METHODS OF ISOLATING T CELLS HAVING ANTIGENIC SPECIFICITY FOR A P53 CANCER-SPECIFIC MUTATION

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 62/565,464, filed September 29, 2017, which is incorporated by reference in its entirety herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government support under project number ZIABC010984 by the National Institutes of Health, National Cancer Institute. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 687,616 Byte ASCII (Text) file named "740175_ST25.txt," dated September 14, 2018.

BACKGROUND OF THE INVENTION

[0004] Adoptive cell therapy (ACT) can produce positive clinical responses in some cancer patients. Nevertheless, obstacles to the successful use of ACT for the widespread treatment of cancer and other diseases remain. For example, T cells that specifically recognize cancer antigens may be difficult to identify and/or isolate from a patient. Accordingly, there is a need for improved methods of obtaining cancer-reactive T cells.

BRIEF SUMMARY

[0005] An aspect of the disclosure provides a method of isolating T cells having antigenic specificity for a mutated p53 amino acid sequence encoded by a cancer-specific p53

mutation, the method comprising: inducing autologous antigen presenting cells (APCs) of a patient to present at least one mutated p53 amino acid sequence; co-culturing autologous T cells of the patient with the autologous APCs that present the mutated p53 amino acid sequence; and selecting the autologous T cells that (a) were co-cultured with the autologous APCs that present the mutated p53 amino acid sequence and (b) have antigenic specificity for the mutated p53 amino acid sequence presented in the context of a major histocompatability complex (MHC) molecule expressed by the patient to provide isolated T cells having antigenic specificity for the mutated p53 amino acid sequence encoded by the cancer-specific p53 mutation.

[0006] Another aspect of the disclosure provides a method of isolating a T cell receptor (TCR), or an antigen-binding portion thereof, having antigenic specificity for a mutated p53 amino acid sequence encoded by a cancer-specific p53 mutation, the method comprising: isolating T cells having antigenic specificity for a mutated p53 amino acid sequence encoded by a cancer-specific p53 mutation according to any of the methods described herein; and isolating a nucleotide sequence that encodes the TCR, or the antigen-binding portion thereof, from the selected autologous T cells, wherein the TCR, or the antigen-binding portion thereof, has antigenic specificity for the mutated p53 amino acid sequence encoded by the cancer-specific p53 mutation.

[0007] Still another aspect of the disclosure provides a method of preparing a population of cells that express a TCR, or an antigen-binding portion thereof, having antigenic specificity for a mutated p53 amino acid sequence encoded by a cancer-specific p53 mutation, the method comprising: isolating a TCR, or an antigen-binding portion thereof, according to any of the methods described herein, and introducing the nucleotide sequence encoding the isolated TCR, or the antigen-binding portion thereof, into peripheral blood mononuclear cells (PBMC) to obtain cells that express the TCR, or the antigen-binding portion thereof.

[0008] Another aspect of the disclosure provides a method of preparing a population of T cells that have antigenic specificity for a mutated p53 amino acid sequence encoded by a cancer-specific p53 mutation, the method comprising: isolating T cells according to any of the methods described herein, and expanding the numbers of selected autologous T cells to obtain a population of T cells that have antigenic specificity for the mutated p53 amino acid sequence encoded by the cancer-specific p53 mutation.

[0008a] In another aspect, the present disclosure provides an isolated population of cells prepared according to the methods as described herein.

[0008b] In yet another aspect, the present disclosure provides a pharmaceutical composition comprising the isolated population of cells as described herein and a pharmaceutically acceptable carrier.

[0008c] In still another aspect, the present disclosure provides the use of the isolated population of cells as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for the treatment or prevention of cancer in a patient.

[0009] Additional examples of the disclosure provide related populations of cells, pharmaceutical compositions, and methods of treating or preventing cancer.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0010] Figure 1 is a graph showing the number of IFN-γ positive spots per $2x10^4$ effector cells measured following co-culture of effector cells and target cells. The effector cells were TIL fragment F12, infusion bag TIL (Rx1) from patient 4141, p53-R175H-specific TCR-transduced cells, or mock transduced T cells from patient 4196. The target cells were HLA-A*02:01 APCs (autologous to patient 4141) that were either (1) electroporated with TMGs composed of irrelevant (IRR; left hatched gray bars), WT p53 (p53wt-TMG; right hatched gray bars) or mutated p53 (p53-mut-TMG; gray bars) sequences or (2) pulsed with peptide vehicle (DMSO; left hatched black bars) or purified (>95% by HPLC) 25 amino acid peptides composed of WT p53-R175 sequence (LP-p53-wt-R175; right hatched black bars) or mutated p53-R175H (LP-p53-mut-R175H; black bars) sequence. T cells only (no target; open bars) was negative control and Phorbol 12-myristate 13-acetate (PMA) and Ionomycin (Iono) was positive control (lattice bars).

[0011] Figure 2 is a graph showing the percentage of 4-1BB+ cells (% of CD4+) detected following co-culture of TIL fragments (F14, F20 or F24) from patient 4130 with autologous APCs (1) electroporated with TMGs composed of irrelevant (IRR; open bars), WT p53 (p53-WT; horizontal black bars) or mutated p53 (p53-MUT; gray bars) sequences or (2) pulsed with peptide vehicle (DMSO; left hatched black bars) or purified (>95% by HPLC) 25 amino acid peptides composed of WT p53-R273 sequence (LP-p53-R273-WT; left hatched gray bars) or mutated p53-R273H (LP-p53-R273H-MUT; black bars) sequence.

[0012] Figure 3 is a graph showing the number of IFN-γ positive spots per $2x10^4$ effector cells measured following co-culture of TIL fragments (F1-6, F9, F13-23, n=18) from patient

4259 with autologous APCs electroporated with TMGs composed of irrelevant (IRR; open bars), WT p53 (p53-WT; gray bars) or mutated p53 (p53-MUT; black bars) sequences.

[0013] Figure 4 is a graph showing the percentage of 4-1BB+ cells (% of CD4+) detected following co-culture of TIL fragments (F1-F6, F9, F13-F23, n=18) from patient 4259 with autologous APCs electroporated with TMGs composed of irrelevant (IRR; open bars), WT p53 (p53-WT; gray bars) or mutated p53 (p53-MUT; black bars) sequences.

[0014] Figure 5 is a graph showing the percentage of 4-1BB+ cells (% of CD8+) detected following co-culture of TIL fragments (F1-F6, F9, F13-F23, n=18) from patient 4259 with

CLAIMS

1. A method of isolating T cells having antigenic specificity for a mutated p53 amino acid sequence encoded by a cancer-specific p53 mutation, the method comprising:

inducing autologous antigen presenting cells (APCs) of a patient to present at least one mutated p53 amino acid sequence;

co-culturing autologous T cells of the patient with the autologous APCs that present the mutated p53 amino acid sequence; and

selecting the autologous T cells that (a) were co-cultured with the autologous APCs that present the mutated p53 amino acid sequence and (b) have antigenic specificity for the mutated p53 amino acid sequence presented in the context of a major histocompatability complex (MHC) molecule expressed by the patient to provide isolated T cells having antigenic specificity for the mutated p53 amino acid sequence encoded by the cancer-specific p53 mutation.

2. The method of claim 1, wherein inducing autologous APCs of the patient to present the mutated p53 amino acid sequence comprises pulsing APCs with a peptide comprising the mutated p53 amino acid sequence or a pool of peptides, each peptide in the pool comprising a different mutated p53 amino acid sequence.

3. The method of claim 2, wherein the peptide or pool of peptides comprise(s) one or more mutated p53 peptides of SEQ ID NOs: 2-13.

4. The method of claim 1, wherein inducing autologous APCs of the patient to present the mutated p53 amino acid sequence comprises introducing a nucleotide sequence encoding the mutated p53 amino acid sequence into the APCs.

5. The method of claim 4, wherein the nucleotide sequence introduced into the autologous APCs is a tandem minigene (TMG) construct, each minigene comprising a p53 gene, each p53 gene including a cancer-specific p53 mutation that encodes a mutated p53 amino acid sequence, wherein each p53 gene in the TMG construct encodes a different mutated p53 amino acid sequence.

6. The method of claim 5, wherein the TMG construct encodes one or more mutated p53 peptides of SEQ ID NOs: 2-13.

7. The method of claim 5, wherein the TMG construct encodes the amino acid sequence of SEQ ID NO: 14.

8. The method of any one of claims 1-7, wherein selecting the autologous T cells that have antigenic specificity for the mutated p53 amino acid sequence comprises selectively growing the autologous T cells that have antigenic specificity for the mutated p53 amino acid sequence.

9. The method of any one of claims 1-8, wherein selecting the autologous T cells that have antigenic specificity for the mutated p53 amino acid sequence comprises selecting the T cells that express any one or more of programmed cell death 1 (PD-1), lymphocyte-activation gene 3 (LAG-3), T cell immunoglobulin and mucin domain 3 (TIM-3), 4-1BB, OX40, and CD107a.

10. The method of any one of claims 1-9, wherein selecting the autologous T cells that have antigenic specificity for the mutated p53 amino acid sequence comprises selecting the T cells (i) that secrete a greater amount of one or more cytokines upon co-culture with APCs that present the mutated p53 amino acid sequence as compared to the amount of the one or more cytokines secreted by a negative control or (ii) in which at least twice as many of the numbers of T cells secrete one or more cytokines upon co-culture with APCs that present the mutated p53 amino acid sequence as compared to the numbers of negative control T cells that secrete the one or more cytokines.

11. The method of claim 10, wherein the one or more cytokines comprise interferon (IFN)-γ, interleukin (IL)-2, tumor necrosis factor alpha (TNF-α), granulocyte/monocyte colony stimulating factor (GM-CSF), IL-4, IL-5, IL-9, IL-10, IL-17, and IL-22.

12. A method of isolating a T cell receptor (TCR), or an antigen-binding portion thereof, having antigenic specificity for a mutated p53 amino acid sequence encoded by a cancer-specific p53 mutation, the method comprising:

isolating T cells having antigenic specificity for a mutated p53 amino acid sequence encoded by a cancer-specific p53 mutation according to the method of any one of claims 1-11; and

isolating a nucleotide sequence that encodes the TCR, or the antigen-binding portion thereof, from the selected autologous T cells, wherein the TCR, or the antigen-binding portion thereof, has antigenic specificity for the mutated p53 amino acid sequence encoded by the cancer-specific p53 mutation.

13. A method of preparing a population of cells that express a TCR, or an antigen-binding portion thereof, having antigenic specificity for a mutated p53 amino acid sequence encoded by a cancer-specific p53 mutation, the method comprising:

isolating a TCR, or an antigen-binding portion thereof, according to the method of claim 12, and

introducing the nucleotide sequence encoding the isolated TCR, or the antigen-binding portion thereof, into peripheral blood mononuclear cells (PBMC) to obtain cells that express the TCR, or the antigen-binding portion thereof.

14. A method of preparing a population of T cells that have antigenic specificity for a mutated p53 amino acid sequence encoded by a cancer-specific p53 mutation, the method comprising:

isolating T cells according to the method of any one of claims 1-11, and

expanding the numbers of selected autologous T cells to obtain a population of T cells that have antigenic specificity for the mutated p53 amino acid sequence encoded by the cancer-specific p53 mutation.

15. The method of claim 14, wherein expanding the numbers of thereof cells comprises culturing the selected cells with feeder PBMC, interleukin (IL)-2, and OKT3 antibody.

16. An isolated population of cells prepared according to the method of any one of claims 13-15.

17. A pharmaceutical composition comprising the isolated population of cells of claim 16 and a pharmaceutically acceptable carrier.

18. Use of the isolated population of cells of claim 16 or the pharmaceutical composition of claim 17 in the manufacture of a medicament for the treatment or prevention of cancer in a patient.

19. The use according to claim 18, wherein the cancer is an epithelial cancer.

20. The use according to claim 18, wherein the cancer is cholangiocarcinoma, melanoma, colon cancer, rectal cancer, ovarian cancer, endometrial cancer, non-small cell lung cancer (NSCLC), glioblastoma, uterine cervical cancer, head and neck cancer, breast cancer, pancreatic cancer, or bladder cancer.

21. The use according to any one of claims 18-20, wherein the isolated population of cells is autologous to the patient.

22. The use according to any one of claims 18-20, wherein the isolated population of cells is allogeneic to the patient.