SUMMARY

[0003a] In one aspect, provided herein is a method of producing an *ex vivo* population of expanded antigen specific T cells, the method comprising:

(a) culturing T cells from a biological sample from a subject in a first cell culture medium comprising antigen presenting cells (APCs) to produce a first population of T cells, wherein the APCs present an epitope of a peptide antigen in complex with an MHC protein;

(b) culturing the first population of T cells in a second cell culture medium to produce a second population of T cells;

(c) enriching CD137 (4-1BB)-expressing T cells from the second population of T cells to produce a third population of T cells; and

expanding the third population of T cells in a third cell culture medium to obtain *ex vivo* population of expanded antigen specific T cells, wherein antigen specific T cells in the third population of T cells is at least ten-fold compared to antigen specific T cells in the first or the second population of T cells.

[0003b] In one aspect, provided herein is a method for producing an *ex vivo* population of expanded antigen specific therapeutic T cells, the method comprising:

(d) culturing T cells from a biological sample from a subject in a first cell culture medium comprising antigen presenting cells (APCs), wherein the APC's present an epitope of a peptide antigen in complex with an MHC protein;

(e) culturing the first population of T cells in a second cell culture medium to produce a second population of T cells; and

expanding the second population of T cells in a third cell culture medium in presence of an increasing concentration of a plurality of peptide antigens comprising the peptide antigen, thereby obtaining the *ex vivo* population of expanded antigen specific therapeutic T cells.

[0003c] In one aspect, provided herein is a pharmaceutical composition comprising a population of T cells comprising the *ex vivo* population of expended antigen-specific T cells as described herein and a therapeutically acceptable excipient.

[0003d] In one aspect, provided herein is a use of a composition comprising the *ex vivo* population of expanded antigen specific T cells as described herein in the manufacture of a medicament for treating cancer in a human subject.

[0003e] In one aspect, provided herein is a composition comprising the *ex vivo* population of expanded antigen specific T cells as described herein for use in therapy.

[0004] Provided herein is a method for producing a therapeutic population of T cells comprising: (a) culturing T cells from a biological sample from a subject in a first cell culture medium comprising antigen presenting cells (APCs) to produce a first population of T cells, wherein the APCs present an epitope of a peptide antigen in complex with an MHC protein; (b) optionally, culturing the first population of T cells in a second cell culture medium to produce a second population of T cells; (c) enriching CD137 (4-1BB)-expressing T cells and/or

CD69 expressing T cells from the first or second population of T cells to produce a third population of T cells; and (d) expanding the third population of T cells in a third cell culture medium to obtain a therapeutic population of T cells comprising antigen-specific T cells.

[0005] In some embodiments, the method comprises culturing the first population of T cells in a second cell culture medium to produce the second population of T cells, and enriching CD137 (4-1BB)-expressing T cells and/or CD69 expressing T cells from the second population of T cells to produce the third population of T cells.

[0006] Provided herein is a method for producing a therapeutic population of T cells comprising: (a) culturing T cells from a biological sample from a subject in a first cell culture medium comprising antigen presenting cells (APCs), wherein the APCs present an epitope of a peptide antigen in complex with an MHC protein; (b) culturing the first population of T cells in a second cell culture medium to produce a second population of T cells; (c) optionally, enriching CD137 (4-1BB)-expressing T cells and/or CD69 expressing T cells from the second population of T cells to produce a third population of T cells; and (d) expanding the second or third population of T cells in a third cell culture medium to obtain a therapeutic population of T cells comprising antigen-specific T cells; wherein the concentration of the peptide antigen in the third culture medium is at least 2-fold lower than the concentration of the peptide antigen in the first culture medium and/or second culture medium.

[0007] In some embodiments, the method comprises enriching CD137 (4-1BB)-expressing T cells and/or CD69 expressing T cells from the second population of T cells to produce a third population of T cells.

[0008] In some embodiments, enriching CD137 (4-1BB)-expressing T cells and/or CD69 expressing T cells from the second population of T cells begins 10, 11, 12, 13, 14, 15, 16, 17 or 18 days after beginning the culturing of the T cells from a biological sample from a subject in a first cell culture medium.

[0009] In some embodiments, the APCs (i) comprise a polynucleotide sequence encoding the peptide antigen, or (ii) are loaded with the epitope of a peptide antigen.

[0010] In some embodiments, the peptide antigen is directly added to the first cell culture medium.

[0011] In some embodiments, the first cell culture medium comprises a first concentration of the peptide antigen

[0012] In some embodiments, the method further comprises supplementing the first cell culture medium with an amount of the peptide antigen such that the first cell culture medium comprises a first concentration of the peptide antigen.

"conventional 5'-cap" refers to a naturally occurring RNA 5'-cap, to the 7-methylguanosine cap (m G). In the context of the present invention, the term "5'-cap" includes a 5'-cap analog that resembles the RNA cap structure and is modified to possess the ability to stabilize RNA and/or enhance translation of RNA if attached thereto, in vivo and/or in a cell. In some embodiments, mRNA is capped cotranscriptionally.

[0373] In some embodiments, the mRNA encoding one or more neoantigenic peptides comprise a 3'-UTR comprising a poly A tail. In some embodiments, the poly A tail is 100-200 bp long (SEQ ID NO: 184). In some embodiments, the poly A tail is longer than 20 nucleotides. In some embodiments, the poly A tail is longer than 50 nucleotides. In some embodiments, the poly A tail is longer than 60 nucleotides. In some embodiments, the poly A tail is longer than 70 nucleotides. In some embodiments, the poly A tail is longer than 80 nucleotides. In some embodiments, the poly A tail is longer than 90 nucleotides. In some embodiments, the poly A tail is longer than 100 nucleotides. In some embodiments, the poly A tail is longer than 110 nucleotides. In some embodiments, the poly A tail is longer than 120 nucleotides. In some embodiments, the poly A tail is longer than 130 nucleotides. In some embodiments, the poly A tail is longer than 140 nucleotides. In some embodiments, the poly A tail is longer than 150 nucleotides. In some embodiments, the poly A tail is longer than 160 nucleotides. In some embodiments, the poly A tail is longer than 170 nucleotides. In some embodiments, the poly A tail is longer than 180 nucleotides. In some embodiments, the poly A tail is longer than 190 nucleotides. In some embodiments, the poly A tail is longer than 200 nucleotides. In some embodiments, the poly A tail is longer than 210 nucleotides. In some embodiments, the poly A tail is longer than 220 nucleotides. In some embodiments, the poly A tail is longer than 230 nucleotides. In some embodiments, the poly A tail is longer than 100 nucleotides. In some embodiments, the poly A tail is longer than 240 nucleotides. In some embodiments, the poly A tail is longer than 100 nucleotides. In some embodiments, the poly A tail is about 250 nucleotides.

[0374] In some embodiments, the poly A tail comprises 100-250 adenosine units (SEQ ID NO: 185). In some embodiments, the poly A tail comprises 120-130 adenine units (SEQ ID NO: 186). In some embodiments, the poly A tail comprises 120 adenine units (SEQ ID NO: 187). In some embodiments, the poly A tail comprises 121 adenine units (SEQ ID NO: 188). In some embodiments, the poly A tail comprises 122 adenine units (SEQ ID NO: 189). In some embodiments, the poly A tail comprises 123 adenine units (SEQ ID NO: 190). In some embodiments, the poly A tail comprises 124 adenine units (SEQ ID NO: 191). In some embodiments, the poly A tail comprises 125 adenine units (SEQ ID NO: 192). In some embodiments, the poly A tail as 129 bases (SEQ ID NO: 193).

[0375] In some embodiments, the coding sequence for two consecutive neoantigenic peptides are separated by a spacer or linker.

[0376] In some embodiments, the spacer or linker comprises up to 5000 nucleotide residues. An exemplary spacer sequence is GGCGGCAGCGGCGGCGGCGGCAGCGGCGGC (SEQ ID NO: 1). Another exemplary spacer sequence is GGCGGCAGCCTGGGCGGCGGCGGCAGCGGC (SEQ ID NO: 2). Another exemplary spacer sequence is GGCGTCGGCACC (SEQ ID NO: 3). Another exemplary spacer sequence is

CAGCTGGGCCTG (SEQ ID NO: 4). Another exemplary spacer is a sequence that encodes a lysine, such as AAA or AAG. Another exemplary spacer sequence is CAACTGGGATTG (SEQ ID NO: 5).

Sterility	BACT/ALERT®	negative

[0657] Safety tests are conducted with rapid methods to minimize time to product release. The current manufacturing process is estimated at 26 calendar days, with an overall turnaround time of 5 to 6 weeks at the initial phase. Due to the nature of autologous therapies and the indication (oncology), manufacturing turnaround is critical.

Example 2. Exemplary peptide antigen:MHCs

[0658] Provided below are exemplary RAS peptide:MHCs that can be used in the methods described herein.

[0659] In some embodiments, a peptide comprises a RAS mutation according to Table 1 below.

Table 1A

Gene	**Exemplary Protein Change**	**Mutation Sequence Context** (Mutated non-native residue underlined)	SEQ ID NO:	**Exemplary Diseases**
KRAS	G12C	MTEYKLVVVGACGVGKSALTIQLIQNHFVD EYDPTIEDSYRKQVVIDGETCLLDILDTAGQE	6	BRCA (breast cancer), CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma), CRC (colorectal cancer), HNSC (head-neck squamous cell carcinoma), LUAD (lung adenocarcinoma), PAAD (pancreatic adenocarcinoma), UCEC (uterine corpus endometrial carcinoma)
KRAS	G12D	MTEYKLVVVGADGVGKSALTIQLIQNHFVD EYDPTIEDSYRKQVVIDGETCLLDILDTAGQE	7	BLCA (urothelial bladder carcinoma), BRCA, CESC, CRC, GBM (glioblastoma), HNSC, KIRP (kidney renal papillary cell carcinoma), LIHC (liver hepatocellular carcinoma), LUAD, PAAD, SKCM (skin cutaneous melanoma), UCEC
KRAS	G12R	MTEYKLVVVGARGVGKSALTIQLIQNHFVD EYDPTIEDSYRKQVVIDGETCLLDILDTAGQE	8	PDAC, LUAD

KRAS	G12V	MTEYKLVVVGAVGVGKSALTIQLIQNHFVD EYDPTIEDSYRKQVVIDGETCLLDILDTAGQE	9	BRCA, CESC, CRC, LUAD, PAAD, THCA (thyroid carcinoma, UCEC
KRAS	Q61H	AGGVGKSALTIQLIQNHFVDEYDPTIEDSYRK QVVIDGETCLLDILDTAGHEEYSAMRDQYMR TGEGFLCVFAINNTKSFEDIHHYREQIKRVKD SEDVPM	10	CRC, LUSC (lung squamous cell carcinoma), PAAD, SKCM, UCEC

KRAS	Q61L	AGGVGKSALTIQLIQNHFVDEYDPTIEDSYRK QVVIDGETCLLDILDTAGLEEYSAMRDQYMR TGEGFLCVFAINNTKSFEDIHHYREQIKRVKD SEDVPM	11	CRC, GBM, HNSC, LUAD, SKCM, UCEC

[0660] Table 1B below shows an exemplary pool of mutated RAS peptides used in the methods described herein.

Table 1B

KRAS Mutation	Peptide name	Sequence	SEQ ID NO:	MS Validated HLA-I Alleles
G12D	AS2	GADGVGKSA	12	C08:02
	AS3	GADGVGKSAL	13	B07:02, C08:02, C03:04
	AS4	VVVGADGVGK	14	A03:01, A11:01, A68:01
	AL1	KKKTEYKLVVVGADGVGKSALTIQL	15	N/A
G12V	BS1	AVGVGKSAL	16	C01:02
	BS2	GAVGVGKSA	17	C03:03, C03:04
	BS3	GAVGVGKSAL	18	C03:03, C03:04
	BS4	VVGAVGVGK	19	A03:01, A11:01, A30:01, A68:01
	BS5	VVVGAVGVGK	20	A03:01, A11:01, A30:01, A68:01
	BL1	KKKKTEYKLVVVGAVGVGKSALTIQL	21	N/A
G12R	CS2	GARGVGKSAL	22	B07:02
	CS3	VVVGARGVGK	23	A03:01, A11:01, A30:01, A68:01
	CL1	KKKTEYKLVVVGARGVGKSALTIQL	24	N/A
G12C	DS1	VVVGACGVGK	25	A03:01, A11:01, A68:01
	DL1	KKKKTEYKLVVVGACGVGKSALTIQL	26	N/A

[0661] In some embodiments, a peptide comprises a RAS Q61H mutation according to Table 2 below.

Table 2

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
ILDTAGHEEY	27	HLA-A36:01	1
ILDTAGHEEY	27	HLA-A01:01	2
DTAGHEEYSAM	28	HLA-A26:01	3
DTAGHEEYSAM	28	HLA-A25:01	4
GHEEYSAM	29	HLA-B15:09	4
DTAGHEEY	30	HLA-A26:01	5

ILDTAGHEE	31	HLA-C08:02	5
AGHEEYSAM	32	HLA-C01:02	6
AGHEEYSAM	32	HLA-B46:01	6

DTAGHEEY	30	HLA-A25:01	6
DTAGHEEY	30	HLA-A01:01	6
DTAGHEEY	30	HLA-B18:01	7
DTAGHEEY	30	HLA-A36:01	7
ILDTAGHEE	31	HLA-C05:01	7
ILDTAGHEE	31	HLA-A02:07	7
ILDTAGHEEY	27	HLA-A29:02	7
ILDTAGHEEY	27	HLA-C08:02	7
HEEYSAMRD	33	HLA-B49:01	8
TAGHEEYSA	34	HLA-B35:03	8
DTAGHEEYS	35	HLA-A68:02	9
DTAGHEEYSAMR	36	HLA-A68:01	9
GHEEYSAM	29	HLA-B39:01	9
ILDTAGHEE	31	HLA-A01:01	9
LDTAGHEEY	37	HLA-B53:01	9
HEEYSAMRD	33	HLA-B41:01	10
ILDTAGHEE	31	HLA-A36:01	10
DTAGHEEY	30	HLA-B58:01	11
LLDILDTAGH	38	HLA-A01:01	12
TAGHEEYSAM	39	HLA-B35:03	12
LDTAGHEEY	37	HLA-B35:01	13
DILDTAGHE	40	HLA-A26:01	14
DTAGHEEY	30	HLA-C12:03	14
ILDTAGHEEY	27	HLA-C05:01	14
AGHEEYSAM	32	HLA-A30:02	15
DILDTAGHEEY	41	HLA-A25:01	15
DTAGHEEY	30	HLA-C02:02	15
ILDTAGHEE	31	HLA-C04:01	15
DILDTAGH	42	HLA-A26:01	16
ILDTAGHEE	31	HLA-A02:01	16
LDTAGHEEY	37	HLA-A29:02	16
ILDTAGHE	43	HLA-A01:01	17
LDTAGHEEY	37	HLA-B18:01	17
AGHEEYSAM	32	HLA-C14:03	18
DILDTAGHEEY	41	HLA-A29:02	18

DTAGHEEYS	35	HLA-A26:01	18
ILDTAGHEEY	27	HLA-B15:01	18
DTAGHEEYSA	44	HLA-A68:02	19
ILDTAGHE	43	HLA-C05:01	19
ILDTAGHEEY	27	HLA-A02:07	19
ILDTAGHEEY	27	HLA-A30:02	19
LDTAGHEEY	37	HLA-A36:01	19
AGHEEYSAM	32	HLA-C14:02	20
AGHEEYSAM	32	HLA-B15:03	20
LLDILDTAGH	38	HLA-A02:07	20

[0662] In some embodiments, a peptide comprises a RAS Q61R mutation according to Table 3 below.

Table 3

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
ILDTAGREEY	45	HLA-A36:01	1
ILDTAGREEY	45	HLA-A01:01	2
DTAGREEYSAM	46	HLA-A26:01	3
DILDTAGR	47	HLA-A33:03	4
DILDTAGR	47	HLA-A68:01	5
DTAGREEY	48	HLA-A26:01	6
DTAGREEYSAM	46	HLA-A25:01	6
CLLDILDTAGR	49	HLA-A74:01	7
DTAGREEY	48	HLA-A01:01	7
REEYSAMRD	50	HLA-B41:01	7
GREEYSAMR	51	HLA-B27:05	8
ILDTAGREE	52	HLA-C08:02	8
ILDTAGREEY	45	HLA-A29:02	8
REEYSAMRD	50	HLA-B49:01	8
AGREEYSAM	53	HLA-B46:01	9
DTAGREEY	48	HLA-B18:01	9
DTAGREEY	48	HLA-A25:01	9
DTAGREEY	48	HLA-A36:01	9
DILDTAGR	47	HLA-A74:01	10
DILDTAGRE	54	HLA-A26:01	10
ILDTAGREE	52	HLA-C05:01	10
DILDTAGR	47	HLA-A26:01	11
GREEYSAM	55	HLA-B39:01	11
AGREEYSAM	53	HLA-B15:03	12
GREEYSAM	55	HLA-C07:02	12
ILDTAGREE	52	HLA-A01:01	12
TAGREEYSA	56	HLA-B35:03	12

ILDTAGREEY	45	HLA-A30:02	13
DTAGREEYS	57	HLA-A68:02	14
ILDTAGRE	58	HLA-A01:01	14
CLLDILDTAGR	49	HLA-A31:01	15
DTAGREEYSAMR	59	HLA-A68:01	15
LLDILDTAGR	60	HLA-A01:01	15
DTAGREEY	48	HLA-B58:01	16
ILDTAGREEY	45	HLA-C08:02	16
DILDTAGR	47	HLA-A31:01	17
ILDTAGREE	52	HLA-C04:01	17
ILDTAGREEY	45	HLA-A32:01	17
LLDILDTAGR	60	HLA-A74:01	17
TAGREEYSAM	61	HLA-B35:03	17
DILDTAGREEY	62	HLA-A32:01	18
ILDTAGRE	58	HLA-C05:01	18
ILDTAGREE	52	HLA-A02:07	18
REEYSAMRD	50	HLA-B40:01	18
AGREEYSAM	53	HLA-B15:01	19
AGREEYSAMR	63	HLA-A31:01	19
ILDTAGRE	58	HLA-A36:01	19
LDILDTAGR	64	HLA-A68:01	19
LDTAGREEY	65	HLA-A29:02	19
LDTAGREEY	65	HLA-B35:01	19
REEYSAMRD	50	HLA-B45:01	19
REEYSAMRDQY	66	HLA-A36:01	19
DTAGREEY	48	HLA-C02:02	20

[0663] In some embodiments, a peptide comprises a RAS Q61K mutation according to Table 4 below.

Table 4

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
ILDTAGKEEY	67	HLA-A36:01	1
ILDTAGKEEY	67	HLA-A01:01	2
DTAGKEEYSAM	68	HLA-A26:01	3
CLLDILDTAGK	69	HLA-A03:01	4
DTAGKEEY	70	HLA-A01:01	5
DTAGKEEY	70	HLA-A26:01	5
DTAGKEEYSAM	68	HLA-A25:01	5
AGKEEYSAM	71	HLA-B46:01	6
DILDTAGKE	72	HLA-A26:01	7
KEEYSAMRD	73	HLA-B41:01	7
DTAGKEEY	70	HLA-B18:01	8
GKEEYSAM	74	HLA-B15:03	8
ILDTAGKEE	75	HLA-C08:02	8
ILDTAGKEEY	67	HLA-A29:02	8

DTAGKEEYS	76	HLA-A68:02	9
LDTAGKEEY	77	HLA-B53:01	9
TAGKEEYSA	78	HLA-B35:03	9
DILDTAGK	79	HLA-A68:01	10
DTAGKEEY	70	HLA-A36:01	10
KEEYSAMRD	73	HLA-B49:01	10
LDTAGKEEY	77	HLA-C07:01	10
DTAGKEEYSAMR	80	HLA-A68:01	11
ILDTAGKEE	75	HLA-C05:01	11
ILDTAGKEEY	67	HLA-C08:02	11
LLDILDTAGK	81	HLA-A01:01	12
AGKEEYSAM	71	HLA-A30:02	13
DTAGKEEY	70	HLA-A25:01	13
DTAGKEEYS	76	HLA-A26:01	13
ILDTAGKE	82	HLA-C05:01	13
LDTAGKEEY	77	HLA-B35:01	13
AGKEEYSAMR	83	HLA-A31:01	14
DILDTAGK	79	HLA-A33:03	14
ILDTAGKE	82	HLA-A01:01	14
ILDTAGKEE	75	HLA-A01:01	14
ILDTAGKEE	75	HLA-A02:07	14
TAGKEEYSAM	84	HLA-B35:03	14
AGKEEYSAM	71	HLA-B15:01	15
ILDTAGKEEY	67	HLA-A30:02	15
LDTAGKEEY	77	HLA-B46:01	15
DTAGKEEY	70	HLA-B58:01	16
ILDTAGKEEY	67	HLA-C05:01	17
AGKEEYSAM	71	HLA-A30:01	18
AGKEEYSAM	71	HLA-B15:03	18
DTAGKEEY	70	HLA-C02:02	18
LDTAGKEEY	77	HLA-A29:02	18

[0664] In some embodiments, a peptide comprises a RAS Q61L mutation according to Table 5 below.

Table 5

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
ILDTAGLEEY	85	HLA-A36:01	1
ILDTAGLEEY	85	HLA-A01:01	2
LLDILDTAGL	86	HLA-A02:07	3
GLEEYSAMRDQY	87	HLA-A36:01	4
DTAGLEEY	88	HLA-A25:01	5
DTAGLEEY	88	HLA-A26:01	5
DTAGLEEYSAM	89	HLA-A26:01	5
DTAGLEEY	88	HLA-A01:01	6
ILDTAGLEE	90	HLA-C08:02	6

ILDTAGLEE	90	HLA-A01:01	6
CLLDILDTAGL	91	HLA-A02:04	7
ILDTAGLEE	90	HLA-A36:01	7
LLDILDTAGL	86	HLA-A01:01	7
DILDTAGL	92	HLA-B14:02	8
DILDTAGLEEY	93	HLA-A25:01	8
DTAGLEEYS	94	HLA-A68:02	8
DTAGLEEYSAM	89	HLA-A25:01	8
GLEEYSAMR	95	HLA-A74:01	8
ILDTAGLE	96	HLA-A01:01	8
DILDTAGLEEY	93	HLA-A26:01	9
DTAGLEEY	88	HLA-A36:01	9
ILDTAGLEEY	85	HLA-A29:02	9
DILDTAGL	92	HLA-B08:01	10
DTAGLEEY	88	HLA-B18:01	10
ILDTAGLEE	90	HLA-A02:07	10
LDTAGLEEY	97	HLA-B35:01	10
CLLDILDTAGL	91	HLA-A02:01	11
DTAGLEEY	88	HLA-C02:02	11
ILDTAGLEE	90	HLA-C05:01	11
ILDTAGLEEY	85	HLA-C08:02	11
ILDTAGLEEY	85	HLA-A02:07	11
LLDILDTAGL	86	HLA-C08:02	11
DILDTAGL	92	HLA-A26:01	12
LDTAGLEEY	97	HLA-B53:01	12
DTAGLEEY	88	HLA-C03:02	13
DTAGLEEY	88	HLA-B58:01	13
ILDTAGLEEY	85	HLA-A30:02	13
LLDILDTAGL	86	HLA-C05:01	13
LLDILDTAGL	86	HLA-C04:01	13
DTAGLEEYSAMR	98	HLA-A68:01	14
ILDTAGLE	96	HLA-A36:01	15
LLDILDTAGL	86	HLA-A02:01	15
AGLEEYSAM	99	HLA-B15:03	16
DTAGLEEYSA	100	HLA-A68:02	16
GLEEYSAMRDQY	87	HLA-A01:01	16
ILDTAGLE	96	HLA-C04:01	16
ILDTAGLEEY	85	HLA-B15:01	16
LDILDTAGL	101	HLA-B37:01	16
AGLEEYSAM	99	HLA-A30:02	17
AGLEEYSAM	99	HLA-B48:01	17
AGLEEYSAMR	102	HLA-A31:01	17
ILDTAGLEE	90	HLA-C04:01	17
LDTAGLEEY	97	HLA-C03:02	17

AGLEEYSAM	99	HLA-C14:02	18
GLEEYSAMR	95	HLA-A31:01	18
LEEYSAMRD	103	HLA-B41:01	18
LLDILDTAGLE	104	HLA-A01:01	18
AGLEEYSAM	99	HLA-C14:03	19
LDILDTAGL	101	HLA-B40:02	19
LDTAGLEEY	97	HLA-A29:02	19
DILDTAGLE	105	HLA-A26:01	20
DTAGLEEY	88	HLA-B15:01	20
ILDTAGLEEY	85	HLA-A02:01	20
LDTAGLEEY	97	HLA-A36:01	20
LDTAGLEEY	97	HLA-B46:01	20
DTAGLEEY	88	HLA-A68:02	21
DTAGLEEY	88	HLA-C12:03	21
ILDTAGLE	96	HLA-C05:01	21
LDTAGLEEY	97	HLA-B18:01	21
LEEYSAMRD	103	HLA-B49:01	21
TAGLEEYSA	106	HLA-B54:01	21
DILDTAGLEEY	93	HLA-A29:02	22
GLEEYSAM	107	HLA-C05:01	22

[0665] In some embodiments, a peptide comprises a RAS G12A mutation according to Table 6 below.

Table 6

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
AAGVGKSAL	108	HLA-C03:04	1
VVVGAAGVGK	109	HLA-A11:01	1
VVGAAGVGK	110	HLA-A11:01	2
TEYKLVVVGAA	111	HLA-B50:01	3
VVGAAGVGK	110	HLA-A03:01	3
VVVGAAGVGK	109	HLA-A68:01	3
AAGVGKSAL	108	HLA-C08:02	4
AAGVGKSAL	108	HLA-C08:01	4
AAGVGKSAL	108	HLA-B46:01	4
AAGVGKSAL	108	HLA-B81:01	5
GAAGVGKSAL	112	HLA-B48:01	5
LVVVGAAGV	113	HLA-A68:02	5
AAGVGKSAL	108	HLA-C03:04	1
VVVGAAGVGK	109	HLA-A11:01	1
VVGAAGVGK	110	HLA-A11:01	2
TEYKLVVVGAA	111	HLA-B50:01	3
VVGAAGVGK	110	HLA-A03:01	3
VVVGAAGVGK	109	HLA-A68:01	3
AAGVGKSAL	108	HLA-C08:02	4
AAGVGKSAL	108	HLA-C08:01	4

AAGVGKSAL	108	HLA-B46:01	4
AAGVGKSAL	108	HLA-B81:01	5
AAGVGKSAL	108	HLA-C03:02	5
AAGVGKSAL	108	HLA-C01:02	5
GAAGVGKSAL	112	HLA-B48:01	5
LVVVGAAGV	113	HLA-A68:02	5
AAGVGKSAL	108	HLA-C03:03	6
VVGAAGVGK	110	HLA-A68:01	6
GAAGVGKSAL	112	HLA-B81:01	7
VVVGAAGVGK	109	HLA-A03:01	7
AAGVGKSAL	108	HLA-C05:01	8
AAGVGKSAL	108	HLA-C12:03	8
GAAGVGKSA	114	HLA-B46:01	8
VVGAAGVGK	110	HLA-A30:01	8
GAAGVGKSA	114	HLA-B55:01	9
KLVVVGAAGV	115	HLA-A02:01	9
AGVGKSAL	116	HLA-B08:01	10
GAAGVGKSAL	112	HLA-C03:04	10
AAGVGKSAL	108	HLA-C17:01	11
GAAGVGKSAL	112	HLA-C03:03	11
VVVGAAGV	117	HLA-A68:02	11
YKLVVVGAA	118	HLA-B54:01	11
AAGVGKSAL	108	HLA-B48:01	12
AGVGKSAL	116	HLA-C03:04	12
AGVGKSAL	116	HLA-C07:01	12
VVVGAAGVGK	109	HLA-A30:01	12
AAGVGKSA	119	HLA-B46:01	13
KLVVVGAAGV	115	HLA-A02:07	13
YKLVVVGAA	118	HLA-B50:01	13
AAGVGKSAL	108	HLA-B07:02	14
GAAGVGKSAL	112	HLA-A68:02	14
VVGAAGVGK	110	HLA-A74:01	14
AGVGKSAL	116	HLA-C08:01	15
GAAGVGKSAL	112	HLA-C17:01	15
GAAGVGKSAL	112	HLA-C08:01	16
GAAGVGKSAL	112	HLA-B35:03	16
AAGVGKSAL	108	HLA-C02:02	17
AAGVGKSAL	108	HLA-B35:03	17
AAGVGKSAL	108	HLA-C12:02	17
AAGVGKSAL	108	HLA-C14:03	17
GAAGVGKSA	114	HLA-B50:01	17
AGVGKSAL	116	HLA-C03:02	18
GAAGVGKSA	114	HLA-C03:04	18
LVVVGAAGV	113	HLA-B55:01	18

TEYKLVVVGAA	111	HLA-B41:01	18
AGVGKSAL	116	HLA-C01:02	19
GAAGVGKSA	114	HLA-B54:01	19
GAAGVGKSAL	112	HLA-B07:02	19
VGAAGVGKSA	120	HLA-B55:01	19
AGVGKSAL	116	HLA-B48:01	20
AGVGKSALTI	121	HLA-B49:01	20
VVVGAAGV	117	HLA-B55:01	20

[0666] In some embodiments, a peptide comprises a RAS G12C mutation according to Table 7 below.

Table 7

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
VVVGACGVGK	25	HLA-A11:01	1
VVGACGVGK	122	HLA-A03:01	2
VVGACGVGK	122	HLA-A11:01	3
VVVGACGVGK	25	HLA-A68:01	4
VVGACGVGK	122	HLA-A68:01	5
VVVGACGVGK	25	HLA-A03:01	5
VVGACGVGK	122	HLA-A30:01	6
ACGVGKSAL	123	HLA-B81:01	7
ACGVGKSAL	123	HLA-C01:02	7
ACGVGKSAL	123	HLA-C14:03	8
ACGVGKSAL	123	HLA-C03:04	9
VVVGACGVGK	25	HLA-A30:01	9
ACGVGKSAL	123	HLA-C14:02	10
CGVGKSAL	124	HLA-B08:01	10
KLVVVGACGV	125	HLA-A02:01	10
ACGVGKSAL	123	HLA-B07:02	11
GACGVGKSAL	126	HLA-B48:01	12
GACGVGKSAL	126	HLA-C03:03	13
ACGVGKSAL	123	HLA-B48:01	14
ACGVGKSAL	123	HLA-B40:01	14
YKLVVVGAC	127	HLA-B48:01	14
YKLVVVGAC	127	HLA-B15:03	14
GACGVGKSA	128	HLA-B46:01	15
GACGVGKSAL	126	HLA-C03:04	15
GACGVGKSAL	126	HLA-C01:02	15
LVVVGACGV	129	HLA-A68:02	15
CGVGKSAL	124	HLA-C03:04	16
GACGVGKSAL	126	HLA-C08:02	16
VVGACGVGK	122	HLA-A74:01	16

[0667] In some embodiments, a peptide comprises a RAS G12D mutation according to Table 8 below.

Table 8

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
GADGVGKSAL	13	HLA-C08:02	1
GADGVGKSAL	13	HLA-C05:01	2
VVVGADGVGK	14	HLA-A11:01	3
DGVGKSAL	130	HLA-B14:02	4
VVGADGVGK	131	HLA-A11:01	4
VVGADGVGK	131	HLA-A03:01	5
DGVGKSAL	130	HLA-B08:01	6
VVVGADGVGK	14	HLA-A68:01	6
GADGVGKSAL	13	HLA-C03:03	7
VVGADGVGK	131	HLA-A30:01	7
ADGVGKSAL	132	HLA-B37:01	8
GADGVGKSAL	13	HLA-C08:01	8
VVGADGVGK	131	HLA-A68:01	8
GADGVGKSA	12	HLA-C08:02	9
GADGVGKSAL	13	HLA-B35:03	9
GADGVGKS	133	HLA-C05:01	10
GADGVGKSA	12	HLA-C05:01	10
ADGVGKSAL	132	HLA-C07:01	11
VVVGADGVGK	14	HLA-A03:01	11
ADGVGKSAL	132	HLA-B40:02	12
ADGVGKSAL	132	HLA-B46:01	13
GADGVGKSAL	13	HLA-C03:04	13
ADGVGKSAL	132	HLA-B81:01	14
GADGVGKSAL	13	HLA-C17:01	14
VVVGADGVGK	14	HLA-A30:01	14
GADGVGKSA	12	HLA-B35:03	15
GADGVGKSA	12	HLA-B46:01	15
GADGVGKSAL	13	HLA-B48:01	15
KLVVVGADGV	134	HLA-A02:01	15
LVVVGADGV	135	HLA-A68:02	15
VGADGVGKSA	136	HLA-B55:01	15
VVGADGVGK	131	HLA-A74:01	16
GADGVGKSA	12	HLA-B53:01	17
KLVVVGADGV	134	HLA-A02:07	17
VGADGVGK	137	HLA-A68:01	17
YKLVVVGAD	138	HLA-B48:01	17
ADGVGKSAL	132	HLA-C14:03	18
DGVGKSALTI	139	HLA-B51:01	18
VGADGVGK	137	HLA-A11:01	18

[0668] In some embodiments, a peptide comprises a RAS G12R mutation according to Table 9 below.

Table 9

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
VVGARGVGK	140	HLA-A03:01	1
EYKLVVVGAR	141	HLA-A33:03	2
VVVGARGVGK	23	HLA-A11:01	3
ARGVGKSAL	142	HLA-C07:02	4
ARGVGKSAL	142	HLA-B39:01	5
ARGVGKSAL	142	HLA-C07:01	5
VVGARGVGK	140	HLA-A11:01	5
VVVGARGVGK	23	HLA-A68:01	5
GARGVGKSA	143	HLA-B46:01	6
ARGVGKSAL	142	HLA-B27:05	7
GARGVGKSA	143	HLA-B55:01	7
RGVGKSAL	144	HLA-C07:01	8
VVGARGVGK	140	HLA-A30:01	9
ARGVGKSAL	142	HLA-B38:01	10
ARGVGKSAL	142	HLA-B14:02	10
VVGARGVGK	140	HLA-A68:01	10
VVVGARGVGK	23	HLA-A03:01	11
GARGVGKSAL	22	HLA-B48:01	12
RGVGKSAL	144	HLA-B48:01	12
RGVGKSALTI	145	HLA-A23:01	12
ARGVGKSAL	142	HLA-C06:02	13
GARGVGKSA	143	HLA-A30:01	13
GARGVGKSAL	22	HLA-B81:01	13
VVVGARGVGK	23	HLA-A30:01	13
GARGVGKSAL	22	HLA-B07:02	14
LVVVGARGV	146	HLA-C06:02	14
RGVGKSAL	144	HLA-B81:01	14
VVGARGVGK	140	HLA-A74:01	15
KLVVVGARGV	147	HLA-A02:01	16
LVVVGARGV	146	HLA-B55:01	16
YKLVVVGAR	148	HLA-A33:03	16
KLVVVGAR	149	HLA-A74:01	17
KLVVVGARGV	147	HLA-B13:02	17
RGVGKSAL	144	HLA-C01:02	17
LVVVGARGV	146	HLA-A68:02	18
VVVGARGV	150	HLA-B55:01	18
ARGVGKSAL	142	HLA-B15:09	19
ARGVGKSAL	142	HLA-C14:03	20
GARGVGKSA	143	HLA-B54:01	20
VVVGARGV	150	HLA-B52:01	20

[0669] In some embodiments, a peptide comprises a RAS G12S mutation according to Table 10 below.

Table 10

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
VVVGASGVGK	151	HLA-A11:01	1
VVGASGVGK	152	HLA-A11:01	2
VVGASGVGK	152	HLA-A03:01	3
VVVGASGVGK	151	HLA-A68:01	4
ASGVGKSAL	153	HLA-C03:04	5
ASGVGKSAL	153	HLA-B46:01	5
VVGASGVGK	152	HLA-A68:01	6
VVVGASGVGK	151	HLA-A03:01	6
ASGVGKSAL	153	HLA-C01:02	7
GASGVGKSAL	154	HLA-B48:01	7
ASGVGKSAL	153	HLA-C07:01	8
ASGVGKSAL	153	HLA-C08:02	9
GASGVGKSAL	154	HLA-B81:01	9
SGVGKSAL	155	HLA-B08:01	9
ASGVGKSAL	153	HLA-C03:03	10
ASGVGKSAL	153	HLA-C03:02	10
SGVGKSAL	155	HLA-B14:02	10
VVGASGVGK	152	HLA-A30:01	10
ASGVGKSAL	153	HLA-C08:01	11
VVVGASGVGK	151	HLA-A30:01	11
GASGVGKSAL	154	HLA-B35:03	12
SGVGKSAL	155	HLA-C07:01	12
ASGVGKSAL	153	HLA-B81:01	13
GASGVGKSA	156	HLA-B55:01	13
GASGVGKSAL	154	HLA-C03:03	13
KLVVVGASGV	157	HLA-A02:01	13
LVVVGASGV	158	HLA-A68:02	13
SGVGKSAL	155	HLA-C01:02	13
ASGVGKSA	159	HLA-B46:01	14
ASGVGKSAL	153	HLA-C15:02	14
GASGVGKSAL	154	HLA-C08:01	15
SGVGKSAL	155	HLA-C03:04	15
ASGVGKSAL	153	HLA-C05:01	16
GASGVGKSAL	154	HLA-C03:04	16
VVGASGVGK	152	HLA-A74:01	16
ASGVGKSAL	153	HLA-B48:01	17
GASGVGKSAL	154	HLA-C01:02	17
SGVGKSAL	155	HLA-C03:02	17
SGVGKSALTI	160	HLA-A23:01	17
VGASGVGKSA	161	HLA-B55:01	18
ASGVGKSAL	153	HLA-C12:03	19
ASGVGKSAL	153	HLA-B57:03	19
KLVVVGASGV	157	HLA-A02:07	19

SGVGKSAL	155	HLA-B81:01	19
ASGVGKSAL	153	HLA-C17:01	20
KLVVVGASG	162	HLA-A32:01	20

[0670] In some embodiments, a peptide comprises a RAS G12V mutation according to Table 11 below.

Table 11

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
VVGAVGVGK	19	HLA-A03:01	1
VVGAVGVGK	19	HLA-A11:01	2
VVVGAVGVGK	20	HLA-A11:01	2
VVVGAVGVGK	20	HLA-A68:01	3
VVGAVGVGK	19	HLA-A68:01	4
LVVVGAVGV	163	HLA-A68:02	5
VVGAVGVGK	19	HLA-A30:01	5
AVGVGKSAL	16	HLA-B81:01	6
KLVVVGAVGV	164	HLA-A02:01	6
AVGVGKSAL	16	HLA-B46:01	7
GAVGVGKSAL	18	HLA-C03:03	7
GAVGVGKSAL	18	HLA-B48:01	7
VVVGAVGVGK	20	HLA-A03:01	7
AVGVGKSAL	16	HLA-C03:04	8
GAVGVGKSAL	18	HLA-C03:04	8
KLVVVGAVGV	164	HLA-A02:07	9
VGVGKSAL	165	HLA-B08:01	9
VVVGAVGV	166	HLA-A68:02	9
AVGVGKSAL	16	HLA-C08:02	10
AVGVGKSAL	16	HLA-B07:02	10
GAVGVGKSAL	18	HLA-B35:03	10
AVGVGKSAL	16	HLA-C08:01	11
AVGVGKSAL	16	HLA-C01:02	11
GAVGVGKSA	17	HLA-B55:01	11
GAVGVGKSAL	18	HLA-B81:01	11
GAVGVGKSAL	18	HLA-C08:01	11
KLVVVGAVGV	164	HLA-B13:02	11
VGVGKSAL	165	HLA-C03:04	11
AVGVGKSAL	16	HLA-A32:01	12
GAVGVGKSA	17	HLA-B46:01	12
VGVGKSAL	165	HLA-C03:02	12
VGVGKSALTI	167	HLA-A23:01	12
GAVGVGKSA	17	HLA-B54:01	13
VGVGKSAL	165	HLA-C01:02	.3
AVGVGKSAL	16	HLA-B48:01	14
AVGVGKSAL	16	HLA-C03:03	14
AVGVGKSAL	16	HLA-B42:01	14

LVVVGAVGV	163	HLA-B55:01	14
VGVGKSAL	165	HLA-C08:01	14
VVGAVGVGK	19	HLA-A74:01	14
AVGVGKSAL	16	HLA-C05:01	15
AVGVGKSAL	16	HLA-C03:02	15
GAVGVGKSA	17	HLA-C03:04	15
KLVVVGAVGV	164	HLA-A02:04	15
LVVVGAVGV	163	HLA-A02:07	15
VGVGKSAL	165	HLA-B14:02	15
VVVGAVGVGK	20	HLA-A30:01	15
VVGAVGVGK	19	HLA-B81:01	16
VVVGAVGV	166	HLA-B55:01	16
AVGVGKSAL	16	HLA-C14:03	17
AVGVGKSAL	16	HLA-B15:01	17
LVVVGAVGV	163	HLA-B54:01	17
AVGVGKSA	168	HLA-B55:01	18
AVGVGKSAL	16	HLA-C17:01	18
GAVGVGKSA	17	HLA-B50:01	19
GAVGVGKSAL	18	HLA-C17:01	19
YKLVVVGAV	169	HLA-A02:04	19
GAVGVGKSAL	18	HLA-B35:01	20
VVGAVGVGK	19	HLA-A31:01	20
YKLVVVGAV	169	HLA-B51:01	20

[0671] In some embodiments, a peptide comprises a RAS G13C mutation according to Table 12 below.

Table 12

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
VVVGAGCVGK	170	HLA-A11:01	1
VVGAGCVGK	171	HLA-A11:01	2
AGCVGKSAL	172	HLA-C01:02	3
VVGAGCVGK	171	HLA-A03:01	4
VVVGAGCVGK	170	HLA-A68:01	4
CVGKSALTI	173	HLA-B13:02	5
VVGAGCVGK	171	HLA-A68:01	5
VVGAGCVGK	171	HLA-A30:01	6
AGCVGKSAL	172	HLA-B48:01	7
AGCVGKSAL	172	HLA-C03:04	8
GCVGKSALTI	174	HLA-B49:01	8
AGCVGKSAL	172	HLA-C08:02	9
VVVGAGCVGK	170	HLA-A03:01	9
KLVVVGAGC	175	HLA-A30:02	10
GCVGKSAL	176	HLA-C07:01	11
VVGAGCVGK	171	HLA-A74:01	12
AGCVGKSAL	172	HLA-C14:03	13

KLVVVGAGC	175	HLA-B15:01	14

[0672] In some embodiments, a peptide comprises a RAS G13D mutation according to Table 13 below.

Table 13

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
AGDVGKSAL	177	HLA-C08:02	1
AGDVGKSAL	177	HLA-C05:01	2
VVGAGDVGK	178	HLA-A11:01	3
VVVGAGDVGK	179	HLA-A11:01	3
VVVGAGDVGK	179	HLA-A68:01	4
GAGDVGKSA	180	HLA-B46:01	5
GAGDVGKSAL	181	HLA-B48:01	5
VVGAGDVGK	178	HLA-A68:01	5
VVGAGDVGK	178	HLA-A03:01	5
AGDVGKSAL	177	HLA-C03:04	6
AGDVGKSAL	177	HLA-C04:01	6
AGDVGKSAL	177	HLA-C01:02	6
DVGKSALTI	182	HLA-B13:02	6
DVGKSALTI	182	HLA-A25:01	6
GDVGKSAL	183	HLA-C07:01	6
GDVGKSAL	183	HLA-B40:02	7
GDVGKSAL	183	HLA-B37:01	8
AGDVGKSAL	177	HLA-B48:01	9
DVGKSALTI	182	HLA-B51:01	10
VVGAGDVGK	178	HLA-A30:01	10
GAGDVGKSAL	181	HLA-C08:01	11
GAGDVGKSAL	181	HLA-B81:01	11
AGDVGKSAL	177	HLA-C08:01	12
GAGDVGKSAL	181	HLA-C03:04	12
DVGKSALTI	182	HLA-B53:01	13
AGDVGKSAL	177	HLA-B07:02	14
AGDVGKSAL	177	HLA-B46:01	14
DVGKSALTI	182	HLA-A26:01	14
VVGAGDVGK	178	HLA-A74:01	14
GAGDVGKSA	180	HLA-B54:01	15
DVGKSALTI	182	HLA-B38:01	16
GAGDVGKSAL	181	HLA-C03:03	16
VVVGAGDVGK	179	HLA-A03:01	16

[0673] In some embodiments, a peptide comprises a mutated RAS peptide according to Table 14 below.

Table 14

Epitope	SEQ ID NO:	MHC protein encoded by allele
GACGVGKSA	128	C03:04

GAVGVGKSA	17	C03:03

Example 3. Method for enrichment and expansion of antigen-specific T cells.

[0674] In this example, a method for increasing the yield of antigen specific T cells is described. An overview of an examplary manufacturing process used to produce large number of KRAS neoantigen-specific T cells is presented in **FIG. 1A**, **FIG. 1B** and **FIG. 2**. In brief, peripheral blood mononuclear cells (PBMCs) derived from leukaphereses from healthy donors or patients were processed to deplete specific cell subsets on day 0. KRAS neoantigen peptides were added on day 1 to the cell culture, and maturation cytokines were added after a period of time. On day 0, human serum was added to the culture. On days 5, 7, 9 and 12, culture maintenance was performed, which entailed addition or changing of media and/or addition of cytokines. On day 13, the KRAS neoantigen peptides were spiked into the culture, which upregulated activation markers on the T cells. On day 14, antigen-specific T cells were enriched by antibody labeling and capturing of the cells expressing one or more of the activation markers. The process on days 13 and 14 are termed the "enrichment process."

Enrichment of antigen specific T cells

[0675] **FIGs. 3** and **4** demonstrate the schematic workflow and results from the feasibility of enrichment at research scale and large-scale runs using the CliniMACS system. For large scale runs, at least 10^9 cells were available to set up the runs. The naïve T cell induction protocol described above is used in large vessels, and the cells are pooled on day 14 after peptide pulsing and were run through the CliniMACS. **FIG. 4** shows results from the studies on feasibility of enrichment. Both the methods tested using MACS buffer yielded viable cells after enrichment which was considerably higher over historical small scale data. Multiple CliniMACS protcols were tested, which have different pressures and flow rates through the magnetic column (e.g., manufacturer specified protocols named Method 2.1, Method 3.2, etc.), and the enrichment was relatively similar. Method 2.1 was selected for further testing. Different running buffers were tested for passing through the CliniMACS columns, including MACS buffer and AIM-V, and the results were relatively similar.

[0676] After the initial stimulation of naïve T cells, the peptide pulse on day 13 can be used to, for example, upregulate cell surface activation markers that can be used to label and enrich antigen-specific T cells. Alternative methods, such as addition of antigen-expressing cells or scaffolds can function in a similar manner. **FIG. 5** shows the upregulation of four different cell surface activation markers (CD39, PD-1, CD137 (4-1BB), and CD69) after peptide pulse on day 13 and analysis on day 14. **FIGs. 5 and 6** demonstrates data that an overnight peptide pulse (up to 24 hours) upregulates specific activation markers on antigen responsive T cells. Of the activation markers shown, 4-1BB (CD137) and CD69 showed great promise (**FIG. 6**). CD137 and CD69 were more substantially increased on multimer positive cells relative to multimer negative cells. Particularly, as shown in **FIG. 5**, CD137 enrichment and peptide spike with 2 uM peptide had a remarkable effect on enriching antigen-specific (multimer positive) T cells (data from lower right compared to the upper right flow cytometry results), exceeding that of CD69. **FIG. 7** shows that KRAS neoantigen-specific CD8+ T cells were enriched using either CD137 or CD69 using this method. Pre-enrichment frequencies were increased on average greater than 10-fold and antigen-specific responses that were not detectable prior to enrichment became detectable after