038] In some examples, a protein comprising the at least one selected epitope sequence is expressed by a cancer cell of the subject.

[0039] In some examples, a protein comprising the at least one selected epitope sequences is expressed by cells in the tumor microenvironment of the subject.

[0040] In some examples, one or more of the at least one selected epitope sequence comprises a mutation.

[0041] In some examples, one or more of the at least one selected epitope sequence comprises a tumor specific mutation.

[0042] In some examples, one or more of the at least one selected epitope sequence is from a protein overexpressed by a cancer cell of the subject.

[0043] In some examples, one or more of the at least one selected epitope sequence comprises a driver mutation.

[0044] In some examples, one or more of the at least one selected epitope sequence comprises a drug resistance mutation.

[0045] In some examples, one or more of the at least one selected epitope sequence is from a tissue-specific protein.

[0046] In some examples, one or more of the at least one selected epitope sequence is from a cancer testes protein.

[0047] In some examples, one or more of the at least one selected epitope sequence is a viral epitope.

[0048] In some examples, one or more of the at least one selected epitope sequence is a minor histocompatibility epitope.

[0049] In some examples, one or more of the at least one selected epitope sequence is from a RAS protein.

[0050] In some examples, one or more of the at least one selected epitope sequence is from a GATA3 protein.

[0051] In some examples, one or more of the at least one selected epitope sequence is from a EGFR protein.

[0052] In some examples, one or more of the at least one selected epitope sequence is from a BTK protein.

[0053] In some examples, one or more of the at least one selected epitope sequence is from a p53 protein.

[0054] In some examples, one or more of the at least one selected epitope sequence is from aTMPRSS2::ERG fusion polypeptide.

[0055] In some examples, one or more of the at least one selected epitope sequence is from a Myc protein.

[0056] In some examples, 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.

[0057] In some examples, at least one of the at least one selected epitope sequence is from a tissue-specific protein that has an expression level in a target tissue of the subject that is at least 2 fold more than an expression level of the tissue-specific protein in each tissue of a plurality of non-target tissues that are different than the target tissue.

[0058] In some examples, 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 comprises contacting the T cell with APCs presenting the epitope.

[0059] In some examples, the APCs presenting the epitope comprises one or more peptides comprising the at least one selected epitope sequence or a polynucleic acid that encodes one or more peptides comprising the at least one selected epitope sequence.

[0060] In some examples, the method comprises depleting CD14+ cells and CD25+ cells from a population of immune cells comprising antigen presenting cells (APCs) and T cells, thereby forming a CD14/CD25 depleted population of immune cells comprising a first population of APCs and T cells.

[0061] In some examples, the population of immune cells is from a biological sample from the subject.

[0062] In some examples, the method further comprises (b) incubating the CD14/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 the at least one selected epitope sequence, or (B) a polynucleotide encoding the polypeptide; thereby forming a population of cells comprising stimulated T cells.

[0063] In some examples, the method further comprises (c) 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 selected epitope sequence and (ii) an MHC protein expressed by the cancer cells or APCs of the subject.

[0064] In some examples, the T cells are expanded in less than 28 days.

[0065] In some examples, 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 biological sample.

[0066] In some examples, 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 biological sample.

[0067] In some examples, 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.

[0068] In some examples, 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.

[0069] In some examples, expanding comprises contacting the population of cells comprising stimulated T cells with a second population of mature APCs, wherein the second population of mature APCs have been incubated with FLT3L and present the at least one selected epitope sequence; and expanding the population of cells comprising stimulated T cells for a second time period, thereby forming an expanded population of T cells.

[0070] In some examples, 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.

[0071] In some examples, expanding further comprises (C) contacting the expanded population of T cells with a third population of mature APCs, wherein the third population of mature APCs (i) have been incubated with FLT3L and (ii) present the at least one selected epitope sequence; and (D) expanding the expanded population of T cells for a third time period, thereby forming the expanded population of cells comprising tumor antigen-specific T cells.

[0072] In some examples, the third population of mature APCs have been incubated with FLT3L for at least 1 day prior to contacting the expanded population of T cells with the third population of mature APCs.

[0073] In some examples, the biological sample is a peripheral blood sample, a leukapheresis sample or an apheresis sample.

[0074] In some examples, the 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.

[0075] In some examples, incubating comprises incubating the CD14/CD25 depleted population of immune cells comprising a first population of APCs and T cells for a first time period in the presence of FLT3L and an RNA encoding the polypeptide.

[0076] In some examples, the method further comprises administering a pharmaceutical composition comprising the expanded population of cells comprising tumor antigen specific T cells to a human subject with cancer.

[0077] In some examples, the human subject with cancer is the human subject from which the biological sample was obtained.

[0078] In some examples, the polypeptide is from 8 to 50 amino acids in length.

[0079] In some examples, the polypeptide comprises at least two of the selected epitope sequence, each expressed by cancer cells of a human subject with cancer.

[0080] In some examples, depleting CD14+ cells and CD25+ cells from the population of immune cells comprising a first population of APCs and T cells comprises contacting the population of immune cells comprising a first population of APCs and T cells with a CD14 binding agent and a CD25 binding agent.

[0081] In some examples, depleting further comprising depleting CD19+ cells from the population of immune cells comprising a first population of APCs and T cells.

[0082] In some examples, depleting further comprising depleting CD11b+ cells from the population of immune cells comprising a first population of APCs and T cells.

[0083] In some examples, the method comprises generating cancer cell nucleic acids from a first biological sample comprising cancer cells obtained from a subject and generating non-cancer cell nucleic acids from a second biological sample comprising non-cancer cells obtained from the same subject.

[0084] In some examples, the protein encoded by an HLA allele of the subject 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.

[0085] In some examples, the method comprises identifying one or two or more different proteins that comprise the at least one selected epitope sequence and that are expressed by cancer cells of the subject

[0086] In some examples, the method comprises identifying one or two or more different proteins that comprise the at least one selected epitope sequence and that are expressed by cancer cells of the subject by measuring levels of RNA encoding the one or two or more different proteins in the cancer cells.

[0087] In some examples, one or more of the at least one selected epitope sequence has a length of from 8 to 12 amino acids.

[0088] In some examples, one or more of the at least one selected epitope sequence has a length of from 13-25 amino acids.

[0089] In some examples, the method comprises isolating genomic DNA or RNA from cancer cells and non-cancer cells of the subject.

[0090] In some examples, one or more of the at least one selected epitope sequence comprises a point mutation or a sequence encoded by a point mutation.

[0091] In some examples, one or more of the at least one selected epitope sequence comprises a sequence encoded by a neoORF mutation.

[0092] In some examples, one or more of the at least one selected epitope sequence comprises a sequence encoded by a gene fusion mutation.

[0093] In some examples, one or more of the at least one selected epitope sequence comprises a sequence encoded by an indel mutation.

[0094] In some examples, one or more of the at least one selected epitope sequence comprises a sequence encoded by a splice site mutation.

[0095] In some examples, at least two of the at least one selected epitope sequence are from a same protein.

[0096] In some examples, at least two of the at least one selected epitope sequence comprise an overlapping sequence.

[0097] In some examples, at least two of the at least one selected epitope sequence are from different proteins.

[0098] In some examples, the one or more peptides comprise at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more peptides.

[0099] In some examples, cancer cells of the subject are cancer cells of a solid cancer.

[0100] In some examples, cancer cells of the subject are cancer cells of a leukemia or a lymphoma.

[0101] In some examples, the mutation is a mutation that occur in a plurality of cancer patients.

[0102] In some examples, the MHC is a Class I MHC.

[0103] In some examples, the MHC is a Class II MHC.

[0104] In some examples, the T cell is a CD8 T cell.

[0105] In some examples, the T cell is a CD4 T cell.

[0106] In some examples, the T cell is a cytotoxic T cell.

[0107] In some examples, the T cell is a memory T cell.

[0108] In some examples, the T cell is a naive T cell.

[0109] In some examples, the method further comprises selecting one or more subpopulation of cells from an expanded population of T cells prior to administering to the subject.

[0110] In some examples, eliciting an immune response in the T cell culture comprises inducing IL2 production from the T cell culture upon contact with the peptide.

[0111] In some examples, eliciting an immune response in the T cell culture comprises inducing a cytokine production from the T cell culture upon contact with the peptide, wherein the cytokine is an Interferon gamma (IFN-γ), Tumor Necrosis Factor (TNF) alpha (α) and/or beta (β) or a combination thereof.

[0112] In some examples, eliciting an immune response in the T cell culture comprises inducing the T cell culture to kill a cell expressing the peptide.

[0113] In some examples, eliciting an immune response in the T cell culture comprises detecting an expression of a Fas ligand, granzyme, perforins, IFN, TNF, or a combination thereof in the T cell culture.

[0114] In some examples, the one or more peptides comprising the at least one selected epitope sequence is purified.

[0115] In some examples, the one or more peptides comprising the at least one selected epitope sequence is lyophilized.

[0116] In some examples, the one or more peptides comprising the at least one selected epitope sequence is in a solution.

[0117] In some examples, the one or more peptides comprising the at least one selected epitope sequence is present in a storage condition such that the integrity of the peptide is ≥99%.

[0118] In some examples, the method comprises stimulating T cells to be cytotoxic against cells loaded with the at least one selected epitope sequences according to a cytotoxicity assay.

[0119] In some examples, the method comprises stimulating T cells to be cytotoxic against cancer cells expressing a protein comprising the at least one selected epitope sequences according to a cytotoxicity assay.

[0120] In some examples, the method comprises stimulating T cells to be cytotoxic against a cancer associated cell expressing a protein comprising the at least one selected epitope sequences according to a cytotoxicity assay.

[0121] In some examples, the at least one selected epitope is expressed by a cancer cell, and an additional selected epitope is expressed by a cancer associated cell.

[0122] In some examples, the additional selected epitope is expressed on a cancer associated fibroblast cell.

[0123] In some examples, the additional selected epitope is selected from Table 8.

[0124] Also provided herein is a pharmaceutical composition comprising a T cell produced by a method provided herein.

[0125] Also provided herein is a library of polypeptides comprising epitope sequences or polynucleotides encoding the polypeptides, wherein each epitope sequence in the library is matched to a protein encoded by an HLA allele; and wherein each epitope sequence in the library is pre-

validated to satisfy at least three of the following criteria: binds to a protein encoded by an HLA allele of a subject with cancer to be treated, is immunogenic according to an immunogenic assay, is presented by antigen presenting cells (APCs) according to a mass spectrometry assay, and/or stimulates T cells to be cytotoxic according to a cytotoxicity assay.

[0126] Also provided herein is a method of treating cancer in a subject comprising administering to the subject (i) a polypeptide comprising a G12R RAS epitope, or (ii) a polynucleotide encoding the polypeptide; wherein: (a) the G12R RAS epitope is vvgaRgvgk and the subject expresses a protein encoded by an HLA-A03:01 allele; (b) the G12R RAS epitope is eyklvvvgaR and the subject expresses a protein encoded by an HLA- A33:03 allele; (c) the G12R RAS epitope is vvvgaRgvgk and the subject expresses a protein encoded by an HLA-A11:01 allele; or (d) the G12R RAS epitope is aRgvgksal and the subject expresses a protein encoded by an HLA-allele selected from the group consisting of HLA-C07:02, HLA- B39:01 and HLA- C07:01.

BRIEF DESCRIPTION OF THE DRAWINGS

[0127] **FIG. 1A** is schematic of an exemplary method provided herein to prime, activate and expand antigen-specific T cells.

CLAIMS

1. A cell population comprising antigen-specific T cells, the cell population comprising T cells that have been contacted with antigen presenting cells (APCs) comprising one or more peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a library of epitope sequences, wherein each epitope sequence in the 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 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;

   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 HLA allele.

2. The cell population 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 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 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 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 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 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 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 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 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 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 a control sample.

12. The cell population 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 of any one of the claims 1-12, 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 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 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 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 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 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 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.

20. A pharmaceutical composition comprising the cell population of any one of claims 1-19 for use in treating cancer in a subject.

21. Use of the cell population of any one of claims 1-19 in the manufacture of a medicament for treating cancer.

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

23. A method of preparing the cell population of any one of claims 1-19, comprising contacting a cell population comprising T cells with antigen presenting cells (APCs) comprising one or more peptides containing at least one selected epitope sequence, wherein the at least one selected epitope sequence is selected from a library of epitope sequences, wherein each epitope sequence in the 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.

24. The method of claim 23, 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 with the APCs comprising the one or more peptides containing the at least one selected epitope sequence.

25. The method of claim 24, wherein the method further comprises 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.