

CLAIMS

What is claimed is:

1. — An *ex vivo* method for preparing antigen-specific T cells, the method comprising contacting T cells with:
 - (a) 1. antigen presenting cells (APCs) comprising one or more peptides containing an epitope with a sequence GACGVGKSA, wherein the APCs express a protein encoded by an HLA-C03:04 allele; ~~or,~~
 - (b) 2. The method of claim 1, further comprising contacting T cells with APCs comprising one or more peptides containing an epitope with a sequence GAVGVGKSA, wherein the APCs express a protein encoded by an HLA-C03:03 allele.
2. 3. The method of claim 1, ~~or 2,~~ wherein the T cells or APCs are from a subject with cancer.
3. — ~~The method of claim 1, wherein the T cells are allogeneic T cells.~~
4. — The method of any one of claims 1-1-3, wherein the T cells or APCs are allogeneic.
4. 5. The method ~~further comprises administering of any one of claims 2-4, wherein the epitope with the T cells~~ sequence GACGVGKSA is capable of binding to a subject in need thereof, wherein the subject expresses a ~~the~~ protein encoded by an the HLA-C03:04 allele, and ~~the T cells have been contacted with APCs comprising one or more peptides containing the wherein the~~ epitope GACGVGKSA with the sequence GAVGVGKSA is capable of binding to the protein encoded by the HLA-C03:03 allele.
5. — ~~The method of any one of claims 1-3, wherein the method further comprises administering the T cells to a subject in need thereof, wherein the subject expresses one or more protein encoded by an HLA-C03:03 allele and the T cells have been contacted with APCs comprising a peptide containing the epitope GAVGVGKSA.~~
6. — ~~The method of any one of claims 1-5, wherein the APCs are from a subject with cancer.~~
7. — ~~The method of any one of claims 1-5, wherein the APCs are allogeneic APCs.~~
8. — The method of any one of claims 1-75, wherein the method comprises obtaining a biological sample comprising T cells and ~~or~~ APCs from a subject.
9. 6. The method of claim 8, and wherein the biological sample is peripheral blood mononuclear cell (PBMC) sample.
10. 7. — The method of claim 8 ~~or 9~~, wherein the method comprises depleting CD14+ cells from the biological sample.
11. 8. — The method ~~of any one of claims 1-10~~ claim 6 or 7, wherein the method comprises depleting CD25+ cells from the biological sample.

~~12.9.~~ The method of any one of claims 1-~~118~~, wherein the method comprises incubating the T cells and APCs in the presence of FMS-like tyrosine kinase 3 receptor ligand (FLT3L).

~~13.10.~~ The method of any one of claims 1-~~129~~, wherein the method comprises stimulating or expanding the T cells in the presence of the APCs for at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more days.

~~14.11.~~ The method of any one of claims 1-~~1310~~, wherein the method comprises expanding the T cells at least 5-fold, 10-fold, ~~20~~-fold, 50-fold, 100-fold, 500-fold or 1,000-fold or more in the presence of the APCs.

~~15.12.~~ The method of any one of claims 1-~~1411~~, wherein the antigen-specific T cells are prepared in less than 28 days.

~~16.13.~~ The method of any one of claims 1-~~1512~~, wherein the epitope:

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

~~17.14.~~ ~~A method~~Use of treating a composition in the manufacture of a medicament for treatment in a subject ~~with cancer, the composition~~ comprising ~~administering to the subject~~:

- (a) a peptide,
- (b) a polynucleotide encoding the peptide,
- (c) antigen presenting cells (APCs) comprising (a) or (b), or
- (d) T cells stimulated with APCs comprising (a) or (b);

wherein:

(i) the peptide comprises an epitope with a sequence GACGVGKSA and the subject expresses a protein encoded by an HLA-C03:04 allele; ~~or~~

~~(ii) the peptide comprises an epitope with a sequence GAVGVGKSA and the subject expresses a protein encoded by an HLA-C03:03 allele.~~

~~18.~~ ~~The method of claim 17,~~ wherein the epitope with the sequence GACGVGKSAGAVGVGKSA is capable of binding to the protein encoded by the HLA-C03:034 allele.

~~19.~~ ~~The method of claim 18, wherein the epitope is capable of being presented by the protein encoded by the HLA-C03:04 allele.~~

~~20.~~ ~~The method of claim 17, wherein the epitope with the sequence GAVGVGKSA is capable of binding to the protein encoded by the HLA-C03:03 allele.~~

- ~~21. The method of claim 20, wherein the epitope is capable of being presented by the protein encoded by the HLA-C03:03 allele.~~
- ~~22.15. The method of any one of claims 1-24~~The use of claim 14, wherein the cancer is selected from the group consisting of pancreatic ductal adenocarcinoma, non-small cell lung cancer, colorectal cancer and cholangiocarcinoma.
- ~~23. The method of any one of claims 1-22~~use of claim 14 or 15, wherein the peptide comprises one or more additional epitopes.
- ~~24.16. The method of claim 23, and~~ wherein the one or more additional epitopes comprise (i) an epitope with a sequence GAVGVGKSA and the subject expresses a protein encoded by an HLA-C03:03 allele or (ii) one or more epitopes of any one of Tables 2-12.
- ~~25. The method~~use of any one of claims ~~4-24~~14-16, wherein the ~~method~~composition further ~~comprises administering an additional anti-cancer therapy to the subject~~
- ~~26.17. The method of claim 25, wherein the additional anti-cancer therapy~~ comprises an additional peptide, a polynucleotide encoding the additional peptide, APCs comprising the additional peptide or the polynucleotide, or T cells stimulated with the APCs, wherein the additional peptide comprises one or more epitopes of any one of Tables 2-12.
- ~~27. The method of any one of claims 17-26, wherein the APCs are from the subject with cancer.~~
- ~~28. The method of any one of claims 17-26, wherein the APCs are allogeneic APCs.~~
- ~~29. The method of any one of claims 17-28, wherein the T cells are from the subject with cancer.~~
- ~~30. The method of any one of claims 17-28, wherein the T cells are allogeneic.~~
- ~~31. The method of any one of claims 17-30, wherein the T cells are stimulated with the APCs in vitro or ex vivo.~~
- ~~32. The method of claim 31, wherein the T cells have been stimulated with the APCs for at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 7, 18, 19 or 20 or more days.~~
- ~~33. The method of any one of claims 17-32, wherein the T cells are expanded in the presence of the APCs in vitro or ex vivo.~~
- ~~34. The method of claim 33, wherein the T cells have been expanded in the presence the APCs for at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 7, 18, 19 or 20 or more days.~~
- ~~35. The method of claim 34, wherein 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 the APCs.~~
- ~~36. The method of any one of claims 1-35~~13, wherein the method comprises assaying the T cells ~~are assayed~~ for expression of a T cell activation marker.

- ~~37.18.~~ The method of any one of claims 1-36, wherein the T cells are assayed or for cytokine production.
- ~~38.~~ The method of claim 36 or 37, wherein the T-cell activation marker or cytokine is selected from the group consisting of cell surface expression of CD107a and/or CD107b, IL2, IFN- γ , TNF α , TNF β and any combination thereof.
- ~~39.~~ The method of any one of claims 1-38, wherein the T cells are antigen-specific T cells.
- ~~40.19.~~ A pharmaceutical composition comprising:
- (a) T cells comprising a population of T cells expressing a T cell receptor (TCR) that binds to a complex of (i) an MHC protein encoded by an HLA-C03:04 allele and (ii) an epitope with a sequence GACGVGKSA; or
 - ~~(b) T cells comprising a population of T cells expressing a T cell receptor (TCR) that binds to a complex of (i) an MHC protein encoded by an HLA-C03:03 allele and (ii) an epitope with a sequence GAVGVGKSA;~~
 - ~~(c)(b)~~ antigen presenting cells (APCs) expressing an MHC protein encoded by an HLA-C03:04 allele, wherein the APCs comprise (i) a peptide having an epitope with a sequence GACGVGKSA or (ii) a polynucleotide encoding the peptide; ~~or~~
 - ~~(d) APCs expressing an MHC protein encoded by an HLA-C03:03 allele, wherein the APCs comprise (i) a peptide having an epitope with a sequence GAVGVGKSA or (ii) a polynucleotide encoding the peptide.~~
- ~~41.~~ The pharmaceutical composition of claim 40, wherein the APCs are from a subject with cancer.
- ~~42.~~ The pharmaceutical composition of claim 40, wherein the APCs are allogeneic APCs.
- ~~43.~~ The pharmaceutical composition of any one of claims 40-42, wherein the T cells are from a subject with cancer.
- ~~44.~~ The pharmaceutical composition of any one of claims 40-43, wherein the T cells are allogeneic.
- ~~45.~~ The pharmaceutical composition of any one of claims 40-44, wherein the population of T cells comprises CD8⁺ T cells.
- ~~46.~~ The pharmaceutical composition of claim 45, wherein at least 0.1% of the CD8⁺ T cells in the population of T cells are derived from naïve CD8⁺ T cells.
- ~~47.~~ The pharmaceutical composition of any one of claims 40-46, wherein the population of T cells comprises CD4⁺ T cells.
- ~~48.~~ The pharmaceutical composition of claim 47, wherein at least 0.1% of the CD4⁺ T cells in the population of T cells are derived from naïve CD4⁺ T cells.

~~49.20. A TCR α T cell receptor (TCR) comprising a TCR alpha chain and a TCR beta chain that binds to complex comprising a mutated RAS epitope with the sequence GACGVGKSA and an MHC encoded by C03:04 allele.~~

~~50. A TCR comprising a TCR alpha chain and a TCR beta chain that binds to a complex comprising a mutated RAS epitope with the sequence GAVGVGKSA and an MHC encoded by C03:03 allele.~~

21. A composition comprising:

(a) a peptide,

(b) a polynucleotide encoding the peptide,

(c) antigen presenting cells (APCs) comprising (a) or (b), or

(d) T cells stimulated with APCs comprising (a) or (b);

wherein:

(i) the peptide comprises an epitope with a sequence GACGVGKSA and the subject expresses a protein encoded by an HLA-C03:04 allele

wherein the epitope with the sequence GAVGVGKSA is capable of binding to the protein encoded by the HLA-C03:03 allele for use in therapy.