

CLAIMS

1. A pharmaceutically acceptable salt of a peptide comprising an amino acid sequence of SEQ ID NO: 22 or SEQ ID NO: 23, wherein the peptide has an overall length of up to 12 amino acids.
2. The pharmaceutically acceptable salt of claim 1, wherein said peptide has the ability to bind to a major histocompatibility complex (MHC) class-I or -II molecule, and wherein said peptide, when bound to said MHC molecule, is capable of being recognized by CD4 and/or CD8 T cells.
3. The pharmaceutically acceptable salt of claim 1 or 2, wherein the peptide consists of an amino acid sequence according to SEQ ID NO: 22 or SEQ ID NO: 23.
4. The pharmaceutically acceptable salt of any of claims 1 to 3, wherein the salt is a chloride, a bromide, a sulfate, a nitrate, a phosphate, an acetate, a trifluoroacetate, a propionate, a glycolate, a pyruvate, an oxalate, a malate, a malonate, a succinate, a maleate, fumarate, a tartrate, a citrate, a benzoate, a cinnamate, a mandelate, a methane sulfonate, an ethane sulfonate, a p-toluenesulfonate or a salicylate.
5. A peptide comprising an amino acid sequence of SEQ ID NO: 22 or SEQ ID NO: 23, or a pharmaceutically acceptable salt thereof, for use in therapy, wherein the peptide has an overall length of up to 12 amino acids.
6. The peptide or the salt thereof for use of claim 5, wherein said peptide has the ability to bind to a MHC class-I or -II molecule, and wherein said peptide, when bound to said MHC, is capable of being recognized by CD4 and/or CD8 T cells.
7. The peptide or the salt thereof for use of claims 5 or 6, wherein the peptide consists of an amino acid sequence according to SEQ ID NO: 22 or SEQ ID NO: 23.
8. A peptide as defined in any of claims 5 to 7, wherein said peptide includes non-peptide bonds.
9. A fusion protein, comprising the peptide defined in any of claims 5 to 8, in particular comprising N-terminal amino acids of the HLA-DR antigen-associated invariant chain (Ii).

10. A binding molecule that specifically recognizes the peptide defined in any of claims 5 to 8, or the peptide of defined in any of claims 5 to 8 when bound to an MHC molecule.
- 5 11. The binding molecule of claim 10, being
(i) an antibody, in particular a soluble or membrane-bound antibody, or
(ii) a T-cell receptor that is reactive with an HLA ligand, wherein the ligand is the peptide defined in any of claims 5 to 8, or the peptide defined in any of claims 5 to 8 when bound to an MHC molecule.
- 10 12. The binding molecule of claim 11, wherein the antibody is (i) a monoclonal immunoglobulin, a bi-specific immunoglobulin and/or a chimeric immunoglobulin, or a fragment thereof and/or (ii) wherein the T-cell receptor is soluble or membrane-bound.
13. The binding molecule of claim 11 or 12, wherein (i) the antibody is a humanized immunoglobulin molecule or a fragment thereof and/or (ii) wherein the antibody or the T-
15 cell receptor, being a soluble T-cell receptor, carries a further effector function such as an immune stimulating domain or toxin.
14. A nucleic acid, encoding a peptide defined in any of claims 5 to 7, or a binding molecule of any of claims 10 to 13, optionally linked to a heterologous promoter sequence, or an expression vector capable of expressing said nucleic acid.
- 20 15. A recombinant host cell comprising the peptide defined in any of claims 5 to 8, the binding molecule of any of claims 11 to 13, the nucleic acid or expression vector of claim 14, wherein said host cell preferably is an antigen presenting cell, such as a dendritic cell, or a T cell or an NK cell.
- 25 16. A method for producing the peptide defined in any of claims 5 to 7, or the binding molecule of any of claims 11 to 13, the method comprising culturing the recombinant host cell of claim 15 that presents the peptide defined in any of claims 5 to 7, or expresses the nucleic acid of claim 14 or bears the expression vector of claim 14, and isolating the peptide from the host cell or its culture medium.
- 30 17. An *in vitro* method for producing activated T lymphocytes, the method comprising contacting *in vitro* T cells with antigen loaded human class I or II MHC molecules expressed

on the surface of a suitable antigen-presenting cell or an artificial construct mimicking an antigen-presenting cell for a period of time sufficient to activate said T cells in an antigen specific manner, wherein said antigen is the peptide defined in any of claims 5 to 8.

- 5 **18.** An activated T lymphocyte, produced by the method of claim 17, that selectively recognizes a cell which presents a polypeptide comprising an amino acid sequence given in any one of claims 1, 3, 5 or 7.
- 10 **19.** A pharmaceutical composition comprising at least one active ingredient selected from the group consisting of the peptide of defined in any of claims 5 to 8, the binding molecule of any of claims 10 to 13, the nucleic acid or the expression vector of claim 14, the recombinant host cell of claim 15 or the activated T lymphocyte of claim 18, or a conjugated or labelled active ingredient, and a pharmaceutically acceptable carrier.
- 15 **20.** The pharmaceutical composition of claim 19, wherein (i) the pharmaceutical composition further comprises pharmaceutically acceptable excipients and/or stabilizers, and/or (ii) wherein the pharmaceutical composition is a vaccine.
- 20 **21.** The pharmaceutical composition according to claim 19 or 20, wherein (i) the pharmaceutical composition further comprises an adjuvant, and/or (ii) wherein the pharmaceutical composition is a vaccine or a cellular therapeutic agent.
- 25 **22.** The pharmaceutical composition according to claim 21, wherein the adjuvant is an interleukin, including IL-2, IL-15 or a combination thereof.
- 30 **23.** The peptide defined in any of claims 5 to 8, the binding molecule of any of claims 10 to 13, the nucleic acid or the expression vector of claim 14, the recombinant host cell of claim 15 the activated T lymphocyte of claim 18 or the pharmaceutical composition of any of claims 19 to 21 for use in diagnosis and/or treatment of cancer.
- 35 **24.** Use of a peptide defined in any of claims 5 to 8, the binding molecule of any of claims 10 to 13, the nucleic acid or expression vector of claim 14, the recombinant host cell of claim 15 or the activated T lymphocyte of claim 18 for the treatment of cancer or in the manufacture of a medicament against cancer.

25. The use of claim 24 or the peptide, binding molecule, nucleic acid, expression vector, recombinant host cell, activated T lymphocyte or pharmaceutical composition for use of claim 23, wherein the treatment is a cellular therapy.

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26. The use of claim 24 or 25 or the peptide, binding molecule, nucleic acid, expression vector, recombinant host cell, activated T lymphocyte or pharmaceutical composition or use of claim 23 or 25, wherein said cancer is selected from the group of lung cancer, brain cancer, hepatic cancer, kidney cancer, colorectal cancer, liver cancer, pancreatic cancer, prostate cancer, leukemia, breast cancer, Merkel cell carcinoma, melanoma, ovarian cancer, and esophageal cancer and other tumors that show an overpresentation of a peptide comprising an amino acid sequence of SEQ ID NO: 22 or SEQ ID NO: 23.

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27. A kit comprising:

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(a) a container comprising a pharmaceutical composition containing the peptide defined in any of claims 5 to 8, the binding molecule of any of claims 10 to 13, the nucleic acid or expression vector of claim 14, the recombinant host cell of claim 15 or the activated T lymphocyte of claim 18, in solution or in lyophilized form;

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(b) optionally, a second container containing a diluent or reconstituting solution for the lyophilized formulation;

(c) optionally, at least one further peptide having an overall length of up to 12 amino acids comprising a sequence selected from the group consisting of SEQ ID NOs: 1 to 265, and

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wherein the kit optionally further comprising one or more of (iii) a buffer, (iv) a diluent, (v) a filter, (vi) a needle, (vii) a syringe, or (viii) an adjuvant.

28. A method for producing a personalized anti-cancer vaccine, said method comprising:

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a) identifying tumor-associated peptides (TUMAPs) presented by a tumor sample from said individual patient;

b) comparing the peptides as identified in a) with a warehouse of peptides that have been pre-screened for immunogenicity and/or over-presentation in tumors as compared to normal tissues

c) selecting at least one peptide from the warehouse that matches a TUMAP identified in the patient; and

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d) formulating the personalized vaccine based on step c).

29. The method according to claim 28, wherein said TUMAPs are identified by:

- a1) comparing expression data from the tumor sample to expression data from a sample of normal tissue corresponding to the tissue type of the tumor sample to identify proteins that are over-expressed or aberrantly expressed in the tumor sample; and
- a2) correlating the expression data with sequences of MHC ligands bound to MHC class I and/or class II molecules in the tumor sample to identify MHC ligands derived from proteins over-expressed or aberrantly expressed by the tumor.

30. The method according to claim 28 or 29, wherein the sequences of MHC ligands are identified by eluting bound peptides from MHC molecules isolated from the tumor sample, and sequencing the eluted ligands.

31. The method according to any of claims 28 to 30, wherein the normal tissue corresponding to the tissue type of the tumor sample is obtained from the same patient.

32. The method according to any of claims 28 to 31, wherein the peptides included in the warehouse are identified based on the following steps:

- aa. Performing genome-wide messenger ribonucleic acid (mRNA) expression analysis by highly parallel methods, such as microarrays or sequencing-based expression profiling, comprising identify genes that over-expressed in a malignant tissue, compared with a normal tissue or tissues;
- ab. Selecting peptides encoded by selectively expressed or over-expressed genes as detected in step aa, and
- ac. Determining an induction of in vivo T-cell responses by the peptides as selected comprising in vitro immunogenicity assays using human T cells from healthy donors or said patient; or
- ba. Identifying HLA ligands from said tumor sample using mass spectrometry;
- bb. Performing genome-wide messenger ribonucleic acid (mRNA) expression analysis by highly parallel methods, such as microarrays or sequencing-based expression profiling, comprising identify genes that over-expressed in a malignant tissue, compared with a normal tissue or tissues;
- bc. Comparing the identified HLA ligands to said gene expression data;
- bd. Selecting peptides encoded by selectively expressed or over-expressed genes as detected in step bc;

be. Re-detecting of selected TUMAPs from step bd on tumor tissue and lack of or infrequent detection on healthy tissues and confirming the relevance of over-expression at the mRNA level; and

5 bf. Determining an induction of in vivo T-cell responses by the peptides as selected comprising in vitro immunogenicity assays using human T cells from healthy donors or said patient.

10 33. The method according to any of claims 28 to 32, wherein the immunogenicity of the peptides included in the warehouse is determined by a method comprising in vitro immunogenicity assays, patient immunomonitoring for individual HLA binding, MHC multimer staining, ELISPOT assays and/or intracellular cytokine staining.

15 34. The method according to any of claims 28 to 33, wherein said warehouse comprises a plurality of peptides selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 265.

20 35. The method according to any of claims 28 to 34, further comprising identifying at least one mutation that is unique to the tumor sample relative to normal corresponding tissue from the individual patient, and selecting a peptide that correlates with the mutation for inclusion in the vaccine or for the generation of cellular therapies.

36. The method according to claim 35, wherein said at least one mutation is identified by whole genome sequencing.