harboring HLA class I as well as class II neo-epitopes provide mechanistically favorable conditions for CTL priming, as $CD4^+$ T cells recognize their ligand on the DC that cross-presents the $CD8^+$ T cell neo-epitope and provide cognate T cell help by CD40L mediated DC activation (Schoenberger, S. P., Toes, R. E., van der Voort, E. I., Offringa, R. & Melief, C. J. Nature 393, 480–3 (1998)). On a related note, we also found that one and the same mutation may give rise to neo-epitopes presented on different HLA class I restriction elements and recognized by independent $CD8^+$ T cells (Fig. 3b). Likewise, we found that one and the same epitope/restriction element complex is recognized by neo-epitope specific T cells with different TCR clonotypes. These findings illustrate that an unexpectedly broad repertoire of mutation specific T cells may be recruited by neo-epitope vaccination and each single mutation leverages a diversity of T-cell specificities.

In summary, the findings presented herein demonstrate that the definition of suitable personalized neo-epitope vaccines, in particular personalized RNA neo-epitope vaccines may unfold a broad neo-antigen specific T-cell repertoire in cancer patients enabling an effective targeting of their mutanome.

DESCRIPTION OF INVENTION

SUMMARY

An example of the disclosure relates to a method for assessing the usefulness of a disease specific amino acid modification within a peptide or polypeptide expressed in a diseased cell for immunotherapy, the method comprising ascertaining whether the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification are presented in the context of MHC molecules of different classes and/or when presented in the context of MHC molecules, preferably MHC molecules of different classes, are reactive with T cells restricted to different MHC classes.

In one example, the MHC molecules of different classes are MHC class I molecules and MHC class II molecules and/or the T cells restricted to different MHC classes are CD4+ and CD8+ T

cells. In one example, presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the disease specific amino acid modification is useful for immunotherapy.

Another example of the disclosure relates to a method for assessing the usefulness of a disease specific amino acid modification within a peptide or polypeptide expressed in a diseased cell for immunotherapy, the method comprising ascertaining whether a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule is reactive with T cells having different T cell receptors.

In one example, the different T cell receptors are of different clonotypes. In one example, reactivity of a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule with T cells having different T cell receptors indicates that the disease specific amino acid modification is useful for immunotherapy.

Another example of the disclosure relates to a method for assessing the usefulness of a disease specific amino acid modification within a peptide or polypeptide expressed in a diseased cell for immunotherapy, the method comprising ascertaining whether the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification are presented in the context of different MHC molecules of the same class and/or when presented in the context of different MHC molecules of the same class are reactive with different T cells restricted to the same MHC class.

In one example, the different MHC molecules of the same class are different MHC class I molecules and/or the different T cells restricted to the same MHC class are different CD8+ T cells. In one example, presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of different

MHC molecules of the same class and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of different MHC molecules of the same class with different T cells restricted to the same MHC class indicates that the disease specific amino acid modification is useful for immunotherapy.

One aspect of the disclosure relates to a method for assessing the usefulness of a disease specific amino acid modification within a peptide or polypeptide expressed in a diseased cell for immunotherapy, the method comprising ascertaining one or more of the following:

(i) ascertaining whether the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification are presented in the context of MHC molecules of different classes and/or when presented in the context of MHC molecules, preferably MHC molecules of different classes, are reactive with T cells restricted to different MHC classes, wherein presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the disease specific amino acid modification is useful for immunotherapy,

(ii) ascertaining whether a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule is reactive with T cells having different T cell receptors, wherein reactivity of a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule with T cells having different T cell receptors indicates that the disease specific amino acid modification is useful for immunotherapy, and/or

(iii) ascertaining whether the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification are presented in the context of different MHC molecules of the same class and/or when presented in the context of different MHC molecules of the same class are reactive with different T cells restricted to the same MHC class, wherein, preferably, (a) the MHC molecules of different classes are MHC class I molecules and MHC class II molecules and/or the T cells restricted to different MHC classes are CD4+ and

CD8+ T cells, and/or (b) the different MHC molecules of the same class are different MHC class I molecules and/or the different T cells restricted to the same MHC class are different CD8+ T cells, wherein presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of different MHC molecules of the same class with different T cells restricted to the same MHC class indicates that the disease specific amino acid modification is useful for immunotherapy.

In one example, the MHC molecules of different classes are MHC class I molecules and MHC class II molecules and/or the T cells restricted to different MHC classes are CD4+ and CD8+ T cells. In one example, presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the disease specific amino acid modification is useful for immunotherapy. In one example, the different T cell receptors are of different clonotypes. In one example, reactivity of a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule with T cells having different T cell receptors indicates that the disease specific amino acid modification is useful for immunotherapy. In one example, the different MHC molecules of the same class are different MHC class I molecules and/or the different T cells restricted to the same MHC class are different CD8+ T cells. In one example, presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of different MHC molecules of the same class with different T cells restricted to the same MHC class indicates that the disease specific amino acid modification is useful for immunotherapy.

Another example of 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 peptides and/or polypeptides expressed in diseased cells each peptide and/or polypeptide comprising at least one disease specific amino acid modification, and

(ii) ascertaining whether the same or different fragments of a peptide or polypeptide comprising the same disease specific amino acid modification are presented in the context of MHC molecules of different classes and/or when presented in the context of MHC molecules, preferably MHC molecules of different classes, are reactive with T cells restricted to different MHC classes, and

(iii) repeating step (ii) for at least one further amino acid modification identified under (i).

In one example, the MHC molecules of different classes are MHC class I molecules and MHC class II molecules and/or the T cells restricted to different MHC classes are CD4+ and CD8+ T cells. In one example, presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the disease specific amino acid modification is useful for immunotherapy.

Another example of 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 peptides and/or polypeptides expressed in diseased cells each peptide and/or polypeptide comprising at least one disease specific amino acid modification, and

(ii) ascertaining whether a fragment of a peptide or polypeptide comprising a disease specific amino acid modification when presented in the context of the same MHC molecule is reactive with T cells having different T cell receptors, and

(iii) repeating step (ii) for at least one further amino acid modification identified under (i).

In one example, the different T cell receptors are of different clonotypes. In one example, reactivity of a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule with T cells having different T cell receptors indicates that the disease specific amino acid modification is useful for immunotherapy.

Another example of 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 peptides and/or polypeptides expressed in diseased cells each peptide and/or polypeptide comprising at least one disease specific amino acid modification, and
(ii) ascertaining whether the same or different fragments of a peptide or polypeptide comprising the same disease specific amino acid modification are presented in the context of different MHC molecules of the same class and/or when presented in the context of different MHC molecules of the same class are reactive with different T cells restricted to the same MHC class, and
(iii) repeating step (ii) for at least one further amino acid modification identified under (i).

In one example, the different MHC molecules of the same class are different MHC class I molecules and/or the different T cells restricted to the same MHC class are different CD8+ T cells. In one example, presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of different MHC molecules of the same class with different T cells restricted to the same MHC class indicates that the disease specific amino acid modification is useful for immunotherapy.

Another aspect of 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 peptides and/or polypeptides expressed in diseased cells each peptide and/or polypeptide comprising at least one disease specific amino acid modification, and

(ii) ascertaining one or more of the following:

(1) ascertaining whether the same or different fragments of a peptide or polypeptide comprising the same disease specific amino acid modification are presented in the context of MHC molecules of different classes and/or when presented in the context of MHC molecules, preferably MHC molecules of different classes, are reactive with T cells restricted to different MHC classes, wherein presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the disease specific amino acid modification is useful for immunotherapy,

(2) ascertaining whether a fragment of a peptide or polypeptide comprising a disease specific amino acid modification when presented in the context of the same MHC molecule is reactive with T cells having different T cell receptors, wherein reactivity of a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule with T cells having different T cell receptors indicates that the disease specific amino acid modification is useful for immunotherapy, and/or

(3) ascertaining whether the same or different fragments of a peptide or polypeptide comprising the same disease specific amino acid modification are presented in the context of different MHC molecules of the same class and/or when presented in the context of different MHC molecules of the same class are reactive with different T cells restricted to the same MHC class, wherein presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of different MHC molecules of the same class with different T cells restricted to the same MHC class indicates that the disease specific amino acid modification is useful for immunotherapy, and

(iii) repeating step (ii) for at least one further amino acid modification identified under (i), wherein, preferably, (a) the MHC molecules of different classes are MHC class I molecules and MHC class II molecules and/or the T cells restricted to different MHC classes are CD4+ and CD8+ T cells, and/or (b) the different MHC molecules of the same class are different MHC class I molecules and/or the different T cells restricted to the same MHC class are different CD8+ T cells.

In one example, the MHC molecules of different classes are MHC class I molecules and MHC class II molecules and/or the T cells restricted to different MHC classes are CD4+ and CD8+ T cells. In one example, presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the disease specific amino acid modification is useful for immunotherapy. In one example, the different T cell receptors are of different clonotypes. In one example, reactivity of a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule with T cells having different T cell receptors indicates that the disease specific amino acid modification is useful for immunotherapy. In one example, the different MHC molecules of the same class are different MHC class I molecules and/or the different T cells restricted to the same MHC class are different CD8+ T cells. In one example, presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of different MHC molecules of the same class with different T cells restricted to the same MHC class indicates that the disease specific amino acid modification is useful for immunotherapy.

In one example, the different amino acid modifications tested in step (ii) are present in the same and/or in different peptides or polypeptides. In one example, the method of the disclosure

comprises comparing the scores obtained for the different amino acid modifications tested in step (ii).

In one example of all examples of the disclosure, the disease specific amino acid modification(s) is (are) due to (a) disease specific somatic mutation(s). In one example of all examples of the disclosure, the disease is cancer and the immunotherapy is anti-cancer immunotherapy. In one example of all examples of the disclosure, the immunotherapy comprises administration of one or more of the following:

(i) a peptide or polypeptide expressed in diseased cells, the peptide or polypeptide comprising at least one disease specific amino acid modification,

(ii) a peptide or polypeptide comprising a fragment of the peptide or polypeptide under (i), the fragment comprising at least one disease specific amino acid modification, and

(iii) a nucleic acid encoding the peptide or polypeptide under (i) or (ii). In one example of all examples of the disclosure, the method of the disclosure is useful in providing a vaccine.

Another aspect of the disclosure relates to a method for providing a vaccine comprising the steps:

(i) identifying one or more disease specific amino acid modifications which are predicted to be useful for immunotherapy by any of the methods of the disclosure,

(ii) providing a vaccine comprising one or more of the following:

(1) a peptide or polypeptide expressed in diseased cells, the peptide or polypeptide comprising at least one of the disease specific amino acid modifications which are predicted to be useful for immunotherapy,

(2) a peptide or polypeptide comprising a fragment of the peptide or polypeptide under (i), the fragment comprising at least one of the disease specific amino acid modifications which are predicted to be useful for immunotherapy, and

(3) a nucleic acid encoding the peptide or polypeptide under (i) or (ii).

In one example of all examples of the disclosure, the fragment is a MHC binding peptide or a potential MHC binding peptide or can be processed to provide a MHC binding peptide or a

potential MHC binding peptide (e.g. MHC binding prediction indicates that the fragment will bind to MHC).

Another example of the disclosure relates to a vaccine produced according to the method of the disclosure. 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.

In one example of all examples of the disclosure, indication of a usefulness of a disease specific amino acid modification for immunotherapy indicates that the peptide or polypeptide expressed in a diseased cell comprising the disease specific amino acid modification or a peptide or polypeptide comprising a fragment thereof such as an epitope or vaccine sequence comprising the disease specific amino acid modification upon administration (optionally in the format of the coding nucleic acid) will induce an immune response.

In one example of all examples of the disclosure, amino acid modifications in peptides or polypeptides are identified by identifying non-synonymous mutations in one or more 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 coding regions. In one example, said mutations are somatic mutations. In one example, said mutations are cancer mutations.

In one example of all examples of the disclosure, 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.

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

(a) providing a vaccine using the methods according to the disclosure; and

(b) administering the vaccine to the patient.

Another example of the disclosure relates to a method of treating a patient comprising administering a vaccine as described herein to the patient.

CLAIMS

1. A method for assessing the usefulness of a disease specific amino acid modification within a peptide or polypeptide expressed in a diseased cell for immunotherapy, the method comprising ascertaining one or more of the following:

(i) ascertaining whether the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification are presented in the context of MHC molecules of different classes and/or when presented in the context of MHC molecules are reactive with T cells restricted to different MHC classes, wherein presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the disease specific amino acid modification is useful for immunotherapy,

(ii) ascertaining whether a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule is reactive with T cells having different T cell receptors, wherein reactivity of a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule with T cells having different T cell receptors indicates that the disease specific amino acid modification is useful for immunotherapy,

and/or

(iii) ascertaining whether the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification are presented in the context of different MHC molecules of the same class and/or when presented in the context of different MHC molecules of the same class are reactive with different T cells restricted to the same MHC class, wherein, preferably, (a) the MHC molecules of different classes are MHC class I molecules and MHC class II molecules and/or the T cells restricted to different MHC classes are CD4+ and CD8+ T cells, and/or (b) the different MHC molecules of the same class are different MHC class I molecules and/or the different T cells restricted to the same MHC class are different CD8+ T cells, wherein presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented

in the context of different MHC molecules of the same class with different T cells restricted to the same MHC class indicates that the disease specific amino acid modification is useful for immunotherapy.

2. 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 peptides and/or polypeptides expressed in diseased cells each peptide and/or polypeptide comprising at least one disease specific amino acid modification, and

(ii) ascertaining one or more of the following:

(1) ascertaining whether the same or different fragments of a peptide or polypeptide comprising the same disease specific amino acid modification are presented in the context of MHC molecules of different classes and/or when presented in the context of MHC molecules are reactive with T cells restricted to different MHC classes, wherein presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the disease specific amino acid modification is useful for immunotherapy,

(2) ascertaining whether a fragment of a peptide or polypeptide comprising a disease specific amino acid modification when presented in the context of the same MHC molecule is reactive with T cells having different T cell receptors, wherein reactivity of a fragment of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the context of the same MHC molecule with T cells having different T cell receptors indicates that the disease specific amino acid modification is useful for immunotherapy,

and/or

(3) ascertaining whether the same or different fragments of a peptide or polypeptide comprising the same disease specific amino acid modification are presented in the context of different MHC molecules of the same class and/or when presented in the context of different MHC molecules of the same class are reactive with different T cells restricted to the same MHC class, wherein presentation of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different fragments of the peptide or polypeptide comprising the disease specific amino acid modification when presented in the

context of different MHC molecules of the same class with different T cells restricted to the same MHC class indicates that the disease specific amino acid modification is useful for immunotherapy,

and

(iii) repeating step (ii) for at least one further amino acid modification identified under (i),

wherein, preferably, (a) the MHC molecules of different classes are MHC class I molecules and MHC class II molecules and/or the T cells restricted to different MHC classes are CD4+ and CD8+ T cells, and/or (b) the different MHC molecules of the same class are different MHC class I molecules and/or the different T cells restricted to the same MHC class are different CD8+ T cells.

3. The method of claim 1 or 2, wherein the different T cell receptors are of different clonotypes.

4. The method of any one of claims 2 or 3, wherein the different amino acid modifications tested in step (ii) are present in the same and/or in different peptides or polypeptides.

5. The method of any one of claims 2 to 4, which comprises comparing the scores obtained for the different amino acid modifications tested in step (ii).

6. The method of any one of claims 1 to 5, wherein the disease specific amino acid modification(s) is (are) due to (a) disease specific somatic mutation(s).

7. The method of any one of claims 1 to 6, wherein the disease is cancer and the immunotherapy is anti-cancer immunotherapy.

8. The method of any one of claims 1 to 7, wherein the immunotherapy comprises administration of one or more of the following:

(i) a peptide or polypeptide expressed in diseased cells, the peptide or polypeptide comprising at least one disease specific amino acid modification,

(ii) a peptide or polypeptide comprising a fragment of the peptide or polypeptide under (i), the fragment comprising at least one disease specific amino acid modification, and

(iii) a nucleic acid encoding the peptide or polypeptide under (i) or (ii).

9. A method for providing a vaccine comprising the steps:

(i) identifying one or more disease specific amino acid modifications which are predicted to be useful for immunotherapy by the method of any one of claims 1 to 8,

(ii) providing a vaccine comprising one or more of the following:

(1) a peptide or polypeptide expressed in diseased cells, the peptide or polypeptide comprising at least one of the disease specific amino acid modifications which are predicted to be useful for immunotherapy,

(2) a peptide or polypeptide comprising a fragment of the peptide or polypeptide under (i), the fragment comprising at least one of the disease specific amino acid modifications which are predicted to be useful for immunotherapy, and

(3) a nucleic acid encoding the peptide or polypeptide under (i) or (ii).

10. The method of any one of claims 1 to 9, wherein the fragment is a MHC binding peptide or a potential MHC binding peptide or can be processed to provide a MHC binding peptide or a potential MHC binding peptide.