

molecules present different peptides. The inventors have demonstrated that an epitope that binds to a single HLA class I allele expressed in a subject is essential, but not sufficient to induce tumor specific T cell responses. Instead tumour specific T cell responses are optimally activated when an epitope is recognised and presented by the HLA molecules encoded by at least three HLA class I genes of an individual (PCT/EP2018/055231, PCT/EP2018/055232, PCT/EP2018/055230, EP 3370065 and EP 3369431).

Based on this discovery the inventors have developed a method for designing and preparing peptides to induce T cell responses in the highest proportion of subjects in a given target human population and have used this method to design a set of peptides for use in treating cancer.

In one aspect, the present disclosure provides a panel of two or more peptides, or one or more polynucleic acids or vectors that encode a panel of two or more peptides, wherein each of the peptides, or each of the encoded peptides, is up to 50 amino acids in length, wherein each peptide or encoded peptide comprises a different amino acid sequence selected from any of SEQ ID NOs: 1 to 2786 and/or 5432-5931, and wherein the amino acid sequence selected from SEQ ID NOs: 1 to 2786 and/or 5432-5931 of each peptide or encoded peptide is a fragment of a different cancer-associated antigen listed in Table 24.

In another aspect, the present disclosure provides a polynucleic acid or a vector that encodes a peptide as described herein that is up to 50 amino acids in length, and that comprises the amino acid sequence of any of SEQ ID NOs: 1 to 2786 and/or 5432-5931. In another aspect, the present

disclosure provides a pharmaceutical composition or kit, comprising a panel of two or more peptides, or one or more polynucleic acids or vectors that encode a panel of two or more peptides as claimed herein~~one or more peptides, or one or more polynucleic acids or vectors that encode one or more peptides, wherein the one or more peptide, or encoded peptide, is up to 50 amino acids in length and comprises the amino acid sequence of any of SEQ ID NOs: 1 to 2786 and/or 5432-5931~~, wherein the

composition or kit optionally comprises at least one pharmaceutically acceptable diluent, carrier, or preservative. In yet another aspect, the present disclosure provides a method of predicting that a specific human subject will have a CD8+ T cell response and/or a CD4+ T cell response to each peptide, or encoded peptide, of a pharmaceutical composition or kit according to claim 5, the method comprising (i) a. determining that each peptide, or encoded peptide comprises at least one

amino acid sequence that is a T cell epitope capable of binding to at least three HLA class I molecules of the subject; and b. predicting that the subject will have a CD8+ T cell response to each peptide or encoded peptide of the pharmaceutical composition or kit; and/or (ii) a. determining that each peptide, or encoded peptide comprises at least one amino acid sequence

CLAIMS

1. A panel of two or more peptides, or one or more polynucleic acids or vectors that encode a panel of two or more peptides, wherein each of the peptides, or each of the encoded peptides, is up to 50 amino acids in length, wherein each peptide or encoded peptide comprises a different amino acid sequence selected from any of SEQ ID NOs: 1 to 2786 and/or 5432-5931, and wherein the amino acid sequence selected from SEQ ID NOs: 1 to 2786 and/or 5432-5931 of each peptide or encoded peptide is a fragment of a different cancer-associated antigen listed in Table 24.

~~2. A polynucleic acids or a vector that encodes a peptide according to claim 1 that is up to 50 amino acids in length, and that comprises the amino acid sequence of any of SEQ ID NOs: 1 to 2786 and/or 5432-5931.~~

- ~~3.2.~~ The panel of peptides, polynucleic acid(s) or vector(s) according to claim 1, wherein each of the peptides or encoded peptides comprises at least one amino acid sequence selected from one of the following groups:
- (a) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a breast cancer-associated antigen listed in Table 24;
 - (b) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a lung cancer-associated antigen listed in Table 24;
 - (c) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a prostate cancer-associated antigen listed in Table 24;
 - (d) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a colorectal cancer-associated antigen listed in Table 24;
 - (e) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a bladder cancer-associated antigen listed in Table 24;

- (f) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a ovarian cancer-associated antigen listed in Table 24;
- (g) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a pancreatic cancer-associated antigen listed in Table 24;
- (h) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a brain cancer-associated antigen listed in Table 24;
- (i) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a leukemia-associated antigen listed in Table 24;
- (j) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a lymphoma-associated antigen listed in Table 24;
- (k) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a hepatocellular cancer-associated antigen listed in Table 24;
- (l) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a melanoma-associated antigen listed in Table 24;
- (m) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a thyroid cancer-associated antigen listed in Table 24;
- (n) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a pediatric cancer-associated antigen listed in Table 24;
- (o) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a gastric cancer-associated antigen listed in Table 24;
- (p) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a kidney cancer-associated antigen listed in Table 24

- (q) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a head and neck cancer-associated antigen listed in Table 24; and
- (r) the sequences listed in Table 25A and/or Table 25B and indicated in Table 25A or 25B to be a fragment of a cervical cancer-associated antigen listed in Table 24.

~~4.3.~~ A pharmaceutical composition or kit, comprising a panel of two or more peptides, or one or more polynucleic acids or vectors that encode a panel of two or more peptides, according to claim 1 or claim 2 ~~one or more peptides, or one or more polynucleic acids or vectors that encode one or more peptides, wherein the one or more peptide, or encoded peptide, is up to 50 amino acids in length and comprises the amino acid sequence of any of SEQ ID NOs: 1 to 2786 and/or 5432-5931,~~ wherein the composition or kit optionally comprises at least one pharmaceutically acceptable diluent, carrier, or preservative.

~~5.4.~~ The pharmaceutical composition or kit of claim 34, comprising at least two peptides, or one or more polynucleic acids or vectors that encode at least two peptides, wherein each peptide, or each encoded peptide, is up to 50 amino acids in length and comprises an amino acid sequence selected from SEQ ID NOs: 1 to 2786 and/or 5432-5931 ~~that~~ comprises a T cell epitope capable of binding to at least three HLA class I alleles and/or a T cell epitope capable of binding to at least three HLA class II alleles of an individual human subject.

~~6.5.~~ A method of predicting that a specific human subject will have a CD8+ T cell response and/or a CD4+ T cell response to each peptide, or encoded peptide, of a pharmaceutical composition or kit according to claim 45, the method comprising

- (i) a. determining that each peptide, or encoded peptide comprises at least one amino acid sequence that is a T cell epitope capable of binding to at least three HLA class I molecules of the subject; and

- b. predicting that the subject will have a CD8+ T cell response to each peptide or encoded peptide of the pharmaceutical composition or kit; and/or
- (ii)
 - a. determining that each peptide, or encoded peptide comprises at least one amino acid sequence that is a T cell epitope capable of binding to at least three HLA class II molecules of the subject; and
 - b. predicting that the subject will have a CD4+ T cell response to each peptide or encoded peptide of the pharmaceutical composition or kit.

~~7.6.~~ The pharmaceutical composition or kit according to claim [34](#) or claim [45](#) for use in therapy for vaccination, providing immunotherapy or inducing a cytotoxic T cell response in a subject, wherein the pharmaceutical composition or the peptides, polynucleic acids or vectors of the kit are to be administered to the subject.

~~8.7.~~ The pharmaceutical composition or kit [for use](#) according to claim [67](#), wherein the therapy is prophylactic.

~~9.8.~~ The pharmaceutical composition or kit [for use](#) according to claim [67](#) or claim [78](#), wherein the pharmaceutical composition or the peptides, polynucleic acids or vectors of the kit have been predicted to induce a cytotoxic T cell response and/or a helper T cell response in the subject using a method according to claim 6.

~~10.9.~~ The pharmaceutical composition or kit [for use](#) according to any one of claims [67](#) to [89](#) wherein the therapy is for treating cancer, optionally bladder cancer, brain cancer, breast cancer, colorectal cancer, gastric cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, melanoma, ovarian cancer, pancreatic cancer, pediatric cancer, thyroid cancer, prostate cancer, kidney cancer, head and neck cancer, esophageal cancer and cervical cancer.

~~11.10.~~ Use of the pharmaceutical composition of claim [34](#) or claim [45](#) in the manufacture of a medicament for treating cancer, optionally bladder cancer, brain

cancer, breast cancer, colorectal cancer, gastric cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, melanoma, ovarian cancer, pancreatic cancer, pediatric cancer, thyroid cancer, prostate cancer, kidney cancer, head and neck cancer, esophageal cancer and cervical cancer.

- ~~12~~.11. A method of preparing a pharmaceutical composition or kit for treating cancer in a specific human subject, the method comprising
- a. selecting two or more peptides, or one or more polynucleic acids or vectors that encode two or more peptides, wherein each peptide, or each encoded peptide, is up to 50 amino acids in length and comprises an amino acid sequence selected from SEQ ID NOs: 1 to 2786 and/or 5432-5931 that comprises a T cell epitope capable of binding to at least three HLA class I alleles and/or a T cell epitope capable of binding to at least three HLA class II alleles of the specific human subject; and
 - b. preparing a pharmaceutical composition or kit comprising the two or more peptides, or one or more polynucleic acids or vectors selected in step a.

- ~~13~~.12. A method of designing or preparing a peptide, or a polynucleic acid or vector that encodes a peptide, or a panel of peptides, or one or more polynucleic acid or vectors that encode a panel of peptides, for use in a method of inducing a T cell response against a target polypeptide, the method comprising
- (i) selecting or defining a model human population comprising a plurality of subjects each defined by HLA class I genotype and/or by HLA class II genotype;
 - (ii) identifying for each subject of the model population:
 - (a) amino acid sequences of the target polypeptide that are a T cell epitope capable of binding to at least three HLA class I molecules of the subject;
 - (b) amino acid sequences of the target polypeptide that are a T cell epitope capable of binding to at least three HLA class II molecules of the subject;

(c) amino acid sequences of the target polypeptide that comprise a T cell epitope capable of binding to at least three HLA class I molecules of the subject and a T cell epitope capable of binding to at least three HLA class II molecules of the subject; or

(d) amino acid sequences of the target polypeptide that both

a. are a T cell epitope capable of binding to at least three HLA class II molecules; and

b. comprise an amino acid sequence that is a T cell epitope capable of binding to at least three HLA class I molecules of the subject;

(iii) selecting a polypeptide fragment window length of between 9 and 50 amino acids;

(iv) identifying a fragment of the target polypeptide that

(a) has the length selected in step (iii); and

(b) comprises an amino acid sequence identified in any one of step (ii) (a) to (d) in the highest proportion of subjects in the model population;

(v) optionally testing the fragment identified in step (iv) against additional pre-defined criteria, rejecting the fragment if the further pre-defined criteria are not met, and repeating step (iv) to identify an alternative fragment of the target polypeptide that

(a) has the length selected in step (iii); and

(b) comprises an amino acid sequence identified in step (iv) in the next highest proportion of subjects in the model population;

(vi) optionally repeating step (iv) and further optionally step (v) in one or more further rounds, wherein a further fragment of the target polypeptide is identified in each round, and wherein in each round subjects are excluded from the model population if any of the fragments selected in step (iv) and not rejected in step (v) of any of the preceding rounds comprises an amino acid sequence identified in step (ii) for that subject; and

(vii) designing or preparing a peptide, a polynucleic acid or vector that encodes a peptide, a panel of peptides, or one or more polynucleic acids or vectors

that encode a panel of peptides, wherein each peptide comprises one or more of the target polypeptide fragments identified in step (iv), (v) or (vi), optionally wherein the polypeptide fragment is flanked at the N and/or C terminus by additional amino acids that are not part of the sequence of the target polypeptide antigen.

~~14.13.~~ 13. The method according to claim ~~12+3~~, wherein the target polypeptide is expressed by a pathogenic organism, a virus or a cancer cell, or is a cancer testes antigen, optionally wherein the target polypeptide is selected from the antigens listed in any of Tables 2 to 5.

~~15.14.~~ 14. The method according to claim ~~12+3~~ or claim ~~13+4~~, the method further comprising selecting one or more peptides, polynucleic acids or vectors designed or prepared according to the method of claim ~~12+3~~ or claim ~~13+4~~ for use in a method of vaccinating, providing immunotherapy to, or inducing a cytotoxic and/or helper T cell response in a subject, optionally wherein each of the one or more peptides, or encoded peptides, comprises an amino acid sequence that is

- (a) a fragment of a polypeptide that is expressed by a pathogenic organism, a virus or a cancer cell; and
- (b) a T cell epitope capable of binding to at least three HLA class I molecules of the subject or a T cell epitope capable of binding to at least three HLA class II molecules of the subject.

~~16.15.~~ 15. A panel of peptides, polynucleic acids or vectors designed and/or prepared according to the method of claim ~~12+3~~ or claim ~~13+4~~, or comprising or encoding two or more peptides designed and/or prepared according to the method of claim ~~12+3~~ or claim ~~13+4~~, or a panel of peptides, polynucleic acids or vectors selected according to the method of claim ~~14+5~~.

~~17~~.16. A pharmaceutical composition or kit comprising a panel of peptides, or one or more polynucleic acids or vectors encoding a panel of peptides, according to claim ~~15~~16, wherein the composition or kit optionally comprises at least one pharmaceutically acceptable diluent, carrier, or preservative.

~~18~~.17. The pharmaceutical composition or kit according to claim ~~16~~17 for use in therapy for vaccination, providing immunotherapy or inducing a cytotoxic T cell response in a subject, wherein the pharmaceutical composition or the panel of peptides, polynucleic acids or vectors of the kit are to be administered to the subject.

~~19~~.18. The pharmaceutical composition or kit according to claim ~~17~~18, wherein one or more or each of the peptides or the encoded peptides comprises an amino acid sequence that is

- (a) a fragment of a polypeptide that is expressed by a pathogenic organism, a virus or a cancer cell; and
- (b) a T cell epitope capable of binding to at least three HLA class I molecules of the subject or a T cell epitope capable of binding to at least three HLA class II molecules of the subject.