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

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

[0010] In some embodiments, 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 embodiments, the epitope that is immunogenic according to an immunogenicity assay is immunogenic according to a multimer assay or a functional assay.

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

[0013] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the functional assay comprises an immunoassay.

[0020] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the method comprises selecting the subject using a circulating tumor DNA assay.

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

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

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

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

[0032] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the method further comprises administering the population of T cells to the subject.

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

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

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

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

[0042] In some embodiments, 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 embodiments, one or more of the at least one selected epitope sequence comprises a driver mutation.

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

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

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

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

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

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

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

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

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

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

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

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

[0056] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the population of immune cells is from a biological sample from the subject.

[0062] In some embodiments, 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 embodiments, 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 embodiments, the T cells are expanded in less than 28 days.

[0065] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the biological sample is a peripheral blood sample, a leukapheresis sample or an apheresis sample.

[0074] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the human subject with cancer is the human subject from which the biological sample was obtained.

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

[0079] In some embodiments, 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 embodiments, 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 embodiments, depleting further comprising depleting CD19+ cells from the population of immune cells comprising a first population of APCs and T cells.

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

[0083] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, one or more of the at least one selected epitope sequence has a length of from 8 to 12 amino acids.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[0109] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the one or more peptides comprising the at least one selected epitope sequence is purified.

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

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

[0117] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the additional selected epitope is expressed on a cancer associated fibroblast cell.

[0123] In some embodiments, 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.

[0128] **FIG. 1B** is schematic of an exemplary method provided herein to prime, activate and expand antigen-specific T cells.

[0129] **FIG. 2** is schematic of an exemplary method for offline characterization of shared epitopes.

[0130] **FIG. 3A** depicts data illustrating that in silico epitope prediction identified multiple neoantigens derived from RAS G12D mutations that are presented according to mass spectrometry.

[0131] **FIG. 3B** depicts data illustrating that in silico epitope prediction identified multiple neoantigens derived from RAS G12V mutations that are presented according to mass spectrometry.

[0132] **FIG. 3C** depicts data illustrating that in silico epitope prediction identified multiple neoantigens derived from RAS G12C mutations that are presented according to mass spectrometry.

[0133] **FIG. 3D** depicts data illustrating that in silico epitope prediction identified multiple neoantigens derived from RAS G12R mutations that are presented according to mass spectrometry.

[0134] **FIG. 4A** depicts data illustrating that presentation of shared neoantigen epitopes can be directly confirmed by mass spectrometry and that RAS neoantigens are targetable in defined patient populations.

[0135] **FIG. 4B** shows head-to-toe plot of MS/MS spectra for the endogenously processed mutant RAS peptide epitope VVVGAVGVGK (top) and its corresponding heavy peptide (bottom). 293T cells were lentivirally transduced with both a polypeptide containing the RAS^{G12V} mutant peptide and an HLA-A*03:01 gene.

[0136] **FIG. 4C** shows head-to-toe plot of MS/MS spectra for the endogenously processed mutant RAS peptide epitope VVVGAVGVGK (top) and its corresponding heavy peptide (bottom). SW620 cells that naturally express the RAS^{G12V} mutant were transduced with a lentiviral vector encoding an HLA-A*03:01 gene.

[0137] **FIG. 4D** shows head-to-toe plot of MS/MS spectra for the endogenously processed mutant RAS peptide epitope VVVGAVGVGK (top) and its corresponding heavy peptide (bottom). NCI-H441 cells naturally expressing both the RAS^{G12V} mutation and the HLA-A*03:01 gene were used for this experiment.

[0138] **FIG. 4E** shows head-to-toe plot of MS/MS spectra for the endogenously processed GATA3 neoORF peptide epitope SMLTGPPARV. Endogenous peptide spectrum is shown in the top panel and corresponding light synthetic spectrum is shown in the bottom panels.

[0139] **FIG. 5** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces de novo CD8 T cell responses against RAS G12 neoantigens on HLA-A11:01 and HLA-A03:01.

[0140] **FIG. 6** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces multiple de novo CD8 T cell responses against RAS G12V neoantigen on HLA-A11:01. As indicated in the pie charts, the frequency of individual T cell clones induced against RAS G12V neoantigen on HLA-A11:01 in 3 independent healthy donors is depicted.

[0141] **FIG. 7** depicts data illustrating that RAS^{G12V}-activated T cells generated ex vivo can kill target cells. A375 target cells expressing GFP were loaded with 2 μM RAS^{G12V} antigen, wild-type RAS antigen, or no peptide as control GFP+ cells. RAS^{G12V}-specific CD8 T cells (effector cells) were incubated with control cells or target cells in a 0.05:1 ratio. In presence of the effector cells, target cells were lysed and depleted more readily that control cells which present either RAS^{WT} antigen or no antigen. Graph of specific cell killing as normalized by target cell growth with no peptide is shown in the left diagram. Representative images are shown on the right.

[0142] **FIG. 8** depicts data illustrating that an exemplary method provided herein to prime, activate and expand RAS G12V -specific T cells with RAS G12V neoantigens on HLA-11:01, but not the corresponding wild-type antigens, induces T cells to become cytotoxic using the indicated effector:target cell ratios and increasing peptide concentration.

[0143] **FIG. 9** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells with one round (1x stimulated) or two rounds (2x stimulated) of FLT3L-treated PBMCs presenting an epitope with the RAS^{G12V} mutation induces T cells to become cytotoxic as measured by AnnexinV positive cells over time after co culturing these T cells with SW620 cells (naturally express the RAS^{G12V} mutant) that were transduced with a lentiviral vector encoding an HLA-A*11:01 gene.

[0144] **FIG. 10** depicts a graph of AnnexinV positive cells over time after co-culturing NCI-H441 cells naturally expressing both the RAS^{G12V} mutation and the HLA-A*03:01 gene with T cells that had been primed and activated and expanded with a peptide containing an epitope with the RAS^{G12V} mutation at the indicated effector:target cell ratio.

[0145] **FIG. 11A** depicts a graph of IL-2 concentration (pg/mL) vs RAS-G12V wild-type or mutant peptide loaded target cells (A375-A11:01) after incubation in the presence of Jurkat cells transduced with a TCR that binds to the RAS-G12V epitope bound to an MHC encoded by the HLA-A11:01 allele.

[0146] **FIG. 11B** depicts graphs of AnnexinV positive cells over time after co culturing TCR-transduced PBMCs with 5,000 SNGM cells with natural G12V and HLA-A11:01 across a range of effector:target cell ratios.

[0147] **FIG. 11C** depicts a graph of IL-2 concentration (pg/mL) vs RAS-G12V wild-type or mutant peptide loaded target cells (A375-A03:01) after incubation in the presence of Jurkat cells transduced with a TCR that binds to RAS-G12V bound to an MHC encoded by the HLA-A03:01 allele.

[0148] **FIG. 11D** depicts a graph of AnnexinV positive cells over time (top) after co-culturing TCR-transduced PBMCs with cells with natural G12V and HLA-A03:01 using an effector:target cell ratio of 0.75:1 and a graph of IFNγ concentration (pg/mL) after 24 hours of coculturing TCR-transduced PBMCs with cells with natural G12V and HLA-A03:01 using an effector:target cell ratio of 0.75:1.

[0149] **FIG. 12A** depicts a graph of IL-2 concentration (pg/mL) vs FLT3L-treated PBMCs contacted with increasing amounts of the indicated RAS-G12V mutant peptides after being co-cultured with Jurkat cells transduced with a TCR that binds to the underlined RAS-G12V epitope bound to an MHC encoded by the HLA-A11:01 allele.

[0150] **FIG. 12B** depicts data illustrating the immunogenicity of the indicated RAS-G12V mutant peptides from **FIG. 12A** both in vitro using PBMCs from healthy donors (top) and in vivo using HLA-A11:01 transgenic mice immunized with the peptides (bottom).

[0151] **FIG. 13** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces de novo CD8 T cell responses against RAS G12V neoantigen on HLA-02:01.

[0152] **FIG. 14** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces de novo CD8 T cell responses against RAS G12 neoantigens on HLA-A68:01.

[0153] **FIG. 15** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces de novo CD8 T cell responses against RAS G12 neoantigens on HLA-B07:02

[0154] **FIG. 16** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces de novo CD8 T cell responses against RAS G12 neoantigens on HLA-B08:01.

[0155] **FIG. 17** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces de novo CD8 T cell responses against RAS G12D neoantigen on HLA-C08:02.

[0156] **FIG. 18** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces de novo CD4 T cell responses against RAS neoantigens.

[0157] **FIG. 19A** depicts data illustrating flow cytometry data demonstrating that enrichment procedures can be used prior to further expansion of antigen-specific T cells. Cells upregulating 4-1BB were enriched using Magnetic-Assisted Cell Separation (MACS; Miltenyi). T cells that were stained by multimers were enriched by MACS on day 14 of stimulation. This approach was able to enrich for multiple antigen-specific T cell populations.

[0158] **FIG. 19B** depicts an exemplary bar graph quantifying the results in **FIG. 19A**.

[0159] **FIG. 20** illustrates a summary of experiments illustrating that predicted GATA3 neoORF epitopes have strong affinity (<500nM), long stability (>0.5hr) and/or can be detected by mass spectrometry analysis of epitopes eluted from HLA molecules from cells expressing the GATA3 neoORF.

[0160] **FIG. 21** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces de novo CD8 T cell responses against GATA3 neoORF neoantigens on HLA-A02:01, HLA-A03:01, HLA-A11:01, HLA-B07:02 and HLA-B08:01.

[0161] **FIG. 22** depicts data illustrating GATA3 neoORF epitope-activated T cells generated ex vivo can kill target cells. 293T target cells expressing GFP were loaded with 2 μM GATA3 neoORF antigen or left unloaded as control GFP+ cells. GATA3-neoORF-specific CD8 T cells (effector cells) were incubated with control cells or target cells in a 1:10 ratio. In presence of the effector cells, target cells were lysed and depleted more readily that control cells which do present GATA3 neoantigen. Graph of GFP+ cells over 100 hours is shown in the top diagram. Images of the control (bottom left image) and target GFP+ cells (bottom right image) in the presence of GATA3 neoantigen activated CD8 cells are shown.

[0162] **FIG. 23** depicts a graph of a comparison of Caspase-3 positive fraction of live target cells in GATA3 neoantigen transduced HEK 293T cells versus non-transduced HEK 293T cells. Two different GATA3 induced healthy donor PBMCs were co-cultured with GATA3 neoantigen transduced HEK 293T cells or non-transduced HEK 293T cells as a negative control group.

[0163] **FIG. 24** depicts flow cytometry data illustrating induction of antigen-specific CD4+ T cells with GATA3 neoORF specific peptide after 20 days in culture, including two stimulations. Antigen-specific T cells are detected by increase in IFNγ and/or TNFα after incubation with GATA3 neoORF peptides (right) relative to no peptides (left)

[0164] **FIG. 25A** depicts a schematic diagram of steps followed through discovery and validation of peptides presented in prostate cancer cell lines or prostate tissue from human donors, and generating validated peptides for a curated validated peptide library.

[0165] **FIG. 25B** depicts data illustrating generation of epitope specific CD8T cells in vitro. The peptides were predicted using T cell epitope prediction software in proteins specific to prostate cancer.

[0166] **FIG. 25C** depicts data illustrating KLK4 epitope-activated T cells generated ex vivo are immunogenic and kill target cells. 293T target cells expressing GFP were loaded with 2 μM KLK4 antigen (LLANGRMPTV) or left unloaded as control GFP+ cells. KLK4 specific CD8 T cells (effector cells) were incubated with control cells or target cells in a 1:10 ratio. In presence of the effector cells, target cell growth was controlled more readily than control cells which do not express KLK4. Also shown is a graph of GFP+ cells over 100 hours (bottom). Images of the control (bottom left image) and target GFP+ cells (bottom right image) in the presence of KLK4 activated CD8 cells are shown.

[0167] **FIG. 26** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces de novo CD8 T cell responses against a BTK C481S neoantigen on HLA-02:01.

[0168] **FIG. 27** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces de novo CD8 T cell responses against EGFR T790M neoantigens on HLA-02:01.

[0169] **FIG. 28A** depicts a schematic of an exemplary method provided herein for application of T cell therapies.

[0170] **FIG. 28B** depicts a schematic of an exemplary method provided herein for application of T cell therapies.

[0171] **FIG. 29** depicts a schematic of an exemplary method for in silico T cell epitope prediction. PPV was determined for a given n number of hits and 5,000 decoys, what fraction of the n top-ranked peptides were hits.

[0172] **FIG. 30** depicts a schematic of allelic coverage of the MHC ligandome using in silico epitope prediction.

[0173] **FIG. 31** depicts a schematic comparing in silico T cell epitope prediction models.

[0174] **FIG. 32** depicts a schematic illustrating identification and validation of immunogenic peptides using in silico T cell epitope prediction and an exemplary method provided herein to prime, activate and expand antigen-specific T cells.

[0175] **FIG. 33** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells can induce and expand multiple neoantigen CD8+ T cell populations. The data shown is representative data from sample from a melanoma patient.

[0176] **FIG. 34** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells generated three CD4+ populations in the same patient. The data shown is representative data from sample from a melanoma patient.

[0177] **FIG. 35** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells repeatedly demonstrates T cell inductions across melanoma patient samples.

[0178] **FIG. 36** depicts representative data from a melanoma patient sample illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces T cells highly specific for mutant epitopes.

[0179] **FIG. 37** depicts representative data from a melanoma patient sample illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces T cells that are highly functional.

[0180] **FIG. 38** depicts data illustrating that an exemplary method provided herein to prime, activate and expand antigen-specific T cells induces CD8+ T cells can kill tumor cells.

DETAILED DESCRIPTION

[0181] Although many epitopes have the potential to bind to an MHC molecule, few are capable of binding to an MHC molecule when tested experimentally. Although many epitopes also have the

potential to potential to be presented by an MHC molecule that can, for example, be detected by mass spectrometry, only a select number of these epitopes can be presented and detected by mass spectrometry. Although many epitopes also have the potential to be immunogenic, when tested experimentally many of these epitopes are not immunogenic, despite being demonstrated to be presented by antigen presenting cells. Many epitopes also have the potential to activate T cells to become cytotoxic; however, many epitopes that have been demonstrated to be presented by antigen presenting cells and/or to be immunogenic are still not capable of activating T cells to become cytotoxic.

[0182] Provided herein are antigens containing T cell epitopes that have been identified and validated as binding to one or more MHC molecules, presented by the one or more MHC molecules, being immunogenic and capable of activating T cells to become cytotoxic. The validated antigens and polynucleotides encoding these antigens can be used in preparing antigen specific T cells for therapeutic uses. In some embodiments, the validated antigens and polynucleotides encoding these antigens can be pre-manufactured and stored for use in a method of manufacturing T cells for therapeutic uses. For example, the validated antigens and polynucleotides encoding these antigens can be pre-manufactured or manufactured quickly to prepare therapeutic T cell compositions for patients quickly. Using validated antigens with T cell epitopes, immunogens such as peptides having HLA binding activity or RNA encoding such peptides can be manufactured. Multiple immunogens can be identified, validated and pre-manufactured in a library. In some embodiments, peptides can be manufactured in a scale suitable for storage, archiving and use for pharmacological intervention on a suitable patient at a suitable time.

[0183] Some, if not all cancers have antigens that are potential targets for immunotherapy. Each peptide antigen may be presented for T cell activation on an antigen presenting cells in association with a specific HLA-encoded MHC molecule. On the other hand, provided herein is a potentially universal approach, where particular epitopes are pre-identified and pre-validated for particular HLAs, and these epitopes can be pre-manufactured for a cell therapy manufacturing process. For example, a number of KRAS epitopes with G12, G13 and Q61 mutations can be identified using a reliable T cell epitope presentation prediction model (see, e.g., PCT/US2018/017849, filed February 12, 2018, and PCT/US2019/068084 filed December 20, 2019, each of which are incorporated by reference in their entirety), with validation of immunogenicity of these epitopes, processing and presentation using mass spectrometry of these epitopes, and ability to generate cytotoxic T cells with TCRs against these epitopes and MHCs encoded by different HLAs. Each epitope is validated with its specific amino acid sequence and relevant HLA. Once these epitopes are validated, a library can be created containing pre-manufactured immunogens, such as peptides containing the epitopes or RNA encoding peptides containing these epitopes.

[0184] The antigens can be non-mutated antigens or mutated antigens. For example, the antigens can be tumor-associated antigens, mutated antigens, tissue-specific antigens or neoantigens. In some embodiments, the antigens are tumor-associated antigens. In some embodiments, the antigens are mutated antigens. In some embodiments, the antigens are tissue-specific antigens. In some embodiments, the antigens are neoantigens. Neoantigens are found in the cancer or the tumor in a subject and is not evident in the germline or expressed in the healthy tissue of the subject. Therefore, for a gene mutation in cancer to satisfy the criteria of generating a neoantigen, the gene mutation in the cancer must be a non-silent mutation that translates into an altered protein product. The altered protein product contains an amino acid sequence with a mutation that can be a mutated epitope for a T cell. The mutated epitope has the potential to bind to an MHC molecule. The mutated epitope also has the potential to be presented by an MHC molecule that can, for example, be detected by mass spectrometry. Furthermore, the mutated epitope has the potential to be immunogenic. Additionally, the mutated epitope has the potential to activate T cells to become cytotoxic.

[0185] 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 two or three or four 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. In some embodiments, the method further comprises administering the population of T cells to the subject.

[0186] In some embodiments, 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. In some embodiments, one or more of the least one selected epitope sequence comprises an epitope that is not expressed by cancer cells of the subject. In some embodiments, the epitope that is not expressed by cancer cells of the subject is expressed by cells in a tumor microenvironment of the subject. In some embodiments, the method comprises selecting the subject using a circulating tumor DNA assay. In some embodiments, the method comprises selecting the subject using a gene panel.

[0187] In some embodiments, the T cell is from a biological sample from the subject. In some embodiments, the T cell is from an apheresis or a leukopheresis sample from the subject. In some embodiments, the T cell is an allogeneic T cell.

[0188] In some embodiments, each of the at least one selected epitope sequence is pre-validated to satisfy one or more or 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.

[0189] In some embodiments, 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. For example, an epitope that binds to a protein encoded by an HLA allele of the subject can bind to an MHC molecule encoded by the HLA allele with an affinity of 400 nM, 300 nM, 200 nM, 150 nM, 100 nM, 75 nM, 50 nM, or 25 nM or less according to a binding assay. In some embodiments, 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. For example, an epitope that binds to a protein encoded by an HLA allele of the subject can be predicted to bind to an MHC molecule encoded by the HLA allele with an affinity of 400 nM, 300 nM, 200 nM, 150 nM, 100 nM, 75 nM, 50 nM, or 25 nM or less using an MHC epitope prediction program implemented on a computer. In some embodiments, the MHC epitope prediction program implemented on a computer is NetMHCpan. In some embodiments, the MHC epitope prediction program implemented on a computer is NetMHCpan version 4.0.

[0190] In some embodiments, the epitope that is presented by antigen presenting cells (APCs) according to a mass spectrometry assay is detected by mass spectrometry after elution from the APCs with a mass accuracy of the detected peptide to be less than 15 Da. For example, the epitope that is presented by antigen presenting cells (APCs) according to a mass spectrometry assay can be detected by mass spectrometry after elution from the APCs with a mass accuracy of the detected peptide to be less than 14 Da, 13 Da, 12 Da, 11 Da, 10 Da, 9 Da, 8 Da, 7 Da, 6 Da, 5 Da, 4 Da, 3 Da, 2 Da, or 1 Da. In some embodiments, the epitope that is presented by antigen presenting cells (APCs) according to a mass spectrometry assay is detected by mass spectrometry after elution from the APCs with a mass accuracy of the detected peptide to be less than 10,000 parts per million (ppm). For example, the epitope that is presented by antigen presenting cells (APCs) according to a mass spectrometry assay can be detected by mass spectrometry after elution from the APCs with a mass accuracy of the detected peptide to be less than 7,500 ppm; 5,000 ppm; 2,500 ppm; 1,000 ppm; 900 ppm; 800 ppm; 700 ppm; 600 ppm; 500 ppm; 400 ppm; 300 ppm; 200 ppm or 100 ppm.

[0191] In some embodiments, the epitope that is immunogenic according to an immunogenicity assay is immunogenic according to a multimer assay. In some embodiments, the multimer assay comprises flow cytometry analysis. In some embodiments, 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. In some embodiments, an 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 0.005% 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. For example, an epitope can be immunogenic according to the multimer assay when (i) at least 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 or more 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 $CD8^+$ 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. For example, an epitope can be 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 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% 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. For example, an epitope can be immunogenic according to the multimer assay when (i) at least 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 or more 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 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% 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.

[0192] In some embodiments, 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. For example, the epitope can be immunogenic according to the multimer assay when at least10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 or more 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. For example, the epitope can be 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 2 out of 6, 7, 8, 9, 10, 11 or 12 stimulations from the same starting sample. For example, the epitope can be 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 2, 3, 4, 5 or 6 out of 6 stimulations from the same starting sample. For example, the epitope can be 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 2,

3, 4, 5, 6 or 7 out of 7 stimulations from the same starting sample. For example, the epitope can be 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 2, 3, 4, 5, 6, 7 or 8 out of 8 stimulations from the same starting sample. For example, the epitope can be 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 2, 3, 4, 5, 6, 7, 8 or 9 out of 9 stimulations from the same starting sample. For example, the epitope can be 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 2, 3, 4, 5, 6, 7, 8, 9 or 10 out of 10 stimulations from the same starting sample. For example, the epitope can be 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 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 out of 11 stimulations from the same starting sample. For example, the epitope can be 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 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 out of 12 stimulations from the same starting sample. For example, the epitope can be 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 3 out of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 stimulations from the same starting sample. For example, the epitope can be 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 4 out of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 stimulations from the same starting sample. For example, the epitope can be immunogenic according to the multimer assay when at least 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 or more 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. For example, the epitope can be immunogenic according to the multimer assay when at least 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 or more T cells that have been stimulated with APCs comprising a peptide containing the at least one selected epitope sequence are detected in at least 2 out of 6, 7, 8, 9, 10, 11 or 12 stimulations from the same starting sample or in at least 3 out of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 stimulations from the same starting sample or in at least 4 out of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 stimulations from the same starting sample. In some embodiments, 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. In some embodiments, 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. In some embodiments,

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.

[0193] In some embodiments, the epitope that is immunogenic according to an immunogenicity assay is immunogenic according to a functional assay. In some embodiments, the functional assay comprises an immunoassay. In some embodiments, 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 In some embodiments, 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 0.005% 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. For example the epitope can be immunogenic according to the functional assay when (i) at least 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 or more 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. For example the epitope can be 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 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% 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. For example the epitope can be immunogenic according to the functional assay when (i) at least10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 or more 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 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% 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.

[0194] In some embodiments, 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. In some embodiments, 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. In some embodiments, 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 In some embodiments, 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. In some embodiments, 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.

[0195] In some embodiments, at least one of the one or more peptides is a synthesized peptide or a peptide expressed from a nucleic acid sequence.

[0196] In some embodiments, 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.

[0197] In some embodiments, the at least one selected epitope sequence is selected from one or more epitope sequences of Table 1-8 and 11-14.

[0198] In some embodiments, 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.

[0199] In some embodiments, a protein comprising the at least one selected epitope sequence is expressed by a cancer cell of the subject. In some embodiments, a protein comprising the at least one selected epitope sequences is expressed by cells in the tumor microenvironment of the subject.

[0200] In some embodiments, one or more of the at least one selected epitope sequence comprises a mutation. In some embodiments, one or more of the at least one selected epitope sequence comprises a tumor specific mutation. In some embodiments, one or more of the at least one selected epitope sequence is from a protein overexpressed by a cancer cell of the subject. In some embodiments, one or more of the at least one selected epitope sequence comprises a driver mutation. In some embodiments, one or more of the at least one selected epitope sequence comprises a drug resistance mutation. In some embodiments, one or more of the at least one selected epitope sequence is from a tissue-specific protein. In some embodiments, one or more of the at least one selected epitope sequence is from a cancer testes protein. In some embodiments, one or more of the at least one selected epitope sequence is a viral epitope. In some embodiments, one or more of the at least one selected epitope sequence is a minor histocompatibility epitope. In some embodiments, one or more of the at least one selected epitope sequence is from a RAS protein. In some embodiments, one or more of the at least one selected epitope sequence is from a GATA3 protein. In some embodiments, one or more of the at least one selected epitope sequence is from a EGFR protein. In some embodiments, one or more of the at least one selected epitope sequence is from a BTK protein. In some embodiments, one or more

of the at least one selected epitope sequence is from a p53 protein. In some embodiments, one or more of the at least one selected epitope sequence is from aTMPRSS2::ERG fusion polypeptide. In some embodiments, one or more of the at least one selected epitope sequence is from a Myc protein. In some embodiments, 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.

[0201] In some embodiments, 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.

[0202] In some embodiments, 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.

[0203] In some embodiments, 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. In some embodiments, the polypeptide comprises at least two of the selected epitope sequence, each expressed by cancer cells of a human subject with cancer.

[0204] In some embodiments, 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. In some embodiments, the population of immune cells is from a biological sample from the subject. In some embodiments, the method further 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 FMS-like tyrosine kinase 3 receptor ligand (FLT3L), and a polypeptide comprising the at least one selected epitope sequence, or a polynucleotide encoding the polypeptide; thereby forming a population of cells comprising stimulated T cells. In some embodiments, the method further comprises 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 the at least one selected epitope sequence and an MHC

protein expressed by the cancer cells or APCs of the subject. In some embodiments, expanding is performed in less than 28 days. In some embodiments, 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. In some embodiments, 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. In some embodiments, depleting further comprising depleting CD19+ cells from the population of immune cells comprising a first population of APCs and T cells. In some embodiments, depleting further comprising depleting CD11b+ cells from the population of immune cells comprising a first population of APCs and T cells.

[0205] In some embodiments, 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. In some embodiments, the human subject with cancer is the human subject from which the biological sample was obtained.

[0206] In some embodiments, 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. In some embodiments, 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. In some embodiments, at least 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% 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. In some embodiments, at least 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% 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 memory CD8+ T cells. In some embodiments, at least 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% 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. In some embodiments, at least 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% 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 memory CD4+ T cells.

[0207] In some embodiments, 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. In some embodiments, the second population of mature APCs has 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. In some embodiments, expanding further comprises contacting the expanded population of T cells with a third population of mature APCs, wherein the third population of mature APCs have been incubated with FLT3L and present the at least one selected epitope sequence; and 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. In some embodiments, 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. In some embodiments, the biological sample is a peripheral blood sample, a leukapheresis sample or an apheresis sample.

[0208] In some embodiments, 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.

[0209] In some embodiments, 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.

[0210] In some embodiments, 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.

[0211] In some embodiments, 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. In some embodiments, 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. In some embodiments, the method comprises isolating genomic DNA or RNA from cancer cells and non-cancer cells of the subject.

[0212] In some embodiments, one or more of the at least one selected epitope sequence comprises a point mutation or a sequence encoded by a point mutation. In some embodiments, one or more of the at least one selected epitope sequence comprises a sequence encoded by a neoORF mutation. In some embodiments, one or more of the at least one selected epitope sequence comprises a sequence encoded by a gene fusion mutation. In some embodiments, one or more of the at least one selected epitope sequence comprises a sequence encoded by an indel mutation. In some embodiments, one or more of the at least one selected epitope sequence comprises a sequence encoded by a splice site mutation. In some embodiments, at least two of the at least one selected epitope sequence are from a same protein. In some embodiments, at least two of the at least one selected epitope sequence comprise an overlapping sequence. In some embodiments, at least two of the at least one selected epitope sequence are from different proteins. In some embodiments, the one or more peptides comprise at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more peptides.

[0213] In some embodiments, cancer cells of the subject are cancer cells of a solid cancer. In some embodiments, cancer cells of the subject are cancer cells of a leukemia or a lymphoma.

[0214] In some embodiments, the mutation is a mutation that occurs in a plurality of cancer patients.

[0215] In some embodiments, the MHC is a Class I MHC. In some embodiments, the MHC is a Class II MHC.

[0216] In some embodiments, the T cell is a CD8 T cell. In some embodiments, the T cell is a CD4 T cell. In some embodiments, the T cell is a cytotoxic T cell. In some embodiments, the T cell t is a memory T cell. In some embodiments, the T cell is a naive T cell.

[0217] In some embodiments, the method further comprises selecting one or more subpopulation of cells from an expanded population of T cells prior to administering to the subject.

[0218] In some embodiments, eliciting an elicit an immune response in the T cell culture comprises inducing IL2 production from the T cell culture upon contact with the peptide. In some embodiments, 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. In some embodiments, eliciting an immune response in the T cell culture comprises inducing the T cell culture to kill a cell expressing the peptide. In some embodiments, 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.

[0219] In some embodiments, the one or more peptides comprising the at least one selected epitope sequence is purified. In some embodiments, the one or more peptides comprising the at least one selected epitope sequence is lyophilized. In some embodiments, the one or more peptides comprising the at least one selected epitope sequence is in a solution. In some embodiments, 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%.

[0220] In some embodiments, 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. In some embodiments, 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. In some embodiments, 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.

[0221] In some embodiments, the at least one selected epitope is expressed by a cancer cell, and an additional selected epitope is expressed by a cancer associated cell. In some embodiments, the additional selected epitope is expressed on a cancer associated fibroblast cell. In some embodiments, the additional selected epitope is selected from Table 8.

[0222] In some embodiments, a method 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; 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 sequences; binds to a protein encoded by an HLA allele of the subject; is immunogenic according to an immunogenic 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.

[0223] In some embodiments, the method comprises selecting the subject using a circulating tumor DNA assay. In some embodiments, the method comprises selecting the subject using a gene panel.

[0224] In some embodiments, the T cell is from a biological sample from the subject. In some embodiments, the T cell is from an apheresis or a leukopheresis sample from the subject.

[0225] In some embodiments, at least one of the one or more peptides a synthesized peptide or a peptide expressed from a nucleic acid sequence.

[0226] In some embodiments, 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. In some embodiments, the method comprises identifying a protein encoded by an HLA allele of the subject that is expressed by the subject. In some embodiments, the method comprises contacting a T cell from the subject with one or more peptides selected from one or more peptides of a table provided herein. In some embodiments, the method comprises contacting a T cell from the subject with one or more peptides comprising an epitope selected from an epitope of a table provided herein. In some embodiments, the method further comprises expanding in vitro or ex vivo the T cell contacted with the one or more peptides to obtain a population of T cells. In some embodiments, the method further comprises administering the

population of T cells to the subject at a dose and a time interval such that the cancer is reduced or eliminated.

[0227] In some embodiments, at least one of the one or more peptides is expressed by a cancer cell of the subject. In some embodiments, at least one of the epitopes of the one or more peptides comprises a mutation.

[0228] In some embodiments, at least one of the epitopes of the one or more peptides comprises a tumor specific mutation. In some embodiments, at least one of the epitopes of the one or more peptides is from a protein overexpressed by a cancer cell of the subject. In some embodiments, at least one of the epitopes of the one or more peptides 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*.

[0229] In some embodiments, at least one of the one or more peptides is from a protein encoded by a tissue-specific antigen epitope gene 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 antigen gene in each tissue of a plurality of non-target tissues that are different than the target tissue.

[0230] In some embodiments, the method comprises: incubating one or more antigen presenting cell (APC) preparations with a population of immune cells from a biological sample depleted of cells expressing CD14 and CD25 for one or more separate time periods; incubating one or more APC preparations with a population of immune cells from a biological sample for one or more separate time periods, wherein the one or more APCs comprise one or more FMS-like tyrosine kinase 3 receptor ligand (FLT3L)-stimulated APCs; or incubating FLT3L and at least one peptide with a population of immune cells from a biological sample, wherein the FLT3L is incubated with the population of immune cells for a first time period and wherein the at least one peptide is incubated with the population of immune cells for a first peptide stimulation time period, thereby obtaining a first stimulated T cell sample, wherein the population of immune cells comprises at least one T cell and at least one APC; wherein at least one antigen specific memory T cell is expanded, or at least one antigen specific naïve T cell is induced.

[0231] In some embodiments, the method comprises 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. In some embodiments, the method comprises incubating a population of

immune cells from a biological sample with 3 or less APC preparations for 3 or less separate time periods. In some embodiments, the method comprises incubating a population of immune cells from a biological sample with 2 or less APC preparations for 2 or less separate time periods. In some embodiments, the method comprises 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. In some embodiments, the total period of preparation of T cells stimulated with an antigen by incubating a population of immune cells from a biological sample with one or more APC preparations for one or more separate time periods is less than 28 days.

[0232] In some embodiments, at least two of the one or more APC preparations comprise a FLT3L-stimulated APC. In some embodiments, at least three of the one or more APC preparations comprise a FLT3L-stimulated APC. In some embodiments, incubating comprises incubating a first APC preparation of the APC preparations to the T cells for more than 7 days. In some embodiments, 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 embodiments, an APC of the APC preparations is an autologous APC or an allogenic APC. In some embodiments, an APC of the APC preparations comprises a dendritic cell (DC). In some embodiments, the DC is a $CD141^+$ DC. In some embodiments, the method comprises depleting cells expressing CD14 and CD25 from the biological sample, thereby obtaining the population of immune cells from a biological sample depleted of cells expressing CD14 and CD25. In some embodiments, the method further comprises depleting cells expressing CD19. In some embodiments, the method further comprises depleting cells expressing CD11b. In some embodiments, depleting cells expressing CD14 and CD25 comprises binding a CD14 or CD25 binding agent to an APC of the one or more APC preparations. In some embodiments, the method further comprises administering one or more of the at least one antigen specific T cell to a subject.

[0233] In some embodiments, incubating comprises incubating a first APC preparation of the one or more APC preparations to the T cells for more than 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days. In some embodiments, the method comprises incubating at least one of the 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 embodiments, the method comprises incubating at least one of the 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 embodiments, the method further comprises removing the one or more cytokines or growth factors of the second medium after the third time period. In some embodiments, an APC of the APC preparations is stimulated with one or more cytokines or growth factors. In some embodiments, 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.

[0234] In some embodiments, the antigen is a neoantigen, a tumor associated antigen, a viral antigen, a minor histocompatibility antigen or a combination thereof.

[0235] In some embodiments, the method is performed *ex vivo*.

[0236] In some embodiments, wherein the method comprises incubating the population of immune cells from a biological sample depleted of cells expressing CD14 and CD25 with FLT3L for a first time period. In some embodiments, the method comprises incubating at least one peptide with the population of immune cells from a biological sample depleted of cells expressing CD14 and CD25 for a second time period, thereby obtaining a first matured APC peptide loaded sample. In some embodiments, the method comprises depleting cells expressing CD14, cells expressing CD19 and cells expressing CD25 from the population of immune cells. In some embodiments, the method comprises depleting cells expressing CD14, cells expressing CD11b and cells expressing CD25 from the population of immune cells. In some embodiments, the method comprises depleting cells expressing CD14, cells expressing CD11b, cells expressing CD19 and cells expressing CD25. In some embodiments, the method comprises depleting at least CD14, CD11b, CD19 and CD25. In some embodiments, the method comprises depleting cells expressing at least one of CD14, CD11b, CD19 and CD25, and at least a fifth cell type expressing a fifth cell surface marker. In some embodiments, the method comprises selectively depleting CD14 and CD25 expressing cells from the population of immune cells, and any one or more of CD19, CD11b expressing cells, from the population of immune cells, at a first incubation period, at a second incubation period, and/or at a third incubation period.

[0237] In some embodiments of the method described herein, 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.

[0238] In some embodiments of the method described herein, 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.

[0239] In some embodiments, 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. In some embodiments, the population of immune cells is from a biological sample from the subject. In some embodiments of the method described herein, the method further 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 FMS-like tyrosine kinase 3 receptor ligand (FLT3L), and a polypeptide comprising the at least one selected epitope sequences, or a polynucleotide encoding the polypeptide; thereby forming a population of cells comprising stimulated T cells. In some

embodiments, the method further comprises 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 the at least one selected epitope sequences and an MHC protein expressed by the cancer cells or APCs of the subject.

[0240] In some embodiments of the method described herein, 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. In some embodiments, the second population of mature APCs has 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. In some embodiments, the expanding further comprises contacting the expanded population of T cells with a third population of mature APCs, wherein the third population of mature APCs have been incubated with FLT3L and present the at least one selected epitope sequence; and 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. In some embodiments, 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. In some embodiments of the method described herein, 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. In some embodiments, the 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.

[0241] In some embodiments, 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. In some embodiments, the human subject with cancer is the human subject from which the biological sample was obtained. In some embodiments, the polypeptide is from 8 to 50 amino acids in length. In some embodiments, the polypeptide comprises at least two of the selected epitope sequence, each expressed by cancer cells of a human subject with cancer.

[0242] In some embodiments, 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. In some embodiments, depleting further comprising depleting CD19+ cells from the population of immune cells comprising a first population of APCs and T cells. In some

embodiments, the method further comprises contacting the population of immune cells with a CD19 binding agent. In some embodiments, depleting further comprising depleting CD11b+ cells from the population of immune cells comprising a first population of APCs and T cells. In some embodiments, the method further comprises contacting the population of immune cells with a CD11b binding agent.

[0243] In some embodiments, the method comprises incubating the first matured APC peptide loaded sample with at least one T cell for a third time period, thereby obtaining a stimulated T cell sample. In some embodiments, the method comprises incubating a T cell of a first stimulated T cell sample with a FLT3L-stimulated APC of a matured APC sample for a fourth time period, FLT3L and a second APC peptide loaded sample of a matured APC sample for a fourth time period or FLT3L and a FLT3L-stimulated APC of a matured APC sample for a fourth time period, thereby obtaining a stimulated T cell sample. In some embodiments, the method comprises incubating a T cell of a second stimulated T cell sample with a FLT3L-stimulated APC of a matured APC sample for a fifth time period, FLT3L and a third APC peptide loaded sample of a matured APC sample for a fifth time period, or FLT3L and a third APC peptide loaded sample of a matured APC sample for a fifth time period, thereby obtaining a stimulated T cell sample.

[0244] In some embodiments, the one or more separate time periods, the 3 or less separate time periods, the first time period, the second time period, the third time period, the fourth time period, or the fifth time period is at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, at least 24 hours, at least 25 hours, at least 26 hours, at least 27 hours, at least 28 hours, at least 29 hours, at least 30 hours, at least 31 hours, at least 32 hours, at least 33 hours, at least 34 hours, at least 35 hours, at least 36 hours, at least 37 hours, at least 38 hours, at least 39 hours, or at least 40 hours.

[0245] In some embodiments, the one or more separate time periods, the 3 or less separate time periods, the first time period, the second time period, the third time period, the fourth time period, or the fifth time period is from 1 to 4 hours, from 1 to 3 hours, from 1 to 2 hours, from 4 to 40 hours, from 7 to 40 hours, from 4 to 35 hours, from 4 to 32 hours, from 7 to 35 hours or from 7 to 32 hours.

[0246] In some embodiments, the population of immune cells comprises the APC or at least one of the one or more APC preparations. In some embodiments, the population of immune cells does not comprise the APC and/or the population of immune cells does not comprise one of the one or more APC preparations.

[0247] In some embodiments, the method comprises incubating FLT3L and at least one peptide with a population of immune cells from a biological sample, wherein the FLT3L is incubated with the population of immune cells for a first time period and wherein the at least one peptide is incubated

with the population of immune cells for a first peptide stimulation time period, thereby obtaining a first stimulated T cell sample, wherein the population of immune cells comprises at least one T cell and at least one APC. In some embodiments, the method comprises incubating FLT3L and at least one peptide with at least one APC, wherein the FLT3L is incubated with the at least one APC for a second time period and wherein the at least one peptide is incubated with the at least one APC for a second peptide stimulation time period, thereby obtaining a first matured APC peptide loaded sample; and incubating the first matured APC peptide loaded sample with the first stimulated T cell sample, thereby obtaining a second stimulated T cell sample. In some embodiments, the method comprises incubating FLT3L and at least one peptide with at least one APC, wherein the FLT3L is incubated with the at least one APC for a third time period and wherein the at least one peptide is incubated with the at least one APC for a third peptide stimulation time period, thereby obtaining a second matured APC peptide loaded sample; and incubating the second matured APC peptide loaded sample with the second stimulated T cell sample, thereby obtaining a third stimulated T cell sample.

[0248] In some embodiments, the method further comprises isolating the first stimulated T cell from the stimulated T cell sample. In some embodiments, isolating as described in the preceding sentence comprises enriching a stimulated T cell from a population of immune cells that have been contacted with the at least one APC incubated with the at least one peptide. In some embodiments, the enriching comprises determining expression of one or more cell markers of at least one the stimulated T cell and isolating the stimulated T cell expressing the one or more cell markers. In some embodiments the cell surface markers may be but not limited to one or more of 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 embodiments, the one or more cell markers comprise a cytokine.

[0249] In some embodiments, the method comprises administering at least one T cell of a first or a second or a third stimulated T cell sample to a subject in need thereof.

[0250] In some embodiments, the method comprises: 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 of 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 CD25 depleted sample comprising T cells for a fourth time period; incubating the T cells with APCs of a matured APC sample for a fifth time period; incubating the T cells with APCs of a matured APC sample for a sixth time period; and administering at least one T cell of the T cells to a subject in need thereof.

[0251] In some embodiments, the method comprises: obtaining a biological sample from a subject comprising at least one APC and at least one T cell; depleting cells expressing CD14 and CD25 from the biological sample, thereby obtaining a CD14 and CD25 cell depleted sample; incubating the CD14 and CD25 cell depleted sample with FLT3L for a first time period; incubating at least one peptide with the CD14 and CD25 cell depleted sample of for a second time period, thereby obtaining an APC peptide loaded sample; incubating the APC peptide loaded sample with the at least one T cell for a third time period, thereby obtaining a first stimulated T cell sample; incubating a T cell of the first stimulated T cell sample with an APC of a matured APC sample for a fourth time period, thereby obtaining a second stimulated T cell sample; optionally, incubating a T cell of the second stimulated T cell sample with an APC of a matured APC sample for a fifth time period, thereby obtaining a third stimulated T cell sample; administering at least one T cell of the first, the second or the third stimulated T cell sample to a subject in need thereof.

[0252] In some embodiments, the method comprises: obtaining a biological sample from a subject comprising at least one APC and at least one T cell; depleting cells expressing CD14 and CD25 from the biological sample, thereby obtaining a CD14 and CD25 cell depleted sample; incubating the CD14 and CD25 cell depleted sample with FLT3L for a first time period; incubating at least one peptide with the CD14 and CD25 cell depleted sample of for a second time period, thereby obtaining an APC peptide loaded sample; incubating the APC peptide loaded sample with the at least one T cell for a third time period, thereby obtaining a first stimulated T cell sample; optionally, incubating a T cell of the first stimulated T cell sample with a FLT3L-stimulated APC of a matured APC sample for a fourth time period, thereby obtaining a second stimulated T cell sample; optionally, incubating a T cell of the second stimulated T cell sample with a FLT3L-stimulated APC of a matured APC sample for a fifth time period, thereby obtaining a third stimulated T cell sample; administering at least one T cell of the first, the second or the third stimulated T cell sample to a subject in need thereof.

[0253] In some embodiments, the method comprises: obtaining a biological sample from a subject comprising at least one APC and at least one T cell ; depleting cells expressing CD14 and CD25 from the biological sample, thereby obtaining a CD14 and CD25 cell depleted sample; incubating the CD14 and CD25 cell depleted sample with FLT3L for a first time period; incubating at least one peptide with the CD14 and CD25 cell depleted sample of 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 T cell for a third time period, thereby obtaining a first stimulated T cell sample; optionally, incubating a T cell of the first stimulated T cell 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 T cell sample; optionally, incubating a T cell of the second stimulated T cell 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 T cell

sample; administering at least one T cell of the first, the second or the third stimulated T cell sample to a subject in need thereof.

[0254] In some embodiments, the method comprises: obtaining a biological sample from a subject comprising at least one APC and at least one T cell ; depleting cells expressing CD14 and CD25 from the biological sample, thereby obtaining a CD14 and CD25 cell depleted sample; incubating the CD14 and CD25 cell depleted sample with FLT3L for a first time period; incubating at least one peptide with the CD14 and CD25 cell depleted sample of 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 T cell for a third time period, thereby obtaining a first stimulated T cell sample; optionally, incubating a T cell of the first stimulated T cell sample with FLT3L and a FLT3L-stimulated APC of a matured APC sample for a fourth time period, thereby obtaining a second stimulated T cell sample; optionally, incubating a T cell of the second stimulated T cell sample with FLT3L and a FLT3L-stimulated APC of a matured APC sample for a fifth time period, thereby obtaining a third stimulated T cell sample; administering at least one T cell of the first, the second or the third stimulated T cell sample to a subject in need thereof.

[0255] In some embodiments, the method comprises: incubating FLT3L and at least one peptide with a population of immune cells from a biological sample, wherein the FLT3L is incubated with the population of immune cells for a first time period and wherein the at least one peptide is incubated with the population of immune cells for a first peptide stimulation time period, thereby obtaining a first stimulated T cell sample, wherein the population of immune cells comprises at least one T cell and at least one APC; optionally, incubating FLT3L and at least one peptide with at least one APC, wherein the FLT3L is incubated with the at least one APC for a second time period and wherein the at least one peptide is incubated with the at least one APC for a second peptide stimulation time period, thereby obtaining a first matured APC peptide loaded sample; and incubating the first matured APC peptide loaded sample with the first stimulated T cell sample, thereby obtaining a second stimulated T cell sample; optionally, incubating FLT3L and at least one peptide with at least one APC, wherein the FLT3L is incubated with the at least one APC for a third time period and wherein the at least one peptide is incubated with the at least one APC for a third peptide stimulation time period, thereby obtaining a second matured APC peptide loaded sample; and incubating the second matured APC peptide loaded sample with the second stimulated T cell sample, thereby obtaining a third stimulated T cell sample; and administering at least one T cell of the first stimulated T cell sample, the second stimulated T cell sample or the third stimulated T cell sample to a subject in need thereof.

[0256] In some embodiments, 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.

[0257] In some embodiments, the method comprises sequencing cancer cell nucleic acids by whole genome sequencing or whole exome sequencing, thereby obtaining a first plurality of nucleic acid sequences comprising cancer cell nucleic acid sequences; and sequencing non-cancer cell nucleic acids by whole genome sequencing or whole exome sequencing, thereby obtaining a second plurality of nucleic acid sequences comprising non-cancer cell nucleic acid sequences. In some embodiments, the method comprises identifying a plurality of cancer specific nucleic acid sequences from a first plurality of nucleic acid sequences that are unique to cancer cells of the subject and that do not include nucleic acid sequences from a second plurality of nucleic acid sequences from non-cancer cells of the subject.

[0258] In some embodiments, the method further comprises selecting one or more subpopulation of cells from the expanded population of T cells prior to administering to the subject. In some embodiments, the selecting one or more subpopulation is performed by cell sorting based on expression of one or more cell surface markers provided herein. In some embodiments, the activated T cells may be sorted based on cell surface markers including but not limited to any one or more of the following: CD27, CD274, CD276, CD8A, CMKLR1, CXCL9, CXCR6, HLA-DQA1, HLA-DRB1, HLA-E, IDO1, LAG3, NKG7, PDCD1LG2, PSMB10, STAT1, CD45RO, CCR7, FLT3LG, IL-6 and others.

[0259] In some embodiments, the method further comprises depleting one or more cells in the subject prior to administering the population of T cells.

[0260] In some embodiments, the one or more subpopulation of cells expressing a cell surface marker provided herein.

[0261] In some embodiments, the amino acid sequence of a peptide provided herein is validated by peptide sequencing. In some embodiments, the amino acid sequence a peptide provided herein is validated by mass spectrometry.

[0262] Also provided herein is a pharmaceutical composition comprising a T cell produced by expanding the T cell in the presence of an antigen presenting cell presenting one or more epitope sequence of any of Tables 1-8 and 11-14.

[0263] Also provided herein is 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 two or three or four 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 stimulates T cells to be cytotoxic according to a cytotoxicity assay. In some embodiments, the library comprises one or two or more peptide sequences comprising an epitope sequence of any of Tables 1-8 and 11-14.

[0264] The peptides and polynucleotides provided herein can be for preparing antigen-specific T cells and include recombinant peptides and polynucleotides and synthetic peptides comprising epitopes, such as a tumor-specific neoepitopes, that have been identified and validated as binding to one or more MHC molecules, presented by the one or more MHC molecules, being immunogenic and/or capable of activating T cells to become cytotoxic. The peptides can be prepared for use in a method to prime T cells ex vivo. The peptides can be prepared for use in a method to activate T cells ex vivo. The peptides can be prepared for use in a method to expand antigen-specific T cells. The peptides can be prepared for use in a method to induce de novo CD8 T cell responses ex vivo. The peptides can be prepared for use in a method to induce de novo CD4 T cell responses ex vivo. The peptides can be prepared for use in a method to stimulate memory CD8 T cell responses ex vivo. The peptides can be prepared for use in a method to stimulate memory CD4 T cell responses ex vivo. The T cells can be obtained from a human subject. The T cells can be allogeneic T cells. The T cells can be T cell lines.

[0265] The epitopes can comprise at least 8 contiguous amino acids of an amino acid sequence encoded by the genome of a cancer cell. The epitopes can comprise from 8-12 contiguous amino acids of an amino acid sequence encoded by the genome of a cancer cell. The epitopes can comprise from 13-25 contiguous amino acids of an amino acid sequence encoded by the genome of a cancer cell. The epitopes can comprise from 8-50 contiguous amino acids of an amino acid sequence encoded by the genome of a cancer cell. In some embodiments, an epitope is from about 8 and about 30 amino acids in length. In some embodiments, an epitope is from about 8 to about 25 amino acids in length. In some embodiments, an epitope is from about 15 to about 24 amino acids in length. In some embodiments, an epitope is from about 9 to about 15 amino acids in length. In some embodiments, an epitope is 8 amino acids in length. In some embodiments, an epitope is 9 amino acids in length. In some embodiments, an epitope is 10 amino acids in length.

[0266] In some embodiments, a peptide containing an epitope is at most 500, at most 250, at most 150, at most 125, or at most 100 amino acids in length In some embodiments, a peptide containing an epitope is at least 8, at least 50, at least 100, at least 200, or at least 300 amino acids in length. In some embodiments, a peptide containing an epitope is from about 8 to about 500 amino acids in length. In some embodiments, a peptide containing an epitope is from about 8 to about 100 amino acids in length. In some embodiments, a peptide containing an epitope is from about 8 to about 50 amino acids in length. In some embodiments, a peptide containing an epitope is from about 15 to about 35 amino acids in length. In some embodiments, a peptide containing an epitope is from about 8 and about 15 amino acids in length. In some embodiments, a peptide containing an epitope is from about 8 and about 11 amino acids in length. In some embodiments, a peptide containing an epitope is 9 or 10 amino acids in length. In some embodiments, a peptide containing an epitope is from about 8 and about 30 amino acids in length. In some embodiments, a peptide containing an epitope is from about 8 to about

25 amino acids in length. In some embodiments, a peptide containing an epitope is from about 15 to about 24 amino acids in length. In some embodiments, a peptide containing an epitope is from about 9 to about 15 amino acids in length.

[0267] In some embodiments, a peptide containing an epitope has a total length of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, or at least 500 amino acids. In some embodiments, a peptide containing an epitope has a total length of at most 8, at most 9, at most 10, at most 11, at most 12, at most 13, at most 14, at most 15, at most 16, at most 17, at most 18, at most 19, at most 20, at most 21, at most 22, at most 23, at most 24, at most 25, at most 26, at most 27, at most 28, at most 29, at most 30, at most 40, at most 50, at most 60, at most 70, at most 80, at most 90, at most 100, at most 150, at most 200, at most 250, at most 300, at most 350, at most 400, at most 450, or at most 500 amino acids. In some embodiments, a peptide containing an epitope comprises a first neoepitope peptide linked to at least a second neoepitope.

[0268] In some embodiments, a peptide contains a validated epitope from one or more of: ABL1, AC011997, ACVR2A, AFP, AKT1, ALK, ALPPL2, ANAPC1, APC, ARID1A, AR, AR-v7, ASCL2, β2M, BRAF, BTK, C15ORF40, CDH1, CLDN6, CNOT1, CT45A5, CTAG1B, DCT, DKK4, EEF1B2, EEF1DP3, EGFR, EIF2B3, env, EPHB2, ERBB3, ESR1, ESRP1, FAM111B, FGFR3, FRG1B, GAGE1, GAGE10, GATA3, GBP3, HER2, IDH1, JAK1, KIT, KRAS, LMAN1, MABEB16, MAGEA1, MAGEA10, MAGEA4, MAGEA8, MAGEB17, MAGEB4, MAGEC1, MEK, MLANA, MLL2, MMP13, MSH3, MSH6, MYC, NDUFC2, NRAS, PAGE2, PAGE5, PDGFRa, PIK3CA, PMEL, pol protein, POLE, PTEN, RAC1, RBM27, RNF43, RPL22, RUNX1, SEC31A, SEC63, SF3B1, SLC35F5, SLC45A2, SMAP1, SMAP1, SPOP, TFAM, TGFBR2, THAP5, TP53, TTK, TYR, UBR5, VHL, XPOT an EEF1DP3:FRY fusion polypeptide, an EGFR:SEPT14 fusion polypeptide, an EGFRVIII deletion polypeptide, an EML4:ALK fusion polypeptide, an NDRG1:ERG fusion polypeptide, an AC011997.1:LRRC69 fusion polypeptide, a RUNX1(ex5)-RUNX1T1fusion polypeptide, a TMPRSS2:ERG fusion polypeptide, a NAB:STAT6 fusion polypeptide, a NDRG1:ERG fusion polypeptide, a PML:RARA fusion polypeptide, a PPP1R1B:STARD3 fusion polypeptide, a MAD1L1:MAFK fusion polypeptide, a FGFR3:TAC fusion polypeptide, a FGFR3:TACC3 fusion polypeptide, a BCR:ABL fusion polypeptide, a C11orf95:RELA fusion polypeptide, a CBFB:MYH11 fusion polypeptide, a CBFB:MYH11 fusion polypeptide, a CD74:ROS1 fusion polypeptide, a CD74:ROS1 fusion polypeptide, ERVE-4: protease, ERVE-4: reverse transcriptase, ERVE-4: reverse transcriptase, ERVE-4: unknown, ERVH-2 matrix protein, ERVH-2: gag, ERVH-2: retroviral matrix, ERVH48-1: coat protein, ERVH48-1: syncytin, ERVI-1

envelope protein, ERVK-5 gag, ERVK-5 env, ERVK-5 pol, EBV A73, EBV BALF3, EBV BALF4, EBV BALF5, EBV BARF0, EBV LF2, EBV RPMS1, HPV-16, HPV-16 E7, and HPV-16 E6. In some embodiments, a neoepitope contains a mutation due to a mutational event in β2M, BTK, EGFR, GATA3, KRAS, MLL2, a TMPRSS2:ERG fusion polypeptide, or TP53 or Myc.

[0269] In some embodiments, an epitope binds a major histocompatibility complex (MHC) class I molecule. In some embodiments, an epitope binds an MHC class I molecule with a binding affinity of about 500 nM or less. In some embodiments an epitope binds an MHC class I molecule with a binding affinity of about 250 nM or less. In some embodiments, an epitope binds an MHC class I molecule with a binding affinity of about 150 nM or less. In some embodiments, an epitope binds an MHC class I molecule with a binding affinity of about 50 nM or less.

[0270] In some embodiments, an epitope binds an binds MHC class I molecule and a peptide containing the class I epitope binds to an MHC class II molecule.

[0271] In some embodiments, an epitope binds an MHC class II molecule. In some embodiments, an epitope binds to human leukocyte antigen (HLA) -A, -B, -C, -DP, -DQ, or -DR. In some embodiments, an epitope binds an MHC class II molecule with a binding affinity of 1000 nM or less. In some embodiments, an epitope binds MHC class II with a binding affinity of 500 nM or less. In some embodiments an epitope binds an MHC class II molecule with a binding affinity of about 250 nM or less. In some embodiments, an epitope binds an MHC class II molecule with a binding affinity of about 150 nM or less. In some embodiments, an epitope binds an MHC class II molecule with a binding affinity of about 50 nM or less.

[0272] In some embodiments, a peptide containing a validated epitope further comprises one or more amino acids flanking the C-terminus of the epitope. In some embodiments, a peptide containing a validated epitope further comprises one or more amino acids flanking the N-terminus of the epitope. In some embodiments, a peptide containing a validated epitope further comprises one or more amino acids flanking the C-terminus of the epitope and one or more amino acids flanking the N-terminus of the epitope. In some embodiments, the flanking amino acids are not native flanking amino acids. In some embodiments, a first epitope used in a method described herein binds an MHC class I molecule and a second epitope binds an MHC class II molecule. In some embodiments, a peptide containing a validated epitope further comprises a modification which increases in vivo half-life of the peptide. In some embodiments, a peptide containing a validated epitope further comprises a modification which increases cellular targeting by the peptide. In some embodiments, a peptide containing a validated epitope further comprises a modification which increases cellular uptake of the peptide. In some embodiments, a peptide containing a validated epitope further comprises a modification which increases peptide processing. In some embodiments, a peptide containing a validated epitope further comprises a modification which increases MHC affinity of the epitope. In some embodiments, a peptide containing a validated epitope further comprises a modification which increases MHC stability

of the epitope. In some embodiments, a peptide containing a validated epitope further comprises a modification which increases presentation of the epitope by an MHC class I molecule, and/or an MHC class II molecule.

[0273] In some embodiments, sequencing methods are used to identify tumor specific mutations. Any suitable sequencing method can be used according to the invention, for example, Next Generation Sequencing (NGS) technologies. Third Generation Sequencing methods might substitute for the NGS technology in the future to speed up the sequencing step of the method. For clarification purposes: the terms "Next Generation Sequencing" or "NGS" in the context of the present invention mean all novel high throughput sequencing technologies which, in contrast to the "conventional" sequencing methodology known as Sanger chemistry, read nucleic acid templates randomly in parallel along the entire genome by breaking the entire genome into small pieces. Such NGS technologies (also known as massively parallel sequencing technologies) are able to deliver nucleic acid sequence information of a whole genome, exome, transcriptome (all transcribed sequences of a genome) or methylome (all methylated sequences of a genome) in very short time periods, e.g. within 1-2 weeks, for example, within 1-7 days or within less than 24 hours and allow, in principle, single cell sequencing approaches. Multiple NGS platforms which are commercially available or which are mentioned in the literature can be used in the context of the invention e.g. those described in detail in WO 2012/159643.

[0274] In some embodiments, a peptide containing a validated epitope is linked to the at least second peptide, such as by a poly-glycine or poly-serine linker. In some embodiments, the modification is conjugation to a carrier protein, conjugation to a ligand, conjugation to an antibody, PEGylation, polysialylation HESylation, recombinant PEG mimetics, Fc fusion, albumin fusion, nanoparticle attachment, nanoparticulate encapsulation, cholesterol fusion, iron fusion, acylation, amidation, glycosylation, side chain oxidation, phosphorylation, biotinylation, the addition of a surface active material, the addition of amino acid mimetics, or the addition of unnatural amino acids. In some embodiments, a peptide containing a validated epitope further comprises a modification which increases cellular targeting to specific organs, tissues, or cell types. In some embodiments, a peptide containing a validated epitope comprises an antigen presenting cell targeting moiety or marker. In some embodiments, the antigen presenting cells are dendritic cells. In some embodiments, the dendritic cells are targeted using DEC205, XCR1, CD197, CD80, CD86, CD123, CD209, CD273, CD283, CD289, CD184, CD85h, CD85j, CD85k, CD85d, CD85g, CD85a, CD141, CD11c, CD83, TSLP receptor, Clec9a, or CD1a marker. In some embodiments, the dendritic cells are targeted using the CD141, DEC205, Clec9a, or XCR1 marker. In some embodiments, the dendritic cells are autologous cells. In some embodiments, one or more of the dendritic cells are bound to a T cell.

[0275] In some embodiments, the method described herein comprises large scale manufacture of and storage of HLA-matched peptides corresponding to shared antigens for treatment of a cancer or a tumor.

[0276] In some embodiments, the method described herein comprises treatment methods, comprising administering to a subject with cancer antigen-specific T cell that are specific to a validated epitope selected from the HLA matched peptide repertoire presented in any of Tables 1-8 and 11-14. In some embodiments, epitope-specific T cells are administered to the patient by infusion. In some embodiments, the T cells are administered to the patient by direct intravenous injection. In some embodiments, the T cell is an autologous T cell. In some embodiments, the T cell is an allogeneic T cell.

[0277] The methods of the disclosure can be used to treat any type of cancer known in the art. In some embodiments, a method of treating cancer comprises treating breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lung cancer, metastatic melanoma, thymoma, lymphoma, sarcoma, mesothelioma, renal cell carcinoma, stomach cancer, gastric cancer, ovarian cancer, NHL, leukemia, uterine cancer, colon cancer, bladder cancer, kidney cancer or endometrial cancer. In some embodiments, the cancer is selected from the group consisting of carcinoma, lymphoma, blastoma, sarcoma, leukemia, squamous cell cancer, lung cancer (including small cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, melanoma, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, head and neck cancer, colorectal cancer, rectal cancer, soft-tissue sarcoma, Kaposi's sarcoma, B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL), small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, mantle cell lymphoma, AIDS-related lymphoma, and Waldenstrom's macroglobulinemia), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), myeloma, Hairy cell leukemia, chronic myeloblasts leukemia, and post-transplant lymphoproliferative disorder (PTLD), abnormal vascular proliferation associated with phakomatoses, edema, Meigs' syndrome. Non-limiting examples of cancers to be treated by the methods of the present disclosure can include melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g., clear cell carcinoma), prostate cancer (e.g., hormone refractory prostate adenocarcinoma), pancreatic adenocarcinoma, breast cancer, colon cancer, lung cancer (e.g., non-small cell lung cancer), esophageal cancer, squamous cell carcinoma of the head and neck, liver cancer, ovarian cancer, cervical cancer, thyroid cancer, glioblastoma, glioma, leukemia, lymphoma, and other neoplastic malignancies. In some embodiments, a cancer to be treated by the methods of treatment of the present disclosure is selected from the group consisting of carcinoma, squamous carcinoma, adenocarcinoma, sarcomata,

endometrial cancer, breast cancer, ovarian cancer, cervical cancer, fallopian tube cancer, primary peritoneal cancer, colon cancer, colorectal cancer, squamous cell carcinoma of the anogenital region, melanoma, renal cell carcinoma, lung cancer, non-small cell lung cancer, squamous cell carcinoma of the lung, stomach cancer, bladder cancer, gall bladder cancer, liver cancer, thyroid cancer, laryngeal cancer, salivary gland cancer, esophageal cancer, head and neck cancer, glioblastoma, glioma, squamous cell carcinoma of the head and neck, prostate cancer, pancreatic cancer, mesothelioma, sarcoma, hematological cancer, leukemia, lymphoma, neuroma, and combinations thereof. In some embodiments, a cancer to be treated by the methods of the present disclosure include, for example, carcinoma, squamous carcinoma (for example, cervical canal, eyelid, tunica conjunctiva, vagina, lung, oral cavity, skin, urinary bladder, tongue, larynx, and gullet), and adenocarcinoma (for example, prostate, small intestine, endometrium, cervical canal, large intestine, lung, pancreas, gullet, rectum, uterus, stomach, mammary gland, and ovary). In some embodiments, a cancer to be treated by the methods of the present disclosure further include sarcomata (for example, myogenic sarcoma), leukosis, neuroma, melanoma, and lymphoma. In some embodiments, a cancer to be treated by the methods of the present disclosure is breast cancer. In some embodiments, a cancer to be treated by the methods of treatment of the present disclosure is triple negative breast cancer (TNBC). In some embodiments, a cancer to be treated by the methods of treatment of the present disclosure is prostate cancer. In some embodiments, a cancer to be treated by the methods of treatment of the present disclosure is colorectal cancer. In some embodiments, a patient or population of patients to be treated with a pharmaceutical composition of the present disclosure have a solid tumor. In some embodiments, a solid tumor is a melanoma, renal cell carcinoma, lung cancer, bladder cancer, breast cancer, cervical cancer, colon cancer, gall bladder cancer, laryngeal cancer, liver cancer, thyroid cancer, stomach cancer, salivary gland cancer, prostate cancer, pancreatic cancer, or Merkel cell carcinoma. In some embodiments, a patient or population of patients to be treated with a pharmaceutical composition of the present disclosure have a hematological cancer. In some embodiments, the patient has a hematological cancer such as diffuse large B cell lymphoma ("DLBCL"), Hodgkin's lymphoma ("HL"), Non-Hodgkin's lymphoma ("NHL"), Follicular lymphoma ("FL"), acute myeloid leukemia ("AML"), or Multiple myeloma ("MM"). In some embodiments, a patient or population of patients to be treated having the cancer selected from the group consisting of ovarian cancer, lung cancer and melanoma.

[0278] The pharmaceutical compositions provided herein may be used alone or in combination with conventional therapeutic regimens such as surgery, irradiation, chemotherapy and/or bone marrow transplantation (autologous, syngeneic, allogeneic or unrelated). In some embodiments, at least one or more chemotherapeutic agents may be administered in addition to the pharmaceutical composition comprising an immunogenic therapy. In some embodiments, the one or more chemotherapeutic agents may belong to different classes of chemotherapeutic agents. In practicing the methods of treatment or

use provided herein, therapeutically-effective amounts of the pharmaceutical compositions can be administered to a subject having a disease or condition. A therapeutically-effective amount can vary widely depending on the severity of the disease, the age and relative health of the subject, the potency of the compounds used, and other factors.

[0279] In some embodiments, the methods for treatment include one or more rounds of leukapheresis prior to transplantation of T cells. The leukapheresis may include collection of peripheral blood mononuclear cells (PBMCs). Leukapheresis may include mobilizing the PBMCs prior to collection. Alternatively, non-mobilized PBMCs may be collected. A large volume of PBMCs may be collected from the subject in one round. Alternatively, the subject may undergo two or more rounds of leukapheresis. The volume of apheresis may be dependent on the number of cells required for transplant. For instance, 12-15 liters of non-mobilized PBMCs may be collected from a subject in one round. The number of PBMCs to be collected from a subject may be between $1x10^8$ to $5x10^{10}$ cells. The number of PBMCs to be collected from a subject may be $1x10^8$, $5x10^8$, $1x10^9$, $5x10^9$, $1x10^{10}$ or $5x10^{10}$ cells. The minimum number of PBMCs to be collected from a subject may be $1x10^6$/kg of the subject's weight. The minimum number of PBMCs to be collected from a subject may be $1x10^6$/kg, $5x10^6$/kg, $1x10^7$/kg, $5x10^7$/kg, $1x10^8$/kg, $5x10^8$/kg of the subject's weight.

[0280] A single infusion may comprise a dose between $1x10^6$ cells per square meter body surface of the subject (cells/m^2) and $5x10^9$ cells/m^2. A single infusion may comprise between about $2.5x10^6$ to about $5x10^9$ cells/m^2. A single infusion may comprise between at least about $2.5x10^6$ cells/m^2. A single infusion may comprise between at most $5x10^9$ cells/m^2. A single infusion may comprise between $1x10^6$ to $2.5x10^6$, $1x10^6$ to $5x10^6$, $1x10^6$ to $7.5x10^6$, $1x10^6$ to $1x10^7$, $1x10^6$ to $5x10^7$, $1x10^6$ to $7.5x10^7$, $1x10^6$ to $1x10^8$, $1x10^6$ to $2.5x10^8$, $1x10^6$ to $5x10^8$, $1x10^6$ to $1x10^9$, $1x10^6$ to $5x10^9$, $2.5x10^6$ to $5x10^6$, $2.5x10^6$ to $7.5x10^6$, $2.5x10^6$ to $1x10^7$, $2.5x10^6$ to $5x10^7$, $2.5x10^6$ to $7.5x10^7$, $2.5x10^6$ to $1x10^8$, $2.5x10^6$ to $2.5x10^8$, $2.5x10^6$ to $5x10^8$, $2.5x10^6$ to $1x10^9$, $2.5x10^6$ to $5x10^9$, $5x10^6$ to $7.5x10^6$, $5x10^6$ to $1x10^7$, $5x10^6$ to $5x10^7$, $5x10^6$ to $7.5x10^7$, $5x10^6$ to $1x10^8$, $5x10^6$ to $2.5x10^8$, $5x10^6$ to $5x10^8$, $5x10^6$ to $1x10^9$, $5x10^6$ to $5x10^9$, $7.5x10^6$ to $1x10^7$, $7.5x10^6$ to $5x10^7$, $7.5x10^6$ to $7.5x10^7$, $7.5x10^6$ to $1x10^8$, $7.5x10^6$ to $2.5x10^8$, $7.5x10^6$ to $5x10^8$, $7.5x10^6$ to $1x10^9$, $7.5x10^6$ to $5x10^9$, $1x10^7$ to $5x10^7$, $1x10^7$ to $7.5x10^7$, $1x10^7$ to $1x10^8$, $1x10^7$ to $2.5x10^8$, $1x10^7$ to $5x10^8$, $1x10^7$ to $1x10^9$, $1x10^7$ to $5x10^9$, $5x10^7$ to $7.5x10^7$, $5x10^7$ to $1x10^8$, $5x10^7$ to $2.5x10^8$, $5x10^7$ to $5x10^8$, $5x10^7$ to $1x10^9$, $5x10^7$ to $5x10^9$, $7.5x10^7$ to $1x10^8$, $7.5x10^7$ to $2.5x10^8$, $7.5x10^7$ to $5x10^8$, $7.5x10^7$ to $1x10^9$, $7.5x10^7$ to $5x10^9$, $1x10^8$ to $2.5x10^8$, $1x10^8$ to $5x10^8$, $1x10^8$ to $1x10^9$, $1x10^8$ to $5x10^9$, $2.5x10^8$ to $5x10^8$, $2.5x10^8$ to $1x10^9$, $2.5x10^8$ to $5x10^9$, $5x10^8$ to $1x10^9$, $5x10^8$ to $5x10^9$, or $1x10^9$ to $5x10^9$ cells/m^2. A single infusion may comprise between $1x10^6$ cells/m^2, $2.5x10^6$ cells/m^2, $5x10^6$ cells/m^2, $7.5x10^6$ cells/m^2, $1x10^7$ cells/m^2, $5x10^7$ cells/m^2, $7.5x10^7$ cells/m^2, $1x10^8$ cells/m^2, $2.5x10^8$ cells/m^2, $5x10^8$ cells/m^2, $1x10^9$ cells/m^2, or $5x10^9$ cells/m^2.

[0281] The methods may include administering chemotherapy to a subject including lymphodepleting chemotherapy using high doses of myeloablative agents. In some embodiments, the

methods include administering a preconditioning agent, such as a lymphodepleting or chemotherapeutic agent, such as cyclophosphamide, fludarabine, or combinations thereof, to a subject prior to the first or subsequent dose. For example, the subject may be administered a preconditioning agent at least 2 days prior, such as at least 3, 4, 5, 6, 7, 8, 9 or 10 days prior, to the first or subsequent dose. In some embodiments, the subject is administered a preconditioning agent no more than 10 days prior, such as no more than 9, 8, 7, 6, 5, 4, 3, or 2 days prior, to the first or subsequent dose.

[0282] In some embodiments, where the lymphodepleting agent comprises cyclophosphamide, the subject is administered between 0.3 grams per square meter of the body surface of the subject (g/m^2) and 5 g/m^2 cyclophosphamide. In some cases, the amount of cyclophosphamide administered to a subject is about at least 0.3 g/m^2. In some cases, the amount of cyclophosphamide administered to a subject is about at most 5 g/m^2. In some cases, the amount of cyclophosphamide administered to a subject is about 0.3 g/m^2 to 0.4 g/m^2, 0.3 g/m^2 to 0.5 g/m^2, 0.3 g/m^2 to 0.6 g/m^2, 0.3 g/m^2 to 0.7 g/m^2, 0.3 g/m^2 to 0.8 g/m^2, 0.3 g/m^2 to 0.9 g/m^2, 0.3 g/m^2 to 1 g/m^2, 0.3 g/m^2 to 2 g/m^2, 0.3 g/m^2 to 3 g/m^2, 0.3 g/m^2 to 4 g/m^2, 0.3 g/m^2 to 5 g/m^2, 0.4 g/m^2 to 0.5 g/m^2, 0.4 g/m^2 to 0.6 g/m^2, 0.4 g/m^2 to 0.7 g/m^2, 0.4 g/m^2 to 0.8 g/m^2, 0.4 g/m^2 to 0.9 g/m^2, 0.4 g/m^2 to 1 g/m^2, 0.4 g/m^2 to 2 g/m^2, 0.4 g/m^2 to 3 g/m^2, 0.4 g/m^2 to 4 g/m^2, 0.4 g/m^2 to 5 g/m^2, 0.5 g/m^2 to 0.6 g/m^2, 0.5 g/m^2 to 0.7 g/m^2, 0.5 g/m^2 to 0.8 g/m^2, 0.5 g/m^2 to 0.9 g/m^2, 0.5 g/m^2 to 1 g/m^2, 0.5 g/m^2 to 2 g/m^2, 0.5 g/m^2 to 3 g/m^2, 0.5 g/m^2 to 4 g/m^2, 0.5 g/m^2 to 5 g/m^2, 0.6 g/m^2 to 0.7 g/m^2, 0.6 g/m^2 to 0.8 g/m^2, 0.6 g/m^2 to 0.9 g/m^2, 0.6 g/m^2 to 1 g/m^2, 0.6 g/m^2 to 2 g/m^2, 0.6 g/m^2 to 3 g/m^2, 0.6 g/m^2 to 4 g/m^2, 0.6 g/m^2 to 5 g/m^2, 0.7 g/m^2 to 0.8 g/m^2, 0.7 g/m^2 to 0.9 g/m^2, 0.7 g/m^2 to 1 g/m^2, 0.7 g/m^2 to 2 g/m^2, 0.7 g/m^2 to 3 g/m^2, 0.7 g/m^2 to 4 g/m^2, 0.7 g/m^2 to 5 g/m^2, 0.8 g/m^2 to 0.9 g/m^2, 0.8 g/m^2 to 1 g/m^2, 0.8 g/m^2 to 2 g/m^2, 0.8 g/m^2 to 3 g/m^2, 0.8 g/m^2 to 4 g/m^2, 0.8 g/m^2 to 5 g/m^2, 0.9 g/m^2 to 1 g/m^2, 0.9 g/m^2 to 2 g/m^2, 0.9 g/m^2 to 3 g/m^2, 0.9 g/m^2 to 4 g/m^2, 0.9 g/m^2 to 5 g/m^2, 1 g/m^2 to 2 g/m^2, 1 g/m^2 to 3 g/m^2, 1 g/m^2 to 4 g/m^2, 1 g/m^2 to 5 g/m^2, 2 g/m^2 to 3 g/m^2, 2 g/m^2 to 4 g/m^2, 2 g/m^2 to 5 g/m^2, 3 g/m^2 to 4 g/m^2, 3 g/m^2 to 5 g/m^2, or 4 g/m^2 to 5 g/m^2. In some cases, the amount of cyclophosphamide administered to a subject is about 0.3 g/m^2, 0.4 g/m^2, 0.5 g/m^2, 0.6 g/m^2, 0.7 g/m^2, 0.8 g/m^2, 0.9 g/m^2, 1 g/m^2, 2 g/m^2, 3 g/m^2, 4 g/m^2, or 5 g/m^2. In some embodiments, the subject is preconditioned with cyclophosphamide at a dose between or between about 20 mg/kg and 100 mg/kg, such as between or between about 40 mg/kg and 80 mg/kg. In some aspects, the subject is preconditioned with or with about 60 mg/kg of cyclophosphamide.

[0283] In some embodiments, where the lymphodepleting agent comprises fludarabine, the subject is administered fludarabine at a dose between or between about 1 milligrams per square meter of the body surface of the subject (mg/m^2) and 100 mg/m^2. In some cases, the amount of fludarabine administered to a subject is about at least 1 mg/m^2. In some cases, the amount of fludarabine administered to a subject is about at most 100 mg/m^2. In some cases, the amount of fludarabine administered to a subject is about 1 mg/m^2 to 5 mg/m^2, 1 mg/m^2 to 10 mg/m^2, 1 mg/m^2 to 15 mg/m^2, 1 mg/m^2 to 20 mg/m^2, 1 mg/m^2 to 30 mg/m^2, 1 mg/m^2 to 40 mg/m^2, 1 mg/m^2 to 50 mg/m^2, 1 mg/m^2 to

70 mg/m^2, 1 mg/m^2 to 90 mg/m^2, 1 mg/m^2 to 100 mg/m^2, 5 mg/m^2 to 10 mg/m^2, 5 mg/m^2 to 15 mg/m^2, 5 mg/m^2 to 20 mg/m^2, 5 mg/m^2 to 30 mg/m^2, 5 mg/m^2 to 40 mg/m^2, 5 mg/m^2 to 50 mg/m^2, 5 mg/m^2 to 70 mg/m^2, 5 mg/m^2 to 90 mg/m^2, 5 mg/m^2 to 100 mg/m^2, 10 mg/m^2 to 15 mg/m^2, 10 mg/m^2 to 20 mg/m^2, 10 mg/m^2 to 30 mg/m^2, 10 mg/m^2 to 40 mg/m^2, 10 mg/m^2 to 50 mg/m^2, 10 mg/m^2 to 70 mg/m^2, 10 mg/m^2 to 90 mg/m^2, 10 mg/m^2 to 100 mg/m^2, 15 mg/m^2 to 20 mg/m^2, 15 mg/m^2 to 30 mg/m^2, 15 mg/m^2 to 40 mg/m^2, 15 mg/m^2 to 50 mg/m^2, 15 mg/m^2 to 70 mg/m^2, 15 mg/m^2 to 90 mg/m^2, 15 mg/m^2 to 100 mg/m^2, 20 mg/m^2 to 30 mg/m^2, 20 mg/m^2 to 40 mg/m^2, 20 mg/m^2 to 50 mg/m^2, 20 mg/m^2 to 70 mg/m^2, 20 mg/m^2 to 90 mg/m^2, 20 mg/m^2 to 100 mg/m^2, 30 mg/m^2 to 40 mg/m^2, 30 mg/m^2 to 50 mg/m^2, 30 mg/m^2 to 70 mg/m^2, 30 mg/m^2 to 90 mg/m^2, 30 mg/m^2 to 100 mg/m^2, 40 mg/m^2 to 50 mg/m^2, 40 mg/m^2 to 70 mg/m^2, 40 mg/m^2 to 90 mg/m^2, 40 mg/m^2 to 100 mg/m^2, 50 mg/m^2 to 70 mg/m^2, 50 mg/m^2 to 90 mg/m^2, 50 mg/m^2 to 100 mg/m^2, 70 mg/m^2 to 90 mg/m^2, 70 mg/m^2 to 100 mg/m^2, or 90 mg/m^2 to 100 mg/m^2. In some cases, the amount of fludarabine administered to a subject is about 1 mg/m^2, 5 mg/m^2, 10 mg/m^2, 15 mg/m^2, 20 mg/m^2, 30 mg/m^2, 40 mg/m^2, 50 mg/m^2, 70 mg/m^2, 90 mg/m^2, or 100 mg/m^2. In some embodiments, the fludarabine can be administered in a single dose or can be administered in a plurality of doses, such as given daily, every other day or every three days. For example, in some instances, the agent, e.g., fludarabine, is administered between or between about 1 and 5 times, such as between or between about 3 and 5 times. In some embodiments, such plurality of doses is administered in the same day, such as 1 to 5 times or 3 to 5 times daily.

[0284] In some embodiments, the lymphodepleting agent comprises a combination of agents, such as a combination of cyclophosphamide and fludarabine. Thus, the combination of agents may include cyclophosphamide at any dose or administration schedule, such as those described above, and fludarabine at any dose or administration schedule, such as those described above. For example, in some aspects, the subject is administered 400 mg/m^2 of cyclophosphamide and one or more doses of 20 mg/m^2 fludarabine prior to the first or subsequent dose of T cells. In some examples, the subject is administered 500 mg/m^2 of cyclophosphamide and one or more doses of 25 mg/m^2 fludarabine prior to the first or subsequent dose of T cells. In some examples, the subject is administered 600 mg/m^2 of cyclophosphamide and one or more doses of 30 mg/m^2 fludarabine prior to the first or subsequent dose of T cells. In some examples, the subject is administered 700 mg/m^2 of cyclophosphamide and one or more doses of 35 mg/m^2 fludarabine prior to the first or subsequent dose of T cells. In some examples, the subject is administered 700 mg/m^2 of cyclophosphamide and one or more doses of 40 mg/m^2 fludarabine prior to the first or subsequent dose of T cells. In some examples, the subject is administered 800 mg/m^2 of cyclophosphamide and one or more doses of 45 mg/m^2 fludarabine prior to the first or subsequent dose of T cells.

[0285] Fludarabine and cyclophosphamide may be administered on alternative days. In some cases, fludarabine and cyclophosphamide may be administered concurrently. In some cases, an initial dose of fludarabine is followed by a dose of cyclophosphamide. In some cases, an initial dose of

cyclophosphamide may be followed by an initial dose of fludarabine. In some examples, a treatment regimen may include treatment of a subject with an initial dose of fludarabine 10 days prior to the transplant, followed by treatment with an initial dose of cyclophosphamide administered 9 days prior to the cell transplant, concurrently with a second dose of fludarabine. In some examples, a treatment regimen may include treatment of a subject with an initial dose of fludarabine 8 days prior to the transplant, followed by treatment with an initial dose of cyclophosphamide administered 7 days prior to the transplant concurrently with a second dose of fludarabine.

[0286] In some embodiments, a peptide comprises an epitope sequence according to any one of Tables 1-8 and 11-14. In some embodiments, a peptide comprises an epitope sequence according to Table 1. In some embodiments, a peptide comprises an epitope sequence according to Table 2. In some embodiments, a peptide comprises an epitope sequence according to Table 3. In some embodiments, a peptide comprises an epitope sequence according to Table 4A. In some embodiments, a peptide comprises an epitope sequence according to Table 4B. In some embodiments, a peptide comprises an epitope sequence according to Table 4C. In some embodiments, a peptide comprises an epitope sequence according to Table 4D. In some embodiments, a peptide comprises an epitope sequence according to Table 4E. In some embodiments, a peptide comprises an epitope sequence according to Table 4F. In some embodiments, a peptide comprises an epitope sequence according to Table 4G. In some embodiments, a peptide comprises an epitope sequence according to Table 4H. In some embodiments, a peptide comprises an epitope sequence according to Table 4I. In some embodiments, a peptide comprises an epitope sequence according to Table 4J. In some embodiments, a peptide comprises an epitope sequence according to Table 4K. In some embodiments, a peptide comprises an epitope sequence according to Table 4L. In some embodiments, a peptide comprises an epitope sequence according to Table 4M. In some embodiments, a peptide comprises an epitope sequence according to Table 5. In some embodiments, a peptide comprises an epitope sequence according to Table 6. In some embodiments, a peptide comprises an epitope sequence according to Table 7. In some embodiments, a peptide comprises an epitope sequence according to Table 8. In some embodiments, a peptide comprises an epitope sequence according to Table 11. In some embodiments, a peptide comprises an epitope sequence according to Table 12. In some embodiments, a peptide comprises an epitope sequence according to Table 13. In some embodiments, a peptide comprises an epitope sequence according to Table 14.

Table 1

Gene	Amino Acid Alteration	Mutation Sequence Context	Peptides (Binding HLA allele example(s))	Exemplary Diseases
TABLE 1A		**POINT MUTATION**		

KRAS	G12C	MTEYKLVVVGACGVGKSA LTIQLIQNHFVDEYDPTIEDS YRKQVVIDGETCLLDILDT AGQE	KLVVVGACGV (A02.01) LVVVGACGV (A02.01) VVGACGVGK (A03.01, A11.01) VVVGACGVGK (A03.01)	BRCA, CESC, CRC, HNSC, LUAD, PAAD, UCEC
KRAS	G12D	MTEYKLVVVGADGVGKSA LTIQLIQNHFVDEYDPTIEDS YRKQVVIDGETCLLDILDT AGQE	VVGADGVGK (A11.01) VVVGADGVGK (A11.01) KLVVVGADGV (A02.01) LVVVGADGV (A02.01)	BLCA, BRCA, CESC, CRC, GBM, HNSC, KIRP, LIHC, LUAD, PAAD, SKCM, UCEC
KRAS	G12V	MTEYKLVVVGAVGVGKSA LTIQLIQNHFVDEYDPTIEDS YRKQVVIDGETCLLDILDT AGQE	KLVVVGAVGV (A02.01) LVVVGAVGV (A02.01) VVGAVGVGK (A03.01, A11.01) VVVGAVGVGK (A03.01, A11.01)	BRCA, CESC, CRC, LUAD, PAAD, THCA, UCEC
KRAS	Q61H	AGGVGKSALTIQLIQNHFV DEYDPTIEDSYRKQVVIDGE TCLLDILDTAGHEEYSAMR DQYMRTGEGFLCVFAINNT KSFEDIHHYREQIKRVKDSE DVPM	ILDTAGHEEY (A01.01)	CRC, LUSC, PAAD, SKCM, UCEC
KRAS	Q61L	AGGVGKSALTIQLIQNHFV DEYDPTIEDSYRKQVVIDGE TCLLDILDTAGLEEYSAMR DQYMRTGEGFLCVFAINNT KSFEDIHHYREQIKRVKDSE DVPM	ILDTAGLEEY (A01.01) LLDILDTAGL (A02.01)	CRC, GBM, HNSC, LUAD, SKCM, UCEC
NRAS	Q61K	AGGVGKSALTIQLIQNHFV DEYDPTIEDSYRKQVVIDGE TCLLDILDTAGKEEYSAMR DQYMRTGEGFLCVFAINNS KSFADINLYREQIKRVKDSD DVPM	ILDTAGKEEY (A01.01)	BLCA, CRC, LIHC, LUAD, LUSC, SKCM, THCA, UCEC
NRAS	Q61R	AGGVGKSALTIQLIQNHFV DEYDPTIEDSYRKQVVIDGE TCLLDILDTAGREEYSAMR DQYMRTGEGFLCVFAINNS KSFADINLYREQIKRVKDSD DVPM	ILDTAGREEY (A01.01)	BLCA, CRC, LUSC, PAAD, PRAD, SKCM, THCA, UCEC
BTK	C481S	MIKEGSMSEDEFIEEAKVM MNLSHEKLVQLYGVCTKQ RPIFIITEYMANGSLLNYLR EMRHRFQTQQLLEMCKDV CEAMEYLESKQFLHRDLA ARNCLVND	EYMANGSLL (A24.02) MANGSLLNY (A01.01, A03.01, A11.01) MANGSLLNYL (A02.01, B07.02, B08.01) SLLNYLREM (A02.01, B07.02, B08.01) YMANGSLLN (A02.01) YMANGSLLNY (A01.01, A03.01, A11.01)	CLL

EGFR	S492R	SLNITSLGLRSLKEISDGDVI ISGNKNLCYANTINWKKLF GTSGQKTKIIRNRGENSCK ATGQVCHALCSPEGCWGP EPRDCVSCRNVSRGRECVD KCNLL	IIRNRGENSCK (A03.01)	CRC
EGFR	T790M	IPVAIKELREATSPKANKEI LDEAYVMASVDNPHVCRL LGICLTSTVQLIMQLMPFGC LLDYVREHKDNIGSQYLLN WCVQIAKGMNYLEDRRLV HRDLAA	CLTSTVQLIM (A01.01, A02.01) IMQLMPFGC (A02.01) IMQLMPFGCL (A02.01, A24.02, B08.01) LIMQLMPFG (A02.01) LIMQLMPFGC (A02.01) LTSTVQLIM (A01.01) MQLMPFGCL (A02.01, B07.02, B08.01) MQLMPFGCLL (A02.01, A24.02, B08.01) QLIMQLMPF (A02.01, A24.02, B08.01) QLIMQLMPFG (A02.01) STVQLIMQL (A02.01) VQLIMQLMPF (A02.01, A24.02, B08.01)	NSCLC, PRAD
ABL1	E255K	VADGLITTLHYPAPKRNKP TVYGVSPNYDKWEMERTD ITMKHKLGGGQYGKVYEG VWKKYSLTVAVKTLKEDT MEVEEFLKEAAVMKEIKHP NLVQLLGVC	GQYGKVYEG (A02.01) GQYGKVYEGV (A02.01) KLGGGQYGK (A03.01) KLGGGQYGKV (A02.01) KVYEGVWKK (A02.01, A03.01) KVYEGVWKKY (A03.01) QYGKVYEGV (A24.02) QYGKVYEGVW (A24.02)	Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), Gastrointestinal stromal tumors (GIST)
ABL1	E255V	VADGLITTLHYPAPKRNKP TVYGVSPNYDKWEMERTD ITMKHKLGGGQYGVVYEG VWKKYSLTVAVKTLKEDT MEVEEFLKEAAVMKEIKHP NLVQLLGVC	GQYGVVYEG (A02.01) GQYGVVYEGV (A02.01) KLGGGQYGV (A02.01) KLGGGQYGVV (A02.01) QYGVVYEGV (A24.02) QYGVVYEGVW (A24.02) VVYEGVWKK (A02.01, A03.01) VVYEGVWKKY (A03.01)	Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), Gastrointestinal stromal tumors (GIST)
ABL1	M351T	LLGVCTREPPFYIITEFMTY GNLLDYLRECNRQEVNAV VLLYMATQISSATEYLEKK NFIHRDLAARNCLVGENHL VKVADFGLSRLMTGDTYT AHAGAKF	ATQISSATEY (A01.01) ISSATEYLEK (A03.01) SSATEYLEK (A03.01) TQISSATEYL (A02.01) YMATQISSAT (A02.01)	Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), Gastrointestinal stromal tumors (GIST)

ABL1	T315I	SLTVAVKTLKEDTMEVEEF LKEAAVMKEIKHPNLVQLL GVCTREPPFYIIIEFMTYGN LLDYLRECNRQEVNAVVL LYMATQISSAMEYLEKKNF IHRDLA	FYIIIEFMTY (A24.02) IIEFMTYGNL (A02.01) IIIEFMTYG (A02.01) IIIEFMTYGN (A02.01) YIIIEFMTYG (A02.01)	Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), Gastrointestinal stromal tumors (GIST)
ABL1	Y253H	STVADGLITTLHYPAPKRN KPTVYGVSPNYDKWEMER TDITMKHKLGGGQHGEVY EGVWKKYSLTVAVKTLKE DTMEVEEFLKEAAVMKEIK HPNLVQLLG	GQHGEVYEGV (A02.01) KLGGGQHGEV (A02.01)	Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), Gastrointestinal stromal tumors (GIST)
ALK	G1269A	SSLAMLDLLHVARDIACGC QYLEENHFIHRDIAARNCL LTCPGPGRVAKIADFGMAR DIYRASYYRKGGCAMLPV KWMPPEAFMEGIFTSKTDT WSFGVLL	KIADFGMAR (A03.01) RVAKIADFGM (A02.01, B07.02)	NSCLC
ALK	L1196M	QVAVKTLPEVCSEQDELDF LMEALIISKFNHQNIVRCIG VSLQSLPRFILMELMAGGD LKSFLRETRPRPSQPSSLAM LDLLHVARDIACGCQYLEE NHFI	FILMELMAGG (A02.01) ILMELMAGG (A02.01) ILMELMAGGD (A02.01) LMELMAGGDL (A02.01) LPRFILMEL (B07.02, B08.01) LPRFILMELM (B07.02) LQSLPRFILM (A02.01, B08.01) SLPRFILMEL (A02.01, A24.02, B07.02, B08.01)	NSCLC
BRAF	V600E	MIKLIDIARQTAQGMDYLH AKSIIHRDLKSNNIFLHEDL TVKIGDFGLATEKSRWSGS HQFEQLSGSILWMAPEVIR MQDKNPYSFQSDVYAFGIV LYELM	LATEKSRWS (A02.01, B08.01) LATEKSRWSG (A02.01, B08.01)	CRC, GBM, KIRP, LUAD, SKCM, THCA
EEF1B2	S43G	MGFGDLKSPAGLQVLNDY LADKSYIEGYVPSQADVAV FEAVSGPPPADLCHALRWY NHIKSYEKEKASLPGVKKA LGKYGPADVEDTTGSGAT	GPPPADLCHAL (B07.02)	BLCA, KIRP, PRAD, SKCM
ERBB3	V104M	ERCEVVMGNLEIVLTGHNA DLSFLQWIREVTGYVLVA MNEFSTLPLPNLRMVRGTQ VYDGKFAIFVMLNYNTNSS HALRQLRLTQLTEILSGGV YIEKNDK		CRC, Stomach Cancer

ESR1	D538G	HLMAKAGLTLQQQHQRLA QLLLILSHIRHMSNKGMEH LYSMKCKNVVPLYGLLLE MLDAHRLHAPTSRGGASV EETDQSHLATAGSTSSHSL QKYYITGEA	GLLLEMLDA (A02.01) LYGLLLEML (A24.02) NVVPLYGLL (A02.01) PLYGLLLEM (A02.01) PLYGLLLEML (A02.01, A24.02) VPLYGLLLEM (B07.02) VVPLYGLLL (A02.01, A24.02)	Breast Cancer
ESR1	S463P	NQGKCVEGMVEIFDMLLA TSSRFRMMNLQGEEFVCLK SIILLNSGVYTFLPSTLKSLE EKDHIHRVLDKITDTLIHLM AKAGLTLQQQHQRLAQLL LILSH	FLPSTLKSL (A02.01, A24.02, B08.01) GVYTFLPST (A02.01) GVYTFLPSTL (A02.01, A24.02) TFLPSTLKSL (A24.02) VYTFLPSTL (A24.02) YTFLPSTLK (A03.01)	Breast Cancer
ESR1	Y537C	IHLMAKAGLTLQQQHQRL AQLLLILSHIRHMSNKGME HLYSMKCKNVVPLCDLLL EMLDAHRLHAPTSRGGAS VEETDQSHLATAGSTSSHS LQKYYITGE	NVVPLCDLL (A02.01) NVVPLCDLLL (A02.01) PLCDLLLEM (A02.01) PLCDLLLEML (A02.01) VPLCDLLLEM (B07.02) VVPLCDLLL (A02.01, A24.02)	Breast Cancer
ESR1	Y537N	IHLMAKAGLTLQQQHQRL AQLLLILSHIRHMSNKGME HLYSMKCKNVVPLNDLLL EMLDAHRLHAPTSRGGAS VEETDQSHLATAGSTSSHS LQKYYITGE	NVVPLNDLL (A02.01) NVVPLNDLLL (A02.01) PLNDLLLEM (A02.01) PLNDLLLEML (A02.01) VPLNDLLLEM (B07.02)	Breast Cancer
ESR1	Y537S	IHLMAKAGLTLQQQHQRL AQLLLILSHIRHMSNKGME HLYSMKCKNVVPLSDLLLE MLDAHRLHAPTSRGGASV EETDQSHLATAGSTSSHSL QKYYITGE	NVVPLSDLL (A02.01) NVVPLSDLLL (A02.01) PLSDLLLEM (A02.01) PLSDLLLEML (A02.01) VPLSDLLLEM (B07.02) VVPLSDLLL (A02.01, A24.02)	Breast Cancer
FGFR3	S249C	HRIGGIKLRHQQWSLVMES VVPSDRGNYTCVVENKFGS IRQTYTLDVLERCPHRPILQ AGLPANQTAVLGSDVEFHC KVYSDAQPHIQWLKHVEV NGSKVG	VLERCPHRPI (A02.01, B08.01) YTLDVLERC (A02.01)	BLCA, HNSC, KIRP, LUSC
FRG1B	L52S	AVKLSDSRIALKSGYGKYL GINSDELVGHSDAIGPREQ WEPVFQNGKMALSASNSC FIRCNEAGDIEAKSKTAGEE EMIKIRSCAEKETKKKDDIP EEDKG	FQNGKMALS (A02.01)	GBM, KIRP, PRAD, SKCM

HER2	V777L (Resistance)	GSGAFGTVYKGIWIPDGEN VKIPVAIKVLRENTSPKAN KEILDEAYVMAGLGSPYVS RLLGICLTSTVQLVTQLMP YGCLLDHVRENRGRLGSQ DLLNWCM	VMAGLGSPYV (A02.01, A03.01)	BRCA
IDH1	R132H	RVEEFKLKQMWKSPNGTIR NILGGTVFREAIICKNIPRLV SGWVKPIIIGHHAYGDQYR ATDFVVPGPGKVEITYTPS DGTQKVTYLVHNFEEGGG VAMGM	KPIIIGHHA (B07.02)	BLCA, GBM, PRAD
IDH1	R132C	RVEEFKLKQMWKSPNGTIR NILGGTVFREAIICKNIPRLV SGWVKPIIIGCHAYGDQYR ATDFVVPGPGKVEITYTPS DGTQKVTYLVHNFEEGGG VAMGM	KPIIIGCHA (B07.02)	BLCA, GBM, PRAD
IDH1	R132G	RVEEFKLKQMWKSPNGTIR NILGGTVFREAIICKNIPRLV SGWVKPIIIGGHAYGDQYR ATDFVVPGPGKVEITYTPS DGTQKVTYLVHNFEEGGG VAMGM	KPIIIGGHA (B07.02)	BLCA, BRCA, CRC, GBM, HNSC, LUAD, PAAD, PRAD, UCEC
IDH1	R132S	RVEEFKLKQMWKSPNGTIR NILGGTVFREAIICKNIPRLV SGWVKPIIIGSHAYGDQYR ATDFVVPGPGKVEITYTPS DGTQKVTYLVHNFEEGGG VAMGM	KPIIIGSHA (B07.02)	BLCA, BRCA, GBM, HNSC, LIHC, LUAD, LUSC, PAAD, SKCM, UCEC
KIT	T670I	VAVKMLKPSAHLTEREAL MSELKVLSYLGNHMNIVN LLGACTIGGPTLVIIEYCCY GDLLNFLRRKRDSFICSKQE DHAEAALYKNLLHSKESSC SDSTNE	IIEYCCYGDL (A02.01) TIGGPTLVII (A02.01) VIIEYCCYG (A02.01)	Gastrointestinal stromal tumors (GIST)
KIT	V654A	VEATAYGLIKSDAAMTVA VKMLKPSAHLTEREALMSE LKVLSYLGNHMNIANLLG ACTIGGPTLVITEYCCYGDL LNFLRRKRDSFICSKQEDH AEAALYK	HMNIANLLGA (A02.01) IANLLGACTI (A02.01) MNIANLLGA (A02.01) YLGNHMNIA (A02.01, B08.01) YLGNHMNIAN (A02.01)	Gastrointestinal stromal tumors (GIST)
MEK	C121S	ISELGAGNGGVVFKVSHKP SGLVMARKLIHLEIKPAIRN QIIRELQVLHESNSPYIVGF YGAFYSDGEISICMEHMDG GSLDQVLKKAGRIPEQILG KVSI	VLHESNSPY (A03.01) VLHESNSPYI (A02.01)	Melanoma

MEK	P124L	LGAGNGGVVFKVSHKPSG LVMARKLIHLEIKPAIRNQII RELQVLHECNSLYIVGFYG AFYSDGEISICMEHMDGGS LDQVLKKAGRIPEQILGKV SIAVI	LQVLHECNSL (A02.01, B08.01) LYIVGFYGAF (A24.02) NSLYIVGFY (A01.01) QVLHECNSL (A02.01, B08.01) SLYIVGFYG (A02.01) SLYIVGFYGA (A02.01) VLHECNSLY (A03.01) VLHECNSLYI (A02.01, A03.01)	Melanoma
MYC	E39D	MPLNVSFTNRNYDLDYDS VQPYFYCDEEENFYQQQQ QSDLQPPAPSEDIWKKFELL PTPPLSPSRRSGLCSPSYVA VTPFSLRGDNDGG	FYQQQQQSDL (A24.02) QQQSDLQPPA (A02.01) QQSDLQPPA (A02.01) YQQQQQSDL (A02.01, B08.01)	Lymphoid Cancer; Burkitt Lymphoma
MYC	P57S	FTNRNYDLDYDSVQPYFYC DEEENFYQQQQQSELQPPA PSEDIWKKFELLSTPPLSPS RRSGLCSPSYVAVTPFSLRG DNDGGGGSFSTADQLEMV TELLG	FELLSTPPL (A02.01, B08.01) LLSTPPLSPS (A02.01)	Lymphoid Cancer
MYC	T58I	TNRNYDLDYDSVQPYFYC DEEENFYQQQQQSELQPPA PSEDIWKKFELLPIPPLSPSR RSGLCSPSYVAVTPFSLRG DNDGGGGSFSTADQLEMV TELLGG	FELLPIPPL (A02.01) IWKKFELLPI (A24.02) LLPIPPLSPS (A02.01, B07.02) LPIPPLSPS (B07.02)	Neuroblastoma
PDGFR a	T674I	VAVKMLKPTARSSEKQAL MSELKIMTHLGPHLNIVNL LGACTKSGPIYIIIEYCFYGD LVNYLHKNRDSFLSHHPEK PKKELDIFGLNPADESTRSY VILS	IIEYCFYGDL (A02.01) IIIEYCFYG (A02.01) IYIIIEYCF (A24.02) IYIIIEYCFY (A24.02) YIIIEYCFYG (A02.01)	Chronic Eosinophilic Leukemia
PIK3CA	E542K	IEEHANWSVSREAGFSYSH AGLSNRLARDNELRENDKE QLKAISTRDPLSKITEQEKD FLWSHRHYCVTIPEILPKLL LSVKWNSRDEVAQMYCLV KDWPP	KITEQEKDFL (A02.01)	BLCA, BRCA, CESC, CRC, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, UCEC
PIK3CA	E545K	HANWSVSREAGFSYSHAG LSNRLARDNELRENDKEQL KAISTRDPLSEITKQEKDFL WSHRHYCVTIPEILPKLLLS VKWNSRDEVAQMYCLVK DWPPIKP	STRDPLSEITK (A03.01) DPLSEITK (A03.01)	BLCA, BRCA, CESC, CRC, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, SKCM, UCEC
PIK3CA	H1047R	LFINLFSMMLGSGMPELQS FDDIAYIRKTLALDKTEQE ALEYFMKQMNDARHGGW TTKMDWIFHTIKQHALN		BRCA, CESC, CRC, GBM, HNSC, LIHC, LUAD, LUSC, PRAD, UCEC

POLE	P286R	QRGGVITDEEETSKKIADQ LDNIVDMREYDVPYHIRLSI DIETTKLPLKFRDAETDQIM MISYMIDGQGYLITNREIVS EDIEDFEFTPKPEYEGPFCV FN	LPLKFRDAET (B07.02)	Colorectal adenocarcinoma ; Uterine/Endometrium Adenocarcinoma; Colorectal adenocarcinoma, MSI+; Uterine/Endometrium Adenocarcinoma, MSI+; Endometrioid carcinoma; Endometrium Serous carcinoma; Endometrium Carcinosarcoma -malignant mesodermal mixed tumor; Glioma; Astrocytoma; GBM
PTEN	R130Q	KFNCRVAQYPFEDHNPPQL ELIKPFCEDLDQWLSEDDN HVAAIHCKAGKGQTGVMI CAYLLHRGKFLKAQEALDF YGEVRTRDKKGVTIPSQRR YVYYYSY	QTGVMICAYL (A02.01)	BRCA, CESC, CRC, GBM, KIRC, LUSC, UCEC
RAC1	P29S	MQAIKCVVVGDGAVGKTC LLISYTTNAFSGEYIPTVFD NYSANVMVDGKPVNLGL WDTAGQEDYDRLRPLSYP QTVGET	AFSGEYIPTV (A02.01, A24.02)	Melanoma
TP53	G245S	IRVEGNLRVEYLDDRNTFR HSVVVPYEPPEVGSDCTTIH YNYMCNSSCMGSMNRRPI LTIITLEDSSGNLLGRNSFE VRVCACPGRDRRTEEENLR KKGEP	SMNRRPILT (A02.01, B08.01) YMCNSSCMGS (A02.01)	BLCA, BRCA, CRC, GBM, HNSC, LUSC, PAAD, PRAD
TP53	R175H	TYSPALNKMFCQLAKTCPV QLWVDSTPPPGTRVRAMAI YKQSQHMTEVVRHCPHHE RCSDSDGLAPPQHLIRVEG NLRVEYLDDRNTFRHSVV VPYEPPEV		BLCA, BRCA, CRC, GBM, HNSC, LUAD, PAAD, PRAD, UCEC

TP53	R248Q	EGNLRVEYLDDRNTFRHSV VVPYEPPEVGSDCTTIHYN YMCNSSCMGGMNQRPILTI ITLEDSSGNLLGRNSFEVRV CACPGRDRRTEEENLRKKG EPHHE	GMNQRPILT (A02.01)	BLCA, BRCA, CRC, GBM, HNSC, KIRC, LIHC, LUSC, PAAD, PRAD, UCEC
TP53	R248W	EGNLRVEYLDDRNTFRHSV VVPYEPPEVGSDCTTIHYN YMCNSSCMGGMNWRPILT IITLEDSSGNLLGRNSFEVR VCACPGRDRRTEEENLRKK GEPHHE	GMNWRPILT (A02.01)	BLCA, BRCA, CRC, GBM, HNSC, LIHC, LUSC, PAAD, SKCM, UCEC
TP53	R273C	PEVGSDCTTIHYNYMCNSS CMGGMNRRPILTIITLEDSS GNLLGRNSFEVCVCACPGR DRRTEEENLRKKGEPHHEL PPGSTKRALPNNTSSSPQPK KKPL	LLGRNSFEVC (A02.01)	BLCA, BRCA, CRC, GBM, HNSC, LUSC, PAAD, UCEC
TABLE 1B		**MSI-ASSOCIATED FRAMESHIFTS**		
ACVR2A	D96fs; +1	GVEPCYGDKDKRRHCFAT WKNISGSIEIVKQGCWLDDI NCYDRTDCVEKKRQP*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
ACVR2A	D96fs; -1	GVEPCYGDKDKRRHCFAT WKNISGSIEIVKQGCWLDDI NCYDRTDCVEKKTALKYIF VAVRAICVMKSFLIFRRWK SHSPLQIQLHLSHPITTSCSI PWCHLC*	ALKYIFVAV (A02.01, B08.01) ALKYIFVAVR (A03.01) AVRAICVMK (A03.01) AVRAICVMKS (A03.01) CVEKKTALK (A03.01) CVEKKTALKY (A01.01) CVMKSFLIF (A24.02, B08.01) CVMKSFLIFR (A03.01) FLIFRRWKS (A02.01, B08.01) FRRWKSHSPL (B08.01) FVAVRAICV (A02.01, B08.01) FVAVRAICVM (B08.01) IQLHLSHPI (A02.01) KSFLIFRRWK (A03.01) KTALKYIFV (A02.01) KYIFVAVRAI (A24.02) RWKSHSPLQI (A24.02) TALKYIFVAV (A02.01, B08.01) VAVRAICVMK (A03.01) VMKSFLIFR (A03.01) VMKSFLIFRR (A03.01) YIFVAVRAI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

C15ORF40	L132fs; +1	TAEAVNVAIAAPPSEGEANAELCRYLSKVLELRKSDVVLDKVGLALFFFFETKSCSVAQAGVQWRSLGSLQPPPPGFKLFSCLSFLSSWDYRRMPPCLANFCIFNRDGVSPCWSGWS*	ALFFFFFET (A02.01) ALFFFFFETK (A03.01) AQAGVQWRSL (A02.01) CLANFCIFNR (A03.01) CLSFLSSWDY (A01.01, A03.01) FFETKSCSV (B08.01) FFFETKSCSV (A02.01) FKLFSCLSFL (A02.01) FLSSWDYRRM (A02.01) GFKLFSCLSF (A24.02) KLFSCLSFL (A02.01, A03.01) KLFSCLSFLS (A02.01, A03.01) LALFFFFFET (A02.01) LFFFFFETK (A03.01) LSFLSSWDY (A01.01) LSFLSSWDYR (A03.01) RMPPCLANF (A24.02) RRMPPCLANF (A24.02) SLQPPPPGFK (A03.01) VQWRSLGSL (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
CNOT1	L1544fs; +1	LSVIIFFFVYIWHWALPLILNNHHICLMSSIILDCNSVRQSIMSVCFFFFSVIFSTRCLTDSRYPNICWFK*	FFFSVIFST (A02.01) MSVCFFFFSV (A02.01) SVCFFFFSV (A02.01, B08.01) SVCFFFFSVI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
CNOT1	L1544fs; -1	LSVIIFFFVYIWHWALPLILNNHHICLMSSIILDCNSVRQSIMSVCFFFFCYILNTMFDR*	FFCYILNTMF (A24.02) MSVCFFFFCY (A01.01) SVCFFFFCYI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
EIF2B3	A151fs; -1	VLVLSCDLITDVALHEVVDLFRAYDASLAMLMRKGQDSIEPVPGQKGKKKQWSSVTSLEWTAQERGCSSWLMKQTWMKSWSLRDPSYRSILEYVSTRVLWMPTSTV*	KQWSSVTSL (A02.01) VLWMPTSTV (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
EPHB2	K1020fs; -1	SIQVMRAQMNQIQSVEGQPLARRPRATGRTKRCQPRDVTKKTCNSNDGKKREWEKRKQILGGGGKYKEYFLKRILIRKAMTVLAGDKKGLGRFMRCVQSETKAVSLQLPLGR*	ILIRKAMTV (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

ESRP1	N512fs; +1	LDFLGEFATDIRTHGVHMV LNHQGRPSGDAFIQMKSAD RAFMAAQKCHKKKHEGQI C*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
ESRP1	N512fs; -1	LDFLGEFATDIRTHGVHMV LNHQGRPSGDAFIQMKSAD RAFMAAQKCHKKT*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
FAM111B	A273fs; -1	GALCKDGRFRSDIGEFEWK LKEGHKKIYGKQSMVDEV SGKVLEMDISKKKHYNRKI SIKKLNRMKVPLMKLITRV *	RMKVPLMK (A03.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
GBP3	T585fs; -1	RERAQLLEEQEKTLTSKLQ EQARVLKERCQGESTQLQN EIQKLQKTLKKKPRDICRIS *	TLKKKPRDI (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
JAK1	P861fs; +1	VNTLKEGKRLPCPPNCPDE VYQLMRKCWEFQPSNRTS FQNLIEGFEALLKTSN*	LIEGFEALLK (A03.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
JAK1	K860fs; -1	CRPVTPSCKELADLMTRCM NYDPNQRPFFRAIMRDINK LEEQNPDIVSEKNQQLKWT PHILKSAS*	QQLKWTPHI (A02.01) QLKWTPHILK (A03.01) IVSEKNQQLK (A03.01) QLKWTPHILK (A03.01) QQLKWTPHI (A24.02) NQQLKWTPHIL (B08.01) NQQLKWTPHI (B08.01) QLKWTPHIL (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
LMAN1	E305fs; +1	DDHDVLSFLTFQLTEPGKE PPTPDKEISEKEKEKYQEEF EHFQQELDKKKRGIPEGPP RPPRAACGGNI*	GPPRPPRAAC (B07.02) PPRPPRAAC (B07.02)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

LMAN1	E305fs; -1	DDHDVLSFLTFQLTEPGKEPPTPDKEISEKEKEKYQEEFEHFQQELDKKKRNSRRATPTSKGSLRRKYLRV*	SLRRKYLRV (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
MSH3	N385fs; +1	TKSTLIGEDVNPLIKLDDAVNVDEIMTDTSTSYLLCISENKENVRDKKKGQHFYWHCGSAACHRRGCV*	SAACHRRGCV (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
MSH3	K383fs; -1	LYTKSTLIGEDVNPLIKLDDAVNVDEIMTDTSTSYLLCISENKENVRDKKRATFLLALWECSLPQARLCLIVSRTLLLVQS*	ALWECSLPQA (A02.01) CLIVSRTLL (B08.01) CLIVSRTLLL (A02.01, B08.01) FLLALWECS (A02.01) FLLALWECSL (A02.01, B08.01) IVSRTLLLV (A02.01) LIVSRTLLL (A02.01, B08.01) LIVSRTLLLV (A02.01) LLALWECSL (A02.01, B08.01) LPQARLCLI (B08.01, B07.02) LPQARLCLIV (B08.01) NVRDKKRATF (B08.01) SLPQARLCLI (A02.01, B08.01)	MSI+CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
NDUFC2	A70fs; +1	LPPPKLTDPRLLYIGFLGYCSGLIDNLIRRRPIATAGLHRQLLYITAFFFCWILSCKT*	FFCWILSCK (A03.01) FFFCWILSCK (A03.01) ITAFFFCWI (A02.01) LYITAFFFCW (A24.02) YITAFFFCWI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
NDUFC2	F69fs; -1	SLPPPKLTDPRLLYIGFLGYCSGLIDNLIRRRPIATAGLHRQLLYITAFFLLDIIL*	ITAFFLLDI (A02.01) LLYITAFFL (A02.01, B08.01) LLYITAFFLL (A02.01, A24.02) LYITAFFLL (A24.02) LYITAFFLLD (A24.02) YITAFFLLDI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

RBM27	Q817; +1	NQSGGAGEDCQIFSTPGHP KMIYSSSNLKTPSKLCSGSK SHDVQEVLKKKTGSNEVTT RYEEKKTGSVRKANRMPK DVNIQVRKKQKHETRRKS KYNEDFERAWREDLTIKR*	GSNEVTTRY (A01.01) MPKDVNIQV (B07.02) TGSNEVTTRY (A01.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
RPL22	K16fs; +1	MAPVKKLVVKGGKKKEAS SEVHS*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
RPL22	K15fs; -1	MAPVKKLVVKGGKKRSKF *		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SEC31A	I462fs; +1	MPSHQGAEQQQQQHHVFIS QVVTEKEFLSRSDQLQQAV QSQGFINYCQKKN*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SEC31A	I462fs; -1	MPSHQGAEQQQQQHHVFIS QVVTEKEFLSRSDQLQQAV QSQGFINYCQKKLMLLRLN LRKMCGPF*	KKLMLLRLNL (A02.01) KLMLLRLNL (A02.01, A03.01, B07.02, B08.01) KLMLLRLNLR (A03.01) LLRLNLRKM (B08.01) LMLLRLNL (B08.01) LMLLRLNLRK (A03.01) LNLRKMCGPF (B08.01) MLLRLNLRK (A03.01) MLLRLNLRKM (A02.01, A03.01, B08.01) NLRKMCGPF (B08.01) NYCQKKLMLL (A24.02) YCQKKLMLL (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SEC63	K530fs; +1	AEVFEKEQSICAAEEQPAE DGQGETNKNRTKGGWQQ KSKGPKKTAKSKKKETFKK KTYTCAITTVKATETKAGK WSRWE*	FKKKTYTCAI (B08.01) ITTVKATETK (A03.01) KSKKKETFK (A03.01) KSKKKETFKK (A03.01) KTYTCAITTV (A02.01, A24.02) TFKKKTYTC (B08.01) TYTCAITTV (A24.02) TYTCAITTVK (A03.01) YTCAITTVK (A03.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

SEC63	K529fs; -1	MAEVFEKEQSICAAEEQPAEDGQGETNKNRTKGGWQQKSKGPKKTAKSKKRNL*	TAKSKKRNL (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SLC35F5	C248fs; -1	NIMEIRQLPSSHALEAKLSRMSYPVKEQESILKTVGKLTATQVAKISFFFALCGFWQICHIKKHFQTHKLL*	FALCGFWQI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SMAP1	K172fs; +1	YEKKKYYDKNAIAITNISSSDAPLQPLVSSPSLQAAVDKNKLEKEKEKKKGREKERKGARKAGKTTYS*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SMAP1	K171fs; -1	KYEKKKYYDKNAIAITNISSSDAPLQPLVSSPSLQAAVDKNKLEKEKEKKRKRKREKRSQKSRQNHLQLKSCRRKISNWSLKKVPALKKLRSPLWIF*	LKKLRSPL (B08.01) SLKKVPAL (B08.01) RKISNWSLKK (A03.01) VPALKKLRSPL (B07.02)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TFAM	E148fs; +1	IYQDAYRAEWQVYKEEISRFKEQLTPSQIMSLEKEIMDKHLKRKAMTKKKRVNTAWKTKKTSFSL*	KRVNTAWKTK (A03.01) MTKKKRVNTA (B08.01) RVNTAWKTK (A03.01) RVNTAWKTKK (A03.01) TKKKRVNTA (B08.01) WKTKKTSFSL (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TFAM	E148fs; -1	IYQDAYRAEWQVYKEEISRFKEQLTPSQIMSLEKEIMDKHLKRKAMTKKKS*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TGFBR2	P129fs; +1	KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKAW*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

TGFBR2	K128fs: -1	EKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKSLVRLSSCVPVALMSAMTTSSSQKNITPAILTCC*	ALMSAMTTS (A02.01) AMTTSSSQK (A03.01, A11.01) AMTTSSSQKN (A03.01) CIMKEKKSL (B08.01) CIMKEKKSLV (B08.01) IMKEKKSL (B08.01) IMKEKKSLV (B08.01) KSLVRLSSCV (A02.01) LVRLSSCVPV (A02.01) RLSSCVPVA (A02.01, A03.01) RLSSCVPVAL (A02.01) SAMTTSSSQK (A03.01, A11.01) SLVRLSSCV (A02.01) VPVALMSAM (B07.02) VRLSSCVPVA (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
THAP5	K99fs; -1	VPSKYQFLCSDHFTPDSLDIRWGIRYLKQTAVPTIFSLPEDNQGKDPSKKNPRRKTWKMRKKYAQKPSQKNHLY*	KMRKKYAQK (A03.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TTK	R854fs; -1	GTTEEMKYVLGQLVGLNSPNSILKAAKTLYEHYSGGESHNSSSSKTFEKKGEKNDLQLFVMSDTTYKIYWTVILLNPCGNLHLKTTSL*	FVMSDTTYK (A03.01) FVMSDTTYKI (A02.01) KTFEKKGEK (A03.01) LFVMSDTTYK (A03.01) MSDTTYKIY (A01.01) VMSDTTYKI (A02.01) VMSDTTYKIY (A01.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
XPOT	F126fs; -1	QQLIRETLISWLQAQMLNPQPEKTFIRNKAAQVFALLFVTEYLTKWPKFFLTFSQ*	YLTKWPKFFL (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TABLE 1C		**FRAMESHIFT**		
APC	V1352fs F1354fs Q1378fs S1398fs	AKFQQCHSTLEPNPADCRVLVYLQNQPGTKLLNFLQERNLPPKVVLRHPKVHLNTMFRRPHSCLADVLLSVHLIVLRVVRLPAPFRVNHAVEW*	FLQERNLPP (A02.01) FRRPHSCLA (B08.01) LIVLRVVRL (B08.01) LLSVHLIVL (A02.01, B08.01)	CRC, LUAD, UCEC, STAD

APC	S1421fs R1435fs T1438fs P1442fs P1443fs V1452fs P1453fs K1462fs E1464fs	APVIFQIALDKPCHQAEVK HLHHLLKQLKPSEKYLKIK HLLLKRERVDLSKLQ*	EVKHLHHLL (B08.01) HLHHLLKQLK (A03.01) HLLLKRERV (B08.01) KIKHLLLKR (A03.01) KPSEKYLKI (B07.02) KYLKIKHLL (A24.02) KYLKIKHLLL (A24.02) LLKQLKPSEK (A03.01) LLKRERVDL (B08.01) LLLKRERVDL (B08.01) QLKPSEKYLK (A03.01) YLKIKHLLL (A02.01, B08.01) YLKIKHLLLK (A03.01)	CRC, LUAD, UCEC, STAD
APC	T1487fs H1490fs L1488fs	MLQFRGSRFFQMLILYYILP RKVLQMDFLVHPA*	ILPRKVLQM (B08.01) KVLQMDFLV (A02.01, A24.02) LPRKVLQMDF (B07.02, B08.01) LQMDFLVHPA (A02.01) QMDFLVHPA (A02.01) YILPRKVLQM (A02.01, B08.01)	CRC, LUAD, UCEC, STAD
ARID1A	Q1306fs S1316fs Y1324fs T1348fs G1351fs G1378fs P1467fs	ALGPHSRISCLPTQTRGCIL LAATPRSSSSSSSNDMIPMA ISSPPKAPLLAAPSPASRLQ CINSNSRITSGQWMAHMAL LPSGTKGRCTACHTALGRG SLSSSSCPQPSPSLPASNKLP SLPLSKMYTTSMAMPILPLP QLLLSADQQAAPRTNFHSS LAETVSLHPLAPMPSKTCH HK*	APSPASRLQC (B07.02) HPLAPMPSKT (B07.02) ILPLPQLLL (A02.01) LLLSADQQA (A02.01) LPTQTRGCI (B07.02) LPTQTRGCIL (B07.02) RISCLPTQTR (A03.01) SLAETVSLH (A03.01) TPRSSSSSS (B07.02) TPRSSSSSSS (B07.02)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS

			ALPPVLLSL (A02.01) ALPPVLLSLA (A02.01) ALPRPLLAL (A02.01) ASRTASCIL (B07.02) EALPRPLLAL (B08.01) HLGHPVASR (A03.01) HPVASRTAS (B07.02) HPVASRTASC (B07.02) IIQLSLLSLL (A02.01) IQLSLLSLL (A02.01) IQLSLLSLLI (A02.01, A24.02)	
ARID1A	S674fs P725fs R727fs I736fs	AHQGFPAAKESRVIQLSLLS LLIPPLTCLASEALPRPLLAL PPVLLSLAQDHSRLLQCQA TRCHLGHPVASRTASCILP*	LLALPPVLL (A02.01) LLIPPLTCL (A02.01) LLIPPLTCLA (A02.01) LLSLLIPPL (A02.01) LLSLLIPPLT (A02.01) LPRPLLALPP (B07.02) QLSLLSLLI (A02.01) RLLQCQATR (A03.01) RPLLALPPV (B07.02) RPLLALPPVL (B07.02) SLAQDHSRL (A02.01) SLAQDHSRLL (A02.01) SLLIPPLTCL (A02.01) SLLSLLIPP (A02.01) SLLSLLIPPL (A02.01, B08.01)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS

ARID1A	G414fs Q473fs H477fs S499fs P504fs Q548fs P549fs	PILAATGTSVRTAARTWVP RAAIRVPDPAAVPDDHAGP GAECHGRPLLYTADSSLWT TRPQRVWSTGPDSILQPAK SSPSAAAATLLPATTVPDPS CPTFVSAAATVSTTTAPVLS ASILPAAIPASTSAVPGSIPL PAVDDTAAPPEPAPLLTAT GSVSLPAAATSAASTLDAL PAGCVSSAPVSAVPANCLF PAALPSTAGAISRFIWVSGI LSPLNDLQ*	AAATSAASTL (B07.02) AAIPASTSAV (B07.02) AIPASTSAV (A02.01) ALPAGCVSSA (A02.01) APLLTATGSV (B07.02) APVLSASIL (B07.02) ATLLPATTV (A02.01) ATVSTTTAPV (A02.01) AVPANCLFPA (A02.01) CLFPAALPST (A02.01) CPTFVSAAA (B07.02) FPAALPSTA (B07.02) FPAALPSTAG (B07.02) GAECHGRPL (B07.02) GAISRFIWV (A02.01) ILPAAIPAST (A02.01) IWVSGILSPL (A24.02) LLTATGSVSL (A02.01) LLYTADSSL (A02.01) LPAAATSAA (B07.02) LPAAATSAAS (B07.02) LPAAIPAST (B07.02) LPAGCVSSA (B07.02) LPAGCVSSAP (B07.02) LYTADSSLW (A24.02) QPAKSSPSA (B07.02) QPAKSSPSAA (B07.02) RFIWVSGIL (A24.02) RPQRVWSTG (B07.02) RVWSTGPDSI (A02.01) SAVPGSIPL (B07.02) SILPAAIPA (A02.01) SLPAAATSA (A02.01) SLPAAATSAA (A02.01) SLWTTRPQR (A03.01) SLWTTRPQRV (A02.01) SPSAAAATL (B07.02) SPSAAAATLL (B07.02) TLDALPAGCV (A02.01) TVSTTTAPV (A02.01) VLSASILPA (A02.01) VLSASILPAA (A02.01) VPANCLFPA (B07.02) VPANCLFPAA (B07.02) VPDPSCPTF (B07.02) VPGSIPLPA (B07.02) VPGSIPLPAV (B07.02) WVSGILSPL (A02.01) YTADSSLWTT (A02.01)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS

ARID1A	T433fs A441fs Y447fs P483fs P484fs P504fs S519fs H544fs P549fs P554fs Q563fs	PCRAGRRVPWAASLIHSRF LLMDNKAPAGMVNRARLH ITTSKVLTLSSSSHPTPSNHR PRPLMPNLRISSSHSLNHHS SSPLSLHTPSSHPSLHISSPR LHTPPSSRRHSSTPRASPPT HSHRLSLLTSSSNLSSQHPR RSPSRLRILSPSLSSPSKLPIP SSASLHRRSYLKIHLGLRHP QPPQ*	APAGMVNRA (B07.02) ASLHRRSYL (B08.01) ASLHRRSYLK (A03.01) FLLMDNKAPA (A02.01) HPRRSPSRL (B07.02, B08.01) HPSLHISSP (B07.02) HRRSYLKIHL (B08.01) HSRFLLMDNK (A03.01) KLPIPSSASL (A02.01) KVLTLSSSSH (A03.01) LIHSRFLLM (B08.01) LLMDNKAPA (A02.01) LMDNKAPAGM (A02.01) LPIPSSASL (B07.02) MPNLRISSS (B07.02, B08.01) MPNLRISSSH (B07.02) NLRISSSHSL (B07.02, B08.01) PPTHSHRLSL (B07.02) RAGRRVPWAA (B08.01) RARLHITTSK (A03.01) RISSSHSLNH (A03.01) RLHTPPSSR (A03.01) RLHTPPSSRR (A03.01) RLRILSPSL (A02.01, B07.02, B08.01) RPLMPNLRI (B07.02) RPRPLMPNL (B07.02) SASLHRRSYL (B07.02, B08.01) SLHISSPRL (A02.01) SLHRRSYLK (A03.01) SLHRRSYLKI (B08.01) SLIHSRFLL (A02.01) SLIHSRFLLM (A02.01, B08.01) SLLTSSSNL (A02.01) SLNHHSSSPL (A02.01, B07.02, B08.01) SLSSPSKLPI (A02.01) SPLSLHTPS (B07.02) SPLSLHTPSS (B07.02) SPPTHSHRL (B07.02) SPRLHTPPS (B07.02) SPRLHTPPSS (B07.02) SPSLSSPSKL (B07.02) SYLKIHLGL (A24.02) TPSNHRPRPL (B07.02, B08.01) TPSSHPSLHI (B07.02)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS

ARID1A	A2137fs P2139fs L1970fs V1994fs	RTNPTVRMRPHCVPFWTGRILLPSAASVCPIPFEACHLCQAMTLRCPNTQGCCSSWAS*	CVPFWTGRIL (B07.02) HCVPFWTGRIL (B07.02) ILLPSAASV (A02.01) ILLPSAASVC (A02.01) LLPSAASVCPI (A02.01) LPSAASVCPI (B07.02) MRPHCVPF (B08.01) RILLPSAASV (A02.01) RMRPHCVPF (A24.02, B07.02, B08.01) RMRPHCVPFW (A24.02) RTNPTVRMR (A03.01) SVCPIPFEA (A02.01) TVRMRPHCV (B08.01) TVRMRPHCVPF (B08.01) VPFWTGRIL (B07.02) VPFWTGRILL (B07.02) VRMRPHCVPF (B08.01)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS
ARID1A	N756fs S764fs T783fs Q799fs A817fs	TNQALPKIEVICRGTPRCPSTVPPSPAQPYLRVSLPEDRYTQAWAPTSRTPWGAMVPRGVSMAHKVATPGSQTIMPCPMPTTPVQAWLEA*	AMVPRGVSM (B07.02, B08.01) AMVPRGVSMA (A02.01) AWAPTSRTPW (A24.02) CPMPTTPVQA (B07.02) CPSTVPPSPA (B07.02) GAMVPRGVSM (B07.02, B08.01) MPCPMPTTPV (B07.02) MPTTPVQAW (B07.02) MPTTPVQAWL (B07.02) SLPEDRYTQA (A02.01) SPAQPYLRV (B07.02) SPAQPYLRVS (B07.02) TIMPCPMPT (A02.01) TPVQAWLEA (B07.02) TSRTPWGAM (B07.02) VPPSPAQPYL (B07.02) VPRGVSMAH (B07.02)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS
β2M	N62fs E67fs L74fs F82fs T91fs E94fs	RMERELKKWSIQTCLSARTGLSISCTTLNSPPLKKMSMPAV*	CLSARTGLSI (B08.01) CTTLNSPPLK (A03.01) GLSISCTTL (A02.01) SPPLKKMSM (B07.02, B08.01) TLNSPPLKK (A03.01) TTLNSPPLK (A03.01) TTLNSPPLKK (A03.01)	CRC, STAD, SKCM, HNSC
β2M	L13fs S14fs	LCSRYSLFLAWRLSSVLQRFRFTHVIQQRMESQIS*	LQRFRFTHV (B08.01) LQRFRFTHVI (B08.01) RLSSVLQRF (A24.02) RLSSVLQRFR (A03.01) VLQRFRFTHV (A02.01, B08.01)	CRC, STAD, SKCM, HNSC

CDH1	A691fs P708fs L711fs	RSACVTVKGPLASVGRHSL SKQDCKFLPFWGFLEEFLL C*	ASVGRHSLSK (A03.01) KFLPFWGFL (A24.02) LASVGRHSL (B07.02) LPFWGFLEEF (B07.02) PFWGFLEEF (A24.02) SVGRHSLSK (A03.01)	ILC LumA Breast Cancer
CDH1	H121fs P126fs H128fs N144fs V157fs P159fs N166fs N181fs F189fs P201fs F205fs	IQWGTTTAPRPIRPPFLESK QNCSHFPTPLLASEDRRET GLFLPSAAQKMKKAHFLK TWFRSNPTKTKKARFSTAS LAKELTHPLLVSLLLKEKQ DG*	APRPIRPPF (B07.02) APRPIRPPFL (B07.02) AQKMKKAHFL (B08.01) FLPSAAQKM (A02.01) GLFLPSAAQK (A03.01) HPLLVSLLL (B07.02) KAHFLKTWFR (A03.01) KARFSTASL (B07.02) KMKKAHFLK (A03.01) KTWFRSNPTK (A03.01) LAKELTHPL (B07.02, B08.01) LAKELTHPLL (B08.01) NPTKTKKARF (B07.02) QKMKKAHFL (B08.01) RFSTASLAK (A03.01) RPIRPPFLES (B07.02) RSNPTKTKK (A03.01) SLAKELTHPL (A02.01, B08.01) TKKARFSTA (B08.01)	ILC LumA Breast Cancer
CDH1	V114fs P127fs V132fs P160fs	PTDPFLGLRLGLHLQKVFH QSHAEYSGAPPPPPAPSGLR FWNPSRIAHISQLLSWPQKT EERLGYSSHQLPRK*	GLRFWNPSR (A03.01) ISQLLSWPQK (A03.01) RIAHISQLL (A02.01) RLGYSSHQL (A02.01) SQLLSWPQK (A03.01) SRIAHISQL (B08.01) WPQKTEERL (B07.02) YSSHQLPRK (A03.01)	ILC LumA Breast Cancer
CDH1	L731fs R749fs E757fs G759fs	FCCSCCFFGGERWSKSPYC PQRMTPGTTFITMMKKEAE KRTRTLT*	CPQRMTPGTT (B07.02) EAEKRTRTL (B08.01) GTTFITMMK (A03.01) GTTFITMMKK (A03.01) ITMMKKEAEK (A03.01) RMTPGTTFI (A02.01) SPYCPQRMT (B07.02) TMMKKEAEK (A03.01) TPGTTFITM (B07.02) TPGTTFITMM (B07.02) TTFITMMKK (A03.01)	ILC LumA Breast Cancer
CDH1	S19fs E24fs S36fs	WRRNCKAPVSLRKSVQTP ARSSPARPDRTRRLPSLGVP GQPWALGAAASRRCCCCC RSPLGSARSRSPATLALTPR ATRSRCPGATWREAASWA E*	CPGATWREA (B07.02) CPGATWREAA (B07.02) RSRCPGATWR (A03.01) TPRATRSRC (B07.02)	ILC LumA Breast Cancer

GATA3	P394fs P387fs S398fs H400fs M401fs S408fs P409fs S408fs P409fs T419fs H424fs P425fs S427fs F431fs S430fs H434fs H435fs S438fs M443fs G444fs *445fs	PGRPLQTHVLPEPHLALQP LQPHADHAHADAPAIQPVL WTTPPLQHGHRHGLEPCS MLTGPPARVPAVPFDLHFC RSSIMKPKRDGYMFLKAES KIMFATLQRSSLWCLCSNH *	HVLPEPHLAL (B07.02) RPLQTHVLPE (B07.02) VLWTTPPLQH (A03.01)	Breast Cancer
GATA3	P426fs H434fs P433fs T441fs	PRPRRCTRHPACPLDHTTPP AWSPPWVRALLDAHRAPS ESPCSPFRLAFLQEQYHEA*	APSESPCSPF (B07.02) CPLDHTTPPA (B07.02) FLQEQYHEA (A02.01, B08.01) RLAFLQEQYH (A03.01) SPCSPFRLAF (B07.02) SPPWVRALL (B07.02) YPACPLDHTT (B07.02)	Breast Cancer

MLL2	P519fs E524fs P647fs S654fs L656fs R755fs L761fs Q773fs	TRRCHCCPHLRSHPCPHHL RNHPRPHHLRHHACHHHL RNCPHPHFLRHCTCPGRWR NRPSLRRLRSLLCLPHLNH HLFLHWRSRPCLHRKSHPH LLHLRRLYPHHLKHRPCPH HLKNLLCPRHLRNCPLPRH LKHLACLHHLRSHPCPLHL KSHPCLHHRRHLVCSHHLK SLLCPLHLRSLPFPHHLRHH ACPHHLRTRLCPHHLKNHL CPPHLRYRAYPPCLWCHAC LHRLRNLPCPHRLRSLPRPL HLRLHASPHHLRTPPHPHH LRTHLLPHHRRTRSCPCRW RSHPCCHYLRSRNSAPGPR GRTCHPGLRSRTCPPGLRS HTYLRRLRSHTCPPSLRSH AYALCLRSHTCPPRLRDHI CPLSLRNCTCPPRLRSRTCL LCLRSHACPPNLRNHTCPPS LRSHACPPGLRNRICPLSLR SHPCPLGLKSPLRSQANAL HLRSCPCSLPLGNHPYLPCL ESQPCLSLGNHLCPLCPRSC RCPHLGSHPCRLS*	ALHLRSCPC (B08.01) CLHHRRHLV (B08.01) CLHHRRHLVC (B08.01) CLHRKSHPHL (B08.01) CLRSHACPP (B08.01) CLRSHTCPP (B08.01) CLWCHACLH (A03.01) CPHHLKNHL (B07.02) CPHHLKNLL (B07.02) CPHHLRTRL (B07.02, B08.01) CPLHLRSLPF (B07.02, B08.01) CPLPRHLKHL (B07.02, B08.01) CPLSLRSHPC (B07.02) CPRHLRNCPL (B07.02, B08.01) FPHHLRHHA (B07.02, B08.01) FPHHLRHHAC (B07.02, B08.01) GLRSRTCPP (B08.01) HACLHRLRNL (B08.01) HLACLHHLR (A03.01) HLCPPHLRY (A03.01) HLCPPHLRYR (A03.01) HLKHLACLH (A03.01) HLKHRPCPH (B08.01) HLKNHLCPP (B08.01) HLKSHPCLH (A03.01) HLKSLLCPL (A02.01, B08.01) HLLHLRRLY (A03.01) HLRNCPLPR (A03.01) HLRNCPLPRH (A03.01) HLRRLYPHHL (B08.01) HLRSHPCPL (B07.02, B08.01) HLRSHPCPLH (A03.01) HLRSLPFPH (A03.01) HLRTRLCPH (A03.01, B08.01) HLVCSHHLK (A03.01) HPCLHHRRHL (B07.02, B08.01) HPGLRSRTC (B07.02) HPHLLHLRRL (B07.02, B08.01) HRKSHPHLL (B08.01) HRRTRSCPC (B08.01) KSHPHLLHLR (A03.01) KSLLCPLHLR (A03.01)	STAD, BLCA, CRC, HNSC, BRCA

			LLCPLHLRSL (A02.01, B08.01) LLHLRRLYPH (B08.01) LPRHLKHLA (B07.02) LPRHLKHLAC (B07.02, B08.01) LRRLRSHTC (B08.01) LRRLYPHHL (B08.01) LVCSHHLKSL (B08.01) NLRNHTCPPS (B08.01) PLHLRSLPF (B08.01) RLCPHHLKNH (A03.01) RLYPHHLKH (A03.01) RLYPHHLKHR (A03.01) RPCPHHLKNL (B07.02) RSHPCPLHLK (A03.01) RSLPFPHHLR (A03.01) RTRLCPHHL (B07.02) RTRLCPHHLK (A03.01) SLLCPLHLR (A03.01) SLRSHACPP (B08.01) SPLRSQANA (B07.02) YLRRLRSHT (B08.01) YPHHLKHRPC (B07.02, B08.01)	
PTEN	I122fs I135fs A148fs L152fs D162fs I168fs	SWKGTNWCNDMCIFITSGQIFKGTRGPRFLWGSKDQRQKGSNYSQSEALCVLL*	FITSGQIFK (A03.01) IFITSGQIF (A24.02) SQSEALCVL (A02.01) SQSEALCVLL (A02.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
PTEN	L265fs K266fs	KRTKCFTFG*		UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
PTEN	A39fs E40fs V45fs R47fs N48fs	PIFIQTLLLWDFLQKDLKAYTGTILMM*	AYTGTILMM (A24.02) DLKAYTGTIL (B08.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
PTEN	T319fs T321fs K327fs A328fs A333fs	QKMILTKQIKTKPTDTFLQILR*	ILTKQIKTK (A03.01) KMILTKQIK (A03.01) KPTDTFLQI (B07.02) KPTDTFLQIL (B07.02) MILTKQIKTK (A03.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM

PTEN	N63fs E73fs A86fs N94fs	GFWIQSIKTITRYTIFVLKDI MTPPNLIAELHNILLKTITH HS*	ITRYTIFVLK (A03.01) LIAELHNIL (A02.01) LIAELHNILL (A02.01) MTPPNLIAEL (A02.01) NLIAELHNI (A02.01) NLIAELHNIL (A02.01) RYTIFVLKDI (A24.02) TITRYTIFVL (A02.01) TPPNLIAEL (B07.02)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
PTEN	T202fs G209fs C211fs I224fs G230fs P231fs R233fs D236fs	NYSNVQWRNLQSSVCGLP AKGEDIFLQFRTHTTGRQV HVL*	FLQFRTHTT (A02.01, B08.01) LPAKGEDIFL (B07.02) LQFRTHTTGR (A03.01) NLQSSVCGL (A02.01) SSVCGLPAK (A03.01) VQWRNLQSSV (A02.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
PTEN	G251fs E256fs K260fs Q261fs L265fs M270fs H272fs T286fs E288fs	YQSRVLPQTEQDAKKGQN VSLLGKYILHTRTRGNLRK SRKWKSM*	GQNVSLLGK (A03.01) HTRTRGNLRK (A03.01) ILHTRTRGNL (B08.01) KGQNVSLLGK (A03.01) LLGKYILHT (A02.01) LRKSRKWKSM (B08.01) SLLGKYILH (A03.01) SLLGKYILHT (A02.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
TP53	A70fs P72fs A76fs A79fs P89fs W91fs S96fs V97fs V97fs G108fs G117fs S121fs V122fs C124fs K139fs V143fs	SSQNARGCSPRGPCTSSSYT GGPCTSPLLAPVIFCPFPEN LPGQLRFPSGLLAFWDSQV CDLHVLPCPQQDVLPTGQD LPCAAVG*	CTSPLLAPV (A02.01) FPENLPGQL (B07.02) GLLAFWDSQV (A02.01) IFCPFPENL (A24.02) LLAFWDSQV (A02.01) LLAPVIFCP (A02.01) LLAPVIFCPF (A02.01, A24.02) LPCPQQDVL (B07.02) RFPSGLLAF (A24.02) RFPSGLLAFW (A24.02) SPLLAPVIF (B07.02) SPRGPCTSS (B07.02) SPRGPCTSSS (B07.02) SQVCDLHVL (A02.01) VIFCPFPENL (A02.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC

TP53	V173fs H178fs D186fs H193fs L194fs E198fs V203fs E204fs L206fs D207fs N210fs T211fs F212fs V225fs S241fs	GAAPTMSAAQIAMVWPLL SILSEWKEICVWSIWMTET LFDIVWWCPMSRLRLALTV PPSTTTTCVTVPAWAA*	AMVWPLLSI (A02.01) AMVWPLLSIL (A02.01) AQIAMVWPL (A02.01, A24.02) AQIAMVWPLL (A02.01) CPMSRLRLA (B07.02, B08.01) CPMSRLRLAL (B07.02, B08.01) IAMVWPLLSI (A02.01, A24.02, B08.01) ILSEWKEICV (A02.01) IVWWCPMSR (A03.01) IVWWCPMSRL (A02.01) IWMTETLFDI (A24.02) LLSILSEWK (A03.01) MSAAQIAMV (A02.01) MSRLRLALT (B08.01) MSRLRLALTV (B08.01) MVWPLLSIL (A02.01) RLALTVPPST (A02.01) TLFDIVWWC (A02.01) TLFDIVWWCP (A02.01) TMSAAQIAMV (A02.01) VWSIWMTETL (A24.02) WMTETLFDI (A02.01, A24.02) WMTETLFDIV (A01.01, A02.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC

TP53	R248fs P250fs S260fs N263fs G266fs N268fs V272fs V274fs P278fs D281fs R282fs T284fs E285fs L289fs K292fs P301fs S303fs T312fs S314fs K319fs K320fs P322fs Y327fs F328fs L330fs R333fs R335fs R337fs E339fs	TGGPSSPSSHWKTPVVIYW DGTALRCVFVPVLGETGAQ RKRISARKGSLTTSCPQGAL SEHCPTTPAPLPSQRRNHW MENISPFRSVGVSASRCSES *	ALRCVFVPV (A02.01, B08.01) ALRCVFVPVL (A02.01, B08.01) ALSEHCPTT (A02.01) AQRKRISARK (A03.01) GAQRKRISA (B08.01) HWMENISPF (A24.02) LPSQRRNHW (B07.02) LPSQRRNHWM (B07.02, B08.01) NISPFRSVGV (A02.01) RISARKGSL (B07.02, B08.01) SPFRSVGVSA (B07.02) SPSSHWKTPV (B07.02, B08.01) TALRCVFVPV (A02.01) VIYWDGTAL (A02.01) VIYWDGTALR (A03.01) VLGETGAQRK (A03.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC
TP53	S149fs P151fs P152fs V157fs Q165fs S166fs H168fs V173fs	FHTPARHPRPRHGHLQAVT AHDGGCEALPPP*	HPRPRHGHL (B07.02, B08.01) HPRPRHGHLQ (B07.02) RPRHGHLQA (B07.02) RPRHGHLQAV (B07.02, B08.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC

TP53	P47fs D48fs D49fs Q52fs F54fs E56fs P58fs P60fs E62fs M66fs P72fs V73fs P75fs A78fs P82fs P85fs S96fs P98fs T102fs Y103fs G108fs F109fs R110fs G117fs	CCPRTILNNGSLKTQVQMK LPECQRLLPPWPLHQQLLH RRPLHQPPPGPCHLLSLPRK PTRAATVSVWASCILGQPS L*	GSLKTQVQMK (A03.01) PPGPCHLLSL (B07.02) RTILNNGSLK (A03.01) SLKTQVQMK (A03.01) SLKTQVQMKL (B08.01) TILNNGSLK (A03.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC
TP53	L26fs P27fs P34fs P36fs A39fs Q38fs	VRKHFQTYGNYFLKTTFCP PCRPKQWMI*	CPPCRPKQWM (B07.02) TTFCPPCRPK (A03.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC
TP53	C124fs L130fs N131fs C135fs K139fs A138fs T140fs V143fs Q144fs V147fs T150fs P151fs P152fs G154fs R156fs R158fs A161fs	LARTPLPSTRCFANWPRPA LCSCGLIPHPRPAPASAPWP STSSHST*	CFANWPRPAL (A24.02) FANWPRPAL (B07.02, B08.01) GLIPHPRPA (A02.01) HPRPAPASA (B07.02, B08.01) HPRPAPASAP (B07.02) IPHPRPAPA (B07.02, B08.01) IPHPRPAPAS (B07.02) RPALCSCGL (B07.02) RPALCSCGLI (B07.02) TPLPSTRCF (B07.02) WPRPALCSC (B07.02) WPRPALCSCG (B07.02)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC

VHL	L178fs D179fs L184fs T202fs R205fs D213fs G212fs	ELQETGHRQVALRRSGRPP KCAERPGAADTGAHCTST DGRLKISVETYTVSSQLLM VLMSLDLDTGLVPSLVSKC LILRVK*	ALRRSGRPPK (A03.01) GLVPSLVSK (A03.01) KISVETYTV (A02.01) LLMVLMSLDL (A02.01, B08.01) LMSLDLDTGL (A02.01) LMVLMSLDL (A02.01) LVSKCLILRV (A02.01) QLLMVLMSL (A02.01, B08.01) RPGAADTGA (B07.02) RPGAADTGAH (B07.02) SLDLDTGLV (A02.01) SLVSKCLIL (A02.01, B08.01) SQLLMVLMSL (A02.01) TVSSQLLMV (A02.01) TYTVSSQLL (A24.02) TYTVSSQLLM (A24.02) VLMSLDLDT (A02.01) VPSLVSKCL (B07.02) VSKCLILRVK (A03.01) YTVSSQLLM (A01.01) YTVSSQLLMV (A02.01)	KIRC, KIRP
VHL	L158fs K159fs R161fs Q164fs	KSDASRLSGA*		KIRC, KIRP
VHL	P146fs I147fs F148fs L158fs	RTAYFCQYHTASVYSERA MPPGCPEPSQA*	FCQYHTASV (B08.01)	KIRC, KIRP
VHL	S68fs S72fs I75fs S80fs P86fs P97fs I109fs H115fs L116fs G123fs T124fs N131fs L135fs V137fs G144fs D143fs I147fs	TRASPPRSSSAIAVRASCCP YGSTSTASRSPTQRCRLAR AAASTATEVTFGSSEMQGH TMGFWLTKLNYLCHLSML TDSLFLPISHCQCIL*	CPYGSTSTA (B07.02) CPYGSTSTAS (B07.02) LARAAASTAT (B07.02) MLTDSLFLP (A02.01) PPRSSSAIAV (B07.02) RAAASTATEV (B07.02) SPPRSSSAI (B07.02) SPPRSSSAIA (B07.02) SPTQRCRLA (B07.02) TQRCRLARA (B08.01) TQRCRLARAA (B08.01)	KIRC, KIRP

VHL	K171fs P172fs N174fs L178fs D179fs L188fs	SSLRITGDWTSSGRSTKIWK TTQMCRKTWSG*	KIWKTTQMCR (A03.01) WTSSGRSTK (A03.01)	KIRC, KIRP
VHL	V62fs V66fs Q73fs V84fs F91fs T100fs P103fs S111fs L116fs H115fs D126fs	RRRRGGVGRRGVRPGRVR PGGTGRRGGDGGRAAAAR AALGELARALPGHLLQSQS ARRAARMAQLRRRAAALP NAAAWHGPPHPQLPRSPLA LQRCRDTRWASG*	ALGELARAL (A02.01) AQLRRRAAA (B08.01) AQLRRRAAAL (B08.01) ARRAARMAQL (B08.01) HPQLPRSPL (B07.02, B08.01) HPQLPRSPLA (B07.02) LARALPGHL (B07.02) LARALPGHLL (B07.02) MAQLRRRAA (B07.02, B08.01) MAQLRRRAAA (B07.02, B08.01) QLRRRAAAL (B07.02, B08.01) RAAALPNAAA (B07.02) RMAQLRRRAA (B07.02, B08.01) SQSARRAARM (B08.01)	KIRC, KIRP
TABLE 1D		**CRYPTIC EXON**		
AR-v7	cryptic final exon	SCKVFFKRAAEGKQKYLC ASRNDCTIDKFRRKNCPSC RLRKCYEAGMTLGEKFRV GNCKHLKMTRP*	GMTLGEKFRV (A02:01) RVGNCKHLK (A03.01)	Prostate Cancer, Castration-resistant Prostate Cancer
TABLE 1E		**OUT OF FRAME FUSIONS**		
AC011997.1:LRRC69	AC011997.1:LRRC69 *out-of-frame	MAGAPPPASLPPCSLISDCC ASNQRDSVGVGPSEP:G:**NNIKICNESASRK***	GPSEPGNNI (B07.02) KICNESASRK (A03.01)	LUSC, Breast Cancer, Head and Neck Cancer, LUAD
EEF1DP3	EEF1DP3:FRY *out-of-frame	HGWRPFLPVRARSRWNRR LDVTVANGR:S:**WKYGWS LLRVPQVNGIQVLNVSLK SSSNVISYE***	GIQVLNVSLK (A03.01) IQVLNVSLK (A03.01) KSSSNVISY (A01.01, A03.01) KYGWSLLRV (A24.02) RSWKYGWSL (A02.01) SLKSSSNVI (B08.01) SWKYGWSLL (A24.02) TVANGRSWK (A03.01) VPQVNGIQV (B07.02) VPQVNGIQVL (B07.02) VTVANGRSWK (A03.01) WSLLRVPQV (B08.01)	Breast Cancer

MAD1L1:MAFK	MAD1L1:MAFK	RLKEVFQTKIQEFRKACYT LTGYQIDITTENQYRLTSLY AEHPGDCLIFK::**LRVPGSS VLVTVPGL***	HPGDCLIFKL (B07.02) KLRVPGSSV (B07.02) KLRVPGSSVL (B07.02) RVPGSSVLV (A02.01) SVLVTVPGL (A02.01) VPGSSVLVTV (B07.02)	CLL
PPP1R1B:STARD3	PPP1R1B:STARD3	AEVLKVIRQSAGQKTTCGQ GLEGPWERPPPLDESERDG GSEDQVEDPALS:A:**LLLRP RPPRPEVGAHQDEQAAQ GADPRLGAQPACRGLPG LLTVPQPEPLLAPPSAA***	ALLLRPRPPR (A03.01) ALSALLLRPR (A03.01)	Breast Cancer
Table 1F		**IN-FRAME DELETIONS and FUSIONS**		
BCR:ABL	BCR:ABL	ERAEWRENIREQQKKCFRS FSLTSVELQMLTNSCVKLQ TVHSIPLTINKE::**EALQRPV ASDFEPQGLSEAARWNSK ENLLAGPSENDPNLFVAL YDFVASG**	LTINKEEAL (A02.01, B08.01)	CML, AML
BCR:ABL	BCR:ABL	ELQMLTNSCVKLQTVHSIP LTINKEDDESPGLYGFLNVI VHSATGFKQSS:K:**ALQRPV ASDFEPQGLSEAARWNSK ENLLAGPSENDPNLFVAL YDFVASGD**	IVHSATGFK (A03.01) ATGFKQSSK (A03.01)	CML, AML
C11orf95:RELA	C11orf95:RELA	ISNSWDAHLGLGACGEAEG LGVQGAEEEEEEEEEEEE GAGVPACPPKGP:E:**LFPLIF PAEPAQASGPYVEIIEQPK QRGMRFRYKCEGRSAGSI PGERSTD**	ELFPLIFPA (A02.01, B08.01) KGPELFPLI (A02.01, A24.02) KGPELFPLIF (A24.02)	Supretentorial ependyomas
CBFB:MYH11	(variant “type a”)	LQRLDGMGCLEFDEERAQ QEDALAQQAFEEARRRTRE FEDRDRSHREEME::**VHELE KSKRALETQMEEMKTQL EELEDELQATEDAKLRLE VNMQALKGQF**		AML
CD74:ROS1	(exon6:exon32)	KGSFPENLRHLKNTMETID WKVFESWMHHWLLFEMS RHSLEQKPTDAPPK::**AGVP NKPGIPKLLEGSKNSIQW EKAEDNGCRITYYILEIRK STSNNLQNQ**	KPTDAPPKAGV (B07.02)	NSCLC, Crizotinib resistance
EGFR	EGFRvIII (internal deletion)	MRPSGTAGAALLALLAAL CPASRALEEKK:G:NYVVTD HGSCVRACGADSYEMEED GVRKCKKCEGPCRKVCNGI GIGEFKD	ALEEKKGNYV (A02.01)	GBM

EGFR:SEPT14	EGFR:SEPT14	LPQPPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQ::**LQDKFEHLKMIQQEEIRKLEEEKKQLEGEIIDFYKMKAASEALQTQLSTD**	IQLQDKFEHL (A02.01, B08.01) QLQDKFEHL (A02.01, B08.01) QLQDKFEHLK (A03.01) YLVIQLQDKF (A02.01, A24.02)	GBM, Glioma, Head and Neck Cancer
EML4:ALK	EML4:ALK	SWENSDDSRNKLSKIPSTPKLIPKVTKTADKHKDVIINQAKMSTREKNSQ:V:**YRRKHQELQAMQMELQSPEYKLSKLRTSTIMTDYNPNYCFAGKTSSISDL**	QVYRRKHQEL (B08.01) STREKNSQV (B08.01) VYRRKHQEL (A24.02, B08.01)	NSCLC
FGFR3:TACC3	FGFR3:TACC3	EGHRMDKPANCTHDLYMIMRECWHAAPSQRPTFKQLVEDLDRVLTVTSTD::**VKATQEENRELRSRCEELHGKNLELGKIMDRFEEVVYQAMEEVQKQKELS**	VLTVTSTDV (A02.01) VLTVTSTDVK (A03.01)	Bladder Cancer, LUSC
NAB:STAT6	NAB:STAT6 ""	RDNTLLLRRVELFSLSRQVARESTYLSSLKGSRLHPEELGGPPLKKLKQE::ATSKSQI**MSLWGLVSKMPPEKVQRLYVDFPQHLRHLLGDWLESQPWEFLVGSDAFCC**	IMSLWGLVS (A02.01) IMSLWGLVSK (A03.01) KLKQEATSK (A03.01) QIMSLWGLV (A02.01) SQIMSLWGL (A02.01, A24.02, B08.01) SQIMSLWGLV (A02.01) TSKSQIMSL (B08.01)	Solitary fibrous tumors
NDRG1:ERG	NDRG1:ERG	MSREMQDVDLAEVKPLVEKGETITGLLQEFDVQ::**EALSVVSEDQSLFECAYGTPHLAKTEMTASSSSDYGQTSKMSPRVPQQDW**	LLQEFDVQEA (A02.01) LQEFDVQEAL (A02.01)	Prostate Cancer
PML:RARA	PML:RARA (exon3:exon3)	VLDMHGFLRQALCRLRQEEPQSLQAAVRTDGFDEFKVRLQDLSSCITQGK:A:**IETQSSSSEEIVPSPPSPPPLPRIYKPCFVCQDKSSGYHYGVSACEGCKG**		Acute promyelocytic leukemia
PML:RARA	PML:RARA (exon6:exon3)	RSSPEQPRPSTSKAVSPPHLDGPPSPRSPVIGSEVFLPNSNHVASGAGEA:**A**:**IETQSSSSEEIVPSPPSPPPLPRIYKPCFVCQDKSSGYHYGVSACEGCKG**		Acute promyelocytic leukemia
RUNX1	RUNX1(ex5)-RUNX1T1(ex2)	VARFNDLRFVGRSGRGKSFTLTITVFTNPPQVATYHRAIKITVDGPREPR:N:**RTEKHSTMPDSPVDVKTQSRLTPPTMPPPPTTQGAPRTSSFTPTTLTNGT**	GPREPRNRT (B07.02) RNRTEKHSTM (B08.01)	AML

TMPRSS2:ERG	TMPRSS2:ERG	MALNS::EALSVVSEDQSLFECAYGTPHLAKTEMTASSSSDYGQTSKMSPRVPQQDW	ALNSEALSV (A02.01) ALNSEALSVV (A02.01) MALNSEALSV (A02.01, B08.01)	Prostate Cancer

Table 2

Gene	Amino Acid Alteration	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
Table 2A		**POINT MUTATIONS** [1]		
AKT1	E17K	MSDVAIVKEGWLHKRGKYIKTWRPRYFLLKNDGTFIGYKERPQDVDQREAPLNNFSVAQCQLMKTER	KYIKTWRPRY (A24.02) WLHKRGKYI (A02.01, B07.02, B08.01) WLHKRGKYIK (A03.01)	BRCA, CESC, HNSC, LUSC, PRAD, SKCM, THCA
ANAPC1	T537A	TMLVLEGSGNLVLYTGVVRVGKVFIPGLPAPSLTMSNTMPRPSTPLDGVSAPKPLSKLLGSLDEVVLLSPVPELRDSSKLHDSLYNEDCTFQQLGTYIHSI	APKPLSKLL (B07.02) GVSAPKPLSK (A03.01) VSAPKPLSK (A03.01)	GBM, LUSC, PAAD, PRAD, SKCM
FGFR3	S249C	HRIGGIKLRHQQWSLVMESVVPSDRGNYTCVVENKFGSIRQTYTLDVLERCPHRPILQAGLPANQTAVLGSDVEFHCKVYSDAQPHIQWLKHVEVNGSKVG	CPHRPILQA (B07.02)	BLCA, HNSC, KIRP, LUSC
FRG1B	I10T	MREPIYMHSTMVFLPWELHTKKGPSPPEQFMAVKLSDSRTALKSGYGKYLGINSDELVGHSDAIGPREQWEPVFQNGKMALLASNSCFIR	KLSDSRTAL (A02.01, B07.02, B08.01) KLSDSRTALK (A03.01) LSDSRTALK (A01.01, A03.01) RTALKSGYGK (A03.01) TALKSGYGK (A03.01)	KIRP, PRAD, SKCM
FRG1B	L52S	AVKLSDSRIALKSGYGKYLGINSDELVGHSDAIGPREQWEPVFQNGKMALSASNSCFIRCNEAGDIEAKSKTAGEEEMIKIRSCAEKETKKKDDIPEEDKG	ALSASNSCF (A02.01, A24.02, B07.02) ALSASNSCFI (A02.01) FQNGKMALSA (A02.01, B08.01)	GBM, KIRP, PRAD, SKCM
HER2	L755S (Resistance)	AMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVSRENTSPKANKEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGC	KVSRENTSPK (A03.01)	BRCA

Gene	Amino Acid Alteration	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
IDH1	R132G	RVEEFKLKQMWKSP NGTIRNILGGTVFRE AIICKNIPRLVSGWV KPIIIGGHAYGDQYR ATDFVVPGPGKVEIT YTPSDGTQKVTYLV HNFEEGGGVAMGM	KPIIIGGHAY (B07.02)	BLCA, BRCA, CRC, GBM, HNSC, LUAD, PAAD, PRAD, UCEC
KRAS	G12C	MTEYKLVVVGACG VGKSALTIQLIQNHF VDEYDPTIEDSYRK QVVIDGETCLLDILD TAGQE	KLVVVGACGV (A02.01) LVVVGACGV (A02.01) VVGACGVGK (A03.01, A11.01) VVVGACGVGK (A03.01)	BRCA, CESC, CRC, HNSC, LUAD, PAAD, UCEC
KRAS	G12D	MTEYKLVVVGADG VGKSALTIQLIQNHF VDEYDPTIEDSYRK QVVIDGETCLLDILD TAGQE	VVGADGVGK (A11.01) VVVGADGVGK (A11.01) KLVVVGADGV (A02.01) LVVVGADGV (A02.01)	BLCA, BRCA, CESC, CRC, GBM, HNSC, KIRP, LIHC, LUAD, PAAD, SKCM, UCEC
KRAS	G12V	MTEYKLVVVGAVG VGKSALTIQLIQNHF VDEYDPTIEDSYRK QVVIDGETCLLDILD TAGQE	KLVVVGAVGV (A02.01) LVVVGAVGV (A02.01) VVGAVGVGK (A03.01, A11.01) VVVGAVGVGK (A03.01, A11.01)	BRCA, CESC, CRC, LUAD, PAAD, THCA, UCEC
KRAS	Q61H	AGGVGKSALTIQLIQ NHFVDEYDPTIEDSY RKQVVIDGETCLLDI LDTAGHEEYSAMRD QYMRTGEGFLCVFA INNTKSFEDIHHYRE QIKRVKDSEDVPM	ILDTAGHEEY (A01.01)	CRC, LUSC, PAAD, SKCM, UCEC
KRAS	Q61L	AGGVGKSALTIQLIQ NHFVDEYDPTIEDSY RKQVVIDGETCLLDI LDTAGLEEYSAMRD QYMRTGEGFLCVFA INNTKSFEDIHHYRE QIKRVKDSEDVPM	ILDTAGLEEY (A01.01) LLDILDTAGL (A02.01)	CRC, GBM, HNSC, LUAD, SKCM, UCEC
NRAS	Q61K	AGGVGKSALTIQLIQ NHFVDEYDPTIEDSY RKQVVIDGETCLLDI LDTAGKEEYSAMRD QYMRTGEGFLCVFA INNSKSFADINLYRE QIKRVKDSDDVPM	ILDTAGKEEY (A01.01)	BLCA, CRC, LIHC, LUAD, LUSC, SKCM, THCA, UCEC
NRAS	Q61R	AGGVGKSALTIQLIQ NHFVDEYDPTIEDSY RKQVVIDGETCLLDI LDTAGREEYSAMRD QYMRTGEGFLCVFA INNSKSFADINLYRE QIKRVKDSDDVPM	ILDTAGREEY (A01.01)	BLCA, CRC, LUSC, PAAD, PRAD, SKCM, THCA, UCEC

Gene	Amino Acid Alteration	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
PIK3CA	E542K	IEEHANWSVSREAG FSYSHAGLSNRLAR DNELRENDKEQLKA ISTRDPLSKITEQEKD FLWSHRHYCVTIPEI LPKLLLSVKWNSRD EVAQMYCLVKDWP P	AISTRDPLSK (A03.01)	BLCA, BRCA, CESC, CRC, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, UCEC
PTEN	R130Q	KFNCRVAQYPFEDH NPPQLELIKPFCEDL DQWLSEDDNHVAAI HCKAGKGQTGVMIC AYLLHRGKFLKAQE ALDFYGEVRTRDKK GVTIPSQRRYVYYY SY	QTGVMICAY (A01.01)	BRCA, CESC, CRC, GBM, KIRC, LUSC, UCEC
RAC1	P29S	MQAIKCVVVGDGA VGKTCLLISYTTNAF SGEYIPTVFDNYSAN VMVDGKPVNLGLW DTAGQEDYDRLRPL SYPQTVGET	FSGEYIPTV (A02.01) TTNAFSGEY (A01.01) YTTNAFSGEY (A01.01)	Melanoma
SF3B1	K700E	AVCKSKKSWQARH TGIKIVQQIAILMGC AILPHLRSLVEIIEHG LVDEQQEVRTISALA IAALAEAATPYGIES FDSVLKPLWKGIRQ HRGKGLAAFLKAI	GLVDEQQEV (A02.01)	AML associated with MDS; Chronic lymphocytic leukaemia-small lymphocytic lymphoma; Myelodysplastic syndrome; AML; Luminal NS carcinoma of breast; Chronic myeloid leukaemia; Ductal carcinoma of pancreas; Chronic myelomonocytic leukaemia; Chronic lymphocytic leukaemia-small lymphocytic lymphoma; Myelofibrosis; Myelodysplastic syndrome; PRAD; Essential thrombocythaemia; Medullomyoblastoma

Gene	Amino Acid Alteration	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
SPOP	F133L	YLSLYLLLVSCPKSEVRAKFKFSILNAKGEETKAMESQRAYRFVQGKDWGLKKFIRRDFLLDEANGLLPDDKLTLFCEVSVVQDSVNISGQNTMNMVKVPE	FVQGKDWGL (A02.01, B08.01)	PRAD
SPOP	F133V	YLSLYLLLVSCPKSEVRAKFKFSILNAKGEETKAMESQRAYRFVQGKDWGVKKFIRRDFLLDEANGLLPDDKLTLFCEVSVVQDSVNISGQNTMNMVKVPE	FVQGKDWGV (A02.01)	PRAD
TP53	G245S	IRVEGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGSMNRRPILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKGEP	CMGSMNRRPI (A02.01, B08.01) GSMNRRPIL (B08.01) MGSMNRRPI (B08.01) MGSMNRRPIL (B08.01) SMNRRPILTI (A02.01, A24.02, B08.01)	BLCA, BRCA, CRC, GBM, HNSC, LUSC, PAAD, PRAD
TP53	R248Q	EGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNQRPILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKGEPHHE	CMGGMNQRPI (A02.01, B08.01) GMNQRPILTI (A02.01, B08.01) NQRPILTII (A02.01, B08.01)	BLCA, BRCA, CRC, GBM, HNSC, KIRC, LIHC, LUSC, PAAD, PRAD, UCEC
TP53	R248W	EGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNWRPILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKGEPHHE	CMGGMNWRPI (A02.01, A24.02, B08.01) GMNWRPILTI (A02.01, B08.01) MNWRPILTI (A02.01, A24.02, B08.01) MNWRPILTII (A02.01, A24.02)	BLCA, BRCA, CRC, GBM, HNSC, LIHC, LUSC, PAAD, SKCM, UCEC
TP53	R273C	PEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVCVCACPGRDRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPL	NSFEVCVCA (A02.01)	BLCA, BRCA, CRC, GBM, HNSC, LUSC, PAAD, UCEC

Gene	Amino Acid Alteration	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
TP53	R273H	PEVGSDCTTIHYNY MCNSSCMGGMNRR PILTIITLEDSSGNLL GRNSFEVHVCACPG RDRRTEEENLRKKG EPHHELPPGSTKRAL PNNTSSSPQPKKKPL	NSFEVHVCA (A02.01)	BRCA, CRC, GBM, HNSC, LIHC, LUSC, PAAD, UCEC
TP53	Y220C	TEVVRRCPHHERCS DSDGLAPPQHLIRVE GNLRVEYLDDRNTF RHSVVVPCEPPEVGS DCTTIHYNYMCNSS CMGGMNRRPILTIIT LEDSSGNLLGRNSF	VVPCEPPEV (A02.01) VVVPCEPPEV (A02.01)	BLCA, BRCA, GBM, HNSC, LIHC, LUAD, LUSC, PAAD, SKCM, UCEC
Table 2B		**MSI-ASSOCIATED FRAMESHIFTS [1]**		
MSH6	F1088fs; +1	YNFDKNYKDWQSA VECIAVLDVLLCLA NYSRGGDGPMCRPV ILLPEDTPPLLRA	ILLPEDTPPL (A02.01) LLPEDTPPL (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
Table 2C		**FRAMESHIFT [1]**		
APC	F1354fs	AKFQQCHSTLEPNP ADCRVLVYLQNQPG TKLLNFLQERNLPPK VVLRHPKVHLNTMF RRPHSCLADVLLSV HLIVLRVVRLPAPFR VNHAVEW*	APFRVNHAV (B07.02) CLADVLLSV (A02.01) FLQERNLPPK (A03.01) HLIVLRVVRL (A02.01, B08.01) HPKVHLNTM (B07.02, B08.01) HPKVHLNTMF (B07.02, B08.01) KVHLNTMFR (A03.01) KVHLNTMFRR (A03.01) LPAPFRVNHA (B07.02) MFRRPHSCL (B07.02, B08.01) MFRRPHSCLA (B08.01) NTMFRRPHSC (B08.01) RPHSCLADV (B07.02) RPHSCLADVL (B07.02) RVVRLPAPFR (A03.01) SVHLIVLRV (A02.01) TMFRRPHSC (B08.01) TMFRRPHSCL (A02.01, B08.01) VLLSVHLIV (A02.01) VLLSVHLIVL (A02.01) VLRVVRLPA (B08.01) VVRLPAPFR (A03.01)	CRC, LUAD, UCEC, STAD

[0287] A subset of peptides from Table 1 (n=562) were synthesized and their affinity for their given HLA class I molecule was measured as described. The values are shown in Table 3. These data show a strong correlation between prediction and measurement (dotted line represents best fit, $R^2 = 0.45$), demonstrating the value of the predictions. However, the outliers demonstrate the importance of these

measurements. Thick vertical and horizontal lines are shown at 500 nM for the predicted affinity and observed affinity, respectively. 500nM is commonly accepted in the field as the maximum affinity for an epitope that is a "weak binder" to HLA class I. Therefore, the points in the lower right quadrant (prediction greater than 500 nM, measurement less than 500 nM) are epitopes that were considered very weak binders but were observed to bind within an acceptable range. Epitopes in this quadrant (n=75) represent 30.5% of epitopes not considered to be binders by prediction (combination of bottom right and top right quadrants, n=246).

Table 3

Mutation	**Allele**	**Peptide**	**Predicted affinity (IC_{50}; (nM))**	**Observed Affinity (IC_{50}; (nM))**	**Stability ($T_{1/2}$ (h))**
ABL1, M351T	A02.01	TQISSATEYL	2921.0	2644.0	0
ABL1, T315I	A02.01	YIIIEFMTYG	3502.0	186.0	0
ABL1, T315I	A02.01	IIIEFMTYG	1991.0	779.0	0
ABL1, T315I	A02.01	IIIEFMTYGN	16793.0	1551.0	0
ABL1, T315I	A02.01	IIEFMTYGNL	2134.0	9702.0	0
ABL1, Y253H	A02.01	KLGGGQHGEV	1705.0	387.0	0.4
AKT1, E17K	B08.01	WLHKRGKYI	47.0	417.0	1.3
AKT1, E17K	A02.01	WLHKRGKYI	4972.0	1250.0	1.2
AKT1, E17K	B07.02	WLHKRGKYI	7185.0	2648.0	0
ALK, G1269A	A02.01	RVAKIADFGM	5258.0	125.0	0.5
ALK, G1269A	B07.02	RVAKIADFGM	7260.0	9723.0	0.2
ALK, L1196M	A02.01	SLPRFILMEL	94.0	26.0	0.5
ALK, L1196M	A02.01	ILMELMAGG	192.0	223.0	0.5
ALK, L1196M	A02.01	LMELMAGGDL	5617.0	311.0	8.9
ALK, L1196M	A02.01	LQSLPRFILM	2519.0	413.0	0
ALK, L1196M	B07.02	SLPRFILMEL	17.0	583.0	0.4
ALK, L1196M	B08.01	LQSLPRFILM	1288.0	1547.0	0
ALK, L1196M	A02.01	FILMELMAGG	189.0	1580.0	0
ALK, L1196M	B08.01	SLPRFILMEL	686.0	1762.0	0
ALK, L1196M	A24.02	SLPRFILMEL	5143.0	2774.0	0.2
ALK, L1196M	A02.01	ILMELMAGGD	5761.0	3451.0	0
APC, AVEW	A02.01	VLLSVHLIV	36.0	72.0	11
APC, AVEW	A02.01	CLADVLLSV	5.0	219.0	24
APC, VHPA	A02.01	KVLQMDFLV	25.0	11.0	6.4
APC, VHPA	A02.01	LQMDFLVHPA	26.0	68.0	1.5
β2M, …MPAV	A03.01	TTLNSPPLKK	62.5	14.3	not measured
β2M, …MPAV	A03.01	TTLNSPPLK	165.4	9.8	not measured
β2M, …MPAV	A03.01	TLNSPPLKK	27.5	5.4	not measured
β2M, …MPAV	A03.01	CTTLNSPPLK	225.3	63.6	not measured

β2M, …MPAV	B08.01	CLSARTGLSI	1106.6	149.6	not measured
β2M, …MPAV	A02.01	GLSISCTTL	669.0	114.5	not measured
β2M, …SIRH	A03.01	LTSSSREWK	413.9	117.8	not measured
β2M, …SIRH	A03.01	LLTSSSREWK	206.1	1769.8	not measured
β2M, …SIRH	B07.02	YPAYSKDSGL	41.1	79.5	not measured
β2M, …SIRH	B08.01	EWKVKFPEL	488.7	538.4	not measured
β2M, …SIRH	A24.02	KFPELLCVW	83.7	13.7	not measured
β2M, …SQIS	B08.01	LQRFRFTHV	55.5	37.3	not measured
β2M, …SQIS	A24.02	RLSSVLQRF	288.9	28.2	not measured
β2M, …SQIS	A02.01	VLQRFRFTHV	163.4	106.7	not measured
β2M, …SQIS	B08.01	VLQRFRFTHV	264.1	480.1	not measured
β2M, …SQIS	A03.01	RLSSVLQRFR	168.1	12.5	not measured
BCR:ABL (e13a2, aka b2a2)	B08.01	LTINKEEAL	4972.0	895.0	0
BCR:ABL (e13a2, aka b2a2)	A02.01	LTINKEEAL	12671.0	4413.0	0
BRAF, V600E	A02.01	LATEKSRWSG	39130.0	23337.0	0
BRAF, V600E	B08.01	LATEKSRWS	24674.0	36995.0	0
BRAF, V600E	B08.01	LATEKSRWSG	13368.0	46582.0	0
BRAF, V600E	A02.01	LATEKSRWS	39109.0	60997.0	0
BTK, C481S	A02.01	SLLNYLREM	48.0	87.0	3
BTK, C481S	A02.01	MANGSLLNYL	2979.0	1082.0	0
BTK, C481S	B07.02	SLLNYLREM	6544.0	1110.0	0
BTK, C481S	B08.01	SLLNYLREM	1091.0	1230.0	0
BTK, C481S	A02.01	YMANGSLLN	7856.0	4444.0	0
BTK, C481S	B07.02	MANGSLLNYL	8921.0	17715.0	0
BTK, C481S	B08.01	MANGSLLNYL	7639.0	19853.0	0
BTK, C481S	A03.01	MANGSLLNY	1030.3	35.6	not measured
BTK, C481S	A01.01	MANGSLLNY	285.7	439.0	not measured
BTK, C481S	A24.02	EYMANGSLL	213.2	5.0	not measured
BTK, C481S	A01.01	YMANGSLLNY	95.7	13.2	not measured
BTK, C481S	A03.01	YMANGSLLNY	109.4	95.9	not measured

C11orf95:RELA	A02.01	ELFPLIFPA	13.0	13.0	5.1
C11orf95:RELA	A24.02	KGPELFPLI	909.0	14.0	1.7
C11orf95:RELA	A02.01	KGPELFPLI	6840.0	101.0	0.3
C11orf95:RELA	B08.01	ELFPLIFPA	7316.0	449.0	0
C15ORF40(+1)	A02.01	KLFSCLSFL	6.0	6.0	14.3
C15ORF40(+1)	A03.01	KLFSCLSFL	1488.0	308.0	0.8
C15ORF40(+1)	A03.01	SLQPPPPGFK	26.3	19.0	not measured
C15ORF40(+1)	A03.01	LFFFFFETK	658.4	413.3	not measured
C15ORF40(+1)	A02.01	ALFFFFFET	28.9	470.7	not measured
C15ORF40(+1)	A03.01	ALFFFFFETK	31.5	216.4	not measured
C15ORF40(+1)	A02.01	FFFETKSCSV	754.5	61.2	not measured
C15ORF40(+1)	B08.01	FFETKSCSV	807.6	7.6	not measured
C15ORF40(+1)	A01.01	LSFLSSWDY	211.1	52.9	not measured
C15ORF40(+1)	A02.01	FLSSWDYRRM	62.2	323.5	not measured
C15ORF40(+1)	A03.01	LSFLSSWDYR	508.7	100.9	not measured
C15ORF40(+1)	A02.01	FKLFSCLSFL	9.9	662.9	not measured
C15ORF40(+1)	A02.01	VQWRSLGSL	986.0	4733.2	not measured
C15ORF40(+1)	A02.01	KLFSCLSFLS	65.1	0.6	not measured
C15ORF40(+1)	A03.01	KLFSCLSFLS	805.1	104.0	not measured
C15ORF40(+1)	A02.01	AQAGVQWRSL	630.2	670.0	not measured
C15ORF40(+1)	A24.02	RRMPPCLANF	253.0	141.1	not measured
C15ORF40(+1)	A03.01	CLSFLSSWDY	890.5	2705.8	not measured
C15ORF40(+1)	A24.02	GFKLFSCLSF	387.4	643.0	not measured
C15ORF40(+1)	A24.02	RMPPCLANF	34.4	8.7	not measured
C15ORF40(+1)	A03.01	CLANFCIFNR	575.4	221.8	not measured
C15ORF40(+1)	A01.01	CLSFLSSWDY	538.7	987.3	not measured
CNOT1(+1)	A02.01	SVCFFFFSV	27.0	175.0	9.4
CNOT1(+1)	B08.01	SVCFFFFSV	4940.0	10599.0	0
CNOT1(+1)	A02.01	MSVCFFFFSV	131.0	1706.4	not measured
CNOT1(+1)	A02.01	FFFSVIFST	608.9	4556.0	not measured

CNOT1(-1)	A01.01	MSVCFFFFCY	310.4	4369.3	not measured
CNOT1(-1)	A02.01	SVCFFFFCYI	237.2	519.8	not measured
CNOT1(-1)	A24.02	FFCYILNTMF	583.4	73.4	not measured
EGFR, T790M	A02.01	MQLMPFGCLL	21.0	20.0	0.4
EGFR, T790M	A02.01	MQLMPFGCL	842.0	166.0	0.4
EGFR, T790M	A02.01	LIMQLMPFGC	1984.0	177.0	0.4
EGFR, T790M	A02.01	QLIMQLMPF	2511.0	227.0	0.3
EGFR, T790M	B08.01	QLIMQLMPF	891.0	388.0	0
EGFR, T790M	B08.01	IMQLMPFGCL	1302.0	548.0	0
EGFR, T790M	A02.01	CLTSTVQLIM	3465.0	716.0	0
EGFR, T790M	A02.01	IMQLMPFGCL	143.0	837.0	0.5
EGFR, T790M	A02.01	IMQLMPFGC	1123.0	1607.0	0.4
EGFR, T790M	B07.02	MQLMPFGCL	10169.0	2270.0	0
EGFR, T790M	A24.02	QLIMQLMPF	3209.0	2389.0	0.4
EGFR, T790M	A02.01	LIMQLMPFG	4961.0	3513.0	0
EGFR, T790M	A24.02	VQLIMQLMPF	1455.0	4559.0	0
EGFR, T790M	A02.01	VQLIMQLMPF	4464.0	5492.0	0
EGFR, T790M	A02.01	QLIMQLMPFG	5751.0	5926.0	0
EGFR, T790M	B08.01	MQLMPFGCL	1105.0	7045.0	0
EGFR, T790M	A02.01	STVQLIMQL	2151.0	8537.0	0
EGFR, T790M	A01.01	CLTSTVQLIM	2998.0	11036.0	0
EGFR, T790M	B08.01	MQLMPFGCLL	970.0	14056.0	0
EGFR, T790M	B08.01	VQLIMQLMPF	3370.0	17898.0	0
EGFR, T790M	A24.02	IMQLMPFGCL	4394.0	18102.0	0
EGFR, T790M	A24.02	MQLMPFGCLL	4168.0	23572.0	0
EGFR, T790M	A01.01	LTSTVQLIM	1000.7	2891.1	not measured
EGFR:SEPT14	B08.01	QLQDKFEHL	917.0	989.0	0
EGFR:SEPT14	A02.01	QLQDKFEHL	422.0	1155.0	0.6
EGFR:SEPT14	A02.01	YLVIQLQDKF	9963.0	2057.0	0
EGFR:SEPT14	A24.02	YLVIQLQDKF	9508.0	2152.0	2.6
EGFR:SEPT14	A02.01	IQLQDKFEHL	820.0	4265.0	0.2
EGFR:SEPT14	B08.01	IQLQDKFEHL	4278.0	10247.0	0
EGFRvIII (internal deletion)	A02.01	ALEEKKGNYV	2445.0	141.0	0
EIF2B3(-1)	A02.01	KQWSSVTSL	54.4	26.5	not measured
EML4:ALK	B08.01	QVYRRKHQEL	194.0	160.0	0
EPHB2(-1)	A02.01	ILIRKAMTV	38.2	19.5	not measured
ESR1, D538G	A24.02	PLYGLLLEML	1519.0	444.0	6.3
ESR1, D538G	A02.01	GLLLEMLDA	705.0	558.0	0.4
ESR1, D538G	A02.01	PLYGLLLEM	349.0	640.0	0.7
ESR1, D538G	A24.02	VVPLYGLLL	2965.0	658.0	0.8

ESR1, D538G	A02.01	PLYGLLLEML	542.0	797.0	0
ESR1, D538G	A02.01	VVPLYGLLL	4432.0	1039.0	0.6
ESR1, D538G	A02.01	NVVPLYGLL	4835.0	10471.0	0
ESR1, D538G	B07.02	VPLYGLLLEM	145.1	27.9	not measured
ESR1, D538G	A24.02	LYGLLLEML	218.3	0.8	not measured
ESR1, S463P	A02.01	FLPSTLKSL	71.0	21.0	2.1
ESR1, S463P	A02.01	GVYTFLPST	307.0	779.0	1.3
ESR1, S463P	A24.02	FLPSTLKSL	10723.0	995.0	1
ESR1, S463P	A02.01	GVYTFLPSTL	248.0	1197.0	0.4
ESR1, S463P	B08.01	FLPSTLKSL	2314.0	1968.0	0
ESR1, S463P	A24.02	GVYTFLPSTL	954.0	7696.0	0
ESR1, Y537C	A02.01	PLCDLLLEM	1067.0	602.0	0.8
ESR1, Y537C	A02.01	VVPLCDLLL	5533.0	1200.0	0
ESR1, Y537C	A02.01	NVVPLCDLLL	1964.0	1373.0	0
ESR1, Y537C	A02.01	PLCDLLLEML	1320.0	2008.0	0.9
ESR1, Y537C	A02.01	NVVPLCDLL	3473.0	3027.0	0
ESR1, Y537C	A24.02	VVPLCDLLL	7992.0	3888.0	0.4
ESR1, Y537N	A02.01	PLNDLLLEM	1062.0	151.0	4.2
ESR1, Y537N	A02.01	NVVPLNDLL	4725.0	2900.0	0
ESR1, Y537N	A02.01	NVVPLNDLLL	2606.0	4190.0	0
ESR1, Y537N	A02.01	PLNDLLLEML	1741.0	11957.0	0
ESR1, Y537S	A02.01	PLSDLLLEM	713.0	404.0	2.7
ESR1, Y537S	A02.01	NVVPLSDLLL	2510.0	741.0	0
ESR1, Y537S	A02.01	NVVPLSDLL	4259.0	916.0	0
ESR1, Y537S	A02.01	VVPLSDLLL	8320.0	2551.0	0
ESR1, Y537S	A02.01	PLSDLLLEML	1138.0	6469.0	0
ESR1, Y537S	A24.02	VVPLSDLLL	8463.0	8252.0	0.5
FAM111B(-1)	A03.01	RMKVPLMK	58.9	33.0	not measured
FGFR3, S249C	A02.01	YTLDVLERC	3309.0	1764.0	6.6
FGFR3, S249C	B08.01	VLERCPHRPI	3629.0	7223.0	0
FGFR3, S249C	A02.01	VLERCPHRPI	4505.0	15321.0	0
FGFR3:TACC3	A02.01	VLTVTSTDV	1255.0	295.0	1.1
FRG1B, I10T	B07.02	KLSDSRTAL	225.0	9.0	6.8
FRG1B, I10T	A02.01	KLSDSRTAL	275.0	111.0	2.8
FRG1B, I10T	B08.01	KLSDSRTAL	3276.0	122.0	0
FRG1B, L52S	A02.01	ALSASNSCFI	327.0	226.0	0.8
FRG1B, L52S	B08.01	FQNGKMALSA	7796.0	425.0	0
FRG1B, L52S	B07.02	ALSASNSCF	13989.0	684.0	0
FRG1B, L52S	A02.01	ALSASNSCF	7913.0	728.0	0.3
FRG1B, L52S	A02.01	FQNGKMALS	2305.0	3276.0	0
FRG1B, L52S	A02.01	FQNGKMALSA	1205.0	6158.0	0
FRG1B, L52S	A24.02	ALSASNSCF	9672.0	16338.0	0.2
GATA3 …CSNH	B08.01	FLKAESKIM	263.4	21.9	not measured

GATA3 …CSNH	B08.01	LQHGHRHGL	693.0	550.4	not measured
GATA3 …CSNH	B07.02	EPHLALQPL	106.6	17.0	not measured
GATA3 …CSNH	B07.02	RPLQTHVLPE	968.0	2534.4	not measured
GATA3 …CSNH	B08.01	FATLQRSSL	138.0	26.6	not measured
GATA3 …CSNH	B07.02	MFATLQRSSL	1285.0	266.9	not measured
GATA3 …CSNH	A24.02	MFLKAESKI	1065.7	332.1	not measured
GATA3 …CSNH	B07.02	FATLQRSSL	261.9	14.0	not measured
GATA3 …CSNH	A02.01	MLTGPPARV	145.4	10.6	not measured
GATA3 …CSNH	B08.01	EPHLALQPL	1128.3	12.4	not measured
GATA3 …CSNH	B07.02	GPPARVPAV	297.6	221.2	not measured
GATA3 …CSNH	B08.01	MFATLQRSSL	220.5	53.4	not measured
GATA3 …CSNH	A02.01	ALQPLQPHA	644.4	603.9	not measured
GATA3 …CSNH	A03.01	VLWTTPPLQH	962.3	16.0	not measured
GATA3 …CSNH	A02.01	VLPEPHLAL	140.7	16.0	not measured
GATA3 …CSNH	B07.02	HVLPEPHLAL	1057.2	1332.6	not measured
GATA3 …CSNH	A03.01	YMFLKAESK	53.1	79.8	not measured
GATA3 …CSNH	B07.02	VPAVPFDLHF	1996.2	2114.2	not measured
GATA3 …CSNH	A02.01	AIQPVLWTT	229.3	8.1	not measured
GATA3 …CSNH	A02.01	TLQRSSLWCL	319.2	117.7	not measured
GATA3 …CSNH	A03.01	KIMFATLQR	62.5	2.5	not measured
GATA3 …CSNH	B07.02	QPVLWTTPPL	54.4	109.3	not measured
GATA3 …CSNH	B08.01	ESKIMFATL	253.7	17.7	not measured
GATA3 …CSNH	B08.01	IMKPKRDGYM	342.1	33.2	not measured
GATA3 …CSNH	B07.02	KPKRDGYMF	109.7	28.2	not measured
GATA3 …CSNH	B08.01	FLKAESKIMF	1539.9	82.3	not measured
GATA3 …CSNH	B07.02	KPKRDGYMFL	32.5	98.1	not measured

GATA3 …CSNH	B08.01	LHFCRSSIM	2141.2	118.7	not measured
GATA3 CSNH	A02.01	SMLTGPPARV	57.0	15.0	21.7
GATA3 CSNH	B08.01	YMFLKAESKI	606.0	32.0	0.4
GATA3 CSNH	A02.01	YMFLKAESKI	163.0	166.0	0.6
GATA3 CSNH	A03.01	YMFLKAESKI	1338.0	21111.0	0
GATA3 YHEA	A02.01	FLQEQYHEA	7.0	11.0	9.5
GATA3 YHEA	B08.01	FLQEQYHEA	1222.0	2285.0	0
GBP3(-1)	B08.01	TLKKKPRDI	286.3	3.3	not measured
HER2, G776insYVMA	A02.01	VMAYVMAGV	6.0	2.0	16.5
HER2, G776insYVMA	A02.01	YVMAYVMAGV	5.0	57.0	20.9
HER2, G776insYVMA	B07.02	YVMAYVMAG	6910.0	170.0	0
HER2, G776insYVMA	B08.01	YVMAYVMAGV	721.0	353.0	0
HER2, G776insYVMA	A02.01	YVMAYVMAG	841.0	11535.0	2.5
HER2, G776insYVMA	B08.01	YVMAYVMAG	836.0	19413.0	1.2
HER2, G776insYVMA	B07.02	YVMAYVMAGV	11445.0	52630.0	0
HER2, L755S	A03.01	KVSRENTSPK	66.0	7.0	13.5
HER2, V777L	A02.01	VMAGLGSPYV	20.0	102.0	2.9
HER2, V777L	A03.01	VMAGLGSPYV	3951.0	11222.0	0
JAK1(-1)	A02.01	SLMPAHWSI	4.0	48.0	5
JAK1(-1)	A02.01	LSLMPAHWSI	21.0	164.0	0.4
JAK1(-1)	B08.01	SLMPAHWSI	282.0	177.0	0
JAK1(-1)	A02.01	FQMQPLSLM	33.0	553.0	0.3
JAK1(-1)	A24.02	SLMPAHWSI	194.0	633.0	0.4
JAK1(-1)	B08.01	LSLMPAHWSI	1914.0	860.0	0
JAK1(-1)	B07.02	SLMPAHWSI	3907.0	1040.0	0
JAK1(-1)	B08.01	FQMQPLSLM	2261.0	6714.0	0
JAK1(-1)	B07.02	FQMQPLSLM	3458.0	10207.0	0
JAK1(-1)	A24.02	LSLMPAHWSI	2125.0	12398.0	0
JAK1(-1)	A24.02	FQMQPLSLM	4021.0	14612.0	0
KIT, T670I	A02.01	VIIEYCCYG	4225.0	191.0	0.5
KIT, T670I	A02.01	IIEYCCYGDL	3918.0	7310.0	0
KIT, T670I	A02.01	TIGGPTLVII	5425.0	10685.0	0
KIT, V654A	A02.01	YLGNHMNIA	92.0	117.0	0.6
KIT, V654A	A02.01	MNIANLLGA	4522.0	128.0	0.3
KIT, V654A	A02.01	HMNIANLLGA	294.0	430.0	0
KIT, V654A	B08.01	YLGNHMNIA	2480.0	872.0	0
KIT, V654A	A02.01	YLGNHMNIAN	7103.0	1342.0	0
KIT, V654A	A02.01	IANLLGACTI	11214.0	6417.0	0
KRAS, G12C	A02.01	KLVVVGACGV	204.0	150.0	1

KRAS, G12C	A02.01	LVVVGACGV	658.0	1213.0	0.6
KRAS, G12C	A03.01	VVVGACGVGK	300.7	1.6	not measured
KRAS, G12C	A03.01	VVGACGVGK	182.0	4.1	not measured
KRAS, G12D	A02.01	KLVVVGADGV	361.0	184.0	0.9
KRAS, G12D	A02.01	LVVVGADGV	2120.0	1192.0	0
KRAS, G12V	A02.01	KLVVVGAVGV	163.0	96.0	0.9
KRAS, G12V	A02.01	LVVVGAVGV	453.0	975.0	0.6
KRAS, G12V	A03.01	VVGAVGVGK	168.9	1.9	not measured
KRAS, Q61H	A01.01	ILDTAGHEEY	131.8	64.2	not measured
KRAS, Q61L	A01.01	ILDTAGLEEY	65.9	8.6	not measured
KRAS, Q61L	A02.01	LLDILDTAGL	113.4	715.7	not measured
LMAN1(+1)	B07.02	GPPRPPRAAC	69.3	48.6	not measured
LMAN1(+1)	B07.02	PPRPPRAAC	263.7	32.8	not measured
LMAN1(-1)	B08.01	SLRRKYLRV	28.0	0.4	not measured
MEK, C121S	A02.01	VLHESNSPYI	189.0	131.0	1.9
MEK, P124L	A02.01	VLHECNSLYI	67.0	10.0	5.1
MEK, P124L	A02.01	SLYIVGFYGA	104.0	390.0	0.4
MEK, P124L	A02.01	SLYIVGFYG	2987.0	1063.0	0
MEK, P124L	A02.01	LQVLHECNSL	5803.0	4723.0	0
MEK, P124L	A02.01	QVLHECNSL	8695.0	7774.0	0.5
MEK, P124L	A03.01	VLHECNSLYI	4733.0	10500.0	0
MEK, P124L	B08.01	QVLHECNSL	6854.0	14532.0	0
MEK, P124L	B08.01	LQVLHECNSL	2782.0	19316.0	0
MLL2, …LSPH	A02.01	LLQVTQTSFA	1935.0	676.9	not measured
MLL2, …LSPH	A02.01	RLWHLLLQV	8.3	1.3	not measured
MLL2, …LSPH	A02.01	LQVTQTSFAL	1147.4	718.3	not measured
MLL2, …LSPH	A02.01	RLWHLLLQVT	140.8	50.9	not measured
MLL2, …LSPH	A02.01	ALAPTLTHM	98.4	59.0	not measured
MLL2, …LSPH	A02.01	ALAPTLTHML	66.4	39.0	not measured
MLL2, CRLS	B08.01	SLGNHLCPL	136.0	6.0	0.5
MLL2, CRLS	A02.01	SLGNHLCPL	28.0	18.0	3.5
MLL2, CRLS	B07.02	SLGNHLCPL	3967.0	2590.0	0
MSH3(-1)	A02.01	LLALWECSL	46.0	15.0	4
MSH3(-1)	A02.01	FLLALWECSL	17.0	114.0	10.8
MSH3(-1)	B08.01	LLALWECSL	1454.0	154.0	0

MSH3(-1)	B08.01	FLLALWECSL	671.0	13100.0	0
MSH3(-1)	A02.01	LIVSRTLLL	755.0	173.5	not measured
MSH3(-1)	A02.01	LIVSRTLLLV	146.6	10920.6	not measured
MSH3(-1)	B08.01	LIVSRTLLL	270.7	881.7	not measured
MSH3(-1)	A02.01	IVSRTLLLV	166.2	12.7	not measured
MSH3(-1)	B08.01	SLPQARLCLI	632.3	4313.9	not measured
MSH3(-1)	B08.01	CLIVSRTLLL	835.7	1100.4	not measured
MSH3(-1)	B08.01	LPQARLCLI	136.5	15.0	not measured
MSH3(-1)	A02.01	SLPQARLCLI	782.4	112.9	not measured
MSH3(-1)	A02.01	CLIVSRTLLL	560.5	2005.1	not measured
MSH3(-1)	A02.01	FLLALWECS	686.6	93.2	not measured
MSH3(-1)	A02.01	FLLALWECSL	16.6	0.9	not measured
MSH3(-1)	A02.01	LLALWECSL	46.1	12.5	not measured
MSH3(-1)	B07.02	LPQARLCLI	134.6	72.6	not measured
MSH3(-1)	B08.01	CLIVSRTLL	915.0	126.7	not measured
MSH3(-1)	A02.01	ALWECSLPQA	24.6	9.0	not measured
MSH3(-1)	B08.01	LPQARLCLIV	591.4	152.1	not measured
MSH6(+1)	A02.01	LLPEDTPPL	8.9	2.8	not measured
MSH6(+1)	A02.01	ILLPEDTPPL	16.3	6.7	not measured
MYC, E39D	A02.01	QQSDLQPPA	4930.0	70.0	0
MYC, E39D	A02.01	QQQSDLQPPA	11835.0	646.0	0
MYC, E39D	A02.01	YQQQQQSDL	8842.0	799.0	0.4
MYC, E39D	B08.01	YQQQQQSDL	5259.0	18868.0	0
MYC, P57S	A02.01	FELLSTPPL	2509.0	225.0	0
MYC, P57S	A02.01	LLSTPPLSPS	5226.0	1770.0	0
MYC, P57S	B08.01	FELLSTPPL	4208.0	3179.0	0
MYC, T58I	A02.01	LLPIPPLSPS	2071.0	449.0	0
MYC, T58I	A02.01	FELLPIPPL	2472.0	553.0	0
NAB:STAT6 (“variant 1” of Chmielecki et al.)	A02.01	SQIMSLWGL	14.0	62.0	1

NAB:STAT6 ("variant 1" of Chmielecki et al.)	A02.01	IMSLWGLVS	3630.0	7321.0	0
NAB:STAT6 ("variant 1" of Chmielecki et al.)	A24.02	SQIMSLWGL	1604.0	8516.0	0
NAB:STAT6 ("variant 1" of Chmielecki et al.)	B08.01	SQIMSLWGL	4587.0	15997.0	0
NDRG1:ERG	A02.01	LLQEFDVQEA	200.0	45.0	2.5
NDRG1:ERG	A02.01	LQEFDVQEAL	2229.0	50280.0	0
NDUFC2(-KCDT14)(+1)	A02.01	ITAFFFCWI	437.7	7490.4	not measured
NDUFC2(-KCDT14)(+1)	A24.02	LYITAFFFCW	46.6	45.3	not measured
NDUFC2(-KCDT14)(+1)	A03.01	FFFCWILSCK	325.9	597.1	not measured
NDUFC2(-KCDT14)(+1)	A03.01	FFCWILSCK	985.7	184.9	not measured
NDUFC2(-KCDT14)(-1)	A02.01	LLYITAFFL	24.0	713.0	17
NDUFC2(-KCDT14)(-1)	B08.01	LLYITAFFL	3588.0	9592.0	0
NDUFC2(-KCDT14)(-1)	A02.01	ITAFFLLDI	699.0	78.7	not measured
NDUFC2(-KCDT14)(-1)	A02.01	YITAFFLLDI	157.0	64.5	not measured
NDUFC2(-KCDT14)(-1)	A24.02	LYITAFFLL	15.6	0.1	not measured
NDUFC2(-KCDT14)(-1)	A02.01	LLYITAFFLL	43.7	323.2	not measured
NDUFC2(-KCDT14)(-1)	A24.02	LLYITAFFLL	59.7	60.1	not measured
NDUFC2(-KCDT14)(-1)	A24.02	LYITAFFLLD	414.3	0.4	not measured
NRAS, Q61K	A01.01	ILDTAGKEEY	272.6	14.3	not measured
NRAS, Q61R	A01.01	ILDTAGREEY	255.8	7.0	not measured
PDGFRa, T674I	A02.01	IIIEYCFYG	693.0	16.0	1.2
PDGFRa, T674I	A02.01	YIIIEYCFYG	1529.0	113.0	0
PDGFRa, T674I	A02.01	IIEYCFYGDL	3049.0	1090.0	0
PIK3CA, E542K	A02.01	KITEQEKDFL	12548.0	1397.0	0
PIK3CA, E542K	A03.01	AISTRDPLSK	41.3	57.5	not measured
PTEN, R130Q	A02.01	QTGVMICAYL	3786.0	9760.0	0
RAC1, P29S	A02.01	FSGEYIPTV	21.0	3.0	6.8
RAC1, P29S	A02.01	AFSGEYIPTV	1008.0	781.0	0

RAC1, P29S	A01.01	TTNAFSGEY	23.0	4.4	not measured
RAC1, P29S	A01.01	YTTNAFSGEY	20.0	10.5	not measured
RBM27(+1)	B07.02	MPKDVNIQV	291.6	12.5	not measured
RBM27(+1)	A01.01	TGSNEVTTRY	343.9	15545.2	not measured
RBM27(+1)	A01.01	GSNEVTTRY	151.6	605.5	not measured
RNF43, RHTP	A02.01	TQLARFFPI	17.0	19.0	0
RNF43, RHTP	A24.02	TQLARFFPI	268.0	52.0	0
RNF43, RHTP	B08.01	TQLARFFPI	41.0	9150.0	0
SEC31A(-1)	A02.01	KLMLLRLNL	58.0	17.0	16.9
SEC31A(-1)	B08.01	KLMLLRLNL	421.0	29.0	0
SEC31A(-1)	B07.02	KLMLLRLNL	2969.0	133.0	1.5
SEC31A(-1)	A03.01	KLMLLRLNL	4664.0	210.0	0
SEC31A(-1)	B08.01	LLRLNLRKM	185.1	68.2	not measured
SEC31A(-1)	A03.01	MLLRLNLRKM	171.4	116.9	not measured
SEC31A(-1)	A03.01	KLMLLRLNLR	95.4	48.4	not measured
SEC31A(-1)	A03.01	MLLRLNLRK	14.6	1.3	not measured
SEC31A(-1)	A03.01	LMLLRLNLRK	23.9	6.0	not measured
SEC31A(-1)	A02.01	MLLRLNLRKM	508.5	2507.4	not measured
SEC31A(-1)	B08.01	MLLRLNLRKM	565.9	95.3	not measured
SEC31A(-1)	A02.01	KLMLLRLNL	57.6	2.6	not measured
SEC31A(-1)	B08.01	LMLLRLNL	116.0	9.3	not measured
SEC31A(-1)	B08.01	KLMLLRLNL	420.6	58.4	not measured
SEC31A(-1)	A02.01	KKLMLLRLNL	275.4	288.9	not measured
SEC31A(-1)	B08.01	NLRKMCGPF	163.5	35.9	not measured
SEC31A(-1)	B08.01	YCQKKLMLL	203.1	222.1	not measured
SEC31A(-1)	B08.01	LNLRKMCGPF	782.2	438.7	not measured
SEC63(+1)	A03.01	YTCAITTVK	279.0	122.4	not measured
SEC63(+1)	A03.01	TYTCAITTVK	556.4	2362.2	not measured
SEC63(+1)	A03.01	ITTVKATETK	795.8	1245.3	not measured

SEC63(+1)	A03.01	KSKKKETFKK	744.0	39.6	not measured
SEC63(+1)	B08.01	TFKKKTYTC	648.2	77.9	not measured
SEC63(+1)	A03.01	KSKKKETFK	411.0	74.3	not measured
SEC63(+1)	B08.01	FKKKTYTCAI	562.8	384.9	not measured
SEC63(-1)	B08.01	TAKSKKRNL	213.8	30.6	not measured
SF3B1, K700E	A02.01	GLVDEQQEV	50.0	44.0	7.4
SLC35F5(-1)	A02.01	FALCGFWQI	10.5	0.4	not measured
SMAP1(-1)	A03.01	KSRQNHLQLK	88.1	4.7	not measured
SMAP1(-1)	B07.02	KSRQNHLQL	329.5	78.0	not measured
SMAP1(-1)	A24.02	KLRSPLWIF	504.5	828.2	not measured
SMAP1(-1)	A03.01	KISNWSLKK	11.5	8.8	not measured
SMAP1(-1)	A11.01	KISNWSLKK	15.3	9.8	not measured
SMAP1(-1)	A11.01	SLKKVPALK	117.6	129.1	not measured
SMAP1(-1)	B08.01	SLKKVPAL	66.8	7.9	not measured
SMAP1(-1)	A03.01	WSLKKVPALK	148.9	94.9	not measured
SMAP1(-1)	A03.01	KISNWSLKKV	168.3	114.6	not measured
SMAP1(-1)	A03.01	RKISNWSLKK	20.8	130.6	not measured
SMAP1(-1)	A03.01	SLKKVPALK	29.6	4.4	not measured
SMAP1(-1)	B08.01	SQKSRQNHL	305.0	44.6	not measured
SMAP1(-1)	B07.02	ALKKLRSPL	355.5	223.2	not measured
SMAP1(-1)	B08.01	ALKKLRSPL	58.9	0.5	not measured
SMAP1(-1)	B08.01	WSLKKVPAL	110.7	12.5	not measured
SMAP1(-1)	A03.01	HLQLKSCRRK	216.7	96.9	not measured
SMAP1(-1)	B08.01	LKKLRSPL	139.6	0.6	not measured
SMAP1(-1)	A03.01	SLKKVPALKK	43.1	9.6	not measured
SPOP, F133L	A02.01	FVQGKDWGL	121.0	34.0	2.1
SPOP, F133L	B08.01	FVQGKDWGL	1401.0	207.0	0

TFAM(+1)	A03.01	RVNTAWKTK	136.4	8.6	not measured
TFAM(+1)	A03.01	RVNTAWKTKK	70.6	2.3	not measured
TFAM(+1)	B08.01	TKKKRVNTA	312.4	159.4	not measured
TFAM(+1)	A03.01	KRVNTAWKTK	304.1	331.6	not measured
TFAM(+1)	B08.01	WKTKKTSFSL	930.6	112.2	not measured
TFAM(+1)	B08.01	MTKKKRVNTA	534.2	186.9	not measured
TGFBR2(-1)	A02.01	RLSSCVPVA	83.0	4.0	18.7
TGFBR2(-1)	A03.01	RLSSCVPVA	4264.0	439.0	0
TGFBR2(-1)	A03.01	AMTTSSSQK	48.5	8.3	not measured
TGFBR2(-1)	A03.01	AMTTSSSQKN	887.2	2336.5	not measured
TGFBR2(-1)	B08.01	IMKEKKSL	69.8	14.1	not measured
TGFBR2(-1)	A02.01	KSLVRLSSCV	903.1	279.8	not measured
TGFBR2(-1)	A02.01	SLVRLSSCV	177.3	29.9	not measured
TGFBR2(-1)	A11.01	SAMTTSSSQK	36.4	15.8	not measured
TGFBR2(-1)	B08.01	IMKEKKSLV	80.8	16.8	not measured
TGFBR2(-1)	A11.01	AMTTSSSQK	89.9	161.6	not measured
TGFBR2(-1)	A03.01	SAMTTSSSQK	96.7	15.7	not measured
TGFBR2(-1)	A02.01	RLSSCVPVAL	84.5	54.2	not measured
TGFBR2(-1)	A02.01	VRLSSCVPVA	640.6	1206.8	not measured
TGFBR2(-1)	A02.01	RLSSCVPVA	82.7	49.5	not measured
TGFBR2(-1)	B08.01	CIMKEKKSL	218.5	7.5	not measured
TGFBR2(-1)	A02.01	ALMSAMTTS	320.4	139.1	not measured
TGFBR2(-1)	A02.01	LVRLSSCVPV	132.7	1237.6	not measured
THAP5(-1)	A03.01	KMRKKYAQK	23.7	5.7	not measured
TMPRSS2:ERG	A02.01	ALNSEALSV	66.0	14.0	9.1
TMPRSS2:ERG	A02.01	ALNSEALSVV	84.0	15.0	2.9
TMPRSS2:ERG	A02.01	MALNSEALSV	198.0	129.0	0.7
TMPRSS2:ERG	B08.01	MALNSEALSV	8512.0	13457.0	0
TP53, AAVG	A02.01	GLLAFWDSQV	57.0	10.0	14.2
TP53, AAVG	A02.01	LLAFWDSQV	13.0	68.0	12.8

TP53, AWAA	A02.01	WMTETLFDI	7.0	14.0	4
TP53, AWAA	A02.01	WMTETLFDIV	15.0	40.0	0.4
TP53, AWAA	A24.02	WMTETLFDI	4936.0	713.0	0
TP53, AWAA	A01.01	WMTETLFDIV	4046.0	14394.0	0
TP53, CSES	B07.02	LPSQRRNHWM	89.0	10.0	6.5
TP53, CSES	B08.01	LPSQRRNHWM	325.0	47.0	0.7
TP53, CSES	A02.01	ALSEHCPTT	208.0	79.0	27.3
TP53, G245S	B08.01	CMGSMNRRPI	1204.0	80.0	0
TP53, G245S	A02.01	YMCNSSCMGS	2485.0	81.0	0.8
TP53, G245S	B08.01	SMNRRPILTI	260.0	337.0	0
TP53, G245S	A02.01	SMNRRPILTI	1644.0	1198.0	0.3
TP53, G245S	B08.01	SMNRRPILT	2536.0	1282.0	0
TP53, G245S	A02.01	CMGSMNRRPI	7822.0	1989.0	0
TP53, G245S	A02.01	SMNRRPILT	7251.0	3839.0	0
TP53, G245S	A24.02	SMNRRPILTI	10308.0	16292.0	0
TP53, G245S	B08.01	GSMNRRPIL	636.7	15.5	not measured
TP53, G245S	B08.01	MGSMNRRPIL	89.1	6.3	not measured
TP53, G245S	B08.01	MGSMNRRPI	324.2	29.1	not measured
TP53, QPSL	B07.02	LPRKPTRAAT	47.0	3.0	3.7
TP53, QPSL	B07.02	LPRKPTRAA	8.0	8.0	5.5
TP53, QPSL	B07.02	KPTRAATVSV	12.0	8.0	3
TP53, QPSL	B08.01	LPRKPTRAA	873.0	1158.0	0
TP53, R248Q	B08.01	NQRPILTII	3433.0	20.0	0
TP53, R248Q	A02.01	GMNQRPILTI	1787.0	709.0	0.4
TP53, R248Q	A02.01	GMNQRPILT	8115.0	3029.0	0
TP53, R248Q	A02.01	CMGGMNQRPI	3025.0	3673.0	0
TP53, R248Q	A02.01	NQRPILTII	10855.0	9606.0	0
TP53, R248Q	B08.01	CMGGMNQRPI	6364.0	18766.0	0
TP53, R248Q	B08.01	GMNQRPILTI	3266.0	29251.0	0
TP53, R248W	B08.01	MNWRPILTI	6447.0	1.0	0
TP53, R248W	A02.01	GMNWRPILTI	189.0	282.0	0.5
TP53, R248W	A02.01	CMGGMNWRPI	354.0	346.0	0.4
TP53, R248W	A02.01	MNWRPILTI	5834.0	516.0	3.8
TP53, R248W	A02.01	MNWRPILTII	8158.0	1026.0	0.4
TP53, R248W	B08.01	GMNWRPILTI	3990.0	1045.0	0
TP53, R248W	A02.01	GMNWRPILT	3416.0	1130.0	0
TP53, R248W	B08.01	CMGGMNWRPI	3218.0	2248.0	0
TP53, R248W	A24.02	CMGGMNWRPI	9521.0	4453.0	0
TP53, R248W	A24.02	MNWRPILTI	3634.0	6977.0	0.2
TP53, R248W	A24.02	MNWRPILTII	1517.0	44901.0	0
TP53, R273C	A02.01	LLGRNSFEVC	1272.0	2081.0	0
TP53, R273C	A02.01	NSFEVCVCA	4239.0	2200.0	0
TP53, R273H	A02.01	NSFEVHVCA	6768.0	503.0	0
TP53, SHST	B07.02	HPRPAPASA	13.0	11.0	4.9

TP53, SHST	B08.01	HPRPAPASA	1718.0	25.0	0
TP53, Y220C	A02.01	VVPCEPPEV	1268.0	187.0	0.9
TTK(-1)	A02.01	VMSDTTYKI	15.8	19.6	not measured
TTK(-1)	A03.01	LFVMSDTTYK	57.9	749.4	not measured
TTK(-1)	A02.01	FVMSDTTYKI	16.0	62.4	not measured
TTK(-1)	A03.01	FVMSDTTYK	63.1	66.9	not measured
TTK(-1)	A03.01	KTFEKKGEK	81.3	32.2	not measured
TTK(-1)	A01.01	VMSDTTYKIY	245.1	375.8	not measured
TTK(-1)	A01.01	MSDTTYKIY	18.9	10.2	not measured
UBR5(-1)	B07.02	RVQNQGHLL	429.1	826.5	not measured
VHL, QCIL	A02.01	MLTDSLFLPI	8.0	16.0	1
VHL, QCIL	A02.01	SMLTDSLFL	14.0	31.0	9.8
VHL, QCIL	B08.01	MLTDSLFLPI	2581.0	110.0	0
VHL, QCIL	A01.01	MLTDSLFLPI	429.0	7673.0	0
XPOT(-1)	A02.01	YLTKWPKFFL	10.7	42.9	not measured

Table 4A

Gene	HLA Allele	Peptide Sequence	Peptide Length	Measured Affinity (nM)	Measured stability (hr.)
KRAS, G12C	A02.01	LVVVGACGV	9	667.1	0.6
KRAS, G12C	A02.01	KLVVVGACGV	10	70.3	1.0
KRAS, G12D	A02.01	LVVVGADGV	9	977.4	0.0
KRAS, G12D	A02.01	KLVVVGADGV	10	137.7	0.9
KRAS, G12V	A02.01	LVVVGAVGV	9	682.5	0.6
KRAS, G12V	A02.01	KLVVVGAVGV	10	57.6	0.9
KRAS, G12C	A03.01	VVGACGVGK	9	4.1	5.0
KRAS, G12C	A03.01	VVVGACGVGK	10	1.6	2.5
KRAS, G12D	A03.01	VVGADGVGK	9	518.7	NB
KRAS, G12D	A03.01	VVVGADGVGK	10	314.9	2.3
KRAS, G12V	A03.01	VVGAVGVGK	9	1.9	1.2
KRAS, G12V	A03.01	VVVGAVGVGK	10	44.2	6.7
KRAS, G12C	A11.01	VVGACGVGK	9	43.2	10.0
KRAS, G12C	A11.01	VVVGACGVGK	10	69.3	15.7

KRAS, G12D	A11.01	VVGADGVGK	9	203.9	3.4
KRAS, G12D	A11.01	VVVGADGVGK	10	33.1	13.0
KRAS, G12V	A11.01	VVGAVGVGK	9	7.7	16.9
KRAS, G12V	A11.01	VVVGAVGVGK	10	26.1	24.3
KRAS, G12D	B08:01	**D**GVGKSAL	8		
KRAS, G12V	B08:01	**V**GVGKSAL	8		
KRAS, G12C	B08:01	**C**GVGKSAL	8		

[0288] Table 4B-4M show peptide sequences comprising RAS mutations, corresponding HLA allele to which it binds, and corresponding predicted binding affinity score with the lowest number (e.g., 1) having the highest affinity and vice-versa.

Table 4B - RAS Q61H Mutation

Peptide	Allele	Rank of Binding Potential
ILDTAGHEEY	HLA-A36:01	1
ILDTAGHEEY	HLA-A01:01	2
DTAGHEEYSAM	HLA-A26:01	3
DTAGHEEYSAM	HLA-A25:01	4
GHEEYSAM	HLA-B15:09	4
DTAGHEEY	HLA-A26:01	5
ILDTAGHEE	HLA-C08:02	5
AGHEEYSAM	HLA-C01:02	6
AGHEEYSAM	HLA-B46:01	6
DTAGHEEY	HLA-A25:01	6
DTAGHEEY	HLA-A01:01	6
DTAGHEEY	HLA-B18:01	7
DTAGHEEY	HLA-A36:01	7
ILDTAGHEE	HLA-C05:01	7
ILDTAGHEE	HLA-A02:07	7
ILDTAGHEEY	HLA-A29:02	7
ILDTAGHEEY	HLA-C08:02	7
HEEYSAMRD	HLA-B49:01	8
TAGHEEYSA	HLA-B35:03	8
DTAGHEEYS	HLA-A68:02	9
DTAGHEEYSAMR	HLA-A68:01	9
GHEEYSAM	HLA-B39:01	9
ILDTAGHEE	HLA-A01:01	9
LDTAGHEEY	HLA-B53:01	9
HEEYSAMRD	HLA-B41:01	10
ILDTAGHEE	HLA-A36:01	10
DTAGHEEY	HLA-B58:01	11
LLDILDTAGH	HLA-A01:01	12
TAGHEEYSAM	HLA-B35:03	12

LDTAGHEEY	HLA-B35:01	13
DILDTAGHE	HLA-A26:01	14
DTAGHEEY	HLA-C12:03	14
ILDTAGHEEY	HLA-C05:01	14
AGHEEYSAM	HLA-A30:02	15
DILDTAGHEEY	HLA-A25:01	15
DTAGHEEY	HLA-C02:02	15
ILDTAGHEE	HLA-C04:01	15
DILDTAGH	HLA-A26:01	16
ILDTAGHEE	HLA-A02:01	16
LDTAGHEEY	HLA-A29:02	16
ILDTAGHE	HLA-A01:01	17
LDTAGHEEY	HLA-B18:01	17
AGHEEYSAM	HLA-C14:03	18
DILDTAGHEEY	HLA-A29:02	18
DTAGHEEYS	HLA-A26:01	18
ILDTAGHEEY	HLA-B15:01	18
DTAGHEEYSA	HLA-A68:02	19
ILDTAGHE	HLA-C05:01	19
ILDTAGHEEY	HLA-A02:07	19
ILDTAGHEEY	HLA-A30:02	19
LDTAGHEEY	HLA-A36:01	19
AGHEEYSAM	HLA-C14:02	20
AGHEEYSAM	HLA-B15:03	20
LLDILDTAGH	HLA-A02:07	20

Table 4C - RAS Q61R Mutation

Peptide	Allele	Rank of Binding Potential
ILDTAGREEY	HLA-A36:01	1
ILDTAGREEY	HLA-A01:01	2
DTAGREEYSAM	HLA-A26:01	3
DILDTAGR	HLA-A33:03	4
DILDTAGR	HLA-A68:01	5
DTAGREEY	HLA-A26:01	6
DTAGREEYSAM	HLA-A25:01	6
CLLDILDTAGR	HLA-A74:01	7
DTAGREEY	HLA-A01:01	7
REEYSAMRD	HLA-B41:01	7
GREEYSAMR	HLA-B27:05	8
ILDTAGREE	HLA-C08:02	8
ILDTAGREEY	HLA-A29:02	8
REEYSAMRD	HLA-B49:01	8
AGREEYSAM	HLA-B46:01	9
DTAGREEY	HLA-B18:01	9
DTAGREEY	HLA-A25:01	9

DTAGREEY	HLA-A36:01	9
DILDTAGR	HLA-A74:01	10
DILDTAGRE	HLA-A26:01	10
ILDTAGREE	HLA-C05:01	10
DILDTAGR	HLA-A26:01	11
GREEYSAM	HLA-B39:01	11
AGREEYSAM	HLA-B15:03	12
GREEYSAM	HLA-C07:02	12
ILDTAGREE	HLA-A01:01	12
TAGREEYSA	HLA-B35:03	12
ILDTAGREEY	HLA-A30:02	13
DTAGREEYS	HLA-A68:02	14
ILDTAGRE	HLA-A01:01	14
CLLDILDTAGR	HLA-A31:01	15
DTAGREEYSAMR	HLA-A68:01	15
LLDILDTAGR	HLA-A01:01	15
DTAGREEY	HLA-B58:01	16
ILDTAGREEY	HLA-C08:02	16
DILDTAGR	HLA-A31:01	17
ILDTAGREE	HLA-C04:01	17
ILDTAGREEY	HLA-A32:01	17
LLDILDTAGR	HLA-A74:01	17
TAGREEYSAM	HLA-B35:03	17
DILDTAGREEY	HLA-A32:01	18
ILDTAGRE	HLA-C05:01	18
ILDTAGREE	HLA-A02:07	18
REEYSAMRD	HLA-B40:01	18
AGREEYSAM	HLA-B15:01	19
AGREEYSAMR	HLA-A31:01	19
ILDTAGRE	HLA-A36:01	19
LDILDTAGR	HLA-A68:01	19
LDTAGREEY	HLA-A29:02	19
LDTAGREEY	HLA-B35:01	19
REEYSAMRD	HLA-B45:01	19
REEYSAMRDQY	HLA-A36:01	19
DTAGREEY	HLA-C02:02	20

Table 4D - RAS Q61K Mutation

Peptide	Allele	Rank of Binding Potential
ILDTAGKEEY	HLA-A36:01	1
ILDTAGKEEY	HLA-A01:01	2
DTAGKEEYSAM	HLA-A26:01	3
CLLDILDTAGK	HLA-A03:01	4
DTAGKEEY	HLA-A01:01	5
DTAGKEEY	HLA-A26:01	5

DTAGKEEYSAM	HLA-A25:01	5
AGKEEYSAM	HLA-B46:01	6
DILDTAGKE	HLA-A26:01	7
KEEYSAMRD	HLA-B41:01	7
DTAGKEEY	HLA-B18:01	8
GKEEYSAM	HLA-B15:03	8
ILDTAGKEE	HLA-C08:02	8
ILDTAGKEEY	HLA-A29:02	8
DTAGKEEYS	HLA-A68:02	9
LDTAGKEEY	HLA-B53:01	9
TAGKEEYSA	HLA-B35:03	9
DILDTAGK	HLA-A68:01	10
DTAGKEEY	HLA-A36:01	10
KEEYSAMRD	HLA-B49:01	10
LDTAGKEEY	HLA-C07:01	10
DTAGKEEYSAMR	HLA-A68:01	11
ILDTAGKEE	HLA-C05:01	11
ILDTAGKEEY	HLA-C08:02	11
LLDILDTAGK	HLA-A01:01	12
AGKEEYSAM	HLA-A30:02	13
DTAGKEEY	HLA-A25:01	13
DTAGKEEYS	HLA-A26:01	13
ILDTAGKE	HLA-C05:01	13
LDTAGKEEY	HLA-B35:01	13
AGKEEYSAMR	HLA-A31:01	14
DILDTAGK	HLA-A33:03	14
ILDTAGKE	HLA-A01:01	14
ILDTAGKEE	HLA-A01:01	14
ILDTAGKEE	HLA-A02:07	14
TAGKEEYSAM	HLA-B35:03	14
AGKEEYSAM	HLA-B15:01	15
ILDTAGKEEY	HLA-A30:02	15
LDTAGKEEY	HLA-B46:01	15
DTAGKEEY	HLA-B58:01	16
ILDTAGKEEY	HLA-C05:01	17
AGKEEYSAM	HLA-A30:01	18
AGKEEYSAM	HLA-B15:03	18
DTAGKEEY	HLA-C02:02	18
LDTAGKEEY	HLA-A29:02	18

Table 4E - RAS Q61L Mutation

Peptide	Allele	Rank of Binding Potential
ILDTAGLEEY	HLA-A36:01	1
ILDTAGLEEY	HLA-A01:01	2
LLDILDTAGL	HLA-A02:07	3

GLEEYSAMRDQY	HLA-A36:01	4
DTAGLEEY	HLA-A25:01	5
DTAGLEEY	HLA-A26:01	5
DTAGLEEYSAM	HLA-A26:01	5
DTAGLEEY	HLA-A01:01	6
ILDTAGLEE	HLA-C08:02	6
ILDTAGLEE	HLA-A01:01	6
CLLDILDTAGL	HLA-A02:04	7
ILDTAGLEE	HLA-A36:01	7
LLDILDTAGL	HLA-A01:01	7
DILDTAGL	HLA-B14:02	8
DILDTAGLEEY	HLA-A25:01	8
DTAGLEEYS	HLA-A68:02	8
DTAGLEEYSAM	HLA-A25:01	8
GLEEYSAMR	HLA-A74:01	8
ILDTAGLE	HLA-A01:01	8
DILDTAGLEEY	HLA-A26:01	9
DTAGLEEY	HLA-A36:01	9
ILDTAGLEEY	HLA-A29:02	9
DILDTAGL	HLA-B08:01	10
DTAGLEEY	HLA-B18:01	10
ILDTAGLEE	HLA-A02:07	10
LDTAGLEEY	HLA-B35:01	10
CLLDILDTAGL	HLA-A02:01	11
DTAGLEEY	HLA-C02:02	11
ILDTAGLEE	HLA-C05:01	11
ILDTAGLEEY	HLA-C08:02	11
ILDTAGLEEY	HLA-A02:07	11
LLDILDTAGL	HLA-C08:02	11
DILDTAGL	HLA-A26:01	12
LDTAGLEEY	HLA-B53:01	12
DTAGLEEY	HLA-C03:02	13
DTAGLEEY	HLA-B58:01	13
ILDTAGLEEY	HLA-A30:02	13
LLDILDTAGL	HLA-C05:01	13
LLDILDTAGL	HLA-C04:01	13
DTAGLEEYSAMR	HLA-A68:01	14
ILDTAGLE	HLA-A36:01	15
LLDILDTAGL	HLA-A02:01	15
AGLEEYSAM	HLA-B15:03	16
DTAGLEEYSA	HLA-A68:02	16
GLEEYSAMRDQY	HLA-A01:01	16
ILDTAGLE	HLA-C04:01	16
ILDTAGLEEY	HLA-B15:01	16
LDILDTAGL	HLA-B37:01	16
AGLEEYSAM	HLA-A30:02	17

AGLEEYSAM	HLA-B48:01	17
AGLEEYSAMR	HLA-A31:01	17
ILDTAGLEE	HLA-C04:01	17
LDTAGLEEY	HLA-C03:02	17
AGLEEYSAM	HLA-C14:02	18
GLEEYSAMR	HLA-A31:01	18
LEEYSAMRD	HLA-B41:01	18
LLDILDTAGLE	HLA-A01:01	18
AGLEEYSAM	HLA-C14:03	19
LDILDTAGL	HLA-B40:02	19
LDTAGLEEY	HLA-A29:02	19
DILDTAGLE	HLA-A26:01	20
DTAGLEEY	HLA-B15:01	20
ILDTAGLEEY	HLA-A02:01	20
LDTAGLEEY	HLA-A36:01	20
LDTAGLEEY	HLA-B46:01	20
DTAGLEEY	HLA-A68:02	21
DTAGLEEY	HLA-C12:03	21
ILDTAGLE	HLA-C05:01	21
LDTAGLEEY	HLA-B18:01	21
LEEYSAMRD	HLA-B49:01	21
TAGLEEYSA	HLA-B54:01	21
DILDTAGLEEY	HLA-A29:02	22
GLEEYSAM	HLA-C05:01	22

Table 4F - RAS G12A Mutation

Peptide	Allele	Rank of Binding Potential
AAGVGKSAL	HLA-C03:04	1
VVVGAAGVGK	HLA-A11:01	1
VVGAAGVGK	HLA-A11:01	2
TEYKLVVVGAA	HLA-B50:01	3
VVGAAGVGK	HLA-A03:01	3
VVVGAAGVGK	HLA-A68:01	3
AAGVGKSAL	HLA-C08:02	4
AAGVGKSAL	HLA-C08:01	4
AAGVGKSAL	HLA-B46:01	4
AAGVGKSAL	HLA-B81:01	5
GAAGVGKSAL	HLA-B48:01	5
LVVVGAAGV	HLA-A68:02	5
AAGVGKSAL	HLA-C03:04	1
VVVGAAGVGK	HLA-A11:01	1
VVGAAGVGK	HLA-A11:01	2
TEYKLVVVGAA	HLA-B50:01	3
VVGAAGVGK	HLA-A03:01	3
VVVGAAGVGK	HLA-A68:01	3

AAGVGKSAL	HLA-C08:02	4
AAGVGKSAL	HLA-C08:01	4
AAGVGKSAL	HLA-B46:01	4
AAGVGKSAL	HLA-B81:01	5
AAGVGKSAL	HLA-C03:02	5
AAGVGKSAL	HLA-C01:02	5
GAAGVGKSAL	HLA-B48:01	5
LVVVGAAGV	HLA-A68:02	5
AAGVGKSAL	HLA-C03:03	6
VVGAAGVGK	HLA-A68:01	6
GAAGVGKSAL	HLA-B81:01	7
VVVGAAGVGK	HLA-A03:01	7
AAGVGKSAL	HLA-C05:01	8
AAGVGKSAL	HLA-C12:03	8
GAAGVGKSA	HLA-B46:01	8
VVGAAGVGK	HLA-A30:01	8
GAAGVGKSA	HLA-B55:01	9
KLVVVGAAGV	HLA-A02:01	9
AGVGKSAL	HLA-B08:01	10
GAAGVGKSAL	HLA-C03:04	10
AAGVGKSAL	HLA-C17:01	11
GAAGVGKSAL	HLA-C03:03	11
VVVGAAGV	HLA-A68:02	11
YKLVVVGAA	HLA-B54:01	11
AAGVGKSAL	HLA-B48:01	12
AGVGKSAL	HLA-C03:04	12
AGVGKSAL	HLA-C07:01	12
VVVGAAGVGK	HLA-A30:01	12
AAGVGKSA	HLA-B46:01	13
KLVVVGAAGV	HLA-A02:07	13
YKLVVVGAA	HLA-B50:01	13
AAGVGKSAL	HLA-B07:02	14
GAAGVGKSAL	HLA-A68:02	14
VVGAAGVGK	HLA-A74:01	14
AGVGKSAL	HLA-C08:01	15
GAAGVGKSAL	HLA-C17:01	15
GAAGVGKSAL	HLA-C08:01	16
GAAGVGKSAL	HLA-B35:03	16
AAGVGKSAL	HLA-C02:02	17
AAGVGKSAL	HLA-B35:03	17
AAGVGKSAL	HLA-C12:02	17
AAGVGKSAL	HLA-C14:03	17
GAAGVGKSA	HLA-B50:01	17
AGVGKSAL	HLA-C03:02	18
GAAGVGKSA	HLA-C03:04	18
LVVVGAAGV	HLA-B55:01	18

TEYKLVVVGAA	HLA-B41:01	18
AGVGKSAL	HLA-C01:02	19
GAAGVGKSA	HLA-B54:01	19
GAAGVGKSAL	HLA-B07:02	19
VGAAGVGKSA	HLA-B55:01	19
AGVGKSAL	HLA-B48:01	20
AGVGKSALTI	HLA-B49:01	20
VVVGAAGV	HLA-B55:01	20

Table 4G - RAS G12C Mutation

Peptide	Allele	Rank of Binding Potential
VVVGACGVGK	HLA-A11:01	1
VVGACGVGK	HLA-A03:01	2
VVGACGVGK	HLA-A11:01	3
VVVGACGVGK	HLA-A68:01	4
VVGACGVGK	HLA-A68:01	5
VVVGACGVGK	HLA-A03:01	5
VVGACGVGK	HLA-A30:01	6
ACGVGKSAL	HLA-B81:01	7
ACGVGKSAL	HLA-C01:02	7
ACGVGKSAL	HLA-C14:03	8
ACGVGKSAL	HLA-C03:04	9
VVVGACGVGK	HLA-A30:01	9
ACGVGKSAL	HLA-C14:02	10
CGVGKSAL	HLA-B08:01	10
KLVVVGACGV	HLA-A02:01	10
ACGVGKSAL	HLA-B07:02	11
GACGVGKSAL	HLA-B48:01	12
GACGVGKSAL	HLA-C03:03	13
ACGVGKSAL	HLA-B48:01	14
ACGVGKSAL	HLA-B40:01	14
YKLVVVGAC	HLA-B48:01	14
YKLVVVGAC	HLA-B15:03	14
GACGVGKSA	HLA-B46:01	15
GACGVGKSAL	HLA-C03:04	15
GACGVGKSAL	HLA-C01:02	15
LVVVGACGV	HLA-A68:02	15
CGVGKSAL	HLA-C03:04	16
GACGVGKSAL	HLA-C08:02	16
VVGACGVGK	HLA-A74:01	16

Table 4H - RAS G12D Mutation

Peptide	Allele	Rank of Binding Potential
GADGVGKSAL	HLA-C08:02	1

GADGVGKSAL	HLA-C05:01	2
VVVGADGVGK	HLA-A11:01	3
DGVGKSAL	HLA-B14:02	4
VVGADGVGK	HLA-A11:01	4
VVGADGVGK	HLA-A03:01	5
DGVGKSAL	HLA-B08:01	6
VVVGADGVGK	HLA-A68:01	6
GADGVGKSAL	HLA-C03:03	7
VVGADGVGK	HLA-A30:01	7
ADGVGKSAL	HLA-B37:01	8
GADGVGKSAL	HLA-C08:01	8
VVGADGVGK	HLA-A68:01	8
GADGVGKSA	HLA-C08:02	9
GADGVGKSAL	HLA-B35:03	9
GADGVGKS	HLA-C05:01	10
GADGVGKSA	HLA-C05:01	10
ADGVGKSAL	HLA-C07:01	11
VVVGADGVGK	HLA-A03:01	11
ADGVGKSAL	HLA-B40:02	12
ADGVGKSAL	HLA-B46:01	13
GADGVGKSAL	HLA-C03:04	13
ADGVGKSAL	HLA-B81:01	14
GADGVGKSAL	HLA-C17:01	14
VVVGADGVGK	HLA-A30:01	14
GADGVGKSA	HLA-B35:03	15
GADGVGKSA	HLA-B46:01	15
GADGVGKSAL	HLA-B48:01	15
KLVVVGADGV	HLA-A02:01	15
LVVVGADGV	HLA-A68:02	15
VGADGVGKSA	HLA-B55:01	15
VVGADGVGK	HLA-A74:01	16
GADGVGKSA	HLA-B53:01	17
KLVVVGADGV	HLA-A02:07	17
VGADGVGK	HLA-A68:01	17
YKLVVVGAD	HLA-B48:01	17
ADGVGKSAL	HLA-C14:03	18
DGVGKSALTI	HLA-B51:01	18
VGADGVGK	HLA-A11:01	18
GADGVGKSAL	HLA-B07:02	19
KLVVVGADGVGK	HLA-A03:01	20

Table 4I - RAS G12R Mutation

Peptide	Allele	Rank of Binding Potential
VVGARGVGK	HLA-A03:01	1
EYKLVVVGAR	HLA-A33:03	2

VVVGARGVGK	HLA-A11:01	3
ARGVGKSAL	HLA-C07:02	4
ARGVGKSAL	HLA-B39:01	5
ARGVGKSAL	HLA-C07:01	5
VVGARGVGK	HLA-A11:01	5
VVVGARGVGK	HLA-A68:01	5
GARGVGKSA	HLA-B46:01	6
ARGVGKSAL	HLA-B27:05	7
GARGVGKSA	HLA-B55:01	7
RGVGKSAL	HLA-C07:01	8
VVGARGVGK	HLA-A30:01	9
ARGVGKSAL	HLA-B38:01	10
ARGVGKSAL	HLA-B14:02	10
VVGARGVGK	HLA-A68:01	10
VVVGARGVGK	HLA-A03:01	11
GARGVGKSAL	HLA-B48:01	12
RGVGKSAL	HLA-B48:01	12
RGVGKSALTI	HLA-A23:01	12
ARGVGKSAL	HLA-C06:02	13
GARGVGKSA	HLA-A30:01	13
GARGVGKSAL	HLA-B81:01	13
VVVGARGVGK	HLA-A30:01	13
GARGVGKSAL	HLA-B07:02	14
LVVVGARGV	HLA-C06:02	14
RGVGKSAL	HLA-B81:01	14
VVGARGVGK	HLA-A74:01	15
KLVVVGARGV	HLA-A02:01	16
LVVVGARGV	HLA-B55:01	16
YKLVVVGAR	HLA-A33:03	16
KLVVVGAR	HLA-A74:01	17
KLVVVGARGV	HLA-B13:02	17
RGVGKSAL	HLA-C01:02	17
LVVVGARGV	HLA-A68:02	18
VVVGARGV	HLA-B55:01	18
ARGVGKSAL	HLA-B15:09	19
ARGVGKSAL	HLA-C14:03	20
GARGVGKSA	HLA-B54:01	20
VVVGARGV	HLA-B52:01	20
KLVVVGARGVGK	HLA-A03:01	21

Table 4J - RAS G12S Mutation

Peptide	Allele	Rank of Binding Potential
VVVGASGVGK	HLA-A11:01	1
VVGASGVGK	HLA-A11:01	2
VVGASGVGK	HLA-A03:01	3

VVVGASGVGK	HLA-A68:01	4
ASGVGKSAL	HLA-C03:04	5
ASGVGKSAL	HLA-B46:01	5
VVGASGVGK	HLA-A68:01	6
VVVGASGVGK	HLA-A03:01	6
ASGVGKSAL	HLA-C01:02	7
GASGVGKSAL	HLA-B48:01	7
ASGVGKSAL	HLA-C07:01	8
ASGVGKSAL	HLA-C08:02	9
GASGVGKSAL	HLA-B81:01	9
SGVGKSAL	HLA-B08:01	9
ASGVGKSAL	HLA-C03:03	10
ASGVGKSAL	HLA-C03:02	10
SGVGKSAL	HLA-B14:02	10
VVGASGVGK	HLA-A30:01	10
ASGVGKSAL	HLA-C08:01	11
VVVGASGVGK	HLA-A30:01	11
GASGVGKSAL	HLA-B35:03	12
SGVGKSAL	HLA-C07:01	12
ASGVGKSAL	HLA-B81:01	13
GASGVGKSA	HLA-B55:01	13
GASGVGKSAL	HLA-C03:03	13
KLVVVGASGV	HLA-A02:01	13
LVVVGASGV	HLA-A68:02	13
SGVGKSAL	HLA-C01:02	13
ASGVGKSA	HLA-B46:01	14
ASGVGKSAL	HLA-C15:02	14
GASGVGKSAL	HLA-C08:01	15
SGVGKSAL	HLA-C03:04	15
ASGVGKSAL	HLA-C05:01	16
GASGVGKSAL	HLA-C03:04	16
VVGASGVGK	HLA-A74:01	16
ASGVGKSAL	HLA-B48:01	17
GASGVGKSAL	HLA-C01:02	17
SGVGKSAL	HLA-C03:02	17
SGVGKSALTI	HLA-A23:01	17
VGASGVGKSA	HLA-B55:01	18
ASGVGKSAL	HLA-C12:03	19
ASGVGKSAL	HLA-B57:03	19
KLVVVGASGV	HLA-A02:07	19
SGVGKSAL	HLA-B81:01	19
ASGVGKSAL	HLA-C17:01	20
KLVVVGASG	HLA-A32:01	20

Table 4K - RAS G12V Mutation

Peptide	Allele	Rank of Binding Potential
VVGAVGVGK	HLA-A03:01	1
VVGAVGVGK	HLA-A11:01	2
VVVGAVGVGK	HLA-A11:01	2
VVVGAVGVGK	HLA-A68:01	3
VVGAVGVGK	HLA-A68:01	4
LVVVGAVGV	HLA-A68:02	5
VVGAVGVGK	HLA-A30:01	5
AVGVGKSAL	HLA-B81:01	6
KLVVVGAVGV	HLA-A02:01	6
AVGVGKSAL	HLA-B46:01	7
GAVGVGKSAL	HLA-C03:03	7
GAVGVGKSAL	HLA-B48:01	7
VVVGAVGVGK	HLA-A03:01	7
AVGVGKSAL	HLA-C03:04	8
GAVGVGKSAL	HLA-C03:04	8
KLVVVGAVGV	HLA-A02:07	9
VGVGKSAL	HLA-B08:01	9
VVVGAVGV	HLA-A68:02	9
AVGVGKSAL	HLA-C08:02	10
AVGVGKSAL	HLA-B07:02	10
GAVGVGKSAL	HLA-B35:03	10
AVGVGKSAL	HLA-C08:01	11
AVGVGKSAL	HLA-C01:02	11
GAVGVGKSA	HLA-B55:01	11
GAVGVGKSAL	HLA-B81:01	11
GAVGVGKSAL	HLA-C08:01	11
KLVVVGAVGV	HLA-B13:02	11
VGVGKSAL	HLA-C03:04	11
AVGVGKSAL	HLA-A32:01	12
GAVGVGKSA	HLA-B46:01	12
VGVGKSAL	HLA-C03:02	12
VGVGKSALTI	HLA-A23:01	12
GAVGVGKSA	HLA-B54:01	13
VGVGKSAL	HLA-C01:02	.3
AVGVGKSAL	HLA-B48:01	14
AVGVGKSAL	HLA-C03:03	14
AVGVGKSAL	HLA-B42:01	14
LVVVGAVGV	HLA-B55:01	14
VGVGKSAL	HLA-C08:01	14
VVGAVGVGK	HLA-A74:01	14
AVGVGKSAL	HLA-C05:01	15
AVGVGKSAL	HLA-C03:02	15
GAVGVGKSA	HLA-C03:04	15
KLVVVGAVGV	HLA-A02:04	15

LVVVGAVGV	HLA-A02:07	15
VGVGKSAL	HLA-B14:02	15
VVVGAVGVGK	HLA-A30:01	15
VVGAVGVGK	HLA-B81:01	16
VVVGAVGV	HLA-B55:01	16
AVGVGKSAL	HLA-C14:03	17
AVGVGKSAL	HLA-B15:01	17
LVVVGAVGV	HLA-B54:01	17
AVGVGKSA	HLA-B55:01	18
AVGVGKSAL	HLA-C17:01	18
GAVGVGKSA	HLA-B50:01	19
GAVGVGKSAL	HLA-C17:01	19
YKLVVVGAV	HLA-A02:04	19
GAVGVGKSAL	HLA-B35:01	20
VVGAVGVGK	HLA-A31:01	20
YKLVVVGAV	HLA-B51:01	20
LVVVGAVGVGK	HLA-A03:01	21
KLVVVGAVGVGK	HLA-A03:01	22

Table 4L RAS G13C Mutation

Peptide	Allele	Rank of Binding Potential
VVVGAGCVGK	HLA-A11:01	1
VVGAGCVGK	HLA-A11:01	2
AGCVGKSAL	HLA-C01:02	3
VVGAGCVGK	HLA-A03:01	4
VVVGAGCVGK	HLA-A68:01	4
CVGKSALTI	HLA-B13:02	5
VVGAGCVGK	HLA-A68:01	5
VVGAGCVGK	HLA-A30:01	6
AGCVGKSAL	HLA-B48:01	7
AGCVGKSAL	HLA-C03:04	8
GCVGKSALTI	HLA-B49:01	8
AGCVGKSAL	HLA-C08:02	9
VVVGAGCVGK	HLA-A03:01	9
KLVVVGAGC	HLA-A30:02	10
GCVGKSAL	HLA-C07:01	11
VVGAGCVGK	HLA-A74:01	12
AGCVGKSAL	HLA-C14:03	13
KLVVVGAGC	HLA-B15:01	14

Table 4M - RAS G13D Mutation

Peptide	Allele	Rank of Binding Potential
AGDVGKSAL	HLA-C08:02	1
AGDVGKSAL	HLA-C05:01	2

VVGAGDVGK	HLA-A11:01	3
VVVGAGDVGK	HLA-A11:01	3
VVVGAGDVGK	HLA-A68:01	4
GAGDVGKSA	HLA-B46:01	5
GAGDVGKSAL	HLA-B48:01	5
VVGAGDVGK	HLA-A68:01	5
VVGAGDVGK	HLA-A03:01	5
AGDVGKSAL	HLA-C03:04	6
AGDVGKSAL	HLA-C04:01	6
AGDVGKSAL	HLA-C01:02	6
DVGKSALTI	HLA-B13:02	6
DVGKSALTI	HLA-A25:01	6
GDVGKSAL	HLA-C07:01	6
GDVGKSAL	HLA-B40:02	7
GDVGKSAL	HLA-B37:01	8
AGDVGKSAL	HLA-B48:01	9
DVGKSALTI	HLA-B51:01	10
VVGAGDVGK	HLA-A30:01	10
GAGDVGKSAL	HLA-C08:01	11
GAGDVGKSAL	HLA-B81:01	11
AGDVGKSAL	HLA-C08:01	12
GAGDVGKSAL	HLA-C03:04	12
DVGKSALTI	HLA-B53:01	13
AGDVGKSAL	HLA-B07:02	14
AGDVGKSAL	HLA-B46:01	14
DVGKSALTI	HLA-A26:01	14
VVGAGDVGK	HLA-A74:01	14
GAGDVGKSA	HLA-B54:01	15
DVGKSALTI	HLA-B38:01	16
GAGDVGKSAL	HLA-C03:03	16
VVVGAGDVGK	HLA-A03:01	16

[0289] 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.

[0290] Table 5 shows GATA peptides and their HLA binding partners.

Table 5

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		FRAMESHIFT [1]		
GATA3	L328fs N334fs	AQAKAVCSQESRDV LCELSDHHNHTLEEE CQWGPCLQCLWALL QASQY*	CLQCLWALL (A02.01) CQWGPCLQCL (A02.01) QWGPCLQCL (A24.02) QWGPCLQCLW (A24.02)	Breast Cancer
GATA3	H400fs S408fs S408fs S430fs H434fs H435fs	PGRPLQTHVLPEPHL ALQPLQPHADHAHA DAPAIQPVLWTTPPL QHGHRHGLEPCSML TGPPARVPAVPFDLH FCRSSIMKPKRDGYM FLKAESKIMFATLQR SSLWCLCSNH*	AIQPVLWTT (A02.01) ALQPLQPHA (A02.01) DLHFCRSSIM (B08.01) EPHLALQPL (B07.02, B08.01) ESKIMFATL (B08.01) FATLQRSSL (B07.02, B08.01) FLKAESKIM (B08.01) FLKAESKIMF (B08.01) GPPARVPAV (B07.02) IMKPKRDGYM (B08.01) KIMFATLQR (A03.01) KPKRDGYMF (B07.02) KPKRDGYMFL (B07.02) LHFCRSSIM (B08.01) LQHGHRHGL (B08.01) MFATLQRSSL (B07.02, B08.01) MFLKAESKI (A24.02) MLTGPPARV (A02.01) QPVLWTTPPL (B07.02) SMLTGPPARV (A02.01) TLQRSSLWCL (A02.01) VLPEPHLAL (A02.01) VPAVPFDLHF (B07.02) YMFLKAESK (A03.01) YMFLKAESKI (A02.01, A03.01, A24.02, B08.01)	Breast Cancer

[0291] Table 6 shows HLA affinity and stability of selected BTK peptides:

Table 6

Gene	HLA Allele	Peptide Sequence	Affinity (nM)	Stability (half-life (hr.))
BTK, C481S	A01.01	YMANGSLLNY	13.24495	0.866167
BTK, C481S	A01.01	MANGSLLNY	439.029	0.216408
BTK, C481S	A03.01	MANGSLLNY	35.62463	0.237963
BTK, C481S	A03.01	YMANGSLLNY	95.93212	0.279088
BTK, C481S	A11.01	MANGSLLNY	535.6333	NB
BTK, C481S	A11.01	YMANGSLLNY	974.2881	NB
BTK, C481S	A24.02	EYMANGSLL	4.961145	5.716141

BTK_C481S	A02.01	SLLNYLREM	67.69132	3.043604
BTK_C481S	A02.01	MANGSLLNYL	1006.566	0
BTK_C481S	A02.01	YMANGSLLN	3999.442	0
BTK_C481S	B07.02	SLLNYLREM	865.8805	0
BTK_C481S	B07.02	MANGSLLNYL	16474.59	0
BTK_C481S	B08.01	SLLNYLREM	959.6542	0
BTK_C481S	B08.01	MANGSLLNYL	18463.09	0

[0292] Table 7 shows HLA affinity and stability of selected EGFR peptides:

Table 7

Gene	HLA Allele	Peptide Sequence	Affinity (nM)	Stability (half-life (hr.))
EGFR, T790M	A01.01	LTSTVQLIM	2891.111	0.103721
EGFR_T790M	A01.01	CLTSTVQLIM	8276.876	0
EGFR_T790M	A02.01	MQLMPFGCLL	16.26147	0.381118
EGFR_T790M	A02.01	MQLMPFGCL	116.3352	0.368273
EGFR_T790M	A02.01	LIMQLMPFGC	132.4766	0.381284
EGFR_T790M	A02.01	QLIMQLMPF	192.8406	0.34067
EGFR_T790M	A02.01	CLTSTVQLIM	537.1391	0
EGFR_T790M	A02.01	IMQLMPFGCL	653.1065	0.515559
EGFR_T790M	A02.01	IMQLMPFGC	1205.368	0.370112
EGFR_T790M	A02.01	LIMQLMPFG	3337.708	0
EGFR_T790M	A02.01	VQLIMQLMPF	4942.892	0
EGFR_T790M	A02.01	QLIMQLMPFG	5214.668	0
EGFR_T790M	A02.01	STVQLIMQL	7256.773	0
EGFR_T790M	A24.02	QLIMQLMPF	2030.807	0.368673
EGFR_T790M	A24.02	VQLIMQLMPF	4103.131	0
EGFR_T790M	A24.02	IMQLMPFGCL	14119.38	0
EGFR_T790M	A24.02	MQLMPFGCLL	18857.47	0
EGFR_T790M	B07.02	MQLMPFGCL	1589.188	0
EGFR_T790M	B08.01	QLIMQLMPF	330.1933	0
EGFR_T790M	B08.01	IMQLMPFGCL	427.3913	0
EGFR_T790M	B08.01	MQLMPFGCL	4931.727	0
EGFR_T790M	B08.01	MQLMPFGCLL	11244.9	0
EGFR_T790M	B08.01	VQLIMQLMPF	16108.18	0

EGFR_T790M	B08.02	QLIMQLMPF	5590.3	ND

Tumor antigens associated with tumor microenvironment

[0293] In many cases, predominant antigens are expressed by cells in the tumor microenvironment that not only serve as excellent biomarkers for the disease, but also can be important vaccine candidates for immunotherapy. Such tumor associated antigens (TAAs) are not necessarily presented on the surface of tumor cells, but on cells that are juxtaposed to the tumor, which could be the stromal cells, connective tissue cells, fibroblasts etc. These are cells that often contribute to the structural integrity of the tumor, feed the tumor and support growth of the tumor. In most cases, TAAs are overexpressed antigens in the tumor microenvironment, however some antigens in the tumor microenvironment may also be unique in the tumor associated cells. As an example, telomerase reverse transcriptase (TERT) is a TAA that is not present in most normal tissues but is activated in most human tumors. Tissue kallikrein-related peptidases, or kallikreins (KLKs), on the other hand are overexpressed in various cancers and comprise a large family of secreted trypsin- or chymotrypsin-like serine proteases. Kallikreins are upregulated in prostrate ovarian and breast cancers. Some TAAs are specific to certain cancers, some are expressed in a large variety of cancers. Carcinoembryonic antigen (CEA) is overexpressed in breast, colon, lung and pancreatic carcinomas, whereas MUC-1 is breast, lung, prostate, colon cancers. Some TAAs are differentiation or tissue specific, for example, MART-1 / melan-A and gp100 are expressed in normal melanocytes and melanoma, and prostate specific membrane antigen (PSMA) and prostate- specific antigen (PSA) are expressed by prostate epithelial cells as well as prostate carcinoma.

[0294] In some embodiments, T cells are developed for adoptive therapy that are directed to overexpressed tissue specific or tumor associated antigens, such as prostrate specific kallikrein proteins KLK2, KLK3, KLK4 in case of prostate cancer therapy, or transglutamase protein 4, TGM4 for adenocarcinoma.

[0295] In some embodiments, the antigenic peptides that are targeted for the adoptive therapy in the methods disclosed herein are effective in modulating the tumor microenvironment. T cells are primed with antigens expressed by cells in the TME, so that the therapy is directed towards weakening and/or breaking down the tumor facilitating TME, oftentimes, in addition to directly targeting the tumor cells for T cell mediated lysis.

[0296] Tumor microenvironment comprises fibroblasts, stromal cells, endothelial cells and connective tissue cells which make up a large proportion of cells that induce or influence tumor growth. Just as T cells can be stimulated and directed attack the tumor cells in a immunosuppressive tumor environment, certain peptides and antigens can be utilized to direct the T cells against cells in the tumor vicinity that help in tumor propagation CD8+ and CD4 + T cells can be generated ex vivo that are directed against antigens on the surface of non-tumor cells in the tumor microenvironment

that promote tumor sustenance and propagation. Cancer/tumor associated fibroblasts (CAFs) are hallmark feature of pancreatic cancers, such as pancreatic adenocarcinoma (PDACs). CAFs express Col10a1 antigen. CAFs are cells that may help perpetuate a tumor. Col10A1 often confers negative prognosis for the tumor. In some embodiments Col10A1 may be considered as a biomarker for tumor sustenance and progression. It is a 680 amino acid long heterodimer protein associated with poor prognosis in breast cancer and colorectal cancers.

[0297] Activation of Col10a1 specific CD8+ T cells and CD4+ T cells may help attack and destruction of Col10A1 specific fibroblasts and help break down the tissue matrix of solid tumors.

[0298] T cells can be generated ex vivo using the method described herein, so that the T cells are activated against cancer-associated fibroblasts (CAFs). For this, Col10a1 peptides comprising epitopes that can specifically activate T cells were generated, and the HLA binding partner determined, using the highly reliable data generated from the in-house generated machine learning epitope presentation software described previously as described in Table 8.

Table 8.

Peptide	HLA Allele	Rank on HLA allele
FTCQIPGIYY	HLA-A01:01	1
GSDGKPGY	HLA-A01:01	2
NAESNGLY	HLA-A01:01	3
LTENDQVWL	HLA-A01:01	4
GTHVWVGLY	HLA-A01:01	5
TYDEYTKGY	HLA-A01:01	6
YTYDEYTKGY	HLA-A01:01	7
FTCQIPGIY	HLA-A01:01	8
NAESNGLYSSEY	HLA-A01:01	9
YLDQASGSA	HLA-A01:01	10
FLLLVSLNL	HLA-A02:01	1
FLLLVSLNLV	HLA-A02:01	2
GLYKNGTPV	HLA-A02:01	3
GLDGPKGNPGL	HLA-A02:01	4
LLLVSLNLV	HLA-A02:01	5
SLSGTPLVSA	HLA-A02:01	6
GLYSSEYV	HLA-A02:01	7
SLSGTPLV	HLA-A02:01	8
MLPQIPFLL	HLA-A02:01	9
GLPGPPGPSA	HLA-A02:01	10

SAFTVILSK	HLA-A03:01	1
AVMPEGFIK	HLA-A03:01	2
GLYKNGTPVMY	HLA-A03:01	3
AIGTPIPFDK	HLA-A03:01	4
GLPGGPGAK	HLA-A03:01	5
ILYNRQQHY	HLA-A03:01	6
AGPPGPPGFGK	HLA-A03:01	7
GIPGFPGSK	HLA-A03:01	8
GTHVWVGLYK	HLA-A03:01	9
GVPGQPGIK	HLA-A03:01	10
AVMPEGFIK	HLA-A11:01	1
SAFTVILSK	HLA-A11:01	2
VSAFTVILSK	HLA-A11:01	3
GTHVWVGLYK	HLA-A11:01	4
AIGTPIPFDK	HLA-A11:01	5
AVMPEGFIKA	HLA-A11:01	6
SSFSGFLVA	HLA-A11:01	7
PVSAFTVILSK	HLA-A11:01	8
GIPGFPGSK	HLA-A11:01	9
GVPGMNGQK	HLA-A11:01	10
AYPAIGTPIPF	HLA-A24:02	1
IGPPGIPGF	HLA-A24:02	2
HYDPRTGIF	HLA-A24:02	3
EYVHSSFSGF	HLA-A24:02	4
AGPPGPPGF	HLA-A24:02	5
YYFSYHVHV	HLA-A24:02	6
AYPAIGTPI	HLA-A24:02	7
PLPNTKTQF	HLA-A24:02	8
MLPQIPFLL	HLA-A24:02	9
CQIPGIYYF	HLA-A24:02	10
RPSLSGTPL	HLA-B07:02	1
LPQIPFLLL	HLA-B07:02	2
IPFLLLVSL	HLA-B07:02	3
LPGPPGPSAV	HLA-B07:02	4
GPIGPPGIPGF	HLA-B07:02	5

IPGPAGISV	HLA-B07:02	6
YPAIGTPIPF	HLA-B07:02	7
SPGPPGPAGI	HLA-B07:02	8
LPGPPGPSA	HLA-B07:02	9
SPGPPGPAG	HLA-B07:02	10
TIKSKGIAV	HLA-B08:01	1
IPFLLLVSL	HLA-B08:01	2
HVHVKGTHV	HLA-B08:01	3
LPNTKTQF	HLA-B08:01	4
LPQIPFLL	HLA-B08:01	5
PFLLLVSL	HLA-B08:01	6
SLNLVHGV	HLA-B08:01	7
LPQIPFLLL	HLA-B08:01	8
TGMPVSAF	HLA-B08:01	9
TPIPFDKIL	HLA-B08:01	10

[0299] Neoantigenic peptides provided herein are prevalidated for HLA binding immunogenicity (Tables 1-8 and 11-14). In some embodiments the neoantigenic peptides, prepared and stored earlier, are used to contact an antigen presenting cell (APC) to then allow presentation to a T cell in vitro for preparation of neoantigen-specific activated T cell. In some embodiments, between 2-80 or more neoantigenic peptides are used to stimulate T cells from a patient at a time.

[0300] In some embodiments the APC is an autologous APC. In some embodiments the APC is a non-autologous APC. In some embodiments the APC is a synthetic cell designed to function as an APC. In some embodiments the T cell is an autologous cell. In some embodiments, an antigen presenting cell is a cell that expresses an antigen. For example, an antigen presenting cell may be a phagocytic cell such as a dendritic cell or myeloid cell, which process an antigen after cellular uptake and presents the antigen in association with an MHC for T cell activation. For certain purposes, an APC as used herein is a cell that normally presents an antigen on its surface. In a non-binding or non-limiting example, relevant to certain cytotoxicity assays as described herein, a tumor cell is an antigen presenting cell, that the T cell can recognize an antigen presenting cell (tumor cell). Similarly, a cell or cell line expressing an antigen can be, for certain purposes as used herein, an antigen presenting cell.

[0301] In some embodiments, one or more polynucleotides encoding one or more neoantigenic peptides may be used to express in a cell to present to a T cell for activation in vitro. The one or more polynucleotides encoding one or more of the neoantigenic peptides are encoded in a vector. In some embodiments, the composition comprises from about 2 to about 80 neoantigenic polynucleotides. In

embodiments, at least one of the additional neoantigenic peptide is specific for an individual subject's tumor. In embodiments, the subject specific neoantigenic peptide is selected by identifying sequence differences between the genome, exome, and/or transcriptome of the subject's tumor sample and the genome, exome, and/or transcriptome of a non-tumor sample. In embodiments, the samples are fresh or formalin-fixed paraffin embedded tumor tissues, freshly isolated cells, or circulating tumor cells. In embodiments, the sequence differences are determined by Next Generation Sequencing.

[0302] In some embodiments the method and compositions provided herein can be used to identify or isolate a T cell receptor (TCR) capable of binding at least one neoantigenic peptide described herein or an MHC-peptide complex comprising at least one neoantigenic peptide described herein. In embodiments, the MHC of the MHC-peptide is MHC class I or class II. In embodiments, TCR is a bispecific TCR further comprising a domain comprising an antibody or antibody fragment capable of binding an antigen. In embodiments, the antigen is a T cell-specific antigen. In embodiments, the antigen is CD3. In embodiments, the antibody or antibody fragment is an anti-CD3 scFv.

[0303] In some embodiments the method and compositions provided herein can be used to prepare a chimeric antigen receptor comprising: (i) a T cell activation molecule; (ii) a transmembrane region; and (iii) an antigen recognition moiety capable of binding at least one neoantigenic peptide described herein or an MHC-peptide complex comprising at least one neoantigenic peptide described herein. In embodiments, CD3-zeta is the T cell activation molecule. In embodiments, the chimeric antigen receptor further comprises at least one costimulatory signaling domain. The In embodiments, the signaling domain is CD28, 4-1BB, ICOS, OX40, ITAM, or Fc epsilon RI-gamma. In embodiments, the antigen recognition moiety is capable of binding the isolated neoantigenic peptide in the context of MHC class I or class II. In embodiments, the CD3-zeta, CD28, CTLA-4, ICOS, BTLA, KIR, LAG3, CD137, OX40, CD27, CD40L, Tim-3, A2aR, or PD-1 transmembrane region. In embodiments, the neoantigenic peptide is located in the extracellular domain of a tumor associated polypeptide. In embodiments, the MHC of the MHC-peptide is MHC class I or class II.

[0304] Provided herein is a T cell comprising the T cell receptor or chimeric antigen receptor described herein, optionally wherein the T cell is a helper or cytotoxic T cell. In embodiments, the T cell is a T cell of a subject.

[0305] Provided herein is a T cell comprising a T cell receptor (TCR) capable of binding at least one neoantigenic peptide described herein or an MHC-peptide complex comprising at least one neoantigenic peptide described herein, wherein the T cell is a T cell isolated from a population of T cells from a subject that has been incubated with antigen presenting cells and one or more of the at least one neoantigenic peptide described herein for a sufficient time to activate the T cells. In embodiments, the T cell is a CD8+ T cell, a helper T cell or cytotoxic T cell. In embodiments, the population of T cells from a subject is a population of CD8+ T cells from the subject. In embodiments, the one or more of the at least one neoantigenic peptide described herein is a subject-specific

neoantigenic peptide. In embodiments, the subject-specific neoantigenic peptide has a different tumor neo-epitope that is an epitope specific to a tumor of the subject. In embodiments, the subject-specific neoantigenic peptide is an expression product of a tumor-specific non-silent mutation that is not present in a non-tumor sample of the subject. In embodiments, the subject-specific neoantigenic peptide binds to a HLA protein of the subject. In embodiments, the subject-specific neoantigenic peptide binds to a HLA protein of the subject with an IC50 less than 500 nM. In embodiments, the activated CD8+ T cells are separated from the antigen presenting cells. In embodiments, the antigen presenting cells are dendritic cells or CD40L-expanded B cells. In embodiments, the antigen presenting cells are non-transformed cells. In embodiments, the antigen presenting cells are non-infected cells. In embodiments, the antigen presenting cells are autologous. In embodiments, the antigen presenting cells have been treated to strip endogenous MHC-associated peptides from their surface. In embodiments, the treatment to strip the endogenous MHC-associated peptides comprises culturing the cells at about 26°C. In embodiments, the treatment to strip the endogenous MHC-associated peptides comprises treating the cells with a mild acid solution. In embodiments, the antigen presenting cells have been pulsed with at least one neoantigenic peptide described herein. In embodiments, pulsing comprises incubating the antigen presenting cells in the presence of at least about 2 μg/mL of each of the at least one neoantigenic peptide described herein. In embodiments, ratio of isolated T cells to antigen presenting cells is between about 30:1 and 300:1. In embodiments, the incubating the isolated population of T cells is in the presence of IL-2 and IL-7. In embodiments, the MHC of the MHC-peptide is MHC class I or class II.

[0306] Provided herein is a method for activating tumor specific T cells comprising: isolating a population of T cells from a subject; and incubating the isolated population of T cells with antigen presenting cells and at least one neoantigenic peptide described herein for a sufficient time to activate the T cells. In embodiments, the T cell is a CD8+ T cell, a helper T cell or cytotoxic T cell. In embodiments, the population of T cells from a subject is a population of CD8+ T cells from the subject. In embodiments, the one or more of the at least one neoantigenic peptide described herein is a subject-specific neoantigenic peptide. In embodiments, the subject-specific neoantigenic peptide has a different tumor neo-epitope that is an epitope specific to a tumor of the subject. In embodiments, the subject-specific neoantigenic peptide is an expression product of a tumor-specific non-silent mutation that is not present in a non-tumor sample of the subject. In embodiments, the subject-specific neoantigenic peptide binds to a HLA protein of the subject. In embodiments, the subject-specific neoantigenic peptide binds to a HLA protein of the subject with an IC50 less than 500 nM. In embodiments, the method further comprises separating the activated T cells from the antigen presenting cells. In embodiments, the method further comprises testing the activated T cells for evidence of reactivity against at least one of neoantigenic peptide of described herein. In embodiments, the antigen presenting cells are dendritic cells or CD40L-expanded B cells. In embodiments, the

antigen presenting cells are non-transformed cells. In embodiments, the antigen presenting cells are non-infected cells. In embodiments, the antigen presenting cells are autologous. In embodiments, the antigen presenting cells have been treated to strip endogenous MHC-associated peptides from their surface. In embodiments, the treatment to strip the endogenous MHC-associated peptides comprises culturing the cells at about 26°C. In embodiments, the treatment to strip the endogenous MHC-associated peptides comprises treating the cells with a mild acid solution. In embodiments, the antigen presenting cells have been pulsed with at least one neoantigenic peptide described herein. In embodiments, pulsing comprises incubating the antigen presenting cells in the presence of at least about 2 μg/ml of each of at least one neoantigenic peptide described herein. In embodiments, ratio of isolated T cells to antigen presenting cells is between about 30:1 and 300:1. In embodiments, the incubating the isolated population of T cells is in the presence of IL-2 and IL-7. In embodiments, the MHC of the MHC-peptide is MHC class I or class II.

[0307] Provided herein is a composition comprising activated tumor specific T cells produced by a method described herein.

[0308] Provided herein is a method of treating cancer in a subject comprising administering to the subject a therapeutically effective amount of activated tumor specific T cell described herein, or produced by a method described herein. In embodiments, the administering comprises administering from about 10^6 to 10^12, from about 10^8 to 10^11, or from about 10^9 to 10^10 of the activated tumor specific T cells.

[0309] Provided herein is a nucleic acid comprising a promoter operably linked to a polynucleotide encoding the T cell receptor described herein. In embodiments, the TCR is capable of binding the at least one neoantigenic peptide in the context of major histocompatibility complex (MHC) class I or class II.

[0310] Provided herein is a nucleic acid comprising a promoter operably linked to a polynucleotide encoding the chimeric antigen receptor described herein. In embodiments, the antigen recognition moiety is capable of binding the at least one neoantigenic peptide in the context of major histocompatibility complex (MHC) class I or class II. In embodiments, the neoantigenic peptide is located in the extracellular domain of a tumor associated polypeptide. In embodiments, the nucleic acid comprises the CD3-zeta, CD28, CTLA-4, ICOS, BTLA, KIR, LAG3, CD137, OX40, CD27, CD40L, Tim-3, A2aR, or PD-1 transmembrane region.

[0311] In some embodiments the autologous immune cells from the peripheral blood of the patient constitute peripheral blood mononuclear cells (PBMC). In some embodiments the autologous immune cells from the peripheral blood of the patient are collected via an apheresis procedure. In some embodiments, the PBMCs are collected from more than one apheresis procedures, or more than one draw of peripheral blood.

[0312] In some embodiments, both CD25+ cells and the CD14+ cells are depleted prior to addition of peptides. In some embodiments, either of CD25+ cells or the CD14+ cells are depleted prior to addition of peptides. In some embodiments, CD25+ cells and not the CD14+ cells are depleted prior to addition of peptides.

[0313] In some embodiments, the depletion procedure is followed by the addition of FMS-like tyrosine kinase 3 receptor ligand (FLT3L) to stimulate the antigen presenting cells (APCs), constituted by the monocytes, macrophages or dendritic cells (DCs) prior to addition of the peptides. In some embodiments, the depletion procedure is followed by selection of DC as suitable PACs for peptide presentation to the T cells, and mature macrophages and other antigen presenting cells are removed from the autologous immune cells from the patient. In some embodiments, the depletion procedure is followed by selection of immature DC as suitable PACs for peptide presentation to the T cells.

[0314] In some embodiments, a selection of 'n' number of neoantigenic peptides is contacted with the APCs for stimulation of the APCs for antigen presentation to the T cells.

[0315] In some embodiments, a first level selection of 'n' number of neoantigenic peptides is based on the binding ability of each of the peptides to at least on HLA haplotype that is predetermined to be present in the recipient patient. In order to determine HLA haplotype that is predetermined to be present in the recipient patient, as is known to one of skill in the art, a patient is subjected to HLA haplotyping assay form a blood sample prior to the commencement of the treatment procedure. In some embodiments, a first level selection of 'n' number of neoantigenic peptides is followed by a second level selection based on the determination of whether the mutation present in the neoantigenic peptide(s) match the neoantigens (or mutations leading to) known to be found in at least 5% of patients known to have the cancer. In some embodiments, the second level of the selection involves further determination of whether the mutation is evident in the patient.

[0316] In some embodiments, a first and the second level selection of 'n' number of neoantigenic peptides for contacting the APCs is followed by a third level of selection, based on the binding affinity of the peptide with the HLA that the peptide is capable of binding to and is at least less than 500 nM, with the determination that higher the binding affinity, the better the choice of the peptide to be selected. In some embodiments, the finally selected 'n' number of peptides can range from 1-200 peptides which are in a mix, for exposing APCs to the peptides in the culture media, and contacting with APCs.

[0317] In some embodiments the 'n' number of peptides can range from 10-190 neoantigenic peptides. In some embodiments the 'n' number of peptides can range from 20-180 neoantigenic peptides. In some embodiments the 'n' number of peptides can range from 30-170 neoantigenic peptides. In some embodiments the 'n' number of peptides can range from 40-160 neoantigenic peptides. In some embodiments the 'n' number of peptides can range from 50-150 neoantigenic peptides. In some embodiments the 'n' number of peptides can range from 60-140 neoantigenic

peptides. In some embodiments the 'n' number of peptides can range from 70-130 neoantigenic peptides. In some embodiments the 'n' number of peptides can range from 80-120 neoantigenic peptides. In some embodiments the 'n' number of peptides can range from 50-100 neoantigenic peptides. In some embodiments the 'n' number of peptides can range from 50-90 neoantigenic peptides. In some embodiments the 'n' number of peptides can range from 50-80 neoantigenic peptides. In some embodiments the 'n' number of peptides comprise at least 60 neoantigenic peptides. In some embodiments the 'n' number of peptides comprise a mixture of (a) neoantigenic peptides that are short, 8-15 amino acids long, comprising the mutated amino acid as described previously, following the formula AxByCz; these peptides are interchangeably called shortmers or short peptides for the purpose of this application; and (b) long peptides that are 15, 30, 50, 60, 80, 100-300 amino acids long and any length in between, which are subject to endogenous processing by dendritic cells for better antigen presentation; these peptides are interchangeably called longmers or long peptides for the purpose of this application. In some embodiments the at least 60 neoantigenic peptides comprise at least 30 shortmers and at least 30 longmers or variations of the same. Exemplary variations of the same include, but are not limited to the following: in some embodiments the at least 60 neoantigenic peptides comprise at least 32 shortmers and at least 32 longmers or variations of the same. In some embodiments the at least 60 neoantigenic peptides comprise at least 34 shortmers and at least 30 longmers or variations of the same. In some embodiments the at least 60 neoantigenic peptides comprise at least 28 shortmers and at least 34 longmers or variations of the same.

[0318] In some embodiments, the 'n' number of peptides are incubated in the medium comprising APCs in culture, where the APCs (DCs) have been isolated from the PBMCs, and previously stimulated with FLT3L. In some embodiments, the 'n' number of peptides are incubated with APCs in presence of FLT3L. In some embodiments, following the step of incubation of the APCs with FLT3L, the cells are added with fresh media containing FL3TL for incubation with peptides. In some embodiments, the maturation of APCs to mature peptide loaded DCs may comprise several steps of culturing the DCs towards maturation, examining the state of maturation by analysis of one or more released substances, (e.g. cytokines, chemokines) in the culture media or obtaining an aliquot of the DCs in culture form time to time. In some embodiments, the maturation of DCs take at least 5 days in culture from onset of the culture. In some embodiments, the maturation of DCs take at least 7 days in culture from onset of the culture. In some embodiments, the maturation of DCs take at least 11 days in culture from onset of the culture, or any number of days in between.

[0319] In some embodiments, the DCs are contacted with T cells after being verified for presence of or absence of maturation factors and peptide tetramer assay for verifying the repertoire of antigens presented.

[0320] In some embodiments, the DCs are contacted with T cells in a T cell media for about 2 days for the first induction. In some embodiments, the DCs are contacted with T cells in a T cell media for

about 3 days for the first induction. In some embodiments, the DCs are contacted with T cells in a T cell media for about 4 days for the first induction. In some embodiments, the DCs are contacted with T cells in a T cell media for at least about 2 days for the second induction. In some embodiments, the DCs are contacted with T cells in a T cell media for at least about 3 days for the second induction. In some embodiments, the DCs are contacted with T cells in a T cell media for at least about 4 days for the second induction. In some embodiments, the DCs are contacted with T cells in a T cell media for 5 days for the second induction. In some embodiments, the DCs are contacted with T cells in a T cell media for about 6 days for the second induction. In some embodiments, the DCs are contacted with T cells in a T cell media for about 7, 8, 9 or 10 days for the second induction. In some embodiments, the DCs are contacted with T cells in a T cell media for about less than 1 days for the third induction. In some embodiments, the DCs are contacted with T cells in a T cell media for at least about 2 or 3 days for the third induction. In some embodiments, the DCs are contacted with T cells in a T cell media for at least about 4 days for the third induction. In some embodiments, the DCs are contacted with T cells in a T cell media for 5 days for the third induction. In some embodiments, the DCs are contacted with T cells in a T cell media for about 6 days for the third induction. In some embodiments, the DCs are contacted with T cells in a T cell media for about 7, 8, 9 or 10 days for the second induction.

[0321] In some embodiments, the T cells are further contacted with one or more shortmer peptides during incubation with DCs (and in addition to the DCs) at either the first induction phase, the second induction phase or the third induction phase. In some embodiments, the T cells are further contacted with one or more shortmer peptides during incubation with DCs at the first induction phase and the second induction phase. In some embodiments, the T cells are further contacted with one or more shortmer peptides during incubation with DCs at the second induction phase and the third induction phase. In some embodiments, the T cells are further contacted with one or more shortmer peptides in all the three induction phases.

[0322] In some embodiments, the APCs and the T cells are comprised in the same autologous immune cells from the peripheral blood of the patient drawn at the first step from the patient. The T cells are isolated and preserved for the time of activation with the DCs at the end of the DC maturation phase. In some embodiments the T cells are cocultured in the presence of a suitable media for activation for the time of activation with the DCs at the end of the DC maturation phase. In some embodiments the T cells are prior cyropreserved cells from the patient, which are thawed and cultured for at least 4 hours to up to about 48 hours for induction at the time of activation with the DCs at the end of the DC maturation phase.

[0323] In some embodiments, the APCs and the T cells are comprised in the same autologous immune cells from the peripheral blood of the patient drawn at the different time periods from the patient, e.g. at different apheresis procedures. In some embodiments the time from apheresis of the patient to the time of harvest, takes between about 20 days to about less than 26 days. In some

embodiments the time from apheresis of the patient to the time of harvest, takes between about 21 days to about less than 25 days. In some embodiments the time from apheresis of the patient to the time of harvest, takes between about 21 days to about less than 24 days. In some embodiments the time from apheresis of the patient to the time of harvest, takes between about 21 days to about less than 23 days. In some embodiments the time from apheresis of the patient to the time of harvest, takes about 21 days. In some embodiments the time from apheresis of the patient to the time of harvest, takes about less than 21 days.

[0324] In some embodiments the release criteria for the activated T cells (the drug substance) comprises any one or more of sterility, endotoxin, cell phenotype, TNC Count, viability, cell concentration, potency. In some embodiments the release criteria for the activated T cells (the drug substance) comprises each one of sterility, endotoxin, cell phenotype, TNC Count, viability, cell concentration, potency.

[0325] In some embodiments the total number of cells is 2 x 10^10. In some embodiments the total number of cells is 2x10^9. In some embodiments the total number of cells is 5 x10^8. In some embodiments the total number of cells is 2 x10^8. In some embodiments the final concentration of the resuspended T cells is 2 x10^5 cells/ml or more. In some embodiments the final concentration of the resuspended T cells is 1 x10^6 cells/ml or more. In some embodiments the final concentration of the resuspended T cells is 2 x10^6 cells/ml or more.

[0326] The following criteria of released cells are described as exemplary non-limiting conditions, particularly because of the reason that the criteria for the cell population and subpopulations in Drug substance (DS) can vary based on the cancer, the state of the cancer, the state of the patient, the availability of the matched HLA haplotype and the growth potential of the APCs and T cells in the presence of the peptide. In some embodiments the activated T cells (the drug substance) comprises at least 2% or at least 3% or at least 4% or at least 5% of CD8+ T cells reactive to a particular neoantigen by tetramer assay. In some embodiments, the activated T cells (the drug substance) comprises at least 2% or at least 3% or at least 4% or at least 5% of CD4+ T cells reactive to a particular neoantigen by tetramer assay. In some embodiments, the activated T cells (the drug substance) comprise at least 5% or at least 6% or at least 7% or at least 8% or at least 9% or at least 10% of cells that are positive for memory T cell phenotype.

[0327] In some embodiments, the activated T cells (the drug substance) are selected based on one or more markers. In some embodiments, the activated T cells (the drug substance) are not selected based on one or more markers. In some embodiments, an aliquot of the activated T cells (the drug substance) are tested for the presence or absence of one or more of the following markers, and the proportions of cells thereof exhibiting each of the tested markers, the one or more markers are selected from a group consisting of: CD19, CD20, CD21, CD22, CD24, CD27, CD38, CD40, CD72, CD3, CD79a, CD79b, IGKC, IGHD, MZB1, TNFRSF17, MS4A1, CD138, TNFRSR13B, GUSPB11, BAFFR, AID, IGHM,

IGHE, IGHA1, IGHA2, IGHA3, IGHA4, BCL6, FCRLA CCR7, CD27, CD45RO, FLT3LG, GRAP2, IL16, IL7R, LTB, S1PR1, SELL, TCF7, CD62L, PLAC8, SORL1, MGAT4A, FAM65B, PXN, A2M, ATM, C20orf112, GPR183, EPB41, ADD3, GRAP2, KLRG1, GIMAP5, TC2N, TXNIP, GIMAP2, TNFAIP8, LMNA, NR4A3, CDKN1A, KDM6B, ELL2, TIPARP, SC5D, PLK3, CD55, NR4A1, REL, PBX4, RGCC, FOSL2, SIK1, CSRNP1, GPR132, GLUL, KIAA1683, RALGAPA1, PRNP, PRMT10, FAM177A1, CHMP1B, ZC3H12A, TSC22D2, P2RY8, NEU1, ZNF683, MYADM, ATP2B1, CREM, OAT, NFE2L2, DNAJB9, SKIL, DENND4A, SERTAD1, YPEL5, BCL6, EGR1, PDE4B, ANXA1, SOD2, RNF125, GADD45B, SELK, RORA, MXD1, IFRD1, PIK3R1, TUBB4B, HECA, MPZL3, USP36, INSIG1, NR4A2, SLC2A3, PER1, S100A10, AIM1, CDC42EP3, NDEL1, IDI1, EIF4A3, BIRC3, TSPYL2, DCTN6, HSPH1, CDK17, DDX21, PPP1R15B, ZNF331, BTG2, AMD1, SLC7A5 POLR3E, JMJD6, CHD1, TAF13, VPS37B, GTF2B, PAF1, BCAS2, RGPD6, TUBA4A, TUBA1A, RASA3, GPCPD1, RASGEF1B, DNAJA1, FAM46C, PTP4A1, KPNA2, ZFAND5, SLC38A2, PLIN2, HEXIM1, TMEM123, JUND, MTRNR2L1, GABARAPL1, STAT4, ALG13, FOSB, GPR65, SDCBP, HBP1, MAP3K8, RANBP2, FAM129A, FOS, DDIT3, CCNH, RGPD5, TUBA1C, ATP1B3, GLIPR1, PRDM2, EMD, HSPD1, MORF4L2, IL21R, NFKBIA, LYAR, DNAJB6, TMBIM1, PFKFB3, MED29, B4GALT1, NXF1, BIRC2, ARHGAP26, SYAP1, DNTTIP2, ETF1, BTG1, PBXIP1, MKNK2, DEDD2, AKIRIN1, HLA-DMA, HLA-DNB, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, CCL18, CCL19, CCL21, CXCL13, LAMP3, LTB, IL7R, MS4A1, CCL2, CCL3, CCL4, CCL5, CCL8, CXCL10, CXCL11, CXCL9, CD3, LTA, IL17, IL23, IL21, IL7, CCL5, CD27, CD274, CD276, CD8A, CMKLR1, CXCL9, CXCR6, HLA-DQA1, HLA-DRB1, HLA-E, IDO1, LAG3, NKG7, PDCD1LG2, PSMB10, STAT1, TIGIT, CD56, CCL2, CCL3, CCL4, CCL5, CXCL8, IFN, IL-2, IL-12, IL-15, IL-18, NCR1, XCL1, XCL2, IL21R, KIR2DL3, KIR3DL1, KIR3DL2, NCAM1, HLA-DMA, HLA-DNB, HLA-DOA, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, and HLA-DRB5.

[0328] In some embodiments, at least 0.01% of naive T cells which were obtained from the obtaining of autologous immune cells from the peripheral blood of the patient were stimulated in response to a neoantigen, and was amplified at the end of the procedure and was harvested. In some embodiments, greater than 0.01% of naive T cells which were obtained from the obtaining of autologous immune cells from the peripheral blood of the patient were stimulated in response to a neoantigen, and was amplified at the end of the procedure and was harvested. In some embodiments, greater than 0.1% of naive T cells which were obtained from the obtaining of autologous immune cells from the peripheral blood of the patient were stimulated in response to a neoantigen, and was amplified at the end of the procedure and was harvested. In some embodiments, greater than 1% of naive T cells which were obtained from the obtaining of autologous immune cells from the peripheral blood of the patient were

stimulated in response to a neoantigen, and was amplified at the end of the procedure and was harvested.

[0329] In some embodiments the total number of cells is harvested from 1, 2, or 3 cycles of the process of DC maturation and T cell activation.

[0330] In some embodiments the harvested cells are cryopreserved in vapor phase of liquid nitrogen in bags.

[0331] As is known to one of skill in the art, all applications described in the preceding paragraphs of this section from obtaining of autologous immune cells from the peripheral blood of the patient to the harvesting of cells is performed in an aseptic closed system, except the steps where aliquots of media or cells are taken out for examination by flow cytometry, mass spectroscopy, cell count, cell sorting or any functional assays, that are terminal to the cells or materials taken out as aliquots. In some embodiments the closed system for aseptic culture of up to the harvesting is proprietary to the applicant's process.

[0332] In some embodiments the T cells are method for culturing and expansion of activated T cells including the steps delineated above, starting from obtaining of autologous immune cells from the peripheral blood of the patient to harvesting, is scalable in an aseptic procedure. In some embodiments, at least 1 Liter of DC cell culture is performed at a time. In some embodiments, at least 1-2 Liters of T cell culture is performed at a time. In some embodiments, at least 5 Liters of DC cell culture is performed at a time. In some embodiments, at least 5-10 Liters of T cell culture is performed at a time. In some embodiments, at least 10 Liter of DC cell culture is performed at a time. In some embodiments, at least 10-40 Liters of T cell culture is performed at a time. In some embodiments, at least 10 Liter of DC cell culture is performed at a time. In some embodiments, at least 10-50 Liters of T cell culture is performed at a time. In some embodiments, simultaneous batch cultures are performed and tested in a system that is a closed system, and that can be manipulated and intervened from outside without introducing non-aseptic means. In some embodiments, a closed system described herein is fully automated.

[0333] When administration is by injection, the active agent can be formulated in aqueous solutions, specifically in physiologically compatible buffers such as Hanks solution, Ringer's solution, or physiological saline buffer. The solution can contain formulation agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active compound can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. In another embodiment, the drug product comprises a substance that further activates or inhibits a component of the host's immune response, for example, a substance to reduce or eliminate the host's immune response to the peptide.

[0334] The disclosure provided herein demonstrates that shared neoantigens can be used for ready therapeutic administration of a patient, thereby reducing the bench-to-bedside time lag considerably.

The composition and methods described herein provide innovative advancements in the field of cancer therapeutics.

EXAMPLES

Example 1. Precision NEOSTIM clinical process

[0335] Provided herein is an adoptive T cell therapy where T cells primed and responsive against curated pre-validated, shelved, antigenic peptides specific for a subject's cancer is administered to the subject. Provided in this example is a method of bypassing lengthy sequencing, identification and manufacture of subject specific neoantigen peptides and thereafter generating T cells having the subject specific TCRs for cancer immunotherapy, at least for the time when a subject undergoes a process of such evaluation and preparations for the personalized therapy. Advantage of this process is that it is fast, targeted and robust. As shown in **FIG. 1A**, patient identified with a cancer or tumor can be administered T cells that are activated ex vivo with warehouse curated peptides having selected, prevalidated collection of epitopes generated from a library of shared antigens known for the identified cancer. The process from patient selection to the T cell therapy may require less than 6 weeks. **FIG. 1B** illustrates the method of generating cancer target specific T cells ex vivo by priming T cells with antigen presenting cells (APCs) expressing putative T cell epitopes and expanding the activated T cells to obtain epitope-specific CD8+ and CD4+ including a population of these cells exhibiting memory phenotype (see, e.g., WO2019094642, incorporated by reference in its entirety). A library of prevalidated epitopes is generated in advance. Such epitopes are collected from prior knowledge in the field, common driver mutations, common drug resistant mutations, tissue specific antigens, and tumor associated antigens. With the help of an efficient computer-based program for epitope prediction, HLA binding and presentation characteristics, pre-validated peptides are generated for storage and stocking as shown in a diagram in **FIG. 2**. Exemplary predictions for common RAS G12 mutations are shown in **FIG. 3A-3D**. Validations are performed using a systematic process as outlined in Examples 2-5. Target tumor cell antigen responsive T cells are generated ex vivo and immunogenicity is validated using an in vitro antigen-specific T cell assay (Example 2). Mass spectrometry is used to validate that cells that express the antigen of interest can process and present the peptides on the relevant HLA molecules (Example 3). Additionally, the ability of these T cells to kill cells presenting the peptide is confirmed using a cytotoxicity assay (Example 4). Exemplary data provided herein demonstrate this validation process for RAS and GATA3 neoantigens, and can be readily applied to other antigens.

Example 2. Generation of target tumor cell antigen responsive T cells ex vivo

[0336] **Materials:**

AIM V media (Invitrogen)

Human FLT3L, preclinical CellGenix #1415-050 Stock 50 ng/μL

TNF-α, preclinical CellGenix #1406-050 Stock 10 ng/μL

IL-1β, preclinical CellGenix #1411-050 Stock 10 ng/μL

PGE1 or Alprostadil – Cayman from Czech republic Stock 0.5 μg/μL

R10 media- RPMI 1640 glutamax + 10% Human serum+ 1% PenStrep

20/80 Media- 18% AIM V + 72% RPMI 1640 glutamax + 10% Human Serum + 1% PenStrep

IL7 Stock 5 ng/μL

IL15 Stock 5 ng/μL

Procedure:

Step 1: Plate 5 million PBMCs (or cells of interest) in each well of 24 well plate with FLT3L in 2 mL AIM V media

Step 2: Peptide loading and maturation- in AIMV

1. Mix peptide pool of interest (except for no peptide condition) with PBMCs (or cells of interest) in respective wells.

2. Incubate for 1 hr.

3. Mix Maturation cocktail (including TNF-α, IL-1β, PGE1, and IL-7) to each well after incubation.

Step 3: Add human serum to each well at a final concentration of 10% by volume and mix.

Step 4: Replace the media with fresh RPMI+ 10% HS media supplemented with IL7 + IL15.

Step 5: Replace the media with fresh 20/80 media supplemented with IL7 + IL15 during the period of incubation every 1-6 days.

Step 6: Plate 5 million PBMCs (or cells of interest) in each well of new 6-well plate with FLT3L in 2 ml AIM V media

Step 7: Peptide loading and maturation for re-stimulation- (new plates)

1. Mix peptide pool of interest (except for no peptide condition) with PBMCs (or cells of interest) in respective wells

2. Incubate for 1 hr.

3. Mix Maturation cocktail to each well after incubation

Step 8: Re-stimulation:

1. Count first stimulation FLT3L cultures and add 5 million cultured cells to the new Re-stimulation plates.

2. Bring the culture volume to 5 mL (AIM V) and add 500 μL of Human serum (10% by volume)

Step 9: Remove 3ml of the media and add 6ml of RPMI+ 10% HS media supplemented with IL7 + IL15.

Step 10: Replace 75% of the media with fresh 20/80 media supplemented with IL7 + IL15.

Step 11: Repeat re-stimulation if needed.

Analysis of antigen-specific induction

[0337] MHC tetramers are purchased or manufactured on-site according to methods known by one of ordinary skill, and are used to measure peptide-specific T cell expansion in the immunogenicity

assays. For the assessment, tetramer is added to 1 x 10^5 cells in PBS containing 1% FCS and 0.1% sodium azide (FACS buffer) according to manufacturer's instructions. Cells are incubated in the dark for 20 minutes at room temperature. Antibodies specific for T cell markers, such as CD8, are then added to a final concentration suggested by the manufacturer, and the cells are incubated in the dark at 4 °C for 20 minutes. Cells are washed with cold FACS buffer and resuspended in buffer containing 1% formaldehyde. Cells are acquired on a LSR Fortessa (Becton Dickinson) instrument, and are analyzed by use of FlowJo software (Becton Dickinson). For analysis of tetramer positive cells, the lymphocyte gate is taken from the forward and side-scatter plots. Data are reported as the percentage of cells that were $CD8^+$/$tetramer^+$.

[0338] Exemplary data for RAS neoantigens on HLA-A03:01 and HLA-A11:01 are shown in **FIG. 5**. Exemplary data across multiple healthy donors for RAS G12V neoantigens on HLA-A11:01 are shown in **FIG. 6**. Exemplary data for RAS G12V neoantigens on HLA-A02:01 are shown in **FIG. 13**. Exemplary data for RAS neoantigens on HLA-A68:01 are shown in **FIG. 14**. Exemplary data for RAS neoantigens on HLA-B07:02 are shown in **FIG. 15**. Exemplary data for RAS neoantigens on HLA-B08:01 are shown in **FIG. 16**. Exemplary data for a RAS G12D neoantigens on HLA-C08:02 are shown in **FIG. 17**. Exemplary data for GATA3 neoantigens on HLA-A02:01, HLA-A03:01, HLA-A11:01, HLA-B07:02, and HLA-B08:01 are shown in **FIG. 21**. Exemplary data for a BTK C481S neoantigen on HLA-A02:01 are shown in **FIG. 26.** Exemplary data for EGFR T790M neoantigens on HLA-A02:01 are shown in **FIG. 27.**

[0339] $CD4^+$ T cell responses towards neoantigens can be induced using the *ex vivo* induction protocol. In this example, $CD4^+$ T cell responses were identified by monitoring IFNγ and/or TNFα production in an antigen specific manner. **FIG.18** shows representative examples of such flow cytometric analysis for CD4+ T cells reactive to a RAS G12D neoantigen. **FIG.24** shows representative examples of such flow cytometric analysis for CD4+ T cells reactive to a GATA3 neoantigen.

Example 3. Evaluation of presentation of antigens

[0340] For a subset of predicted antigens, the affinity of the neoepitopes for the indicated HLA alleles and stability of the neoepitopes with the HLA alleles was determined. Exemplary data for a subset of RAS neoantigens and GATA3 neoantigens are shown in **FIGS. 4A and 20**, respectively.

[0341] An exemplary detailed description of the protocol utilized to measure the binding affinity of peptides to Class I MHC has been published (Sette et al, Mol. Immunol. 31(11):813-22, 1994). In brief, MHCI complexes were prepared and bound to radiolabeled reference peptides. Peptides were incubated at varying concentrations with these complexes for 2 days, and the amount of remaining radiolabeled peptide bound to MHCI was measured using size exclusion gel-filtration. The lower the concentration of test peptide needed to displace the reference radiolabeled peptide demonstrates a stronger affinity of the test peptide for MHCI. Peptides with affinities to MHCI <50nM are generally

considered strong binders while those with affinities <150nM are considered intermediate binders and those <500nM are considered weak binders (Fritsch et al, 2014).

[0342] An exemplary detailed description of the protocol utilized to measure the binding stability of peptides to Class I MHC has been published (Harndahl et al. J Immunol Methods. 374:5-12, 2011). Briefly, synthetic genes encoding biotinylated MHC-I heavy and light chains are expressed in E. coli and purified from inclusion bodies using standard methods. The light chain (β2m) is radio-labeled with iodine (125I), and combined with the purified MHC-I heavy chain and peptide of interest at 18°C to initiate pMHC-I complex formation. These reactions are carried out in streptavidin coated microplates to bind the biotinylated MHC-I heavy chains to the surface and allow measurement of radiolabeled light chain to monitor complex formation. Dissociation is initiated by addition of higher concentrations of unlabeled light-chain and incubation at 37°C. Stability is defined as the length of time in hours it takes for half of the complexes to dissociate, as measured by scintillation counts.

[0343] To assess whether antigens could be processed and presented from the larger polypeptide context, peptides eluted from HLA molecules isolated from cells expressing the genes of interest were analyzed by tandem mass spectrometry (MS/MS).

[0344] For analysis of presentation of RAS neoantigens, cell lines were utilized that have RAS mutations naturally or were lentivirally transduced to express the mutated RAS gene. HLA molecules were either isolated based on the natural expression of the cell lines or the cell lines were lentivirally transduced or transiently transfected to express the HLA of interest. 293T cells were transduced with a lentiviral vector encoding various regions of a mutant RAS peptide. Greater than 50 million cells expressing peptides encoded by a mutant RAS peptide were cultured and peptides were eluted from HLA-peptide complexes using an acid wash. Eluted peptides were then analyzed by targeted MS/MS with parallel reaction monitoring (PRM). For 293T cells lentivirally transduced with both a RAS^{G12V} mutation and an HLA-A*03:01 gene, the peptide with amino acid sequence vvvgaVgvgk was detected by mass spectrometry. Spectral comparison to its corresponding stable heavy-isotope labeled synthetic peptide (**FIG. 4B**) showed mass accuracy of the detected peptide to be less than 5 parts per million (ppm). Endogenous peptide spectra are shown in the top panels and corresponding stable heavy-isotope labeled spectra are shown in the bottom panels. For SW620 cells naturally expressing a RAS^{G12V} mutation and lentivirally transduced with the HLA-A*03:01 gene, the peptide with amino sequence vvvgaVgvgk was detected by mass spectrometry. Spectral comparison to its corresponding stable heavy-isotope labeled synthetic peptide showed mass accuracy of the detected peptide to be less than 5 ppm (**FIG. 4C**). Endogenous peptide spectra are shown in the top panels and corresponding stable heavy-isotope labeled spectra are shown in the bottom panels. For NCI-H441 cells naturally expressing both the RAS^{G12V} mutation and the HLA-A*03:01 gene, the peptide with amino acid sequence vvvgaVgvgk was detected by mass spectrometry. Spectral comparison to its corresponding stable heavy-isotope labeled synthetic peptide showed mass accuracy of the detected peptide to be less than

5 ppm (**FIG. 4D**). Endogenous peptide spectra are shown in the top panels and corresponding stable heavy-isotope labeled spectra are shown in the bottom panels. A similar procedure was performed to analyze peptides derived from multiple RAS^{G12} mutations on HLA-A*03:01, HLA-A*11:01, HLA-A*30:01, HLA-A*68:01 and HLA-B*07:02 and Table 13 lists those peptides that were detected by mass spectrometry.

Table 13

Allele	Mutation	Neoantigen	Length
A*03:01	G12C	vvvgaCgvgk	10
	G12D	vvvgaDgvgk	10
	G12D	klvvvgaDgvgk	12
	G12R	vvvgaRgvgk	10
	G12R	klvvvgaRgvgk	12
	G12V	vvvgaVgvgk	10
	G12V	vvgaVgvgk	9
	G12V	klvvvgaVgvgk	12
A*11:01	G12C	vvvgaCgvgk	10
	G12D	vvvgaDgvgk	10
	G12R	vvvgaRgvgk	10
	G12V	vvvgaVgvgk	10
	G12V	vvgaVgvgk	9
A*30:01	G12R	vvvgaRgvgk	10
A*68:01	G12C	vvvgaCgvgk	10
	G12D	vvvgaDgvgk	10
	G12R	vvvgaRgvgk	10
	G12V	vvvgaVgvgk	10
	G12V	vvgaVgvgk	9
	G12V	lvvvgaVgvgk	11
B*07:02	G12D	gaDgvgksal	10
	G12R	gaRgvgksal	10

[0345] For analysis of presentation of GATA3 neoantigens, 293T cells were transduced with a lentiviral vector encoding various regions of peptides encoded by the GATA3 neoORF. Between 50 and 700 million of the transduced cells expressing peptides encoded by the GATA3 neoORF sequence were cultured and peptides were eluted from HLA-peptide complexes using an acid wash. Eluted peptides were then analyzed by targeted MS/MS using PRM. Spectral comparison between peptides

derived from GATA3 neoORF and corresponding synthetic peptides were performed to confirm each detection. For 293T cells expressing an HLA-A*02:01 protein, the peptides VLPEPHLAL, SMLTGPPARV and MLTGPPARV were detected by mass spectrometry (Table 14 and **FIG. 20**). Spectral comparison to corresponding synthetic peptides showed mass accuracy of the detected peptide (SMLTGPPARV) to be less than 5 ppm (**FIG. 4E**). For 293T cells expressing an HLA-A*03:01 or HLA-A*11:01 protein, the peptide KIMFATLQR was detected by mass spectrometry (**FIG. 20**). For 293T cells expressing an HLA-A*30:02 protein, the peptides IMKPKRDGY and SIMKPKRDGY were detected by mass spectrometry (Table 14). For 293T cells expressing an HLA-B*07:02 protein, the peptides KPKRDGYMF and KPKRDGYMFL were detected by mass spectrometry (Table 14 and **FIG. 20**). For 293T cells expressing an HLA-B*08:01 protein, the peptide ESKIMFATL was detected by mass spectrometry (Table 14 and **FIG. 20**). For 293T cells expressing an HLA-B*40:02 protein, the peptides AESKIMFATL and AESKIMFAT were detected by mass spectrometry (Table 14). For 293T cells expressing an HLA-C*03:03 protein, the peptide FATLQRSSL was detected by mass spectrometry (Table 14).

Table 14

Allele	**Mutation**	**Neoantigen**	**Length**
A*02:01	GATA3 neoORF	VLPEPHLAL	9
	GATA3 neoORF	SMLTGPPARV	10
	GATA3 neoORF	MLTGPPARV	9
A*03:01	GATA3 neoORF	KIMFATLQR	9
A*11:01	GATA3 neoORF	KIMFATLQR	9
A*30:02	GATA3 neoORF	IMKPKRDGY	9
	GATA3 neoORF	SIMKPKRDGY	10
B*07:02	GATA3 neoORF	KPKRDGYMF	9
	GATA3 neoORF	KPKRDGYMFL	10
B*08:01	GATA3 neoORF	ESKIMFATL	9
B*40:02	GATA3 neoORF	AESKIMFATL	10
	GATA3 neoORF	AESKIMFAT	9
C*03:03	GATA3 neoORF	FATLQRSSL	9

HLA Class I Binding and Stability

[0346] A subset of the peptides used for affinity measurements were also used for stability measurements using the assay described (n=275). These data are shown in Table 3. Less than 50 nM was considered by the field as a strong binder, 50-150 nM was considered an intermediate binder, 150-500 nM was considered a weak binder, and greater than 500 nM was considered a very weak binder. The connection between the observed stability and observed affinity was evident by the decreasing

median stability across these binned stability intervals. However, there is considerable overlap between the bins, and importantly there are epitopes in all bins with observed stability in the multiple hour range, including the very weak binders.

[0347] Immunogenicity assays are used to test the ability of each test peptide to expand T cells. Mature professional APCs are prepared for these assays in the following way. Monocytes are enriched from healthy human donor PBMCs using a bead-based kit (Miltenyi). Enriched cells are plated in GM-CSF and IL-4 to induce immature DCs. After 5 days, immature DCs are incubated at 37°C with each peptide for 1 hour before addition of a cytokine maturation cocktail (GM-CSF, IL-1β, IL-4, IL-6, TNFα, PGE1β). Cells are incubated at 37°C to mature DCs. In some embodiments the peptides, when administered into a patient is required to elicit an immune response.

[0348] Table 4A shows peptide sequences comprising RAS mutations, corresponding HLA allele to which it binds, and measured stability and affinity.

Example 4. Assessment of cytotoxic capacity of antigen-specific T cells in vitro

[0349] Cytotoxicity activity can be measured with the detection of cleaved Caspase 3 in target cells by Flow cytometry. Target cancer cells are engineered to express the mutant peptide along and the proper MHC-I allele. Mock-transduced target cells (i.e. not expressing the mutant peptide) are used as a negative control. The cells are labeled with CFSE to distinguish them from the stimulated PBMCs used as effector cells. The target and effector cells are co-cultured for 6 hours before being harvested. Intracellular staining is performed to detect the cleaved form of Caspase 3 in the CFSE-positive target cancer cells. The percentage of specific lysis is calculated as: Experimental cleavage of Caspase 3/spontaneous cleavage of Caspase 3 (measured in the absence of mutant peptide expression) x 100. Exemplary data showing that T cells induced against GATA3 neoantigens can kill target cells expressing the GATA3 neoORF is shown in **FIG. 23**.

[0350] In some examples, cytotoxicity activity is assessed by co-culturing induced T cells with a population of antigen-specific T cells with target cells expressing the corresponding HLA, and by determining the relative growth of the target cells, along with measuring the apoptotic marker Annexin V in the target cancer cells specifically. Target cancer cells are engineered to express the mutant peptide or the peptide is exogenously loaded. Mock-transduced target cells (i.e. not expressing the mutant peptide), target cells loaded with wild-type peptides, or target cells with no peptide loaded are used as a negative control. The cells are also transduced to stably express GFP allowing the tracking of target cell growth. The GFP signal or Annexin-V signal are measured over time with an IncuCyte S3 apparatus. Annexin V signal originating from effector cells is filtered out by size exclusion. Target cell growth and death is expressed as GFP and Annexin-V area (mm^2) over time, respectively.

[0351] Exemplary data demonstrating that T cells stimulated to recognize a RAS^{G12V} neoantigen on HLA-A11:01 specifically recognize and kill target cells loaded with the mutant peptide but not the wild-type peptide is shown in **FIG. 7**. Exemplary data demonstrating that T cells stimulated to

recognize a RAS^{G12V} neoantigen on HLA-A11:01 kill target cells loaded with nanomolar amounts of peptide at E:T ratios of <0.2:1 are shown in **FIG. 8.** Exemplary data demonstrating that T cells stimulated to recognize a RAS^{G12V} neoantigen on HLA-A11:01 kill SW620 cells that naturally have the RASG12V mutation and are transduced with HLA-A11:01 are shown in **FIG. 9**. Exemplary data demonstrating that T cells stimulated to recognize a RAS^{G12V} neoantigen on HLA-A03:01 kill NCI-H441 cells that naturally have the RASG12V mutation and HLA-A03:01 are shown in **FIG. 10**. Exemplary data demonstrating that T cells stimulated to recognize a GATA3 neoantigen on HLA-A02:01 kill 293T cells that naturally have HLA-A02:01 and are transduced with the GATA3 neoORF are shown in **FIGS. 22 and 23**.

Example 5. Precision NEO-STIM process applied to tissue-specific antigens

[0352] Antigens that are specifically expressed in a non-essential tissue can be targeted if a tumor arises in such a tissue. For example, antigens specifically expressed in prostate tissues can be targeted in the context of metastatic prostate cancer in which the primary tumor was resected, because the only cells expressing these antigens are metastatic cancer cells. There are multiple such non-essential tissues. As an example, prostate cells were evaluated using two methodologies to discover potential prostate-specific antigens. In one approach, prostate tissue or prostate cancer cell lines were evaluated using HLA-MS as outlined in Example 3. This approach can lead to identification of antigens that are validated to be processed and presented. Exemplary data from this approach is shown in **FIG. 25A**. In another approach, genes known to be expressed specifically in prostate cells can be evaluated through one or more MHC binding and presentation prediction software. A peptide-MHC prediction algorithm was generated and was used for these studies. As in Examples 2, 3 and 4, mass spectrometry, cellular and immunological assays further help validate a predicted peptide – HLA pair. Exemplary results from this analysis on 4 genes known to be specifically expressed in the prostate (KLK2, KLK3, KLK4, TGM4) are shown in the table below. These epitopes were further subjected to immunogenicity studies as in Example 2. The epitopes that are prefixed with '*', were shown to induce positive CD8+ T cell response in either one or both the donors (marked as 1 or 2 in column 6 respectively) and also demonstrated in **FIG. 25B**.

Table 12

Peptide	Allele	Gene	Predicted Affinity (nM)	RECON Percent Rank	Immunogenicity (# donors/2)
SLQCVSLHL	HLA-A02:01	KLK2	39.4	0.4	
LVLSIALSV	HLA-A02:01	KLK2	54.9	1.1	
VILGVHLSV	HLA-A02:01	KLK2	62.1	0.4	
VLAPQESSV	HLA-A02:01	KLK2	65.7	0.08	

SLQCVSLHLL	HLA-A02:01	KLK2	90.3	0.4	
MLLRLSEPA	HLA-A02:01	KLK2;KLK3	56	2.5	
LTMPALPMV	HLA-A02:01	KLK3	14.3	1.1	
FLTLSVTWIA	HLA-A02:01	KLK3	16.9	3.5	
KLQCVDLHV	HLA-A02:01	KLK3	21.2	0.3	
*FLTPKKLQCV	HLA-A02:01	KLK3	126.4	0.17	1
FLRPGDDSTL	HLA-A02:01	KLK3	982.7	0.4	
*FLGYLILGV	HLA-A02:01	KLK4	6.3	0.05	1
*LLANDLMLI	HLA-A02:01	KLK4	10.7	0.4	2
*FQNSYTIGL	HLA-A02:01	KLK4	15.1	1.6	2
MLIKLDESV	HLA-A02:01	KLK4	17.6	0.25	
VLQCVNVSV	HLA-A02:01	KLK4	19.2	0.1	
*LLANGRMPTV	HLA-A02:01	KLK4	25.9	0.25	2
*ILNDTGCHYV	HLA-A02:01	TGM4	17.2	0.1	1
*FQYPEFSIEL	HLA-A02:01	TGM4	21.2	1	1
ILGKYQLNV	HLA-A02:01	TGM4	22	0.3	
LLGNSVNFTV	HLA-A02:01	TGM4	27.8	0.7	
*VLDCCISLL	HLA-A02:01	TGM4	30.6	0.4	1
ILGSFELQL	HLA-A02:01	TGM4	31.2	0.25	
*RLIWLVKMV	HLA-A02:01	TGM4	64.4	0.17	1
VLLGNSVNFTV	HLA-A02:01	TGM4	83.7	0.6	
TLAIPLTDV	HLA-A02:01	TGM4	149.2	0.25	

[0353] In a further assay, T cells that are specific for the peptides indicated in the table were tested for ability to kill target cells as described in Example 4. An exemplary data is presented in **FIG. 25C**, where KLK4 prostate specific epitope were co-cultured with 293T-GFP cells either loaded with 2uM of peptide or not loaded. Peptide loaded target cells were killed to a much greater extent (right image) compared to the no peptide control (left image).

Example 6. Enrichment of target antigen activated T cells

[0354] Tumor antigen responsive T cells may be further enriched. In this example, multiple avenues for enrichment of antigen responsive T cells are explored and results presented. After the initial stimulation of antigen-specific T cells (Example 2, Steps 1-5), an enrichment procedure can be used prior to further expansion of these cells. As an example, stimulated cultures and pulsed with the same peptides used for the initial stimulation on day 13, and cells upregulating 4-1BB are enriched using Magnetic-Assisted Cell Separation (MACS; Miltenyi). These cells can then be further expanded, for

example, using anti-CD3 and anti-CD28 microbeads and low-dose IL-2. As shown in **FIG. 19A** (middle row) and **FIG. 19B** (middle column), this approach can enrich for multiple antigen-specific T cell populations. As another example, T cells that are stained by multimers can be enriched by MACS on day 14 of stimulation and further expanded, for example, using anti-CD3 and anti-CD28 microbeads and low-dose IL-2. As shown in **FIG. 19A** (bottom row) and **FIG. 19B** (right column), this approach can enrich for multiple antigen-specific T cell populations.

Example 7. Immunogenicity assays for selected peptides

[0355] After maturation of DCs, PBMCs (either bulk or enriched for T cells) are added to mature dendritic cells with proliferation cytokines. Cultures are monitored for peptide-specific T cells using a combination of functional assays and/or tetramer staining. Parallel immunogenicity assays with the modified and parent peptides allowed for comparisons of the relative efficiency with which the peptides expanded peptide-specific T cells. In some embodiments, the peptides elicit 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.

[0356] Immunogenicity can be measured by a tetramer assay. MHC tetramers are purchased or manufactured on-site, and are used to measure peptide-specific T cell expansion in the immunogenicity assays. For the assessment, tetramer is added to 1x10^5 cells in PBS containing 1% FCS and 0.1% sodium azide (FACS buffer) according to manufacturer's instructions. Cells are incubated in the dark for 20 minutes at room temperature. Antibodies specific for T cell markers, such as CD8, are then added to a final concentration suggested by the manufacturer, and the cells are incubated in the dark at 4 degrees Celsius for 20 minutes. Cells are washed with cold FACS buffer and resuspended in buffer containing 1% formaldehyde. Cells are acquired on a FACS Calibur (Becton Dickinson) instrument, and are analyzed by use of Cellquest software (Becton Dickinson). For analysis of tetramer positive cells, the lymphocyte gate is taken from the forward and side-scatter plots. Data are reported as the percentage of cells that were $CD8^+/Tetramer^+$.

[0357] Immunogenicity can be measured by intracellular cytokine staining. In the absence of well-established tetramer staining to identify antigen-specific T cell populations, antigen-specificity can be estimated using assessment of cytokine production using well-established flow cytometry assays. Briefly, T cells are stimulated with the peptide of interest and compared to a control. After stimulation, production of cytokines by CD4+ T cells (e.g., IFNγ and TNFα) are assessed by intracellular staining. These cytokines, especially IFNγ, used to identify stimulated cells.

[0358] In some embodiments the immunogenicity is measured by measuring a protein or peptide expressed by the T cell, using ELISpot assay. Peptide-specific T cells are functionally enumerated using the ELISpot assay (BD Biosciences), which measures the release of IFNγ from T cells on a single cell basis. Target cells (T2 or HLA-A0201 transfected C1Rs) were pulsed with 10 μM peptide for one hour at 37 degrees C, and washed three times. 1x10^5 peptide-pulsed targets are co-cultured

in the ELISPOT plate wells with varying concentrations of T cells (5x10^2 to 2x10^3) taken from the immunogenicity culture. Plates are developed according to the manufacturer's protocol, and analyzed on an ELISPOT reader (Cellular Technology Ltd.) with accompanying software. Spots corresponding to the number of IFN gamma-producing T cells are reported as the absolute number of spots per number of T cells plated. T cells expanded on modified peptides are tested not only for their ability to recognize targets pulsed with the modified peptide, but also for their ability to recognize targets pulsed with the parent peptide.

[0359] CD107a and b are expressed on the cell surface of CD8+ T cells following activation with cognate peptide. The lytic granules of T cells have a lipid bilayer that contains lysosomal-associated membrane glycoproteins ("LAMPs"), which include the molecules CD107a and b. When cytotoxic T cells are activated through the T cell receptor, the membranes of these lytic granules mobilize and fuse with the plasma membrane of the T cell. The granule contents are released, and this leads to the death of the target cell. As the granule membrane fuses with the plasma membrane, C107a and b are exposed on the cell surface, and therefore are markers of degranulation. Because degranulation as measured by CD107a and b staining is reported on a single cell basis, the assay is used to functionally enumerate peptide-specific T cells. To perform the assay, peptide is added to HLA-A0201-transfected cells C1R to a final concentration of 20 μM, the cells were incubated for 1 hour at 37 degrees C, and washed three times. 1x10^5 of the peptide-pulsed C1R cells were aliquoted into tubes, and antibodies specific for CD107a and b are added to a final concentration suggested by the manufacturer (Becton Dickinson). Antibodies are added prior to the addition of T cells in order to "capture" the CD107 molecules as they transiently appear on the surface during the course of the assay. 1x10^5 T cells from the immunogenicity culture are added next, and the samples were incubated for 4 hours at 37 degrees C. The T cells are further stained for additional cell surface molecules such as CD8 and acquired on a FACS Calibur instrument (Becton Dickinson). Data is analyzed using the accompanying Cellquest software, and results were reported as the percentage of CD8+ CD107 a and b+ cells.

[0360] Cytotoxic activity is measured using a chromium release assay. Target T2 cells are labeled for 1 hour at 37 degrees C with Na51Cr and washed 5x10^3 target T2 cells were then added to varying numbers of T cells from the immunogenicity culture. Chromium release is measured in supernatant harvested after 4 hours of incubation at 37 degrees C. The percentage of specific lysis is calculated as:

Experimental release-spontaneous release/Total release-spontaneous release x 100

[0361] Immunogenicity assays were carried out to assess whether each peptide can elicit a T cell response by antigen-specific expansion. Though current methods are imperfect, and therefore negative results do not imply a peptide is incapable of inducing a response, a positive result demonstrates that a peptide can induce a T cell response. Several peptides from Table 3 were tested for their capacity to elicit CD8+ T cell responses with multimer readouts as described. Each positive result was measured with a second multimer preparation to avoid any preparation biases. In an exemplary assay, HLA-

A02:01+ T cells were co-cultured with monocyte-derived dendritic cells loaded with TMPRSS2::ERG fusion neoepitope (ALNSEALSV; HLA-A02:01) for 10 days. CD8+ T cells were analyzed for antigen-specificity for TMPRSS2::ERG fusion neoepitope using multimers (initial: BV421 and PE; validation: APC and BUV396).

[0362] While antigen-specific CD8+ T cell responses are readily assessed using well-established HLA Class I multimer technology, CD4+ T cell responses require a separate assay to evaluate because HLA Class II multimer technology is not well-established. In order to assess CD4+ T cell responses, T cells were re-stimulated with the peptide of interest and compared to a control. In the case of a completely novel sequence (e.g., arising from a frame-shift or fusion), the control was no peptide. In the case of a point-mutation, the control was the WT peptide. After stimulation, production of cytokines by CD4+ T cells (e.g., IFNγ and TNFα) were assessed by intracellular staining. These cytokines, especially IFNγ, used to identify stimulated cells. Antigen-specific CD4+ T cell responses showed increased cytokine production relative to control.

Example 8. Cell Expansion and preparation

[0363] To prepare APCs, the following method was employed (a) obtain of autologous immune cells from the peripheral blood of the patient; enrich monocytes and dendritic cells in culture; load peptides and mature DCs.

T cell Induction (Protocol 1)

[0364] First induction: (a) Obtaining autologous T cells from an apheresis bag; (b) Depleting CD25+ cells and CD14+ cells, alternatively, depleting only CD25+ cells; (c) Washing the peptide loaded and mature DC cells, resuspending in the T cell culture media; (d) Incubating T cells with the matured DC.

[0365] Second induction: (a) Washing T cells, and resuspending in T cell media, and optionally evaluating a small aliquot from the cell culture to determine the cell growth, comparative growth and induction of T cell subtypes and antigen specificity and monitoring loss of cell population; (b) Incubating T cells with mature DC.

[0366] Third induction: (a) Washing T cells, and resuspending in T cell media, and optionally evaluating a small aliquot from the cell culture to determine the cell growth, comparative growth and induction of T cell subtypes and antigen specificity and monitoring loss of cell population; (b) Incubating T cells with mature DC.

[0367] To harvest peptide activated t cells and cryopreserve the T cells, the following method was employed (a) Washing and resuspension of the final formulation comprising the activated T cells which are at an optimum cell number and proportion of cell types that constitutes the desired characteristics of the Drug Substance (DS). The release criteria testing include inter alia, Sterility, Endotoxin, Cell Phenotype, TNC Count, Viability, Cell Concentration, Potency; (b) Filling drug substance in suitable enclosed infusion bags; (c) Preservation until time of use.

Example 9. Methods of functional characterization of the CD4+ and CD8+ neoantigen-specific T cells.

[0368] Neoantigens, which arise in cancer cells from somatic mutations that alter protein-coding gene sequences, are emerging as an attractive target for immunotherapy. They are uniquely expressed on tumor cells as opposed to healthy tissue and may be recognized as foreign antigens by the immune system, increasing immunogenicity. T cell manufacturing processes were developed to raise memory and de novo CD4+ and CD8+ T cell responses to patient-specific neoantigens through multiple rounds of ex-vivo T cell stimulation, generating a neoantigen-reactive T cell product for use in adoptive cell therapy. Detailed characterization of the stimulated T cell product can be used to test the many potential variables these processes utilize.

[0369] To probe T cell functionality and/or specificity, an assay was developed to simultaneously detect antigen-specific T cell responses and characterize their magnitude and function. This assay employs the following steps. First T cell-APC co-cultures were used to elicit reactivity in antigen-specific T cells. Optionally, sample multiplexing using fluorescent cell barcoding is employed. To identify antigen-specific CD8+ T cells and to examine T cell functionality, staining of peptide-MHC multimers and multiparameter intracellular and/or cell surface cell marker staining were probed simultaneously using FACS analysis. The results of this streamlined assay demonstrated its application to study T cell responses induced from a healthy donor. Neoantigen-specific T cell responses induced toward peptides were identified in a healthy donor. The magnitude, specificity and functionality of the induced T cell responses were also compared. Briefly, different T cell samples were barcoded with different fluorescent dyes at different concentrations (see, e.g., Example 19). Each sample received a different concentration of fluorescent dye or combination of multiple dyes at different concentrations. Samples were resuspended in phosphate-buffered saline (PBS) and then fluorophores dissolved in DMSO (typically at 1:50 dilution) were added to a maximum final concentration of 5 μM. After labeling for 5 min at 37 °C, excess fluorescent dye was quenched by the addition of protein-containing medium (e.g. RPMI medium containing 10% pooled human type AB serum). Uniquely barcoded T cell cultures were challenged with autologous APC pulsed with the antigen peptides as described above.

[0370] The differentially labeled samples were combined into one FACS tube or well, and pelleted again if the resulting volume is greater than 100 μL. The combined, barcoded sample (typically 100 μL) was stained with surface marker antibodies including fluorochrome conjugated peptide-MHC multimers. After fixation and permeabilization, the sample was additionally stained intracellularly with antibodies targeting TNF-α and IFN-γ.

[0371] The cell marker profile and MHC tetramer staining of the combined, barcoded T cell sample were then analyzed simultaneously by flow cytometry on flow cytometer. Unlike other methods that analyze cell marker profiles and MHC tetramer staining of a T cell sample separately, the simultaneous

analysis of the cell marker profile and MHC tetramer staining of a T cell sample described in this example provides information about the percentage of T cells that are both antigen specific and that have increased cell marker staining. Other methods that analyze cell marker profiles and MHC tetramer staining of a T cell sample, separately determine the percentage of T cells of a sample that are antigen specific, and separately determine the percentage of T cells that have increased cell marker staining, only allowing correlation of these frequencies.

[0372] The simultaneous analysis of the cell marker profile and MHC tetramer staining of a T cell sample described in this example does not rely on correlation of the frequency of antigen specific T cells and the frequency of T cells that have increased cell marker staining; rather, it provides a frequency of T cells that are both antigen specific and that have increased cell marker staining. The simultaneous analysis of the cell marker profile and MHC tetramer staining of a T cell sample described in this example allows for determination on a single cell level, those cells that are both antigen specific and that have increased cell marker staining.

[0373] To evaluate the success of a given induction process, a recall response assay was used followed by a multiplexed, multiparameter flow cytometry panel analysis. A sample taken from an induction culture was labeled with a unique two-color fluorescent cell barcode. The labeled cells were incubated on antigen-loaded DCs or unloaded DCs overnight to stimulate a functional response in the antigen-specific cells. The next day, uniquely labeled cells were combined prior to antibody and multimer staining according to Table 9 below.

Table 9

Marker	**Fluorochrome**	**Purpose**
CD19/CD16/CD14	BUV395	Cell exclusion
Live/Dead	Near-IR	Dead cell exclusion
CD3	BUV805	Lineage gating
CD4	Alexa Fluor 700	Lineage gating
CD8	PerCP-Cy5.5	Lineage gating
Barcode 1	CFSE	Sample multiplexing
Barcode 2	TagIT Violet	Sample multiplexing
Multimer 1	PE	CD8+antigen specificity
Multimer 2	BV650	CD8+antigen specificity
IFNγ	APC	Functionality
TNFα	BV711	Functionality

CD107a	BV786	Cytotoxicity
4-1BB	PE/Dazzle 594	Activation

[0374] Patient-specific neoantigens were predicted using bioinformatics engine. Synthetic long peptides covering the predicted neoantigens were used as immunogens in the stimulation protocol to assess the immunogenic capacity. The stimulation protocol involves feeding these neoantigen-encoding peptides to patient-derived APCs, which are then co-cultured with patient-derived T cells to prime neoantigen specific T cells.

[0375] Multiple rounds of stimulations are incorporated in the stimulation protocol to prime, activate and expand memory and de novo T cell responses. The specificity, phenotype and functionality of these neoantigen-specific T cells was analyzed by characterizing these responses with the following assays: Combinatorial coding analysis using pMHC multimers was used to detect multiple neoantigen-specific CD8+ T cell responses. A recall response assay using multiplexed, multiparameter flow cytometry was used to identify and validate CD4+ T cell responses. The functionality of CD8+ and CD4+ T cell responses was assessed by measuring production of pro-inflammatory cytokines including IFN-γ and TNFα, and upregulation of the CD107a as a marker of degranulation. A cytotoxicity assay using neoantigen-expressing tumor lines was used to understand the ability of CD8+ T cell responses to recognize and kill target cells in response to naturally processed and presented antigen. The cytotoxicity was measured by the cell surface upregulation of CD107a on the T cells and upregulation of active Caspase3 on neoantigen- expressing tumor cells. The stimulation protocol was successful in the expansion of pre-existing CD8+ T cell responses, as well as the induction of *de novo* CD8+ T cell responses (Table 10).

Table 10

Patient	HUGO Symbol	Full Gene Name	Type
NV10	$SRSF1_{E>K}$ $ARAP1_{Y>H}$ $PKDREJ_{G>R}$	Serine And Arginine Rich Splicing Factor 1 Ankyrin Repeat And PH Domain Polycystin Family Receptor For Egg Jelly	CD8
	$MKRN1_{S>L}$ $CREBBP_{S>L}$ $TPCN1_{K>E}$	Makorin Ring Finger Protein 1 CREB Binding Protein Two Pore Segment Channel 1	CD4
NV6	$AASDH_{neoORF}$ $ACTN4_{K>N}$ $CSNK1A1_{S>L}$ $DHX40_{neoORF}$ $GLI3_{P>L}$ $QARS_{R>W}$ $FAM178B_{P>L}$ $RPS26_{P>L}$	Aminoadipate-Semialdehyde Dehydrogenase Actinin Alpha 4 Casein Kinase 1 Alpha 1 DEAH-Box Helicase 40 GLI Family Zinc Finger 3 Glutaminyl-TRNA Synthetase Family With Sequence Similarity 178 Member B Ribosomal Protein S26	CD8

[0376] Using PBMCs from a melanoma patient a clinical study performed by Applicant's group, expansion of a pre-existing CD8+ T cell response was observed from 4.5% of CD8+ T cells to 72.1% of CD8+ T cells ($SRSF1E_{>K}$). Moreover, the stimulation protocol was effective in inducing two presumed de novo CD8+ T cell responses towards patient- specific neoantigens (exemplary de novo CD8+ T cell responses: $ARAP1_{Y>H}$: 6.5% of CD8+ T cells and $PKDREJ_{G>R}$: 13.4% of CD8+ T cells; no cells were detectable prior to the stimulation process). The stimulation protocol successfully induced seven de novo CD8+ T cell responses towards both previously described and novel model neoantigens using PBMCs from another melanoma patient, NV6, up to varying magnitudes ($ACTN4_{K>N}$ $CSNK1A1_{S>L}$ DHX40neoORF 7, $GLI3_{P>L}$, $QARS_{R>W}$, $FAM178B_{P>L}$ and $RPS26_{P>L}$, range: 0.2% of CD8+ T cells up to 52% of CD8+ T cells). Additionally, a CD8+ memory T cell response towards a patient-specific neoantigen was expanded (AASDHneoORF, up to 13% of CD8+ T cells post stimulation).

[0377] The induced CD8+ T cells from the patient was characterized in more detail. Upon re-challenge with mutant peptide loaded DCs, neoantigen-specific CD8+ T cells exhibited one, two and/or all three functions (16.9% and 65.5% functional CD8+ pMHC+ T cells for SRSF1E>K and ARAP1Y>H, respectively. When re-challenged with different concentrations of neoantigen peptides, the induced CD8+ T cells responded significantly to mutant neoantigen peptide but not to the wildtype peptide. In said patient, CD4+ T cell responses were identified using a recall response assay with mutant neoantigen loaded DCs. Three CD4+ T cell responses were identified (MKRN1S>L, CREBBPS>L and TPCN1K>E) based on the reactivity to DCs loaded with mutant neoantigen peptide. These CD4+ T cell responses also showed a polyfunctional profile when re-challenged with mutant neoantigen peptide. 31.3%, 34.5% & 41.9% of CD4+ T cells exhibited one, two and/or three functions; MKRN1S>L, CREBBPS>L and TPCN1K>E responses, respectively.

[0378] The cytotoxic capacity of the induced CD8+ responses from said patient was also assessed. Both SRSF1E>K and ARAP1Y>H responses showed a significant upregulation of CD107a on the CD8+ T cells and active Caspase3 on the tumor cells transduced with the mutant construct after co-culture.

[0379] Using the stimulation protocol, predicted patient-specific neoantigens, as well as model neoantigens, were confirmed to be immunogenic by the induction of multiple neoantigen-specific CD8+ and CD4+ T cell responses in patient material. The ability to induce polyfunctional and mutant- specific CD8+ and CD4+ T cell responses proves the capability of predicting high-quality neoantigens and generating potent T cell responses. The presence of multiple enriched neoantigen-specific T cell populations (memory and de novo) at the end of the stimulation process demonstrates the ability to raise new T cell responses and generate effective cancer immunotherapies to treat cancer patients.

[0380] Exemplary materials for T cell culture are provided below:

Materials: AIM V media (Invitrogen)Human FLT3L; preclinical CellGenix #1415-050 Stock 50 ng/μL TNFα; preclinical CellGenix #1406-050 Stock 10 ng/μL; IL-1β, preclinical CellGenix #1411-050 Stock 10 ng/μL; PGE1 or Alprostadil – Cayman from Czech republic Stock 0.5 μg/μL; R10 media- RPMI 1640 glutamax + 10% Human serum+ 1% PenStrep; 20/80 Media- 18% AIM V + 72% RPMI 1640 glutamax + 10% Human Serum + 1% PenStrep; IL7 Stock 5 ng/μL; IL15 Stock 5 ng/μL; DC media (Cellgenix); CD14 microbeads, human, Miltenyi #130-050-201, Cytokines and/or growth factors, T cell media (AIM V + RPMI 1640 glutamax + serum + PenStrep), Peptide stocks - 1 mM per peptide (HIV A02 – 5-10 peptides, HIV B07- 5-10 peptides, DOM - 4-8 peptides, PIN - 6-12 peptides).

CLAIMS

What is claimed is:

1. A method for treating cancer in a subject in need thereof comprising:
   (a) 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
   (b) contacting a T cell from the subject or an allogeneic T cell with antigen presenting cells (APCs) comprising one or more peptides containing the at least one selected epitope sequence, 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 an HLA allele of the subject,
      (ii) is immunogenic according to an immunogenicity assay,
      (iii)is presented by APCs according to a mass spectrometry assay, and
      (iv)stimulates T cells to be cytotoxic according to a cytotoxicity assay.
2. The method of claim 1, wherein
   (a) 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;
   (b) 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
   (c) the at least one epitope sequence comprises a protein expressed by a cell in a tumor microenvironment.
3. The method of claim 1 or 2, wherein one or more of the least one selected epitope sequence comprises an epitope that is not expressed by cancer cells of the subject.
4. The method of claim 3, wherein the epitope that is not expressed by cancer cells of the subject is expressed by cells in a tumor microenvironment of the subject.
5. The method of any one of claims 1-4, wherein 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.
6. The method of any one of claims 1-4, wherein 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.
7. The method of any one of claims 1-6, wherein 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).

8. The method of any one of claims 1-7, wherein the epitope that is immunogenic according to an immunogenicity assay is immunogenic according to a multimer assay or a functional assay.
9. The method of claim 8, wherein the multimer assay comprises flow cytometry analysis.
10. The method of claim 8 or 9, wherein 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.
11. The method of claim 10, wherein the 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.
12. The method of claim 11, wherein 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.
13. The method of claim 11 or 12, 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.
14. The method of any one of claims 10-13, wherein 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.
15. The method of any one of claims 10-14, wherein 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.
16. The method of claim 8, wherein the functional assay comprises an immunoassay.
17. The method of claim 8 or 16, wherein 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
18. The method of claim 17, wherein 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.

19. The method of any one of claims 1-18, 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 epitope.
20. The method of claim 19, wherein 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.
21. The method of claim 19, wherein 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.
22. The method of claim 19, wherein 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
23. The method of any one of claims 1-22, wherein 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.
24. The method of any one of claims 1-23, wherein the method comprises selecting the subject using a circulating tumor DNA assay or a gene panel.
25. The method of any one of claims 1-24, wherein the T cell is from a biological sample from the subject.
26. The method of claim 25, wherein the T cell is from a peripheral blood mononuclear cell (PBMC) sample from the subject or an apheresis sample from the subject or a leukopheresis sample from the subject.
27. The method of any one of claims 1-24, wherein the T cell is an allogeneic T cell.
28. The method of any one of claims 1-27, wherein each of the at least one selected epitope sequence satisfies each of the following criteria:

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

(ii) is immunogenic according to an immunogenicity assay,

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

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

29. The method of any one of claims 1-28, wherein at least one of the one or more peptides is a synthesized peptide or a peptide expressed from a nucleic acid sequence.
30. The method of any one of claims 1-29, wherein 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.
31. The method of any one of claims 1-30, 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.
32. The method of any one of claims 1-31, wherein 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.
33. The method of any one of claims 1-32, wherein the method further comprises administering the population of T cells to the subject.
34. The method of any one of claims 1-33, wherein a protein comprising the at least one selected epitope sequence is expressed by a cancer cell of the subject.
35. The method of any one of claims 1-34, wherein a protein comprising the at least one selected epitope sequences is expressed by cells in the tumor microenvironment of the subject.
36. The method of any one of claims 1-34, wherein one or more of the at least one selected epitope sequence comprises a mutation.
37. The method of any one of claims 1-36, 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.
38. The method of any one of claims 1-37, 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.*
39. The method of any one of claims 1-38, wherein 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.

40. The method of any one of claims 1-39, wherein 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.
41. The method of claim 40, wherein 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.
42. The method of any one of claims 1-41, wherein 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.
43. The method of claim 42, wherein the population of immune cells is from a biological sample from the subject.
44. The method of claim 42 or 43, wherein 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

(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; thereby forming a population of cells comprising stimulated T cells.

45. The method of claim 44, wherein 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.
46. The method of claim 45, wherein steps (b) and (c) are performed in less than 28 days.
47. The method of any one of claims 42-46, wherein 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.
48. The method of any one of claims 42-47, wherein 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.

49. The method of any one of claims 42-48, wherein 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.
50. The method of any one of claims 42-49, wherein 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.
51. The method of any one of claims 42-50, wherein expanding comprises:
    (A) contacting the population of cells comprising stimulated T cells with a second population of mature APCs, wherein the second population of mature APCs
        (i) have been incubated with FLT3L and
        (ii) present the at least one selected epitope sequence; and
    (B) expanding the population of cells comprising stimulated T cells for a second time period, thereby forming an expanded population of T cells.
52. The method of claim 51, wherein the second population of mature APCs have been incubated with FLT3L for at least 1 day prior to contacting the population of cells comprising stimulated T cells with the second population of mature APCs.
53. The method of claim 51, 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) 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.
54. The method of claim 53, wherein 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.
55. The method of any one of claims 42-54, wherein the biological sample is a peripheral blood sample, a leukapheresis sample or an apheresis sample.
56. The method of any one of claims 42-55, further comprising 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.
57. The method of any one of claims 42-56, wherein 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.
58. The method of any one of claims 42-57, further comprising administering a pharmaceutical composition comprising the expanded population of cells comprising tumor antigen specific T cells to a human subject with cancer.

59. The method of claim 58, wherein the human subject with cancer is the human subject from which the biological sample was obtained.
60. The method of any one of claims 42-59, wherein the polypeptide is from 8 to 50 amino acids in length.
61. The method of any one of claims 42-60, wherein the polypeptide comprises at least two of the selected epitope sequence, each expressed by cancer cells of a human subject with cancer.
62. The method of any one of claims 42-61, wherein depleting further comprising depleting CD19+ cells from the population of immune cells comprising a first population of APCs and T cells.
63. The method of any one of claims 42-62, wherein depleting further comprising depleting CD11b+ cells from the population of immune cells comprising a first population of APCs and T cells.
64. The method of any one of claims 1-63, wherein 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.
65. The method of any one of claims 1-64, wherein 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.
66. The method of any one of claims 1-65, wherein 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
67. The method of any one of claims 1-66, wherein 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.
68. The method of any one of claims 1-67, wherein one or more of the at least one selected epitope sequence has a length of from 8 to 12 amino acids.
69. The method of any one of claims 1-68, wherein one or more of the at least one selected epitope sequence has a length of from 13-25 amino acids.
70. The method of any one of claims 1-69, wherein the method further comprises selecting one or more subpopulation of cells from an expanded population of T cells prior to administering to the subject.
71. The method of any one of claims 1-70, wherein eliciting an immune response in the T cell culture comprises inducing cytokine or IL2 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.

72. The method of any one of claims 1-71, wherein the method comprises stimulating T cells to be cytotoxic against cells loaded with the at least one selected epitope sequences or cancer cells expressing a protein comprising the at least one selected epitope sequences according to a cytotoxicity assay.
73. The method of any one of claims 1-72, wherein 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.
74. The method of any one of claims 1-73, wherein the at least one selected epitope is expressed by a cancer cell, and an additional selected epitope is expressed by a cancer associated cell.
75. The method of claim 74, wherein the additional selected epitope is expressed on a cancer associated fibroblast cell.
76. The method of claim 75, wherein the additional selected epitope is selected from Table 8.