SUMMARY

[0003a] In one aspect, provided herein is a method of preparing tumor antigen-specific T cells ex vivo suitable for use as an autologous therapy, the method comprising: (a) 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, wherein the population of immune cells is from a biological sample from a human subject with cancer; and (b) incubating the CD14/CD25 depleted population of immune 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 tumor antigen epitope sequences expressed by cancer cells of a human subject, wherein each of the at least two tumor antigen epitope sequences contains a mutation and binds to an MHC protein expressed by the subject with a stronger affinity than a corresponding wild-type epitope sequence, or (B) a polynucleotide encoding the polypeptide; thereby forming a population of stimulated T cells; and (c) expanding the population of stimulated T cells, thereby forming an expanded population of T cells, wherein expanding the population of stimulated T cells comprises expanding T cells derived from naïve CD8+ T cells or naïve CD4+ T cells, wherein the expanded population of T cells comprises (i) at least 1x10^6 total CD8+ T cells, (ii) at least 1x10^ 6 total CD4+ T cells and (iii) T cells that are specific to (A) a first complex comprising (i) a first tumor antigen epitope sequence of the at least two tumor antigen epitope sequences and (ii) an MHC protein expressed by the cancer cells or APCs of the human subject, and (B) a second complex comprising (i) a second tumor antigen epitope sequence of the at least two tumor antigen epitope sequences (ii) an MHC protein expressed by the cancer cells or APCs of the human subject; and wherein at least 0.1% of the CD8+ T cells that are specific to the first complex or the second complex in the expanded population of T cells are derived from the naïve CD8+ T cells, and at least 0.1% of the CD4+ T cells that are specific to the first complex or the second complex in the expanded population of T cells are derived from the naïve CD4+ T cells.

[0003b] In one aspect, provided herein is a pharmaceutical composition comprising the expanded population of T cells as described herein, and a pharmaceutically acceptable excipient.

[0003c] In one aspect, provided herein is a use of the pharmaceutical composition as described herein in the manufacture of a medicament for treating a human subject with cancer.

[0003d] In one aspect, provided herein is a pharmaceutical composition as described herein for use in therapy.

[0004] In some examples, provided herein is a pharmaceutical composition comprising: a population of immune cells from a biological sample comprising at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein an amount of immune cells expressing CD14 and/or CD25 in the population is proportionally different from an amount of immune cells expressing CD14 and/or CD25 in the biological sample. In some examples, provided herein is a pharmaceutical composition comprising: a population of immune cells

from a biological sample comprising at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein an amount of immune cells expressing CD14 and/or CD25 in the population is different from an amount of immune cells expressing CD14 and/or CD25 in the biological sample. In some examples, provided herein is a pharmaceutical composition comprising: a population of immune cells from a biological sample comprising at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein a percentage of immune cells expressing CD14 and/or CD25 in the population is different from a percentage of immune cells expressing CD14 and/or CD25 in the biological sample. In some examples, provided herein is a pharmaceutical composition comprising: a population of immune cells from a biological sample comprising at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein a concentration of immune cells expressing CD14 and/or CD25 in the population is different from a concentration of immune cells expressing CD14 and/or CD25 in the biological sample.

[0005] In some examples, provided herein is a composition comprising a population of immune cells from a biological sample, wherein an amount of immune cells expressing CD14 and CD25 in the population is proportionally less than an amount of immune cells expressing CD14 and CD25 in the biological sample. In some examples, provided herein is a composition comprising a population of immune cells from a biological sample, wherein an amount of immune cells expressing CD14 and CD25 in the population is less than an amount of immune cells expressing CD14 and CD25 in the biological sample. In some examples, provided herein is a composition comprising a population of immune cells from a biological sample, wherein a percentage of immune cells expressing CD14 and CD25 in the population is less than a percentage of immune cells expressing CD14 and CD25 in the biological sample. In some examples, provided herein is a composition comprising a population of immune cells from a biological sample, wherein a concentration of immune cells expressing CD14 and CD25 in the population is less than a concentration of immune cells expressing CD14 and CD25 in the biological sample.

[0006] In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising T cells from a biological sample, wherein the T cells comprise at least one antigen specific T cell that is an antigen presenting cell (APC) stimulated T cell and comprises a T cell receptor (TCR) specific to at least one antigen peptide sequence, wherein the APC is a FLT3L-stimulated APC; and a pharmaceutically acceptable excipient.

[0007] In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising T cells from a biological sample, wherein the T cells comprise at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein the at least one antigen specific T cell is at least about 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total T cells, total $CD4^+$ T cells, total $CD8^+$ T cells, or total immune cells; and wherein the biological sample comprises one or more antigen specific T cells, wherein the one or more antigen specific T cells in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, or 0.05% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells of total T cells, total $CD4^+$ T cells, total $CD8^+$ T cells, or total immune cells in the biological sample. In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising T cells expanded or induced from a biological sample, wherein the T cells expanded or induced from the biological sample comprise at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein an amount, concentration or percentage of the at least one antigen specific T cell is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, or 1000 greater than an amount, concentration or percentage of antigen specific T cells or total T cells in the biological sample. In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising T cells from a biological sample, wherein the T cells comprise at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein the at least one antigen specific T cell comprises expanded or induced antigen specific T cells that are expanded or induced at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, or 1000 fold from the biological sample. In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising $CD4^+$ T cells from a biological sample, wherein the $CD4^+$ T cells comprise at least one antigen specific $CD4^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein the at least one antigen specific $CD4^+$ T cell is at least about 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total T cells, total $CD4^+$ T cells, total $CD8^+$ T cells, or total immune cells; and wherein the biological sample comprises one or more antigen specific $CD4^+$ T cells, wherein the one or more antigen specific $CD4^+$ T cells in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, or

0.05% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells of total T cells, total $CD4^+$ T cells, total $CD8^+$ T cells, or total immune cells in the biological sample. In some examples, a pharmaceutical composition comprises a population of immune cells comprising $CD4^+$ T cells from a biological sample, wherein the $CD4^+$ T cells comprise at least one antigen specific $CD4^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein the at least one antigen specific $CD4^+$ T cell is at least about 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total $CD4^+$ T cells; and wherein the biological sample comprises one or more antigen specific $CD4^+$ T cells, wherein the one or more antigen specific $CD4^+$ T cells in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, or 0.05% of total $CD4^+$ T cells in the biological sample. In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising $CD4^+$ T cells expanded or induced from a biological sample, wherein the $CD4^+$ T cells expanded or induced from the biological sample comprise at least one antigen specific $CD4^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein an amount, concentration or percentage of the at least one antigen specific T cell is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, or 1000 greater than an amount, concentration or percentage of antigen specific $CD4^+$ T cells, total $CD4^+$ T cells, or total T cells in the biological sample. In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising $CD4^+$ T cells from a biological sample, wherein the $CD4^+$ T cells comprise at least one antigen specific $CD4^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein the at least one antigen specific $CD4^+$ T cell comprises expanded or induced antigen specific $CD4^+$ T cells that are expanded or induced at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, or 1000 fold from the biological sample. In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising $CD4^+$ T cells from a biological sample depleted of CD25 expressing cells or CD25 and CD14 expressing cells, wherein the $CD4^+$ T cells comprise at least one antigen specific $CD4^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein the at least one antigen specific $CD4^+$ T cell comprises expanded or induced antigen specific $CD4^+$ T cells that are expanded or induced at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or 500 fold higher compared from the biological sample. In some examples,

provided herein is a pharmaceutical composition comprising a population of immune cells comprising $CD8^+$ T cells from a biological sample, wherein the $CD8^+$ T cells comprise at least one antigen specific $CD8^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein the at least one antigen specific $CD8^+$ T cell is at least about 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total T cells, total $CD4^+$ T cells, total $CD8^+$ T cells, or total immune cells; and wherein the biological sample comprises one or more antigen specific $CD8^+$ T cells, wherein the one or more antigen specific $CD8^+$ T cells in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, or 0.05% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells of total T cells, total $CD4^+$ T cells, total $CD8^+$ T cells, or total immune cells in the biological sample. In some examples, a pharmaceutical composition comprises a population of immune cells comprising $CD8^+$ T cells from a biological sample, wherein the $CD8^+$ T cells comprise at least one antigen specific $CD8^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein the at least one antigen specific $CD8^+$ T cell is at least about 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total $CD8^+$ T cells; and wherein the biological sample comprises one or more antigen specific $CD4^+$ T cells, wherein the one or more antigen specific $CD8^+$ T cells in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, or 0.05% of total $CD8^+$ T cells in the biological sample. In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising $CD8^+$ T cells expanded or induced from a biological sample, wherein the $CD8^+$ T cells expanded or induced from the biological sample comprise at least one antigen specific $CD8^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein an amount, concentration or percentage of the at least one antigen specific T cell is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, or 1000 greater than an amount, concentration or percentage of antigen specific $CD8^+$ T cells, total $CD8^+$ T cells, or total T cells in the biological sample. In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising $CD8^+$ T cells from a biological sample, wherein the $CD8^+$ T cells comprise at least one antigen specific $CD8^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein the at least one antigen specific $CD8^+$ T cell comprises expanded or induced antigen specific $CD4^+$ T cells that are expanded or induced at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900,

or 1000 fold from the biological sample. In some examples, provided herein is a pharmaceutical composition comprising a population of immune cells comprising $CD8^+$ T cells from a biological sample, wherein the $CD8^+$ T cells comprise at least one antigen specific $CD8^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein the at least one antigen specific $CD8^+$ T cell comprises expanded or induced antigen specific $CD8^+$ T cells that are expanded or induced at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, or fold from the biological sample.

[0008] In some examples, the at least one antigen specific T cell comprises at least one APC-stimulated T cell. In some examples, the amount of immune cells expressing CD14 and/or CD25 in the population is proportionally less than the amount of immune cells expressing CD14 and/or CD25 in the biological sample. In some examples, the amount of immune cells expressing CD14 and/or CD25 in the population is proportionally more than the amount of immune cells expressing CD14 and/or CD25 in the biological sample. In some examples, the percentage of immune cells expressing CD14 and/or CD25 in the population is less than the percentage of immune cells expressing CD14 and/or CD25 in the biological sample. In some examples, the percentage of immune cells expressing CD14 and/or CD25 in the population is more than the percentage of immune cells expressing CD14 and/or CD25 in the biological sample. In some examples, the concentration of immune cells expressing CD14 and/or CD25 in the population is less than the concentration of immune cells expressing CD14 and/or CD25 in the biological sample. In some examples, the concentration of immune cells expressing CD14 and/or CD25 in the population is more than the concentration of immune cells expressing CD14 and/or CD25 in the biological sample.

[0009] In some examples, the biological sample is from a subject. In some examples, the subject is a human. In some examples, the subject has a disease or disorder. In some examples, the disease or disorder is cancer. In some examples, the cancer is selected from the group consisting of ovarian cancer, lung cancer and melanoma.

[0010] In some examples, the at least one antigen specific T cell comprises at least one $CD4^+$ T cell. In some examples, the at least one antigen specific T cell comprises at least one $CD8^+$ T cell. In some examples, the at least one antigen specific T cell comprises at least one CD4 enriched T cell. In some examples, the at least one antigen specific T cell comprises at least one CD8 enriched T cell. In some examples, the at least one antigen specific T cell comprises a memory T cell. In some examples, the at least one antigen specific T cell comprises a naïve T cell. In some examples, the at least one antigen specific T cell comprises a memory $CD4^+$ T cell. In some examples, the at least one antigen specific T cell comprises an expanded memory $CD4^+$ T cell. In some examples, the at least one antigen specific T cell comprises a naïve $CD4^+$ T cell. In some examples, the at least one antigen specific T cell comprises an induced naïve $CD4^+$ T cell. In some examples, the at least one antigen specific T cell comprises a memory $CD8^+$ T cell. In some examples, the at least one antigen

specific T cell comprises an expanded memory $CD8^+$ T cell. In some examples, the at least one antigen specific T cell comprises a naïve $CD8^+$ T cell. In some examples, the at least one antigen specific T cell comprises an induced naïve $CD8^+$ T cell.

[0011] In some examples, the at least one antigen-specific T cell is stimulated in a medium comprising IL-7, IL-15, an indoleamine 2,3-dioxygenase-1 (IDO) inhibitor, an anti-PD-1 antibody, IL-12, or a combination thereof. In some examples, the IDO inhibitor is epacadostat, navoximod, 1-methyltryptophan, or a combination thereof.

[0012] In some examples, the at least one antigen peptide sequence comprises a mutation selected from (A) a point mutation, (B) a splice-site mutation, (C) a frameshift mutation, (D) a read-through mutation, (E) a gene-fusion mutation, and combinations thereof. In some examples, the at least one antigen peptide sequence binds to the HLA protein of a subject with a greater affinity than a corresponding wild-type peptide. In some examples, the at least one antigen peptide sequence binds to the HLA protein of a subject with a K_D or an IC_{50} less than 500 nM, 250 nM, 150 nM, 100 nM, 50 nM, 25 nM or 10 nM. In some examples, each of the at least one antigen peptide sequence binds to a protein encoded by an HLA allele expressed by a subject. In some examples, the TCR binds to a peptide-HLA complex with a K_D or an IC_{50} of less than 500 nM, 250 nM, 150 nM, 100 nM, 50 nM, 25 nM or 10 nM. In some examples, each of the at least one antigen peptide sequence comprises a mutation that is not present in non-cancer cells of a subject. In some examples, each of the at least one antigen peptide sequences is encoded by a gene or an expressed gene of a subject's cancer cells.

[0013] In some examples, one or more of the at least one antigen peptide sequence has a length of at least 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18; 19; 20; 21; 22; 23; 24; 25; 26; 27; 28; 29; 30; 40; 50; 60; 70; 80; 90; 100; 150; 200; 250; 300; 350; 400; 450; 500; 600; 700; 800; 900; 1,000; 1,500; 2,000; 2,500; 3,000; 4,000; 5,000; 7,500; or 10,000 naturally occurring amino acids. In some examples, one or more of the at least one antigen peptide sequence binds to a protein encoded by a class I HLA allele and has a length of from 8-12 naturally occurring amino acids. In some examples, one or more of the at least one antigen peptide sequence binds to a protein encoded by a class II HLA allele and has a length of from 16-25 naturally occurring amino acids.

[0014] In some examples, the at least one antigen peptide sequence comprises a plurality of antigen peptide sequences. In some examples, the plurality of antigen peptide sequences comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, or 500 antigen peptide sequences. In some examples, the antigen is a neoantigen, a tumor associated antigen, an overexpressed antigen, a viral antigen, a minor histocompatibility antigen or a combination thereof.

[0015] In some examples, the APC is one or more APC preparations. In some examples, the APC comprises an APC loaded with one or more antigen peptides comprising one or more of the at least one antigen peptide sequence. In some examples, the APC is an autologous APC, an allogenic APC, or an artificial APC. In some examples, the APC comprises a dendritic cell (DC). In some examples, the APC is derived from a $CD14^+$ monocyte. In some examples, the APC is a CD14 enriched APC. In some examples, the APC is a CD141

enriched APC. In some examples, the $CD14^+$ monocyte is enriched from a biological sample from a subject comprising PBMCs. In some examples, the $CD14^+$ monocyte is stimulated with one or more cytokines or growth factors. In some examples, the one or more cytokines or growth factors comprise GM-CSF, IL-4, FLT3L, TNF-α, IL-1β, PGE1, IL-6, IL-7, IFN-α, R848, LPS, ss-rna40, poly I:C, or a combination thereof.

[0016] In some examples, the $CD14^+$ monocyte is from a second biological sample comprising PBMCs. In some examples, the second biological sample is from the same subject.

[0017] In some examples, the biological sample comprises peripheral blood mononuclear cells (PBMCs).

[0018] In some examples, the at least one antigen specific T cell comprises a plurality of antigen specific T cells. In some examples, a percentage of the at least one antigen specific T cell in the composition is at least about 0.00001%, 0.00002%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total T cells or total immune cells. In some examples, a percentage of at least one antigen specific $CD8^+$ T cell in the composition is at least about 0.00001%, 0.00002%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells. In some examples, a percentage of at least one antigen specific $CD4^+$ T cell in the composition is at least about 0.00001%, 0.00002%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells. In some examples, a percentage of the at least one antigen specific T cell in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1% or 0.5% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells. In some examples, a percentage of at least one antigen specific $CD8^+$ T cell in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1% or 0.5% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells. In some examples, a percentage of at least one antigen specific $CD4^+$ T cell in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1% or 0.5% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells.

[0019] In some examples, the pharmaceutical composition comprises: a population of immune cells from a biological sample comprising at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a pharmaceutically acceptable excipient; wherein an amount of immune cells expressing CD19 and/or CD16 in the population is different from an amount of immune cells expressing CD19 and/or CD16 in the biological sample. In some examples, the pharmaceutical composition comprises: a population of immune cells from a biological sample comprising at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, and a

pharmaceutically acceptable excipient; wherein an amount of immune cells expressing CD19 and/or CD16 in the population is less than an amount of immune cells expressing CD19 and/or CD16 in the biological sample.

[0020] In some examples, provided herein is a method of treatment comprising administering a composition described herein to a subject with a disease or disorder.

[0021] In some examples, provided herein is a method of using a composition described herein, for the manufacture of a medicament for use in therapy.

[0022] In one example, provided herein is a method of preparing at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising incubating an APC with a population of immune cells from a biological sample depleted of cells expressing CD25 or CD14 and CD25, wherein the at least one antigen specific T cell comprises an amount of expanded or induced antigen specific T cells that is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or 500 fold higher than an amount of antigen specific T cells expanded or induced using a method comprising incubating an APC with a population of immune cells from a biological sample depleted of cells expressing CD14. In one example, provided herein is a method of preparing at least one antigen specific $CD4^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising incubating an APC with a population of immune cells from a biological sample depleted of cells expressing CD25 or CD14 and CD25, wherein the at least one antigen specific $CD4^+$ T cell comprises an amount of expanded or induced antigen specific $CD4^+$ T cells that is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or 500 fold higher than an amount of antigen specific $CD4^+$ T cells expanded or induced using a method comprising incubating an APC with a population of immune cells from a biological sample depleted of cells expressing CD14. In one example, provided herein is a method of preparing at least one antigen specific $CD8^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising incubating an APC with a population of immune cells from a biological sample depleted of cells expressing CD25 or CD14 and CD25, wherein the at least one antigen specific $CD8^+$ T cell comprises an amount of expanded or induced antigen specific $CD8^+$ T cells that is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or 500 fold higher than an amount of antigen specific $CD8^+$ T cells expanded or induced using a method comprising incubating an APC with a population of immune cells from a biological sample depleted of cells expressing CD14.

[0023] In some examples, the biological sample is further depleted of CD19 expressing cells. In some examples, the biological sample is further depleted of CD19 expressing cells. In some examples, the APC is a

FLT3L-stimulated APC. In some examples, incubating the population of immune cells is performed in a medium containing IL-7, IL-15, or a combination thereof. In some examples, the medium further comprises an indoleamine 2,3-dioxygenase-1 (IDO) inhibitor, an anti-PD-1 antibody, IL-12, or a combination thereof. In some examples, the IDO inhibitor is epacadostat, navoximod, 1-methyltryptophan, or a combination thereof.

[0024] In one example, provided herein is a method of preparing a pharmaceutical composition comprising at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising: incubating FMS-like tyrosine kinase 3 receptor ligand (FLT3L) with a population of immune cells from a biological sample for a first time period; and thereafter incubating at least one T cell of the biological sample with an APC, wherein the at least one antigen specific T cell comprises an amount of expanded or induced antigen specific T cells that is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or 500 fold higher than an amount of antigen specific T cells expanded or induced using a method that does not comprise incubating FMS-like tyrosine kinase 3 receptor ligand (FLT3L) with a population of immune cells from a biological sample for a first time period; and that does comprise incubating at least one T cell of the biological sample with an APC thereafter. In one example, provided herein is a method of preparing a pharmaceutical composition comprising at least one antigen specific $CD4^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising: incubating FMS-like tyrosine kinase 3 receptor ligand (FLT3L) with a population of immune cells from a biological sample for a first time period; and thereafter incubating at least one $CD4^+$ T cell of the biological sample with an APC, wherein the at least one antigen specific $CD4^+$ T cell comprises an amount of expanded or induced antigen specific $CD4^+$ T cells that is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or 500 fold higher than an amount of antigen specific $CD4^+$ T cells expanded or induced using a method that does not comprise incubating FMS-like tyrosine kinase 3 receptor ligand (FLT3L) with a population of immune cells from a biological sample for a first time period; and that does comprise incubating at least one $CD4^+$ T cell of the biological sample with an APC thereafter. In one example, provided herein is a method of preparing a pharmaceutical composition comprising at least one antigen specific $CD8^+$ T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising: incubating FMS-like tyrosine kinase 3 receptor ligand (FLT3L) with a population of immune cells from a biological sample for a first time period; and thereafter incubating at least one $CD8^+$ T cell of the biological sample with an APC, wherein the at least one antigen specific $CD8^+$ T cell comprises an amount of expanded or induced antigen specific $CD8^+$ T cells that is at least about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75,

80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or 500 fold higher than an amount of antigen specific $CD8^+$ T cells expanded or induced using a method that does not comprise incubating FMS-like tyrosine kinase 3 receptor ligand (FLT3L) with a population of immune cells from a biological sample for a first time period; and that does comprise incubating at least one $CD8^+$ T cell of the biological sample with an APC thereafter.

[0025] In one example, provided herein is a method of preparing at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising incubating an APC with a population of immune cells from a biological sample depleted of cells expressing CD14 and/or CD25.

[0026] In one example, provided herein is a method of preparing at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising incubating a FMS-like tyrosine kinase 3 receptor ligand (FLT3L)-stimulated APC with a population of immune cells from a biological sample.

[0027] In one example, provided herein is a method of preparing a pharmaceutical composition comprising at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising: incubating FMS-like tyrosine kinase 3 receptor ligand (FLT3L) with a population of immune cells from a biological sample for a first time period; and thereafter incubating at least one T cell of the biological sample with an APC.

[0028] In one example, provided herein is a method of preparing at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising incubating a population of immune cells from a biological sample with one or more APC preparations for one or more separate time periods of less than 28 days from incubating the population of immune cells with a first APC preparation of the one or more APC preparations, wherein at least one antigen specific memory T cell is expanded, or at least one antigen specific naïve T cell is induced.

[0029] In one example, provided herein is a method of preparing at least one antigen specific T cell comprising a T cell receptor (TCR) specific to at least one antigen peptide sequence, the method comprising incubating a population of immune cells from a biological sample with 3 or less APC preparations for 3 or less separate time periods, wherein at least one antigen specific memory T cell is expanded or at least one antigen specific naïve T cell is induced.

[0030] In some examples, the population of immune cells is from a biological sample depleted of CD14 and/or CD25 expressing cells.

[0031] In some examples, the APC is a FLT3L-stimulated APC. In some examples, at least one of the APC preparations comprises a FLT3L-stimulated APC. In some examples, at least two of the APC preparations comprise a FLT3L-stimulated APC. In some examples, at least three of the APC preparations comprise a FLT3L-stimulated APC. In some examples, each of the APC preparations comprises a FLT3L-stimulated APC.

[0032] In some examples, the APC comprises one or more APC preparations. In some examples, the APC preparations comprise 3 or less APC preparations. In some examples, the APC preparations are incubated with the immune cells sequentially within one or more separate time periods. In some examples, the biological sample is from a subject. In some examples, the subject is a human. In some examples, the subject has a disease or disorder. In some examples, the disease or disorder is cancer. In some examples, the cancer is selected from the group consisting of ovarian cancer, lung cancer and melanoma.

[0033] In some examples, the at least one antigen specific T cell comprises at least one $CD4^+$ T cell. In some examples, the at least one antigen specific T cell comprises at least one $CD8^+$ T cell. In some examples, at least one antigen specific T cell comprises at least one CD4 enriched T cell. In some examples, at least one antigen specific T cell comprises at least one CD8 enriched T cell. In some examples, the at least one antigen specific T cell comprises at least one memory T cell. In some examples, the at least one antigen specific T cell comprises at least one naïve T cell. In some examples, the at least one antigen specific T cell comprises at least one memory $CD4^+$ T cell. In some examples, the at least one antigen specific T cell comprises at least one naïve $CD4^+$ T cell. In some examples, the at least one antigen specific T cell comprises at least one memory $CD8^+$ T cell. In some examples, the at least one antigen specific T cell comprises at least one naïve $CD8^+$ T cell.

[0034] In some examples, the at least one antigen peptide sequence comprises a mutation selected from (A) a point mutation, (B) a splice-site mutation, (C) a frameshift mutation, (D) a read-through mutation, (E) a gene-fusion mutation, and combinations thereof. In some examples, the at least one antigen peptide sequence comprises a point mutation and binds to the HLA protein of a subject with a greater affinity than a corresponding wild-type peptide. In some examples, the at least one antigen peptide sequence binds to the HLA protein of a subject with a K_D or an IC_{50} of less than 500 nM, 250 nM, 150 nM, 100 nM, 50 nM, 25 nM or 10 nM. In some examples, each of the at least one antigen peptide sequence binds to a protein encoded by an HLA allele expressed by a subject. In some examples, the TCR binds to a peptide-HLA complex with a K_D or an IC_{50} of less than 500 nM, 250 nM, 150 nM, 100 nM, 50 nM, 25 nM or 10 nM. In some examples, each of the at least one antigen peptide sequences comprises a mutation that is not present in non-cancer cells of a subject. In some examples, each of the at least one antigen peptide sequences is encoded by gene or an expressed gene of a subject's cancer cells. In some examples, one or more of the at least one antigen peptide sequence has a length of at least 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18; 19; 20; 21; 22; 23; 24; 25; 26; 27; 28; 29; 30; 40; 50; 60; 70; 80; 90; 100; 150; 200; 250; 300; 350; 400; 450; 500; 600; 700; 800; 900; 1,000; 1,500; 2,000; 2,500; 3,000; 4,000; 5,000; 7,500; or 10,000 naturally occurring amino acids. In some examples, one or more of the at least one antigen peptide sequence binds to a protein encoded by a class I HLA allele and has a length of from 8-12 naturally occurring amino acids. In some examples, one or more of the at least one antigen peptide sequence binds to a protein encoded by a class II HLA allele and has a length of from 16-25 naturally occurring amino acids. In some examples, the at least one antigen peptide sequence comprises a plurality of antigen peptide sequences. In some examples, the plurality of antigen peptide sequences

comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, or 500 antigen peptide sequences. In some examples, the antigen is a neoantigen, a tumor associated antigen, a viral antigen, a minor histocompatibility antigen or a combination thereof.

[0035] In some examples, a method comprises depleting cells expressing CD14 and/or CD25 from the biological sample. In some examples, depleting cells expressing CD14 and/or CD25 comprises binding a CD14 and/or CD25 binding agent to an APC or an APC of the APC preparations. In some examples, the CD14 and/or CD25 binding agent is biotinylated. In some examples, depleting cells expressing CD14 and/or CD25 further comprises binding an anti-biotin reagent on a solid support to the CD14 and/or CD25 binding agent. In some examples, the CD14 and/or CD25 binding agent is attached to a solid support.

[0036] In some examples, the APC or an APC of the APC preparations comprises an APC loaded with one or more antigen peptides comprising one or more of the at least one antigen peptide sequence. In some examples, the APC or an APC of the APC preparations is an autologous APC or an allogenic APC. In some examples, the APC or an APC of the APC preparations comprises a dendritic cell (DC). In some examples, the APC or an APC of the APC preparations is derived from a $CD14^+$ monocyte. In some examples, the APC or an APC of the APC preparations is enriched from a biological sample. In some examples, the APC or an APC of the APC preparations is stimulated with one or more cytokines or growth factors. In some examples, the one or more cytokines or growth factors comprise GM-CSF, IL-4, FLT3L, TNF-α, IL-1β, PGE1, IL-6, IFN-α, R848, LPS, ss-rna40, poly I:C, or a combination thereof.

[0037] In some examples, the APC or an APC of the APC preparations is from a second biological sample. In some examples, the second biological sample is from the same subject. In some examples, the biological sample comprises peripheral blood mononuclear cells (PBMCs).

[0038] In some examples, the at least one antigen specific T cell comprises a plurality of antigen specific T cells. In some examples, a percentage of the at least one antigen specific T cell is at least about 0.00001%, 0.00002%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells. In some examples, a percentage of at least one antigen specific $CD8^+$ T cell is at least about 0.00001%, 0.00002%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells. In some examples, a percentage of at least one antigen specific $CD4^+$ T cell is at least about 0.00001%, 0.00002%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells. In some examples, a

percentage of the at least one antigen specific T cell in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1% or 0.5% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells. In some examples, a percentage of at least one antigen specific $CD8^+$ T cell in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1% or 0.5% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells. In some examples, a percentage of at least one antigen specific $CD4^+$ T cell in the biological sample is at most about 0.00001%, 0.00005%, 0.0001%, 0.0005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1% or 0.5% of total $CD4^+$ T cells, total $CD8^+$ T cells, total T cells or total immune cells.

[0039] In some examples, the method further comprises administering one or more of the at least one antigen specific T cell to a subject.

[0040] In some examples, a total time period of the separate time periods is less than 28 days. In some examples, incubating comprises incubating a first APC preparation of the APC preparations to the T cells for more than 7 days. In some examples, a method comprises incubating the APC or one or more of the APC preparations with a first medium comprising at least one cytokine or growth factor for a first time period. In some examples, the at least one cytokine or growth factor comprises GM-CSF, IL-4, FLT3L, TNF-α, IL-1β, PGE1, IL-6, IFN-α, R848, LPS, ss-rna40, poly I:C, or any combination thereof. In some examples, a method comprises incubating one or more of the APC preparations with at least one peptide for a second time period. In some examples, a method comprises incubating the APC or one or more of the APC preparations with a second medium comprising one or more cytokines or growth factors for a third time period, thereby obtaining a matured APC. In some examples, the one or more cytokines or growth factors comprises GM-CSF, IL-4, FLT3L, TNF-α, IL-1β, PGE1, IL-6, IFN-α, R848, LPS, ss-rna40, poly I:C, or a combination thereof. In some examples, the method further comprises removing the one or more cytokines or growth factors of the second medium after the third time period and before a start of the fourth time period.

[0041] In some examples, the method is performed *ex vivo*.

[0042] In some examples, the biological sample is freshly obtained from a subject or is a frozen sample.

[0043] In some examples, the method comprises obtaining a biological sample from a subject comprising at least one APC and at least one PBMC.

[0044] In some examples, the method comprises depleting cells expressing CD14 and/or CD25 from a biological sample, thereby obtaining a CD14 and/or CD25 cell depleted sample.

[0045] In some examples, the method comprises incubating a CD14 and/or CD25 cell depleted sample with FLT3L for a first time period.

[0046] In some examples, the method comprises incubating at least one peptide with a CD14 and/or CD25 cell depleted sample for a second time period, thereby obtaining a first matured APC peptide loaded sample.

[0047] In some examples, the method comprises incubating a first matured APC peptide loaded sample with at least one PBMC for a third time period, thereby obtaining a first stimulated PBMC sample.

[0048] In some examples, the method comprises incubating a PBMC of a first stimulated PBMC sample with a FLT3L-stimulated APC of a matured APC sample for a fourth time period, thereby obtaining a second stimulated PBMC sample.

[0049] In some examples, the method comprises incubating a PBMC of a first stimulated PBMC sample with FLT3L and a second APC peptide loaded sample of a matured APC sample for a fourth time period, thereby obtaining a second stimulated PBMC sample.

[0050] In some examples, the method comprises incubating a PBMC of a first stimulated PBMC sample with FLT3L and a FLT3L-stimulated APC of a matured APC sample for a fourth time period, thereby obtaining a second stimulated PBMC sample.

[0051] In some examples, the method comprises incubating a PBMC of a second stimulated PBMC sample with a FLT3L-stimulated APC of a matured APC sample for a fifth time period, thereby obtaining a third stimulated PBMC sample.

[0052] In some examples, the method comprises incubating a PBMC of a second stimulated PBMC sample with FLT3L and a third APC peptide loaded sample of a matured APC sample for a fifth time period, thereby obtaining a third stimulated PBMC sample

[0053] In some examples, the method comprises incubating a PBMC of a second stimulated PBMC sample with FLT3L and a FLT3L-stimulated APC of a matured APC sample for a fifth time period, thereby obtaining a third stimulated PBMC sample.

[0054] In some examples, the method comprises administering at least one T cell of a first stimulated PBMC sample to a subject in need thereof. In some examples, the method comprises administering at least one T cell of a second stimulated PBMC sample to a subject in need thereof. In some examples, the method comprises administering at least one T cell of a third stimulated PBMC sample to a subject in need thereof.

[0055] In some examples, incubating the PBMC of the first stimulated PBMC sample is performed in the presence of IL-7, IL-15, or a combination thereof. In some examples, incubating the PBMC of the first stimulated PBMC sample is performed in the presence of an indoleamine 2,3-dioxygenase-1 (IDO) inhibitor, an anti-PD-1 antibody, IL-12, or a combination thereof. In some examples, incubating the PBMC of the second stimulated PBMC sample is performed in the presence of IL-7, IL-15, or a combination thereof. In some examples, incubating the PBMC of the second stimulated PBMC sample is performed in the presence of an indoleamine 2,3-dioxygenase-1 (IDO) inhibitor, an anti-PD-1 antibody, IL-12, or a combination thereof.

[0056]

[0057] In one example, provided herein is a method comprising: obtaining a biological sample from a subject comprising at least one antigen presenting cell (APC); enriching cells expressing CD14 from the biological sample, thereby obtaining a $CD14^+$ cell enriched sample; incubating the $CD14^+$ cell enriched sample with at least one cytokine or growth factor for a first time period; incubating at least one peptide with the $CD14^+$ cell enriched sample for a second time period, thereby obtaining an APC peptide loaded sample; incubating the APC peptide loaded sample with one or more cytokines or growth factors for a third time period, thereby

obtaining a matured APC sample; incubating APCs of the matured APC sample with a CD14 and/or CD25 depleted sample comprising PBMCs for a fourth time period; incubating the PBMCs with APCs of a matured APC sample for a fifth time period; incubating the PBMCs with APCs of a matured APC sample for a sixth time period; and administering at least one T cell of the PBMCs to a subject in need thereof.

[0058] In one example, provided herein is a method comprising: obtaining a biological sample from a subject comprising at least one APC and at least one PBMC; depleting cells expressing CD14 and/or CD25 and/or CD19 from the biological sample, thereby obtaining a CD14 and/or CD25 and/or CD19 cell depleted sample; incubating the CD14 and/or CD25 and/or CD19 cell depleted sample with FLT3L for a first time period; incubating at least one peptide with the CD14 and/or CD25 and/or CD19 cell depleted sample for a second time period, thereby obtaining an APC peptide loaded sample; incubating the APC peptide loaded sample with the at least one PBMC for a third time period, thereby obtaining a first stimulated PBMC sample; incubating a PBMC of the first stimulated PBMC sample with an APC of a matured APC sample for a fourth time period, thereby obtaining a second stimulated PBMC sample; optionally, incubating a PBMC of the second stimulated PBMC sample with an APC of a matured APC sample for a fifth time period, thereby obtaining a third stimulated PBMC sample; administering at least one T cell of the first, the second or the third stimulated PBMC sample to a subject in need thereof.

[0059] In one example, prvided herein is a method comprising obtaining a biological sample from a subject comprising at least one APC and at least one PBMC; depleting cells expressing CD14 and/or CD25 and/or CD19 from the biological sample, thereby obtaining a CD14 and/or CD25 and/or CD19 cell depleted sample; incubating the CD14 and/or CD25 and/or CD19 cell depleted sample with FLT3L for a first time period; incubating at least one peptide with the CD14 and/or CD25 and/or CD19 cell depleted sample for a second time period, thereby obtaining an APC peptide loaded sample; incubating the APC peptide loaded sample with the at least one PBMC for a third time period, thereby obtaining a first stimulated PBMC sample; optionally, incubating a PBMC of the first stimulated PBMC sample with a FLT3L-stimulated APC of a matured APC sample for a fourth time period, thereby obtaining a second stimulated PBMC sample; optionally, incubating a PBMC of the second stimulated PBMC sample with a FLT3L-stimulated APC of a matured APC sample for a fifth time period, thereby obtaining a third stimulated PBMC sample; and administering at least one T cell of the first, the second or the third stimulated PBMC sample to a subject in need thereof.

[0060] In one example, prvided herein is a method comprising: obtaining a biological sample from a subject comprising at least one APC and at least one PBMC; depleting cells expressing CD14 and/or CD25 and/or CD19 from the biological sample, thereby obtaining a CD14 and/or CD25 and/or CD19 cell depleted sample; incubating the CD14 and/or CD25 and/or CD19 cell depleted sample with FLT3L for a first time period; incubating at least one peptide with the CD14 and/or CD25 and/or CD19 cell depleted sample for a second time period, thereby obtaining a first APC peptide loaded sample; incubating the first APC peptide loaded sample with the at least one PBMC for a third time period, thereby obtaining a first stimulated PBMC sample;

optionally, incubating a PBMC of the first stimulated PBMC sample with FLT3L and a second APC peptide loaded sample of a matured APC sample for a fourth time period, thereby obtaining a second stimulated PBMC sample; optionally, incubating a PBMC of the second stimulated PBMC sample with FLT3L and a third APC peptide loaded sample of a matured APC sample for a fifth time period, thereby obtaining a third stimulated PBMC sample; and administering at least one T cell of the first, the second or the third stimulated PBMC sample to a subject in need thereof

[0061] In one example, prvided herein is a method comprising: obtaining a biological sample from a subject comprising at least one APC and at least one PBMC; depleting cells expressing CD14 and/or CD25 and/or CD19 from the biological sample, thereby obtaining a CD14 and/or CD25 and/or CD19 cell depleted sample; incubating the CD14 and/or CD25 and/or CD19 cell depleted sample with FLT3L for a first time period; incubating at least one peptide with the CD14 and/or CD25 and/or CD19 cell depleted sample for a second time period, thereby obtaining a first APC peptide loaded sample; incubating the first APC peptide loaded sample with the at least one PBMC for a third time period, thereby obtaining a first stimulated PBMC sample; optionally, incubating a PBMC of the first stimulated PBMC sample with FLT3L and a FLT3L-stimulated APC of a matured APC sample for a fourth time period, thereby obtaining a second stimulated PBMC sample; optionally, incubating a PBMC of the second stimulated PBMC sample with FLT3L and a FLT3L-stimulated APC of a matured APC sample for a fifth time period, thereby obtaining a third stimulated PBMC sample; administering at least one T cell of the first, the second or the third stimulated PBMC sample to a subject in need thereof.

[0062] In one example, provided herein is a method comprising determining expression of one or more cell markers of at least one immune cell of a stimulated immune cell sample; and determining binding of the at least one immune cell of the stimulated immune cell sample to a peptide-MHC complex; wherein determining expression and determining binding are performed simultaneously. In some examples, the stimulated immune cell sample is a population of immune cells stimulated with APCs comprising a peptide-MHC complex. In some examples, the population of immune cells is from a biological sample.

[0063] In one example, provided herein is a method comprising incubating a population of immune cells from a biological sample with APCs comprising a peptide-MHC complex, thereby obtaining a stimulated immune cell sample; determining expression of one or more cell markers of at least one immune cell of the stimulated immune cell sample; and determining binding of the at least one immune cell of the stimulated immune cell sample to a peptide-MHC complex; wherein determining expression and determining binding are performed simultaneously.

[0064] In some examples, the one or more cell markers comprise TNF-α, IFN-γ, LAMP-1, 4-1BB, IL-2, IL-17A, Granzyme B, PD-1, CD25, CD69, TIM3, LAG3, CTLA-4, CD62L, CD45RA, CD45RO, FoxP3, or any combination thereof. In some examples, the one or more cell markers comprise a cytokine. In some examples, the one or more cell markers comprise a degranulation marker. In some examples, the one or more cell markers comprise a cell-surface marker. In some examples, the one or more cell markers comprise a protein.

In some examples, determining binding of the at least one immune cell of the stimulated immune cell sample to the peptide-MHC complex comprises determining binding of the at least one immune cell of the stimulated immune cell sample to a MHC tetramer comprising the peptide and the MHC of the peptide-MHC complex. In some examples, the MHC is a class I MHC or a class II MHC. In some examples, the peptide-MHC complex comprises one or more labels. In some examples, the population of immune cells from a biological sample comprises two or more samples each comprising a population of immune cells from one or more biological samples. In some examples, the two or more samples are labeled with two or more sample labels. In some examples, determining expression and determining binding comprises fluorescent activated cell sorting (FACS). In some examples, determining expression and determining binding comprises single cell analysis. In some examples, determining expression and determining binding comprises determining a percentage of immune cells that both express the one or more cell markers and that bind to the peptide-MHC complex. In some examples, the labels comprise a fluorophore. In some examples, the population of immune cells comprises a population of immune cells representative of the population of immune cells of a composition described herein.

[0065] Where examples or examples of the disclosure are described in terms of a Markush group or other grouping of alternatives, the present disclosure encompasses not only the entire group listed as a whole, but also each member of the group individually and all possible subgroups of the main group, and also the main group absent one or more of the group members. The present disclosure also envisages the explicit exclusion of one or more of any of the group members in the claimed disclosure.

INCORPORATION BY REFERENCE

[0066] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference in their entirety for all purposes, to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. For example, all publications and patents mentioned herein are incorporated herein by reference in their entirety for the purpose of describing and disclosing the kits, compositions, and methodologies that are described in the publications, which might be used in connection with the methods, kits, and compositions described herein. The documents discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors described herein are not entitled to antedate such disclosure by virtue of prior invention or for any other reason.

BRIEF DESCRIPTION OF THE DRAWINGS

[0067] **FIG. 1A** depicts an example schematic of an antigen specific T cell manufacturing protocol.

[0068] **FIG. 1B** depicts an example schematic of an antigen specific T cell manufacturing protocol.

[0069] **FIG. 2** depicts an example result showing fraction of antigen specific $CD8^+$ memory T cells induced by long peptide or short peptide. “Bulk” indicates the sample containing T cells used for induction is whole peripheral blood mononuclear cell (PBMC). “$Treg^-$” indicates the sample containing T cells used for induction is PBMCs depleted of CD25 expressing cells.

[0070] FIG. 3 depicts an example flow cytometry analysis showing the fraction of antigen specific $CD8^+$ naïve T cells induced with a GAS7 peptide.

[0071] FIG. 4 depicts an example result showing antigen specific $CD8^+$ T cell responses to a peptide pool of HIV short peptides, short previously identified neoantigens (PINs), or long PINs. "Whole PBMC" indicates

CLAIMS

What is claimed is:

1. A method of preparing tumor antigen-specific T cells ex vivo suitable for use as an autologous therapy, the method comprising:

(a) 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, wherein the population of immune cells is from a biological sample from a human subject with cancer; and

(b) incubating the CD14/CD25 depleted population of immune 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 tumor antigen epitope sequences expressed by cancer cells of a human subject, wherein each of the at least two tumor antigen epitope sequences contains a mutation and binds to an MHC protein expressed by the subject with a stronger affinity than a corresponding wild-type epitope sequence, or (B) a polynucleotide encoding the polypeptide; thereby forming a population of stimulated T cells; and

(c) expanding the population of stimulated T cells, thereby forming an expanded population of T cells, wherein expanding the population of stimulated T cells comprises expanding T cells derived from naïve CD8+ T cells or naïve CD4+ T cells, wherein the expanded population of T cells comprises (i) at least 1x10^6 total CD8+ T cells, (ii) at least 1x10^ 6 total CD4+ T cells and (iii) T cells that are specific to

> (A) a first complex comprising (i) a first tumor antigen epitope sequence of the at least two tumor antigen epitope sequences and (ii) an MHC protein expressed by the cancer cells or APCs of the human subject, and
>
> (B) a second complex comprising (i) a second tumor antigen epitope sequence of the at least two tumor antigen epitope sequences (ii) an MHC protein expressed by the cancer cells or APCs of the human subject; and

wherein at least 0.1% of the CD8+ T cells that are specific to the first complex or the second complex in the expanded population of T cells are derived from the naïve CD8+ T cells, and at least 0.1% of the CD4+ T cells that are specific to the first complex or the second complex in the expanded population of T cells are derived from the naïve CD4+ T cells.

2. The method of claim 1, wherein steps (b) and (c) are performed in less than 28 days.

3. The method of claim 1, wherein the fraction of CD8+ T cells that are specific to the first complex or the second complex of the total number of CD8+ T cells in the expanded population of T cells is at least two-fold higher than the fraction of CD8+ T cells that are specific to the first complex or the second complex of the total number of CD8+ T cells in the biological sample.

4. The method of claim 1, wherein the fraction of CD4+ T cells that are specific to the first complex or the second complex of the total number of CD4+ T cells in the expanded population of T cells is at least two-fold higher than the fraction of CD4+ T cells that are specific to the first complex or the second complex of the total number of CD4+ T cells in the biological sample.

5. The method of claim 1, wherein expanding comprises (A) contacting the population of stimulated T cells with a second population of mature APCs, wherein the second population of mature APCs (i) has been incubated with FLT3L and (ii) presents the at least two tumor antigen epitope sequences; and (B) expanding the population of stimulated T cells for a second time period, thereby forming an expanded population of T cells.

6. The method of claim 5, wherein the second population of mature APCs has been incubated with FLT3L for at least 1 day prior to contacting the population of stimulated T cells with the second population of mature APCs.

7. The method of claim 5, wherein 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) has been incubated with FLT3L and (ii) presents the at least two tumor antigen epitope sequences; and (D) expanding the expanded population of T cells for a third time period, thereby forming the expanded population of T cells of (c).

8. The method of claim 7, wherein the third population of mature APCs has 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.

9. The method of claim 1, wherein the biological sample is a peripheral blood sample, a leukapheresis sample or an apheresis sample.

10. The method of claim 1, further comprising harvesting the expanded population of T cells, cryopreserving the expanded population of T cells or preparing a pharmaceutical composition containing the expanded population of T cells.

11. The method of claim 1, wherein incubating comprises incubating the CD14/CD25 depleted population of immune cells in the presence of FLT3L and an RNA encoding the polypeptide.

12. The method of claim 1, wherein the polypeptide is from 8 to 50 amino acids in length.

13. The method of claim 1, wherein the polypeptide comprises at least three tumor antigen epitope sequences, each expressed by cancer cells of the human subject with cancer.

14. The method of claim 1, wherein depleting CD14+ cells and CD25+ cells from the population of immune cells comprises contacting the population of immune cells with a CD14 binding agent and a CD25 binding agent.

15. The method of claim 1, wherein depleting further comprises depleting CD19+ cells from the population of immune cells.

16. A pharmaceutical composition comprising the expanded population of T cells of claim 1, and a pharmaceutically acceptable excipient.

17. Use of the pharmaceutical composition according to claim 16 in the manufacture of a medicament for treating a human subject with cancer.

18. The pharmaceutical composition according to claim 16 for use in therapy.