[0013] In some embodiments, the protein comprises CYP11A1, CYP11B1, CYP11B2, MC2R, STAR, or any combination thereof, and wherein the cancer comprises adrenal cancer.

[0014] In some embodiments, the epitope sequence has from 70% to 100% sequence identity to a peptide sequence selected from the group consisting of SEQ ID NOs 2212-2523, 4817-4915, and 7449-7538, and wherein the cancer comprises adrenal cancer.

[0015] In some embodiments, the protein comprises ALPPL2, POTEE, PRAME, or any combination thereof, and wherein the cancer comprises uterine cancer.

[0016] In some embodiments, the epitope sequence has from 70% to 100% sequence identity to a peptide sequence selected from the group consisting of SEQ ID NOs 627-719, 5285-5431, and 6085-6183, and wherein the cancer comprises uterine cancer.

[0017] In some embodiments, the protein comprises KLK2, KLK3, KLK4, POTEH, POTEG, TGM4, RLN1, POTEE, PPIAL4G or any combination thereof, and wherein the cancer comprises prostate cancer.

[0018] In some embodiments, the epitope sequence has from 70% to 100% sequence identity to a peptide sequence selected from the group consisting of SEQ ID NOs 3441-4274, 5285-6084, 6580-6845, and 8100-8434, and wherein the cancer comprises prostate cancer.

[0019] In some embodiments, the protein comprises ANKRD30A, COL10A1, or a combination thereof and wherein the cancer comprises breast cancer.

[0020] In some embodiments, the epitope sequence has from 70% to 100% sequence identity to a peptide sequence selected from the group consisting of SEQ ID NOs 815-988, and 1749-1867, and wherein the cancer comprises breast cancer.

[0021] In some embodiments, the protein comprises CTCFL, PRAME, CLDN6, EPYC, or any combination thereof, and wherein the cancer comprises ovarian cancer.

[0022] In some embodiments, the epitope sequence has from 70% to 100% sequence identity to a peptide sequence selected from the group consisting of SEQ ID NOs 1659-1748, 1964-2119, 2827-2919, and 6085-6183, and wherein the cancer comprises ovarian cancer.

[0023] In some embodiments, the protein comprises CTCFL, and wherein the cancer comprises cervical cancer.

[0024] In some embodiments, the epitope sequence has from 70% to 100% sequence identity to a peptide sequence selected from the group consisting of SEQ ID NOs 1964-2119, and wherein the cancer comprises cervical cancer.

[0025] In some embodiments, the protein comprises POTEE, PPIAL4G, or a combination thereof, and wherein the cancer comprises colorectal cancer.

[0026] In some embodiments, the epitope sequence has from 70% to 100% sequence identity to a peptide sequence selected from the group consisting of SEQ ID Nos 5285-5431, and 5996-6084, and wherein the cancer comprises colorectal cancer.

epitope sequence binds to or is predicted to bind to a protein encoded by a MHC allele expressed by the human subject in need of the autologous T cell therapy, and the TCR binds to the epitope when presented in a complex by the protein encoded by a MHC allele expressed by the human subject, wherein the epitope is a tissue specific epitope that is encoded by a tissue-specific antigen epitope gene that has an expression level in the target tissue 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. In some embodiments, the T cell is a non-engineered cell. In some embodiments, the T cell is autologous to the subject. In some embodiments, the T cell is modified ex vivo.

[0054] In some embodiments, the TCR is specific to the tissue-specific antigen peptide in a complex with a class I MHC protein or a class II MHC protein.

[0055] In some embodiments, the at least one antigen specific T cell expresses CD8 or CD4.

[0056] In some embodiments, the at least one antigen specific T cell comprise an exogenous polynucleotide encoding the TCR.

[0057] In some embodiments, the at least one antigen specific T cell comprises a TCR that is specific for an epitope sequence that has from 70% to 100% sequence identity to a peptide sequence selected from the group consisting of SEQ ID NOs 6846-7061, 7359-7448, 7629-8099, and 8619-8744, and wherein the cancer comprises thyroid cancer.

[0058] In some embodiments, the at least one antigen specific T cell comprises a TCR that is specific for an epitope sequence from the protein RBPJL, AQP12A, AQP12B, IAPP, CELA2A, CELA2B, AMY2A, CTRC, G6PC2, KIRREL2, PNLIPRP1, SERPINI2, SYNC, or any combination thereof, and wherein the cancer comprises pancreatic cancer.

[0059] In some embodiments, the at least one antigen specific T cell comprises a TCR that is specific for an epitope sequence that has from 70% to 100% sequence identity to a peptide sequence selected from the group consisting of SEQ ID NOs 720-814, 989-1182, 1373-1565, 2120-2211, 2920-3009, 3101-3196, 3320-3440, 5193-5284, 6487-6579, 7062-7150, and 7539-7628, and wherein the cancer comprises pancreatic cancer.

[0060] In some embodiments, the at least one antigen specific T cell comprises a TCR that is specific for an epitope sequence from the protein: CYP11A1, CYP11B1, CYP11B2, MC2R, STAR, or any combination thereof, and wherein the cancer comprises adrenal cancer.

[0061] In some embodiments, the at least one antigen specific T cell comprises a TCR that is specific for an epitope sequence that has from 70% to 100% sequence identity to a peptide sequence selected from the group consisting of SEQ ID NOs 2212-2523, 4817-4915, and 7449-7538, and wherein the cancer comprises adrenal cancer.

from a target tissue that is at least 2 fold greater than an expression level of the tissue-specific antigen epitope gene in each tissue of a plurality of non-target tissues that are different than the target tissue.

[0095] Provided herein is a method of preparing T cells comprising a T cell receptor (TCR) specific to a complex of (i) a epitope sequence of a tissue specific antigen peptide of a protein and (ii) a protein encoded by an HLA allele of a human subject, the method comprising: incubating T cells in the presence of antigen presenting cells (APCs) comprising the epitope sequence, wherein the APCs express the protein encoded by an HLA allele of a human subject.

[0096] In some embodiments, the APCs comprise a polypeptide comprising the epitope sequence or a polynucleotide encoding a polypeptide comprising the epitope sequence. In some embodiments, the APCs are APCs from a human subject. In some embodiments, the T cells are T cells from a human subject. In some embodiments, the method further comprises administering the T cells to a human subject in need thereof.

[0097] Provided herein is a method of treatment, comprising: administering a composition to a human subject in need thereof, wherein the composition comprises: a tissue-specific antigen peptide comprising an epitope sequence of a protein, wherein the epitope sequence is expressed by the tumor; a polynucleotide encoding the tissue-specific antigen peptide; one or more antigen presenting cells (APCs) presenting the tissue-specific antigen peptide; a T cell receptor (TCR) specific to the tissue-specific antigen peptide; or a population of immune cells from a biological sample comprising at least one antigen specific T cell comprising the TCR. wherein the epitope sequence binds to or is predicted to bind to a protein encoded by a MHC allele expressed by the human subject, and wherein the protein is encoded by a tissue-specific antigen epitope gene that has an expression level in the tumor 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.

[0097a] Provided herein is the use of a composition in the manufacture of a medicament for treating a disease or a condition in a human subject in need thereof, wherein the composition comprises: (a) a tissue-specific antigen peptide comprising an epitope sequence of a protein, wherein the epitope sequence is expressed by a diseased cell of a target tissue of the human subject; (b) a polynucleotide encoding the tissue-specific antigen peptide; (c) one or more antigen presenting cells (APCs) presenting the epitope sequence; (d) a T cell receptor (TCR) specific to a complex of (i) the epitope sequence and (ii) a protein encoded by an HLA allele of the human subject; or (e) a population of immune cells from a biological sample comprising at least one antigen specific T cell comprising the TCR; wherein the epitope sequence binds to or is predicted to bind to a protein encoded by an HLA allele expressed by the human subject, and wherein the tissue-specific antigen epitope gene has an expression level in the diseased cell of a target tissue of the human subject that is at least 2 fold more than an expression level of the tissue-specific antigen epitope gene in cells of each non-target tissue of the subject, wherein the tissue-specific antigen is specific to a non-essential tissue, and wherein the non-essential tissue is a tissue not required for survival of a human.

[0097b] Provided herein is a method of preparing T cells comprising a T cell receptor (TCR) specific to a complex of (i) an epitope sequence of a tissue specific antigen peptide and (ii) a protein encoded by an HLA

allele of a human subject, the method comprising: (a) contacting a T cell ex vivo with an antigen peptide in complex with an HLA of an APC, wherein the T cell has reduced on no tolerance for the antigenic peptide, (b) determining a sequence of a TCR of the T cell that recognizes the antigen peptide in complex with the HLA, wherein the T cell has reduced or no immune tolerance to a tissue of origin of the antigen peptide; (c) (i) expanding the T cell in presence of the antigen peptide in complex with the HLA, or (ii) expressing a recombinant polynucleic acid encoding the sequence of the TCR in isolated T cells; thereby preparing the T cells comprising the TCR; and wherein the T cell is from a subject, wherein the tissue-specific antigen is specific to a non-essential tissue, and wherein the non-essential tissue is a tissue not required for survival of a human.

[0097c] Provided herein is a composition comprising: (a) a tissue-specific antigen peptide comprising an epitope sequence of a polypeptide encoded by a gene selected from the group consisting of KLK2, KLK3 and KLK4; (b) a polynucleotide encoding the tissue-specific antigen peptide; (c) one or more antigen presenting cells (APCs) presenting the epitope sequence; (d) a T cell receptor (TCR) or an antibody, or a functional part thereof that is specific to a complex of (i) the epitope sequence and (ii) a protein encoded by an HLA allele; or (e) a population of immune cells from a biological sample comprising at least one antigen specific T cell comprising the TCR.

[0098] In some embodiments, each tissue of the plurality of tissues is an essential tissue.

[0099] In some embodiments, the plurality of tissues comprise skeletal muscle, coronary artery, heart, adipose, uterus, vagina, skin, salivary gland, brain, lung, esophagus, stomach, colon, small intestine, nerve, or any combination thereof.

[00100] In some embodiments, each non-target tissue of the plurality of non-target tissues is a non-essential tissue.

[00101] In some embodiments, the MHC allele is a class I MHC allele or a class II MHC allele.

[00102] Provided herein is a method of treating a cancer, comprising: administering a composition described herein to a subject in need thereof.

[00103] In some embodiments, the cancer comprises adrenal gland cancer, breast cancer, cervical cancer, colorectal cancer, fallopian tube cancer, glioma, head and neck cancer, liver cancer, squamous cell lung

[00174] **FIG. 65** is a boxplot illustrating expression levels of gene KIRREL2 in a number of different normal tissues and tumors.

[00175] **FIG. 66** is a boxplot illustrating expression levels of gene G6PC2 in a number of different normal tissues and tumors.

[00176] **FIG. 67** is a boxplot illustrating expression levels of gene AQP12B in a number of different normal tissues and tumors.

[00177] **FIG. 68** is a boxplot illustrating expression levels of gene CYP11B1 in a number of different normal tissues and tumors.

[00178] **FIG. 69** is a boxplot illustrating expression levels of gene CYP11B2 in a number of different normal tissues and tumors.

[00179] **FIG. 70** is a boxplot illustrating expression levels of gene STAR in a number of different normal tissues and tumors.

[00180] **FIG. 71** is a boxplot illustrating expression levels of gene CYP11A1 in a number of different normal tissues and tumors.

[00181] **FIG. 72** is a boxplot illustrating expression levels of gene MC2R in a number of different normal tissues and tumors.

[00182] **FIG. 73** shows a schematic of an exemplary workflow for epitope mapping using targeted proteomics. FIG. 73 discloses SEQ ID NO: 8970.

[00183] **FIG. 74** depicts exemplary graphs demonstrating spectral validation of class I HLA epitopes by mass spectrometry of endogenous peptides using targeted proteomics. Chromatographs of 6 characteristic fragment ions for the light (endogenous) ("HPEYNRPLL" disclosed as SEQ ID NO: 4143) and heavy isotope-labeled synthetic peptide sequence “HPEYNRPLL (SEQ ID NO: 8969)” derived from KLK4 (HLA*B-07:02, where the endogenous peptide was identified in a human prostate specimen) are shown. Matched chromatography retention times and a high dot product similarity score (0.992, calculated using Skyline software) of peptide fragment ions provide validation that this epitope is processed and presented on the HLA-B*07:02 molecule.

[00184] **FIG. 75** depicts two exemplary spectrums showing spectral validation of endogenous peptides using targeted proteomics. Spectrum for the light (endogenous) HPEYNRPLL (SEQ ID NO: 4143) epitope identified on a human prostate specimen (left) and the corresponding heavy isotope-labeled synthetic peptide (right) ("HPEYNRPLL" disclosed as SEQ ID NO: 8969) are shown. B and Y fragment ions are shown, and display high spectral similarity, confirming detection of the endogenous epitope. For each peptide, the top 200 more intense ions were plotted, and corresponding mass error of highlighted b and y ions are plotted below spectrum plots.

[00185] **FIG. 76** depicts exemplary flow cytometry plots of peptide-MHC multimer staining of target epitopes after naive T cell inductions in healthy donors with the indicated HLA-I molecules. Multimer positive populations and the percentage of multimer positive cells is shown. The top panel displays positive sample identifications using a combinatorial multimer analysis. The bottom panel displays results from a

confirmation combinatorial analysis performed on frozen samples following the initial identification from the top panel. Multimer positive cells from analyses in the bottom panel are sorted for downstream TCR identification. FIG. 76 discloses SEQ ID NOS 4143, 3757 and 6095, respectively, in order of appearance.

[00186] **FIG. 77** depicts graphs showing exemplary TCR clonotypes identified from the 10X genomics pipeline. Each graph originated from a single sorted, multimer positive, population. The samples in this case all contained two unique TCR clonotypes, identified by a paired alpha and beta sequence. In the case where the 10X genomics pipeline identified a clonotype that contained multiple of either alpha or beta sequences, all possible combinations were synthesized for antigen specificity and avidity.

[00187] **FIG. 78** depicts exemplary plots showing avidity of exemplary TCRs. The plots reflect the CD69 expression on transduced Jurkat cells (identified by the co-expression of murine TCR, CD8, and CD3) following an overnight coculture with a target cell line presenting the HLA and loaded with a variable amount of peptide. Of the seven TCRs tested, five of them show increased expression of CD69 in a peptide dependent manner. The concentration required to achieve a 50% activation (EC50) is calculated from these plots and the results are shown on the plot. Target cells were previously transduced to overexpress the allele of interest. A375s were plated at 50K / well 2-5 hours prior to pulsing with peptide for 1 hour prior to the addition of effector cells. T2s were plated at 10K / well prior to pulsing with peptide for 1 hour prior to the addition of effector cells. Peptide was pulsed with a final concentration between 10e3 and 10e-1 nM. The cells were co-cultured overnight before harvest and staining for CD69 expression via flow using a CD8, CD3, and murine TCR constant antibodies as lineage markers for effector cells.

[00188] **FIG. 79** depicts exemplary plots showing endogenous activity of two different exemplary TcRs. avidity of exemplary TcRs. the plots here reflect the activation of two different TCR sequences (hereafter named mTCR21-033 and mTCR-034) following a coculture with the cell line mDA-PCa-2b which is endogenous for both HLA-B07 and KLK4. These plots are showing an increase in activation of mTCR21-033, but not mTCR21-034 following a 24-hour treatment with a cocktail of interferons (IFN). The IFN treatment increases the expression of surface HLA on cell lines, and the increased surface expression of the HLA can provide more expression of HLA-B07 bound to the KLK4 epitope. mDA-PCa-2b cells were plated at 50K / well in F12K media. The next day the cultures were treated with a cocktail of interferon alpha, beta, and gamma all at 1 U/μL final concentration. The next day the cells were washed with RPMI supplemented with 10% FBS and Glutamax. The cultures were then pulsed with peptide at a final concentration of 2 μM for 1 hour before the addition of effector cells. The cells were co-cultured overnight before harvest and staining for CD69 expression via flow using a CD8, CD3, and murine TCR constant antibodies as lineage markers for effector cells and HLA-B07 as a lineage marker for the target cells.

peptide	allele	gene	cancer	SEQ	peptide	allele	gene	cancer	SEQ
FVISGVLTLIPV	HLA-A02:01	CLDN6	Ovarian	1672	FAMPMQDIKM	HLA-B46:01	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6153
ILGVVLTLL	HLA-A02:01	CLDN6	Ovarian	1673	VLYPVPLESY	HLA-B46:01	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6154
GLLVYLAGA	HLA-A02:01	CLDN6	Ovarian	1674	AAFDGRHSQ	HLA-B46:01	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6155
LVSCALPMWK	HLA-A03:01	CLDN6	Ovarian	1675	VSPEPLQAL	HLA-C01:02	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6156
VSCALPMWK	HLA-A03:01	CLDN6	Ovarian	1676	YLHARLREL	HLA-C01:02	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6157
GLLVYLAGAK	HLA-A03:01	CLDN6	Ovarian	1677	ITDDQLLAL	HLA-C01:02	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6158
ALFGLLVYL	HLA-A03:01	CLDN6	Ovarian	1678	TSPRRLVEL	HLA-C01:02	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6159
ALFGLLVY	HLA-A03:01	CLDN6	Ovarian	1679	AWPFTCLPL	HLA-C01:02	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6160
LVALFGLLVY	HLA-A03:01	CLDN6	Ovarian	1680	ISISALQSL	HLA-C03:04	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6161
VSCALPMWK	HLA-A11:01	CLDN6	Ovarian	1681	LAIAALELL	HLA-C03:04	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6162
STSAPAISR	HLA-A11:01	CLDN6	Ovarian	1682	HVMNPLETL	HLA-C03:04	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6163
LVSCALPMWK	HLA-A11:01	CLDN6	Ovarian	1683	LAIAALEL	HLA-C03:04	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6164
ALFGLLVYL	HLA-A11:01	CLDN6	Ovarian	1684	FTSQFLSL	HLA-C03:04	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6165
MWKALLFLTL	HLA-A24:02	CLDN6	Ovarian	1685	FYDPEPILCPCF	HLA-C04:01	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	8964
RYSTSAPAI	HLA-A24:02	CLDN6	Ovarian	1686	SYEDIHGTL	HLA-C04:01	PRAME	Lung squam.;Melanoma;Ovarian;Uterine	6167

peptide	allele	gene	cancer	SEQ	peptide	allele	gene	cancer	SEQ
KKLQCVDLHV	HLA-A02:01	KLK3	Prostate	3748	RAMCAMMSFEK	HLA-A30:01	TGM4	Prostate	8229
FLTPKKLQCV	HLA-A02:01	KLK3	Prostate	3749	KQVKEINAQK	HLA-A30:01	TGM4	Prostate	8230
LITELTMPA	HLA-A02:01	KLK3	Prostate	3750	AIRKGDIFI	HLA-A30:01	TGM4	Prostate	8231
FLSLPAPL	HLA-A02:01	KLK3	Prostate	3751	KTGPKKFIVK	HLA-A30:01	TGM4	Prostate	8232
LMLLRLSEPA	HLA-A02:01	KLK2; KLK3	Prostate	3752	VLRALGIPA	HLA-A30:01	TGM4	Prostate	8233
HLPQNFLPI	HLA-A02:01	KLK3	Prostate	3753	RPVKENFLHM	HLA-A30:01	TGM4	Prostate	8234
KLQCVDLHVI	HLA-A02:01	KLK3	Prostate	3754	SVNFTVILK	HLA-A30:01	TGM4	Prostate	8235
SLVPWRGGV	HLA-A02:01	KLK3	Prostate	3755	KMAKLCDLNK	HLA-A30:01	TGM4	Prostate	8236
LITELTMPAL	HLA-A02:01	KLK3	Prostate	3756	QVKEINAQK	HLA-A30:01	TGM4	Prostate	8237
VLVHPQWVL	HLA-A02:01	KLK2; KLK3; KLK4	Prostate	3757	RRRDITYEYK	HLA-A30:01	TGM4	Prostate	8238
LTLAVCGGV	HLA-A02:01	KLK3	Prostate	3758	RRRDITYEY	HLA-A30:01	TGM4	Prostate	8239
FLTLAVCGGVLV	HLA-A02:01	KLK3	Prostate	3759	RLIWLVKMV	HLA-A30:01	TGM4	Prostate	8240
FLPIAQPA	HLA-A02:01	KLK3	Prostate	3760	RKGDIFIVY	HLA-A30:01	TGM4	Prostate	8241
VFLTLSVTWIA	HLA-A02:01	KLK3	Prostate	3761	DTRFVFSEV	HLA-A30:01	TGM4	Prostate	8242
LLRLSEPAEL	HLA-A02:01	KLK3	Prostate	3762	NVKTGNHILK	HLA-A30:01	TGM4	Prostate	8243
TLAVCGGVLV	HLA-A02:01	KLK3	Prostate	3763	GDRLIWLVK	HLA-A30:01	TGM4	Prostate	8244
VISNDVCAQV	HLA-A02:01	KLK3	Prostate	3764	HTLVVLDPR	HLA-A33:03	TGM4	Prostate	8245
FMLCAGRWT	HLA-A02:01	KLK3	Prostate	3765	HVWTDAWMKR	HLA-A33:03	TGM4	Prostate	8246
ILITELTMPAL	HLA-A02:01	KLK3	Prostate	3766	HAFLLLSSER	HLA-A33:03	TGM4	Prostate	8247
FLRPGDDSTL	HLA-A02:01	KLK3	Prostate	8963	EFQTSSPVFR	HLA-A33:03	TGM4	Prostate	8248
RLSEPAEL	HLA-A02:01	KLK3	Prostate	3768	QTSSPVFRR	HLA-A33:03	TGM4	Prostate	8249
VLTPKKLQCV	HLA-A02:01	KLK3	Prostate	3769	DIFIVYDTR	HLA-A33:03	TGM4	Prostate	8250
SLYTKVVHY	HLA-A02:01	KLK3	Prostate	3770	SVNFTVILKR	HLA-A33:03	TGM4	Prostate	8251
ALPERPSL	HLA-A02:01	KLK3	Prostate	3771	FQTSSPVFRR	HLA-A33:03	TGM4	Prostate	8252
ILLGRHSL	HLA-A02:01	KLK3	Prostate	3772	MVFMPDEDER	HLA-A33:03	TGM4	Prostate	8253
FLTPKKLQC	HLA-A02:01	KLK3	Prostate	3773	NFHVWTDAWMKR	HLA-A33:03	TGM4	Prostate	8254
SGDSGGPLV	HLA-A02:01	KLK3	Prostate	3774	STKAVGQDR	HLA-A33:03	TGM4	Prostate	8255
SLFHPEDTGQV	HLA-A02:01	KLK3	Prostate	3775	AFLLLSSER	HLA-A33:03	TGM4	Prostate	8256
RLSEPAELT	HLA-A02:01	KLK3	Prostate	3776	EFQTSSPVFRR	HLA-A33:03	TGM4	Prostate	8257
VLHGSLVPW	HLA-A02:01	KLK3	Prostate	3777	ESSLKPTDR	HLA-A33:03	TGM4	Prostate	8258
GVLVHPQWV	HLA-A02:01	KLK2; KLK3; KLK4	Prostate	3778	EVFTSFQYP	HLA-A33:03	TGM4	Prostate	8259
WIAPPLQVL	HLA-A02:01	KLK3	Prostate	3779	SIELPNTGR	HLA-A33:03	TGM4	Prostate	8260
TLSVTWIGA	HLA-A02:01	KLK3	Prostate	3780	EFSIELPNTGR	HLA-A33:03	TGM4	Prostate	8261
ELTDAVKV	HLA-A02:01	KLK3	Prostate	3781	VNFTVILKR	HLA-A33:03	TGM4	Prostate	8262
FLRPGDDST	HLA-A02:01	KLK3	Prostate	3782	SPVFRRGQV	HLA-B07:02	TGM4	Prostate	8263
FHPEDTGQV	HLA-A02:01	KLK3	Prostate	3783	RDPVLVCRAM	HLA-B07:02	TGM4	Prostate	8264
TWIAPPLQV	HLA-A02:01	KLK3	Prostate	3784	SPVFRRGQVF	HLA-B07:02	TGM4	Prostate	8265
HPLYDMSLLK	HLA-A03:01	KLK3	Prostate	3785	LPNTGRIGQL	HLA-B07:02	TGM4	Prostate	8266
PLYDMSLLK	HLA-A03:01	KLK3	Prostate	3786	KPTDRRDPV	HLA-B07:02	TGM4	Prostate	8267
VLTAAHCIRK	HLA-A03:01	KLK3	Prostate	3787	KPTDRRDPVL	HLA-B07:02	TGM4	Prostate	8268
LTAAHCIRK	HLA-A03:01	KLK3	Prostate	3788	APYKWTGSA	HLA-B07:02	TGM4	Prostate	8269

Table 1B - Tumor Epitope Sequences

Epitope	SEQ ID NO:	Target	Sample	HLA class	MS validation	Allele (MS)	Allele (predicted)	Cancer
AYSEKVTEF	3534	KLK2	MDAPCa2b	I	X	HLA-C*06:02	HLA-A24:02	Prostate
GLWTGGKDTCGV	3468	KLK2	MDAPCa2b	I			HLA-A02:01	Prostate
HPEDTGQVF	3988	KLK3	MDAPCa2b, human prostate	I	X	HLA-C*07:01 HLA-C*04:01	HLA-C07:01	Prostate
HPEYNRPLL	4143	KLK4	MDAPCa2b, human prostate, breast cancer	I	X	HLA-C*07:01 HLA-B*07:02	HLA-B07:02	Prostate
QRVPVSHSF	3544	KLK2	MDAPCa2b, human prostate	I	X	HLA-C*07:01 HLA-C*07:02	HLA-A24:02	Prostate
SESDTIRSI	4176	KLK4	mantle cell lymphoma, breast cancer	I			HLA-B13:02	Prostate
SLFHPEDTGQV	3775	KLK3	MDAPCa2b	I			HLA-A02:01	Prostate
SLQCVSLHL	3456	KLK2	MDAPCa2b, human prostate	I			HLA-A02:01	Prostate
VILLGRHSL	3891	KLK3	PXD004023	I			HLA-B08:01	Prostate
VLVHPQWVL	3757	KLK3	MDAPCa2b	I			HLA-A02:01	Prostate
LFHPEDTGQVF	3827	KLK3	Human prostate	I	X	HLA-A*24:02	HLA-A24:02	Prostate
RPRSLQCVSL	3578	KLK2	Human prostate	I			HLA-B07:02	Prostate
GYLQGLVSF	4094	KLK4	Human prostate	I	X	HLA-A*24:02	HLA-A24:02	Prostate
IRNKSVILL	3974	KLK3	Human prostate	I	X	HLA-C*06:02 HLA-C*07:02	HLA-C07:01	Prostate
KLQCVDLHV	3740	KLK3	Human prostate	I	X	HLA-A*02:01	HLA-A02:01	Prostate
LLANGRMPTV	4029	KLK4	Human prostate	I	X	HLA-A*02:01	HLA-A02:01	Prostate
LRPGDDSTL	3767	KLK3	Human prostate	I	X	HLA-C*07:02	HLA-C07:02	Prostate
MPALPMVL	3874	KLK3	Human prostate	I	X	HLA-B*07:02	HLA-B07:02	Prostate
NRPLLANDL	4216	KLK4	Human prostate	I	X	HLA-C*06:02 HLA-C*07:02	HLA-C01:02	Prostate
SLQCVSLHL	3456	KLK2	Human prostate	I	X	HLA-A*02:01	HLA-A02:01	Prostate
TWIAPPLQV	3784	KLK3	Human prostate	I	X	HLA-C*04:01	HLA-A02:01	Prostate
VFQVSHSF	3828	KLK3	Human prostate	I	X	HLA-C*07:02	HLA-A24:02	Prostate
YSEKVTEFML	3454	KLK2	Human prostate	I	X	HLA-A*01:01	HLA-A01:01	Prostate

LLSHGAVIEV	831	ANKRD30A	CD165, HCC1500, CAMA1	I	X	HLA-A*02:01	HLA-A02:01	Breast
SIPTKALEL	942	ANKRD30A	B721, A375, CAMA1, HCC1500	I			HLA-C01:02	Breast
SQYSGQLKV	927	ANKRD30A	B721	I			HLA-B13:02	Breast
SVPNKALEL	941	ANKRD30A	B721, A375	I	X	HLA-C*04:01	HLA-C01:02	Breast
SLSKILDTV	826	ANKRD30A	CAMA1, HCC1500	I	X	HLA-A*02:01	HLA-A02:01	Breast
SIPTKALEL	942	ANKRD30A	CAMA1	I	X	HLA-C*04:01	HLA-C01:02	Breast
SLDQKLFQL	827	ANKRD30A	CAMA1, HCC1500	I	X	HLA-A*02:01	HLA-A02:01	Breast
SVPNKALEL	941	ANKRD30A	HCC1500	I			HLA-C01:02	Breast
DSLFFLRGR	6132	PRAME	expi293	I			HLA-A33:03	Lung squam.; Melanoma; Ovarian; Uterine
ELFSYLIEK	6108	PRAME	Human melanoma, expi293	I			HLA-A03:01	Lung squam.; Melanoma; Ovarian; Uterine
FYDPEPILC	6166	PRAME	Jurkat	I			HLA-C04:01	Lung squam.; Melanoma; Ovarian; Uterine
ISISALQSL	6161	PRAME	A375	I			HLA-C03:04	Lung squam.; Melanoma; Ovarian; Uterine
ITDDQLLAL	6088	PRAME	A375	I			HLA-A01:01	Lung squam.; Melanoma; Ovarian; Uterine
KRKKNVLRL	6173	PRAME	Human melanoma	I			HLA-C07:01	Lung squam.; Melanoma; Ovarian; Uterine
LQSLLQHLI	6146	PRAME	Human melanoma	I			HLA-B13:02	Lung squam.; Melanoma; Ovarian; Uterine
LSHIHASSY	6152	PRAME	Human melanoma	I			HLA-B46:01	Lung squam.; Melanoma; Ovarian; Uterine
PYLGQMINL	6120	PRAME	Human melanoma, A375, expi293	I			HLA-A24:02	Lung squam.; Melanoma; Ovarian; Uterine

QLLALLPSL	6093	PRAME	H1	I			HLA-A02:01	Lung squam.; Melanoma; Ovarian; Uterine
SFYGNSISI	6174	PRAME	expi293	I			HLA-C07:01	Lung squam.; Melanoma; Ovarian; Uterine
SLLQHLIGL	6095	PRAME	Expi293, MDAPCa2b, A375, human melanoma, JEKO, ovarian carcinoma, H1, LNZ308, JY, Hek293, HeLa, Roja, THP1	I	X	HLA-A*02:01	HLA-A02:01	Lung squam.; Melanoma; Ovarian; Uterine
SPSVSQLSVL	6139	PRAME	Human melanoma, lung cancer, JY, H1, A375	I			HLA-B07:02	Lung squam.; Melanoma; Ovarian; Uterine
SPYLGQMINL	6138	PRAME	melanoma	I			HLA-B07:02	Lung squam.; Melanoma; Ovarian; Uterine
TSPRRLVEL	6159	PRAME	Human melanoma, A375	I			HLA-C01:02	Lung squam.; Melanoma; Ovarian; Uterine
VLYPVPLESY	6105	PRAME	Human melanoma, lung cancer	I			HLA-A03:01	Lung squam.; Melanoma; Ovarian; Uterine
VSPEPLQAL	6156	PRAME	A375	I			HLA-C01:02	Lung squam.; Melanoma; Ovarian; Uterine
YLHARLREL	6140	PRAME	Human melanoma, expi293	I			HLA-B08:01	Lung squam.; Melanoma; Ovarian; Uterine
RLDQLLRHV	6104	PRAME	Human melanoma	I			HLA-A02:01	Lung squam.; Melanoma; Ovarian; Uterine
EVDPASNTY	4638	MAGEA4	GBM, A375	I	X	HLA-A*01:01	HLA-A01:01	Lung squam.
GVYDGREHTV	4653	MAGEA4	GBM, OvCa13, Breast tumor, Lung tumor, A375, MSV000082648	I	X	HLA-A*02:01	HLA-A02:01	Lung squam.

KEVDPASNTY	4640	MAGEA4	OvCa114, A375, Lung tumor	I			HLA-A01:01	Lung squam.

KVDELAHFL	4648	MAGEA4	A375	I	X	HLA-A*02:01	HLA-A02:01	Lung squam.
QIFPKTGL	4692	MAGEA4	A375	I			HLA-B08:01	Lung squam.
QSPQGASAL	4707	MAGEA4	A375	I			HLA-C01:02	Lung squam.
SALPTTISF	4699	MAGEA4	Lung tumor	I			HLA-B46:01	Lung squam.
TVYGEPRKL	4722	MAGEA4	Human melanoma, A375	I			HLA-C07:01	Lung squam.
VYGEPRKL	4727	MAGEA4	A375	I			HLA-C07:02	Lung squam.
YPSLREAAL	4682	MAGEA4	A375	I			HLA-B07:02	Lung squam.
ALLEEEEGV	4644	MAGEA4	A375	I			HLA-A02:01	Lung squam.
KVLEHVVRV	4643	MAGEA4	A375	I	X	HLA-A*02:01	HLA-A02:01	Lung squam.

Table 1C - Tumor Epitope Sequences

Epitope	SEQ ID NO:	Target	Sample	HLA class	MS validation	Allele (MS)	Allele (predicted)	Cancer
AYSEKVTEF	3534	KLK2	MDAPCa2b	I	X	HLA-C*06:02	HLA-A24:02	Prostate
HPEDTGQVF	3988	KLK3	MDAPCa2b, human prostate	I	X	HLA-C*07:01 HLA-C*04:01	HLA-C07:01	Prostate
HPEYNRPLL	4143	KLK4	MDAPCa2b, human prostate, breast cancer	I	X	HLA-C*07:01 HLA-B*07:02	HLA-B07:02	Prostate
QRVPVSHSF	3544	KLK2	MDAPCa2b, human prostate	I	X	HLA-C*07:01 HLA-C*07:02	HLA-A24:02	Prostate
LFHPEDTGQVF	3827	KLK3	Human prostate	I	X	HLA-A*24:02	HLA-A24:02	Prostate
GYLQGLVSF	4094	KLK4	Human prostate	I	X	HLA-A*24:02	HLA-A24:02	Prostate
IRNKSVILL	3974	KLK3	Human prostate	I	X	HLA-C*06:02 HLA-C*07:02	HLA-C07:01	Prostate
KLQCVDLHV	3740	KLK3	Human prostate	I	X	HLA-A*02:01	HLA-A02:01	Prostate
LLANGRMPTV	4029	KLK4	Human prostate	I	X	HLA-A*02:01	HLA-A02:01	Prostate
LRPGDDSTL	3767	KLK3	Human prostate	I	X	HLA-C*07:02	HLA-C07:02	Prostate
MPALPMVL	3874	KLK3	Human prostate	I	X	HLA-B*07:02	HLA-B07:02	Prostate
NRPLLANDL	4216	KLK4	Human prostate	I	X	HLA-C*06:02 HLA-C*07:02	HLA-C01:02	Prostate
SLQCVSLHL	3456	KLK2	Human prostate	I	X	HLA-A*02:01	HLA-A02:01	Prostate
TWIAPPLQV	3784	KLK3	Human prostate	I	X	HLA-C*04:01	HLA-A02:01	Prostate

VFQVSHSF	3828	KLK3	Human prostate	I	X	HLA-C*07:02	HLA-A24:02	Prostate
YSEKVTEFML	3454	KLK2	Human prostate	I	X	HLA-A*01:01	HLA-A01:01	Prostate
LLSHGAVIEV	831	ANKRD30A	CD165, HCC1500, CAMA1	I	X	HLA-A*02:01	HLA-A02:01	Breast
SVPNKALEL	941	ANKRD30A	B721, A375	I	X	HLA-C*04:01	HLA-C01:02	Breast
SLSKILDTV	826	ANKRD30A	CAMA1, HCC1500	I	X	HLA-A*02:01	HLA-A02:01	Breast
SIPTKALEL	942	ANKRD30A	CAMA1	I	X	HLA-C*04:01	HLA-C01:02	Breast
SLDQKLFQL	827	ANKRD30A	CAMA1, HCC1500	I	X	HLA-A*02:01	HLA-A02:01	Breast
SLLQHLIGL	6095	PRAME	Expi293, MDAPCa2b, A375, human melanoma, JEKO, ovarian carcinoma, H1, LNZ308, JY, Hek293, HeLa, Roja, THP1	I	X	HLA-A*02:01	HLA-A02:01	Lung squam.; Melanoma; Ovarian; Uterine
EVDPASNTY	4638	MAGEA4	GBM, A375	I	X	HLA-A*01:01	HLA-A01:01	Lung squam.
GVYDGREHTV	4653	MAGEA4	GBM, OvCa13, Breast tumor, Lung tumor, A375, MSV000082648	I	X	HLA-A*02:01	HLA-A02:01	Lung squam.
KVDELAHFL	4648	MAGEA4	A375	I	X	HLA-A*02:01	HLA-A02:01	Lung squam.
KVLEHVVRV	4643	MAGEA4	A375	I	X	HLA-A*02:01	HLA-A02:01	Lung squam.

[00246] In aspects, provided herein are compositions comprising tissue-specific antigens. In some embodiments, the compositions comprise antigenic peptides, including tissue-specific antigens. In some embodiments, the tissue-specific antigens comprise tumor epitope sequence(s) as provided herein. In some embodiments, also provided herein are compositions comprising polynucleotides that code for the tissue-specific antigens.

[00247] In some embodiments, the size of the antigenic peptides provided herein comprise, but is not limited to, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about

(see, *e.g.*, Sambrook et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

[00390] Expression vectors comprising the isolated polynucleotides, as well as host cells containing the expression vectors, are also contemplated. The antigenic peptides can be provided in the form of RNA or cDNA molecules encoding the desired antigenic peptides. One or more antigenic peptides of the disclosure can be encoded by a single expression vector.

[00391] In some embodiments, the polynucleotides can comprise the coding sequence for the tissue-specific antigenic peptide fused in the same reading frame to a polynucleotide which aids, for example, in expression and/or secretion of a polypeptide from a host cell (*e.g.*, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell). The polypeptide having a leader sequence is a preprotein and can have the leader sequence cleaved by the host cell to form the mature form of the polypeptide.

[00392] In some embodiments, the polynucleotides can comprise the coding sequence for the antigenic peptide of the present disclosure fused in the same reading frame to a marker sequence that allows, for example, for purification of the encoded polypeptide, which can then be incorporated into a personalized vaccine or immunogenic composition. For example, the marker sequence can be a hexa-histidine tag (SEQ ID NO: 8965) supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or the marker sequence can be a hemagglutinin (HA) tag derived from the influenza hemagglutinin protein when a mammalian host (*e.g.*, COS-7 cells) is used. Additional tags include, but are not limited to, Calmodulin tags, FLAG tags, Myc tags, S tags, SBP tags, Softag 1, Softag 3, V5 tag, Xpress tag, Isopeptag, SpyTag, Biotin Carboxyl Carrier Protein (BCCP) tags, GST tags, fluorescent protein tags (*e.g.*, green fluorescent protein tags), maltose binding protein tags, Nus tags, Strep-tag, thioredoxin tag, TC tag, Ty tag, and the like.

[00393] In some embodiments, the polynucleotides can comprise the coding sequence for one or more of the tissue-specific antigenic peptides fused in the same reading frame to create a single concatamerized antigenic peptide construct capable of producing multiple antigenic peptides.

[00394] In some embodiments, isolated nucleic acid molecules having a nucleotide sequence at least 60% identical, at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, or at least 96%, 97%, 98% or 99% identical to a polynucleotide encoding a tissue-specific antigenic peptide of the present disclosure, can be provided.

[00395] Isolated tissue-specific antigenic peptides described herein can be produced *in vitro* (*e.g.*, in the laboratory) by any suitable method known in the art. Such methods range from direct protein synthetic methods to constructing a DNA sequence encoding isolated polypeptide sequences and expressing those sequences in a suitable transformed host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of

column chromatography, and the like), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexahistidine (SEQ ID NO: 8965), maltose binding domain, influenza coat sequence, glutathione-S-transferase, and the like can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Isolated proteins can also be physically characterized using such techniques as proteolysis, nuclear magnetic resonance and x-ray crystallography. For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a cancer stem cell protein-Fc composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

[00403] Recombinant protein as described herein produced in bacterial culture can be isolated, for example, by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. High performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of a recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

[00404] *In Vivo* Peptide / Polypeptide Synthesis

[00405] The present disclosure also contemplates the use of nucleic acid molecules as vehicles for delivering antigenic peptides/polypeptides to the subject in need thereof, *in vivo*, in the form of, *e.g.*, DNA/RNA vaccines (see, *e.g.*, WO2012/159643, and WO2012/159754, hereby incorporated by reference in their entireties).

[00406] In some embodiments, antigens can be administered to a patient in need thereof by use of a plasmid. These are plasmids which usually consist of a strong viral promoter to drive the *in vivo* transcription and translation of the gene (or complementary DNA) of interest (Mor, et al., (1995). The Journal of Immunology 155 (4): 2039–2046). Intron A can sometimes be included to improve mRNA stability and hence increase protein expression (Leitner, et al. (1997).The Journal of Immunology 159 (12): 6112–6119). Plasmids also include a strong polyadenylation/transcriptional termination signal, such as bovine growth hormone or rabbit beta-globulin polyadenylation sequences (Alarcon et al., (1999). Adv.

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 in a number of different normal tissues and tumors, respectively.

[00499] As illustrated in the plots, these gene were identified as specific to the respective tissues as indicated on the top of each plot.

[00500] Next, the sequences of the genes identified in the first step were scanned through by the same bioinformatic program to find short peptide sequences with high likelihood of being presented on a common MHC I allele. Table 1A summarizes the findings on tissue-specific antigens and their corresponding cancer type, in which the respective tissue-specific gene from which the respective tissue-specific antigen was identified has relatively high expression level.

[00501] To validate this exemplary approach, a bioinformatics approach was used to identify of tissue-specific antigens. Table 2 summarizes a list of tissue-specific antigens ranked based on use of two different exemplary algorithms that predict binding affinity of the peptides to HLA molecules. As can be seen in Table 2, for each peptide, their rank ranges by both programs were comparable. The total number of peptides in the data set was 8,962.

Table 2 – Predictions of candidate tissue-specific antigens using two different algorithms

Peptide Sequence	SEQ ID NO:	Allele	Rank range peptides fall within (algorithm 1)	Rank range peptides fall within (algorithm 2)
YAITFAMRL	8711	HLA-C03:04	0001-0100	0001-0100
SLYEHLVNL	6852	HLA-A02:01	0001-0100	0001-0100
HTYPFGWYQK	7726	HLA-A11:01	0001-0100	0001-0100
FAINSEMFL	2980	HLA-C03:04	0001-0100	0001-0100
RPAPPGRYL	7008	HLA-B07:02	0001-0100	0001-0100
FVISGVLTL	1668	HLA-C03:04	0001-0100	0001-0100
SSLYSYFQK	7179	HLA-A11:01	0050-0150	0050-0150
RLFFRVCLK	2641	HLA-A03:01	0050-0150	0050-0150
VLTSGIVFV	1665	HLA-A02:01	0150-0250	0150-0250
YAGNNMHSL	2066	HLA-C03:04	0200-0300	0200-0300
RLFRTKTWK	2229	HLA-A03:01	0250-0350	0250-0350
VASDMMVSL	575	HLA-C03:04	0300-0400	0300-0400
YRKPGISLL	8045	HLA-C07:02	0350-0450	0350-0450

Peptide Sequence	SEQ ID NO:	Allele	Rank range peptides fall within (algorithm 1)	Rank range peptides fall within (algorithm 2)
RLYFGTSGY	7725	HLA-A03:01	0400-0500	0400-0500
FIANLPPEL	6601	HLA-C03:04	0400-0500	0400-0500
SYPPLHEWAF	4489	HLA-A24:02	0450-0550	0450-0550
FAGILTTVL	8323	HLA-C03:04	0550-0650	0550-0650
VLDGLDVLL	6098	HLA-A02:01	0800-0900	0800-0900
YRYLCISHL	7252	HLA-C07:02	1100-1200	1100-1200
YMIPAKTLVQV	2220	HLA-A02:01	1100-1200	1100-1200
CVDAEGMEVY	7629	HLA-A01:01	1300-1400	1300-1400
HLMQKFEKV	7691	HLA-A02:01	1350-1450	1350-1450
IAHDLRLLL	1262	HLA-C03:04	1550-1650	1550-1650
VTLPPFMCNK	7382	HLA-A03:01	1650-1750	1650-1750
SSFSTTINY	4573	HLA-A11:01	1800-1900	1800-1900
LSDQAVLAL	287	HLA-C03:04	1850-1950	1850-1950
FLQQPEDLV	3335	HLA-A02:01	2000-2100	2000-2100
NYLSLPRPR	1229	HLA-A33:03	2100-2200	2100-2200
LYRDTGLVK	1909	HLA-A30:01	2150-2250	2150-2250
FSGNQVWRY	4916	HLA-A01:01	2150-2250	2150-2250
MRLCWAWEL	1074	HLA-C07:01	2350-2450	2350-2450
YAYASGNL	6947	HLA-C03:04	2450-2550	2450-2550
DIFIVYDTR	8250	HLA-A33:03	2600-2700	2600-2700
STFTETTLY	7635	HLA-A01:01	2600-2700	2600-2700
AMILPSSSL	2185	HLA-C03:04	2750-2850	2750-2850
TMDDDTAVLV	5305	HLA-A02:01	2750-2850	2750-2850
TFFFDPENF	2243	HLA-A24:02	2800-2900	2800-2900
RLHDGIADI	4931	HLA-A02:01	2850-2950	2850-2950
YRAYYTFLNF	2999	HLA-C07:02	3100-3200	3100-3200
SYKSFESDK	5233	HLA-A30:01	3200-3300	3200-3300
AYYTFLNFM	3005	HLA-C07:02	3250-3350	3250-3350
GPQNDGKQL	7403	HLA-B07:02	3500-3600	3500-3600
NIKHHYCYV	227	HLA-A30:01	3500-3600	3500-3600
NRGQRLLAF	1360	HLA-C07:02	3500-3600	3500-3600
APDMSRML	1518	HLA-B07:02	4300-4400	4300-4400

Peptide Sequence	SEQ ID NO:	Allele	Rank range peptides fall within (algorithm 1)	Rank range peptides fall within (algorithm 2)
IYAGNNMHSLL	2016	HLA-A24:02	4400-4500	4400-4500
RALGFEPRK	1591	HLA-A03:01	4400-4500	4400-4500
VPNKALEL	908	HLA-B07:02	4450-4550	4450-4550
SAWSPTLPL	7511	HLA-C01:02	4600-4700	4600-4700
SSCPPQPCTK	4298	HLA-A30:01	4600-4700	4600-4700
GPGSGPLLRL	2042	HLA-B07:02	4700-4800	4700-4800
SVSESDTIR	4077	HLA-A33:03	4800-4900	4800-4900
SVVQVAKATGK	3039	HLA-A11:01	4950-5050	4950-5050
FQQELALLK	5025	HLA-A03:01	5150-5250	5150-5250
RALCVIALLV	1711	HLA-B13:02	5200-5300	5200-5300
LTSGIVFVI	1712	HLA-B13:02	5300-5400	5300-5400
LAFLVLEAV	8800	HLA-B13:02	5350-5450	5350-5450
FTREFLGAL	6759	HLA-B46:01	5600-5700	5600-5700
DRRCQLNIL	5594	HLA-C07:01	5750-5850	5750-5850
RLFDDDETGKI	1583	HLA-A02:01	6200-6300	6200-6300
AAMANPRAL	8920	HLA-B46:01	6250-6350	6250-6350
LFHPEDTGQVF	3827	HLA-A24:02	6450-6550	6450-6550
QLSEEQNTGI	5480	HLA-A02:01	6550-6650	6550-6650
FTSFQYPEF	8324	HLA-B46:01	6600-6700	6600-6700
LSDYKEKQILKV	5721	HLA-A01:01	6950-7050	6950-7050
LAIPLTDVKF	8330	HLA-B46:01	6950-7050	6950-7050
PSCLKKLLQR	4318	HLA-A33:03	7200-7300	7200-7300
CQPSCLKKL	4287	HLA-C07:01	7250-7350	7250-7350
FSRAVAAKW	6763	HLA-B46:01	7300-7400	7300-7400
HRDFSGHPNF	5674	HLA-C04:01	7350-7450	7350-7450
MSATTVSSL	1932	HLA-C04:01	7550-7650	7550-7650
KLLQRCFEK	4285	HLA-A02:01	7800-7900	7800-7900
KLGFKVTLP	7377	HLA-A02:01	7800-7900	7800-7900
GVACKGREQL	7283	HLA-A02:01	8200-8300	8200-8300
ACWPAFTVL	1885	HLA-A24:02	8300-8400	8300-8400
RHNVICQLL	5775	HLA-B07:02	8300-8400	8300-8400

Peptide Sequence	SEQ ID NO:	Allele	Rank range peptides fall within (algorithm 1)	Rank range peptides fall within (algorithm 2)
RAMRCCRPRYRP	6370	HLA-B13:02	8450-8550	8450-8550
RHNVICQL	5868	HLA-B07:02	8600-8700	8600-8700
SKSRSPHKGV	8445	HLA-A02:01	8750-8850	8750-8850
RYYRQRQRS	6327	HLA-A02:01	8800-8900	8800-8900
SCQTRRRAM	6323	HLA-A02:01	8850-8950	8850-8950

Example 2. Confirmation of HLA-binding Affinity and Immunogenicity

[00502] The following example demonstrates quantification of binding affinities of HLA class I and class II peptides (HLA binding assays), and test of the ability of each test peptide to expand T cells (immunogenicity assays). Experimental protocol described below are exemplary and non-limiting, other protocols following similar principle can also be used to test HLA binding affinity and immunogenicity of the peptide as described herein.

[00503] HLA binding assays can be performed with peptides that are either motif-bearing or not motif-bearing. 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 th 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 unlabled 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

[00504] Live cell/flow cytometry-based assays can also be used, e.g., an assay utilizing a TAP-deficient hybridoma cell line T2 (American Type Culture Collection (ATCC Accession No. CRL-1992), Manassas, Va.). TAP deficiency in this cell line leads to inefficient loading of MHCI in the ER and an excess of empty MHCIs. Salter and Cresswell, EMBO J. 5:943-49 (1986); Salter, Immunogenetics 21:235-46 (1985). Empty MHCIs are highly unstable and short-lived. When T2 cells are cultured at reduced temperatures, empty MHCIs appear transiently on the cell surface, where they can be stabilized by exogenous addition of MHCI-binding peptides. To perform this binding assay, peptide-receptive MHCIs were induced by culturing aliquots of 107 T2 cells overnight at 26 oC in serum free AIM-V medium alone, or in medium

contaminants. Searches included oxidated methionine as a variable modification in all searches, and carboxymethylation of cystine residues as a variable modification when sample processing included cystine reduction and alkylation steps. A minimum scored peak intensity (SPI) of 50% & PSM FDR estimate <1% was used to filter results. All sequences between 7 and 17 amino acids in length were considered.

Targeted mass spectrometry MHC-peptide analysis

[00569] For targeted analyses, isolated MHC-I peptides were labeled using an isobaric labeling reagent from the tandem mass tag (TMT) 10-plex reagent set (Thermo Fisher). Dried peptides were resuspended in 50 mM HEPES buffer pH 8.5 and combined with 33.3 µg of TMT solubilized in 100% anhydrous acetonitrile. Peptides were incubated for 1 hour at room temperature after which the reaction was quenched with hydroxylamine. Peptides were subsequently dried by vacuum centrifugation, and resuspended in 3% acetonitrile, 5% formic acid. Prior to analysis, heavy isotope-labeled synthetic peptides corresponding to epitope targets of interest were labeled with Super Heavy TMT labeling reagent (Thermo Fisher) as previously described. Dried, labeled synthetic peptides were resuspended in 3% acetonitrile, 5% formic acid and 100 fmol of each peptide was added to the isolated, TMT-10plex labeled enriched peptide mixture.

[00570] Peptides were analyzed using SureQuant targeted data acquisition strategy, where the heavy isotope labeled synthetic peptide serves as a trigger to guide the acquisition of spectrum corresponding to the light (unlabeled) endogenous MHC peptide using mass offset triggering and pseudo-spectral matching. All analyses were analyzed in Skyline, where the detection of an endogenous peptide was verified by matching retention times and spectral similarity between the heavy synthetic peptide and the light endogenous peptide (**FIG. 74**). Spectral similarity metrics include the dot product score and manual validation by comparing intensity distributions of 6 pre-selected product ions. Chromatographs of 6 characteristic fragment ions for the light (endogenous) and heavy isotope-labeled synthetic peptide sequence "HPEYNRPLL (SEQ ID NO: 8969)" derived from KLK4 (HLA*B-07:02, where the endogenous peptide was identified in a human prostate specimen) are shown. Matched chromatography retention times and a high dot product similarity score (0.992, calculated using Skyline software) of peptide fragment ions provide validation that this epitope is processed and presented on the HLA-B*07:02 molecule. Two exemplary spectrums showing spectral validation of endogenous peptides using targeted proteomics are shown in **FIG. 75**. Spectrum for the light (endogenous) HPEYNRPLL (SEQ ID NO: 4143) epitope identified on a human prostate specimen (left) and the corresponding heavy isotope-labeled synthetic peptide (right) are shown. B and Y fragment ions are shown, and display high spectral similarity, confirming detection of the endogenous epitope. For each peptide, the top 200 more intense ions were plotted, and corresponding mass error of highlighted b and y ions are plotted below spectrum plots.

NeoStim TCR identification protocol

[00571] In vitro T cell inductions were used to prime, enrich, and expand antigen specific T cells. Healthy human donor PMBCs were seeded into multiple wells of a GREX 24 well flask with FLT3-L in AIM-V

expression of CD69 in a peptide dependent manner. The concentration required to achieve a 50% activation (EC50) is calculated from these plots and the results are shown on the plot.

[00584] The following Table 5 shows exemplary results of TCR discovery using the protocols above.

Table 5. TCR discovery results

Epitope	SEQ ID NO:	Allele	Gene	Reactive T cell population generated?
SLSKILDTV	826	A02:01	ANKRD30A	Y
LLSHGAVIEV	831	A02:01	ANKRD30A	Y
SLQCVSLHL	3456	A02:01	KLK2	Y
VLVHPQWVL	3757	A02:01	KLK2/3/4	Y
SLFHPEDTGQV	3775	A02:01	KLK3	Y
HPEYNRPLL	4143	B07:02	KLK4	Y
KIWEELSVLEV	8966	A02:01	MAGEA3	Y
KVLEHVVRV	4643	A02:01	MAGEA4	Y
GLSNLTHVL	8967	A02:01	PRAME	Y
SLLQHLIGL	6095	A02:01	PRAME	Y
PYLGQMINL	6120	A24:02	PRAME	Y
SPSVSQLSVL	6139	B07:02	PRAME	Y
MPMQDIKMIL	8968	B07:02	PRAME	Y

A T cell population reactive to each of the above epitope:MHC complexes has been generated.

Endogenous TCR activity assay

[00585] MDA-PCa-2b cells were plated at 50K / well in F12K media. The next day the cultures were treated with a cocktail of interferon alpha, beta, and gamma all at 1 U/uL final concentration. The next day the cells were washed with RPMI supplemented with 10% FBS and Glutamax. The cultures were then pulsed with peptide at a final concentration of 2 µM for 1 hour before the addition of effector cells.

[00586] The cells were co-cultured overnight before harvest and staining for CD69 expression via flow using a CD8, CD3, and murine TCR constant antibodies as lineage markers for effector cells and HLA-B07 as a lineage marker for the target cells. **FIG. 79** depicts exemplary plots showing endogenous activity of two different exemplary TCRs. avidity of exemplary TCRs. the plots here reflect the activation of two different TCR sequences (hereafter named mTCR21-033 and mTCR-034) following a coculture with the cell line MDA-PCa-2b which is endogenous for both HLA-B07 and KLK4. These plots are showing an increase in activation of mTCR21-033, but not mTCR21-034 following a 24-hour treatment with a cocktail of interferons (IFN). The IFN treatment increases the expression of surface HLA on cell lines, and the increased surface expression of the HLA can provide more expression of HLA-B07 bound to the KLK4 epitope.