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(54) **Titre : PEPTIDES ET COMBINAISON DE PEPTIDES A UTILISER EN IMMUNOTHERAPIE CONTRE LE CANCER DU POUMON NON A PETITES CELLULES ET D'AUTRES CANCERS**

(54) **Title: PEPTIDES AND COMBINATION OF PEPTIDES FOR USE IN IMMUNOTHERAPY AGAINST NON-SMALL CELL LUNG CANCER AND OTHER CANCERS**

(57) **Abrégé/Abstract:**
The present invention relates to peptides, proteins, nucleic acids and cells for use in immunotherapeutic methods. In particular, the present invention relates to the immunotherapy of cancer. The present invention furthermore relates to tumor-associated T-cell peptide epitopes, alone or in combination with other tumor-associated peptides that can for example serve as active pharmaceutical ingredients of vaccine compositions that stimulate anti-tumor immune responses, or to stimulate T cells ex vivo and transfer into patients. Peptides bound to molecules of the major histocompatibility complex (MHC), or peptides as such, can also be targets of antibodies, soluble T-cell receptors, and other binding molecules.

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(57) **Abstract**: The present invention relates to peptides, proteins, nucleic acids and cells for use in immunotherapeutic methods. In particular, the present invention relates to the immunotherapy of cancer. The present invention furthermore relates to tumor-associated T-cell peptide epitopes, alone or in combination with other tumor-associated peptides that can for example serve as active pharmaceutical ingredients of vaccine compositions that stimulate anti-tumor immune responses, or to stimulate T cells ex vivo and transfer into patients. Peptides bound to molecules of the major histocompatibility complex (MHC), or peptides as such, can also be targets of antibodies, soluble T-cell receptors, and other binding molecules.

CLAIMS

1. A peptide comprising an amino acid sequence selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 24, and variant sequences thereof which are at least 88% homologous to SEQ ID No. 1 to SEQ ID No. 24, and wherein said variant binds to molecule(s) of the major histocompatibility complex (MHC) and/or induces T cells cross-reacting with said variant peptide; and a pharmaceutical acceptable salt thereof, wherein said peptide is not a full-length polypeptide.

2. The peptide according to claim 1, wherein said peptide has the ability to bind to an 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.

3. The peptide or variant thereof according to claim 1 or 2, wherein the amino acid sequence thereof comprises a continuous stretch of amino acids according to any one of SEQ ID No. 1 to SEQ ID No. 24.

4. The peptide or variant thereof according to any of claims 1 to 3, wherein said peptide or variant thereof has an overall length of from 8 to 100, preferably from 8 to 30, and more preferred from 8 to 16 amino acids, and most preferred wherein the peptide consists or consists essentially of an amino acid sequence according to any of SEQ ID No. 1 to SEQ ID No. 24.

5. The peptide or variant thereof according to any of Claims 1 to 4, wherein said peptide is modified and/or includes non-peptide bonds.

6. The peptide or variant thereof according to any of Claims 1 to 5, wherein said peptide is part of a fusion protein, in particular comprising N-terminal amino acids of the HLA-DR antigen-associated invariant chain (Ii).

7. A nucleic acid, encoding a peptide or variant thereof according to any one of claims 1 to 6, optionally linked to a heterologous promoter sequence.

8. An expression vector capable of expressing or expressing the nucleic acid according to claim 7.

9. A recombinant host cell comprising the peptide according to claim 1 to 6, the nucleic acid according to claim 7 or the expression vector according to claim 8, wherein said host cell preferably is an antigen presenting cell, such as a dendritic cell.

10. A method for producing the peptide or variant thereof according to any one of claims 1 to 6, the method comprising culturing the host cell according to claim 9 that presents the peptide according to claim 1 to 6, or expresses the nucleic acid according to claim 7 or comprises the expression vector according to claim 8, and isolating the peptide or variant thereof from the host cell or its culture medium.

11. 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 a peptide according to any one of claims 1 to 4.

12. An activated T lymphocyte, produced by the method according to claim 11, that selectively recognizes a cell which presents a polypeptide comprising an amino acid sequence given in any one of claims 1 to 4.

13. A method for killing target cells in a patient which target cells present a polypeptide comprising an amino acid sequence given in any one of claims 1 to 4, the method comprising administering to the patient an effective number of activated T cells as defined in claim 12.

14. An antibody, in particular a soluble or membrane-bound antibody, that specifically recognizes the peptide or variant thereof according to any of claims

1 to 5, preferably the peptide or variant thereof according to any of claims 1 to 5 when bound to an MHC molecule.

15. The peptide or variant thereof according to any one of claims 1 to 6, the nucleic acid according to claim 7, the expression vector according to claim 8, the host cell according to claim 9, the activated T lymphocyte according to claim 12 or the antibody according to claim 14 for use in medicine.

16. The peptide or variant thereof according to any one of claims 1 to 6, the nucleic acid according to claim 7, the expression vector according to claim 8, the host cell according to claim 9, the activated T lymphocyte according to claim 12 or the antibody according to claim 14 for use in diagnosis and/or treatment of cancer, or for use in the manufacture of a medicament against cancer.

17. The peptide or variant thereof according to any one of claims 1 to 6, the nucleic acid according to claim 7, the expression vector according to claim 8, the host cell according to claim 9, the activated T lymphocyte according to claim 12 or the antibody according to claim 14 for use according to claim 16, wherein said cancer is selected from the group of wherein said cancer is selected from the group of non-small cell lung cancer, small cell lung cancer, renal cell cancer, brain cancer, gastric cancer, colorectal cancer, hepatocellular cancer, pancreatic cancer, prostate cancer, leukemia, breast cancer, Merkel cell carcinoma, melanoma, ovarian cancer, urinary bladder cancer, uterine cancer, gallbladder and bile duct cancer and esophageal cancer, and other tumors that show an overexpression of a protein from which a peptide SEQ ID No. 1 to SEQ ID No. 24 is derived from.

18. A kit comprising:

(a) a container comprising a pharmaceutical composition containing the peptide(s) or the variant according to any one of claims 1 to 6, the nucleic acid(s) according to claim 7, the expression vector(s) according to claim 8, the cell(s) according to claim 9, the activated T lymphocyte(s) according to claim 12 or the antibody according to claim 14, in solution or in lyophilized form;

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

(c) optionally, at least one more peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 24, and

(d) optionally, instructions for (i) use of the solution or (ii) reconstitution and/or use of the lyophilized formulation.

19. The kit according to claim 18, further comprising one or more of (iii) a buffer, (iv) a diluent, (v) a filter, (vi) a needle, or (v) a syringe.

20. The kit according to claim 18 or 19, wherein said peptide is selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 24.

21. A T-cell receptor (TCR), preferably a soluble or membrane-bound TCR or functional fragment thereof that is reactive with an HLA ligand, wherein said ligand has at least 85% identity to an amino acid sequence selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 24, or wherein said amino acid sequence consists any of SEQ ID No. 1 to SEQ ID No. 24.

22. The T-cell receptor according to claim 21, wherein said T-cell receptor is provided as a soluble molecule and optionally carries a further effector function such as an antibody fragment, an immune stimulating domain and/or a toxin.

23. The T-cell receptor according to claim 21 or 22, wherein the TCR is an alpha/beta heterodimeric TCR comprising alpha and beta chain constant domain sequences in which the constant domain sequences are linked by a native disulfide bond, e.g. between Cys4 of exon 2 either of TRAC and Cys2 of exon 2 of either TRBC1 or TRBC2.

24. The T-cell receptor according to any one of claims 21 to 23, wherein the TCR is associated with a detectable label, a therapeutic agent, a PK modifying moiety or any combination thereof.

25. The T-cell receptor according to claim 24, wherein the therapeutic agent is an anti-CD3 antibody covalently linked to the C- or N-terminus of an alpha or beta chain of the TCR.

26. A nucleic acid, encoding for a T-cell receptor according to any one of claims 21 to 25, optionally linked to a heterologous promoter sequence, or an expression vector capable of expressing said nucleic acid.

27. A host cell comprising the nucleic acid according to claim 26 or the nucleic acid encoding an antibody according to claim 14 or the expression vector according to claim 26, wherein said host cell preferably is a T cell or NK cell.

28. A method for producing the T-cell receptor according to any one of claims 21 to 24, said method comprising culturing a host cell according to claim 27 and isolating said T cell receptor from said host cell and/or its culture medium.

29. A method for producing a personalized anti-cancer vaccine or a compound-based and/or cellular therapy for an individual patient, said method comprising:

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

d) producing and/or formulating the personalized vaccine or compound-based or cellular therapy based on step c).

30. The method according to claim 29, 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.

31. The method according to claim 29 or 30, 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.

32. The method according to any one of claims 29 to 31, wherein the normal tissue corresponding to the tissue type of the tumor sample is obtained from the same patient.

33. The method according to any one of claims 29 to 32, 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

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.

34. The method according to any one of claims 29 to 33, 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.

35. The method according to any of claims 29 to 34, wherein said warehouse comprises a plurality of peptides selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 24.

36. The method according to any of claims 29 to 35, 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.

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

38. A pharmaceutical composition comprising at least one active ingredient selected from the group consisting of

a) a peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 24;

b) a T-cell receptor reactive with a peptide and/or the peptide-MHC complex according to a);

c) a fusion protein comprising a peptide according to a), and the N-terminal amino acids 1 to 80 of the HLA-DR antigen-associated invariant chain (Ii);

d) a nucleic acid encoding for any of a) to c) or an expression vector comprising said nucleic acid,

e) a host cell comprising the expression vector of d,

f) an activated T-lymphocyte, obtained by a method comprising contacting in vitro T cells with a peptide according to a) expressed on the surface of a suitable antigen presenting cell for a period of time sufficient to activate said T cell in an antigen specific manner, as well as a method to transfer these activated T cells into the autologous or other patients;

g) an antibody, or soluble T-cell receptor, reactive to a peptide and/or the peptide – MHC complex according to a) and/or a cell presenting a peptide according to a), and potentially modified by fusion with for example immune-activating domains or toxins,

h) an aptamer recognizing a peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 24 and/or a complex of a peptide selected from the group consisting of SEQ ID No. 1 to SEQ ID No. 24 with an MHC molecule,

i) a conjugated or labelled peptide or scaffold according to any of a) to h) and a pharmaceutically acceptable carrier, and optionally, pharmaceutically acceptable excipients and/or stabilizers.

39. An aptamer that specifically recognizes the peptide or variant thereof according to any of claims 1 to 5, preferably the peptide or variant thereof according to any of claims 1 to 5 that is bound to an MHC molecule.

40. A method of treating a cancer, comprising administering to a subject in need thereof the pharmaceutical composition according to claim 38, wherein the cancer is non-small cell lung cancer, small cell lung cancer, renal cell cancer, brain cancer, gastric cancer, colorectal cancer, hepatocellular cancer, pancreatic cancer, prostate cancer, leukemia, breast cancer, Merkel cell carcinoma, melanoma, ovarian cancer, urinary bladder cancer, uterine cancer, gallbladder and bile duct cancer, esophageal cancer, or a combination thereof.

41. The method according to claim 41, wherein the cancer is non-small cell lung cancer.