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 cancer-specific amino acid modification within a neo-antigen expressed in a cancer cell for anti-cancer immunotherapy, the method comprising ascertaining one or more of the following:

(i) ascertaining whether the same or different neo-epitopes of the neo-antigen comprising the cancer-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 neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the cancer-specific amino acid modification is useful for anti-cancer immunotherapy,

(ii) ascertaining whether a neo-epitope of the neo-antigen comprising the cancer-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 neo-epitope of the neo-antigen comprising the cancer-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 cancer-specific amino acid modification is useful for anti-cancer immunotherapy, and/or

(iii) ascertaining whether the same or different neo-epitopes of the neo-antigen comprising the cancer-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 neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different neo-epitopes of the neo-antigen comprising the cancer-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 cancer-specific amino acid modification is useful for anti-cancer 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.

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 cancer-specific amino acid modifications for their usefulness in anti-cancer immunotherapy, the method comprising the steps of:

(i) identifying peptides and/or polypeptides expressed in cancer cells each peptide and/or polypeptide comprising at least one cancer-specific amino acid modification, and

(ii) ascertaining one or more of the following:

(1) ascertaining whether the same or different neo-epitopes of the neo-antigen comprising the same cancer-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 neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the cancer-specific amino acid modification is useful for anti-cancer immunotherapy,

(2) ascertaining whether a neo-epitope of the neo-antigen comprising the cancer-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 neo-epitope of the neo-antigen comprising the cancer-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 cancer-specific amino acid modification is useful for anti-cancer immunotherapy, and/or

(3) ascertaining whether the same or different neo-epitopes of the neo-antigen comprising the same cancer-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 neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different neo-epitopes of the neo-antigen comprising the cancer-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 cancer-specific amino acid modification is useful for anti-cancer immunotherapy, and

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 cancer-specific amino acid modifications which are predicted to be useful for anti-cancer immunotherapy by any of the methods of the disclosure,

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

(1) a neo-antigen expressed in cancer cells, the neo-antigen comprising at least one of the cancer-specific amino acid modifications which are predicted to be useful for anti-cancer immunotherapy,

(2) a neo-antigen comprising a neo-epitope of the neo-antigen under (i), the neo-epitope comprising at least one of the cancer-specific amino acid modifications which are predicted to be useful for anti-cancer immunotherapy, and

(3) a nucleic acid encoding the neo-antigen 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

CLAIMS

1. A method for assessing the usefulness of a cancer-specific amino acid modification within a neo-antigen expressed in a cancer cell for anti-cancer immunotherapy, the method comprising ascertaining one or more of the following:

(i) ascertaining whether the same or different neo-epitopes of the neo-antigen comprising the cancer-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 neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the cancer-specific amino acid modification is useful for anti-cancer immunotherapy,

(ii) ascertaining whether a neo-epitope of the neo-antigen comprising the cancer-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 neo-epitope of the neo-antigen comprising the cancer-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 cancer-specific amino acid modification is useful for anti-cancer immunotherapy,

and/or

(iii) ascertaining whether the same or different neo-epitopes of the neo-antigen comprising the cancer-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 neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different neo-epitopes of the neo-antigen comprising the cancer-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 cancer-specific amino acid modification is useful for anti-cancer immunotherapy.

2. A method for selecting and/or ranking cancer-specific amino acid modifications for their usefulness in anti-cancer immunotherapy, the method comprising the steps of:

(i) identifying peptides and/or polypeptides expressed in cancer cells each peptide and/or polypeptide comprising at least one cancer-specific amino acid modification, and

(ii) ascertaining one or more of the following:

(1) ascertaining whether the same or different neo-epitopes of the neo-antigen comprising the same cancer-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 neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification in the context of MHC molecules of different classes and/or reactivity of the same or different neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification when presented in the context of MHC molecules with T cells restricted to different MHC classes indicates that the cancer-specific amino acid modification is useful for anti-cancer immunotherapy,

(2) ascertaining whether a neo-epitope of the neo-antigen comprising the cancer-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 neo-epitope of the neo-antigen comprising the cancer-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 cancer-specific amino acid modification is useful for anti-cancer immunotherapy,

and/or

(3) ascertaining whether the same or different neo-epitopes of the neo-antigen comprising the same cancer-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 neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification in the context of different MHC molecules of the same class and/or reactivity of the same or different neo-epitopes of the neo-antigen comprising the cancer-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 cancer-specific amino acid modification is useful for anti-cancer 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 cancer-specific amino acid modification(s) is (are) due to (a) cancer-specific somatic mutation(s).

7. The method of any one of claims 1 to 6, wherein presentation of the same or different neo-epitopes of the neo-antigen comprising the cancer-specific amino acid modification in the context of MHC molecules may be ascertained by using any peptide:MHC binding predictive tools and/or by determining binding of the neo-epitope to MHC molecules experimentally.

8. The method of any one of claims 1 to 6, wherein reactivity of the same or different neo-epitopes of the neo-antigen comprising the cancer specific amino acid modification with T cells when presented in the context of MHC molecules may be ascertained experimentally.

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

(i) a neo-antigen expressed in cancer cells, the neo-antigen comprising at least one cancer-specific amino acid modification,

(ii) a neo-antigen comprising a neo-epitope of the neo-antigen under (i), the neo-epitope comprising at least one cancer-specific amino acid modification, and

(iii) a nucleic acid encoding the neo-antigen under (i) or (ii).

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

(i) identifying one or more cancer-specific amino acid modifications which are predicted to be useful for anti-cancer immunotherapy by the method of any one of claims 1 to 9,

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

(1) a neo-antigen expressed in cancer cells, the neo-antigen comprising at least one of the cancer-specific amino acid modifications which are predicted to be useful for anti-cancer immunotherapy,

(2) a neo-antigen comprising a neo-epitope of the neo-antigen under (i), the neo-epitope comprising at least one of the cancer-specific amino acid modifications which are predicted to be useful for anti-cancer immunotherapy, and

(3) a nucleic acid encoding the neo-antigen under (i) or (ii).

11. The method of any one of claims 1 to 10, wherein the neo-epitope is an MHC binding peptide or a potential MHC binding peptide or can be processed to provide an MHC binding peptide or a potential MHC binding peptide.