

components to antigen presenting cells. Exosomes are produced by invagination and thus, contain besides endocytic membrane molecules mainly cytosolic components. Thus, it is believed that cytosolic components such as proteins are enriched in exosomes and can be transferred to antigen presenting cells. Exosomes can also productively transfer mRNA, which can be translated in the cells which take up the RNA. Thus, without wishing to be bound to a particular theory, it is believed that peptides or polypeptides which are included in exosomes, in particular cytosolic peptides or proteins, or peptides or polypeptides the coding RNA of which is included in exosomes according to the invention are particularly useful for immunotherapy because exosomes are taken up by antigen presenting cells and the peptides and proteins (optionally following translation of the coding RNA) are presented by the antigen presenting cells. The exosomes are thus transport vehicles for the peptides, proteins or RNA to antigen presenting cells and protect the peptides, proteins or RNA against degradation by proteases and ribonucleases. Alternatively, it is possible that peptides and proteins are taken up by antigen presenting cells as complexes with other molecules such as antibodies through a receptor dependent mechanism.

DESCRIPTION OF INVENTION

SUMMARY

In one aspect, the present disclosure refers to a method for predicting the usefulness of a cancer-associated neo-antigen or neo-epitope thereof comprising one or more cancer specific amino acid modifications for anti-cancer immunotherapy, the method comprising ascertaining the distribution or localization of the neo-antigen comprising ascertaining in a computational database whether the neo-antigen is located or abundant in the cytosol and/or within exosomes *in vivo*, wherein localization or abundance of the neo-antigen or a nucleic acid coding therefor, or a neo-epitope of the neo-antigen in the cytosol and/or within exosomes *in vivo* indicates that the neo-antigen or neo-epitope thereof is useful for anti-cancer immunotherapy, wherein processing and presentation of the neo-antigen in the MHC I pathway results in recognition of complexes formed by MHC I and neo-epitopes of the neo-antigen by CD8⁺ T cells.

In one example, the disclosure relates to a method for predicting the usefulness of a protein or a fragment thereof expressed by diseased cells for immunotherapy, the method comprising ascertaining the distribution or localization of the protein or a nucleic acid coding therefor, or of a fragment of the protein.

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In one example, the method comprises ascertaining whether the protein or a nucleic acid coding therefor, or a fragment of the protein is located or abundant in the cytosol and/or within exosomes *in vivo*.

10 In one example, localization or abundance of the protein or a nucleic acid coding therefor, or of a fragment of the protein in the cytosol and/or within exosomes indicates that the protein or a fragment thereof is useful for immunotherapy.

In a further example, the disclosure relates to a method for predicting the usefulness of a protein
15 or a fragment thereof expressed by diseased cells for immunotherapy, the method comprising ascertaining whether the protein or a fragment thereof is cross-presented by antigen presenting cells, preferably professional antigen presenting cells.

In one example, cross-presentation of the protein or a fragment thereof by antigen presenting cells
20 indicates that the protein or fragment thereof is useful for immunotherapy.

In one example, ascertaining whether the protein or a fragment thereof is cross-presented by antigen presenting cells comprises ascertaining whether the protein or a nucleic acid coding therefor, or a fragment of the protein is located or abundant in the cytosol and/or within exosomes
25 *in vivo*.

In one example, localization or abundance of the protein or a nucleic acid coding therefor, or of a fragment of the protein in the cytosol and/or within exosomes indicates that the protein or a fragment thereof is cross-presented by antigen presenting cells.

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In one example, ascertaining whether the protein or a fragment thereof is cross-presented by antigen presenting cells comprises ascertaining an existing antibody response to the protein or a fragment thereof.

- 5 In one example, an existing antibody response to the protein or a fragment thereof indicates that the protein or a fragment thereof is cross-presented by antigen presenting cells.

In one example, ascertaining whether the protein or a fragment thereof is cross-presented by antigen presenting cells comprises ascertaining whether the protein or a fragment thereof binds to
10 F actin.

In one example, binding of the protein or a fragment thereof to F actin indicates that the protein or a fragment thereof is cross-presented by antigen presenting cells.

- 15 In one example, ascertaining whether the protein or a fragment thereof is cross-presented by antigen presenting cells comprises ascertaining whether the protein or a fragment thereof binds to RNA.

In one example, binding of the protein or a fragment thereof to RNA indicates that the protein or
20 a fragment thereof is cross-presented by antigen presenting cells.

In one example of the disclosure, the protein fragment is present within exosomes as MHC peptide complex, preferably on the surface of exosomes.

- 25 In one example of the disclosure, localization or abundance of the protein or a nucleic acid coding therefor, or of a fragment of the protein in the cytosol indicates processing and presentation of the protein in the MHC I pathway, preferably of diseased cells. In one example, processing and presentation of the protein in the MHC I pathway results in recognition of complexes formed by MHC I and fragments of the protein by CD8⁺ T cells.

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In one example of the disclosure, localization or abundance of the protein or a nucleic acid coding therefor, or of a fragment of the protein within exosomes indicates accumulation of the protein or a nucleic acid coding therefor, or of a fragment of the protein in antigen presenting cells, preferably professional antigen presenting cells. In one example, accumulation of the protein or a nucleic acid coding therefor, or of a fragment of the protein in antigen presenting cells indicates processing and presentation of the protein in the MHC I and/or MHC II pathway, preferably of the antigen presenting cells. In one example, processing and presentation of the protein in the MHC I pathway results in recognition of complexes formed by MHC I and fragments of the protein by CD8+ T cells.

In a further example, the disclosure relates to a method for predicting the usefulness of a protein or a fragment thereof expressed by diseased cells for immunotherapy, the method comprising ascertaining one or more of the following:

- (a) ascertaining an existing antibody response to the protein or a fragment thereof,
- (b) ascertaining whether the protein or a fragment thereof binds to F actin, and/or
- (c) ascertaining whether the protein or a fragment thereof binds to RNA.

In one example, an existing antibody response to the protein or a fragment thereof indicates that the protein or a fragment thereof is useful for immunotherapy.

In one example, binding of the protein or a fragment thereof to F actin indicates that the protein or a fragment thereof is useful for immunotherapy.

In one example, binding of the protein or a fragment thereof to RNA indicates that the protein or a fragment thereof is useful for immunotherapy.

In one example of the disclosure, the protein or a fragment thereof comprises a disease specific amino acid modification. In one example, the amino acid modification is due to a disease specific somatic mutation.

In one example of the disclosure, the disease is cancer and the immunotherapy is anti-cancer immunotherapy.

5 In one example of the disclosure, the protein fragment is a MHC binding peptide or a potential MHC binding peptide.

In a further example, the disclosure relates to a method for selecting and/or ranking disease specific amino acid modifications for their usefulness in immunotherapy, the method comprising the steps of:

- 10 (i) identifying proteins expressed by diseased cells each protein comprising at least one disease specific amino acid modification, and
(ii) ascertaining the distribution or localization of a protein identified under (i) or a nucleic acid coding therefor, or of a fragment of the protein, and
(iii) repeating step (ii) for at least one further protein identified under (i).

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In one example, step (ii) comprises ascertaining whether the protein or a nucleic acid coding therefor, or a fragment of the protein is located or abundant in the cytosol and/or within exosomes *in vivo*.

20 In one example, localization or abundance of the protein or a nucleic acid coding therefor, or of a fragment of the protein in the cytosol and/or within exosomes indicates that the disease specific amino acid modification is useful for immunotherapy.

25 In a further example, the disclosure relates to a method for selecting and/or ranking disease specific amino acid modifications for their usefulness in immunotherapy, the method comprising the steps of:

- (i) identifying proteins expressed by diseased cells each protein comprising at least one disease specific amino acid modification, and
(ii) ascertaining whether a protein identified under (i) or a fragment of the protein is cross-
30 presented by antigen presenting cells, preferably professional antigen presenting cells, and
(iii) repeating step (ii) for at least one further protein identified under (i).

In one example, cross-presentation of the protein or a fragment thereof by antigen presenting cells indicates that the disease specific amino acid modification is useful for immunotherapy.

- 5 In a further example, the disclosure relates to a method for selecting and/or ranking disease specific amino acid modifications for their usefulness in immunotherapy, the method comprising the steps of:
- (i) identifying proteins expressed by diseased cells each protein comprising at least one disease specific amino acid modification, and
 - 10 (ii) ascertaining for a protein identified under (i) or a fragment of the protein one or more of the following:
 - (a) ascertaining an existing antibody response to the protein or a fragment thereof,
 - (b) ascertaining whether the protein or a fragment thereof binds to F actin, and/or
 - (c) ascertaining whether the protein or a fragment thereof binds to RNA, and
 - 15 (iii) repeating step (ii) for at least one further protein identified under (i).

In one example, an existing antibody response to the protein or a fragment thereof indicates that the disease specific amino acid modification is useful for immunotherapy.

- 20 In one example, binding of the protein or a fragment thereof to F actin indicates that the disease specific amino acid modification is useful for immunotherapy.

In one example, binding of the protein or a fragment thereof to RNA indicates that the disease specific amino acid modification is useful for immunotherapy.

- 25 In one example of the disclosure, the disease specific amino acid modification is comprised by a protein fragment which is a MHC binding peptide or a potential MHC binding peptide.

- 30 In one example of the disclosure, the method is used in the manufacture of a vaccine. In one example, the vaccine is derived from one or more proteins or fragments thereof or one or more disease specific amino acid modifications which are predicted as being useful for immunotherapy.

In a further example, the disclosure relates to a method for providing a vaccine comprising the step:

identifying one or more proteins or fragments thereof or one or more disease specific amino acid modifications which are predicted as being useful for immunotherapy by the method of any of the examples described herein. In one example, the method further comprises the step: providing a vaccine comprising a peptide or polypeptide comprising one or more proteins or fragments thereof or one or more disease specific amino acid modifications which are predicted as being useful for immunotherapy, or a nucleic acid encoding the peptide or polypeptide.

In a further example, the disclosure relates to a vaccine produced according to the method of any of the examples described herein.

In one example of the disclosure, indication of a usefulness of a protein or a fragment thereof expressed by diseased cells for immunotherapy indicates that the protein or a fragment thereof upon administration (optionally in the format of the coding nucleic acid) will be immunogenic.

In one example of the disclosure, a protein fragment described herein is an MHC binding peptide or a potential MHC binding peptide (e.g. MHC binding prediction indicates that the protein fragment will bind to MHC). In one example, the MHC binding peptide is a modified peptide which is a fragment of a modified protein.

In one example, amino acid modifications in proteins or peptides are identified by identifying non-synonymous mutations in one or more protein-coding regions.

In one example, amino acid modifications are identified by partially or completely sequencing the genome or transcriptome of one or more cells such as one or more cancer cells and optionally one or more non-cancerous cells and identifying mutations in one or more protein-coding regions. In one example, said mutations are somatic mutations. In one example, said mutations are cancer mutations.

In one example, in particular in order to provide a personalized vaccine for a patient such as a cancer patient, the modification(s) are present in said patient and the methods of the disclosure are performed for said patient. In a further example, the present disclosure provides a vaccine which is obtainable using the methods according to the disclosure. Preferred examples of such vaccines are described herein.

A vaccine provided according to the disclosure may comprise a pharmaceutically acceptable carrier and may optionally comprise one or more adjuvants, stabilizers etc. The vaccine may in the form of a therapeutic or prophylactic vaccine.

Another example relates to a method for inducing an immune response in a patient, comprising administering to the patient a vaccine provided according to the disclosure.

Another example relates to a method of treating a patient comprising the steps:

- (a) providing an immunotherapeutic agent described herein such as a vaccine using the methods according to the disclosure; and
- (b) administering said immunotherapeutic agent to the patient.

Another example relates to a method of treating a patient comprising administering an immunotherapeutic agent described herein such as a vaccine to the patient.

In one example, the patient is a cancer patient and the vaccine is an anti-cancer vaccine such as a vaccine the administration of which provides cancer specific neo-epitopes.

In further examples, the disclosure provides the vaccines described herein for use in the methods of treatment described herein, in particular for use in treating or preventing cancer.

The treatments of cancer described herein can be combined with surgical resection and/or radiation and/or traditional chemotherapy.

Other features and advantages of the instant disclosure will be apparent from the following detailed description and claims.

DETAILED DESCRIPTION OF THE INVENTION

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Although the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodologies, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which

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will be limited only by the appended claims. Unless defined otherwise, all

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CLAIMS

1. A method for predicting the usefulness of a cancer-associated neo-antigen or neo-epitope thereof comprising one or more cancer specific amino acid modifications for anti-cancer immunotherapy, the method comprising
- 5 ascertaining the distribution or localization of the neo-antigen comprising ascertaining in a computational database whether the neo-antigen is located or abundant in the cytosol and/or within exosomes *in vivo*,
- 10 wherein localization or abundance of the neo-antigen or a nucleic acid coding therefor, or a neo-epitope of the neo-antigen in the cytosol and/or within exosomes *in vivo* indicates that the neo-antigen or neo-epitope thereof is useful for anti-cancer immunotherapy,
- 15 wherein processing and presentation of the neo-antigen in the MHC I pathway results in recognition of complexes formed by MHC I and neo-epitopes of the neo-antigen by CD8⁺ T cells.
2. The method of claim 1, wherein the one or more amino acid modifications are due to cancer specific somatic mutations.