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 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 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 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 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 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 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.

Another 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,

(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, 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.

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 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 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 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 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 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 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,

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

example of all aspects 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 aspects 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 aspects 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 aspect 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 aspects 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 aspects 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 aspects of the discclosure, 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.

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 example s, the disclosure provides a vaccine as 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.

The disclosure also relates to the following:

1. A method for predicting whether a disease-specific amino acid modification within a polypeptide expressed in a diseased cell is useful for immunotherapy, the method comprising determining whether the same or different fragments of the polypeptide comprising the disease-specific amino acid modification are presented in the context of MHC molecules of different classes.

2. The method of item 1, wherein the MHC molecules of different classes are MHC class I molecules and MHC class II molecules.

3. The method of item 1 or 2, wherein presentation of the same or different fragments of the polypeptide comprising the disease-specific amino acid modification in the context of MHC molecules of different classes indicates that the disease-specific amino acid modification is useful for immunotherapy.

4. The method of any one of items 1 to 3 further comprising determining whether the same or different fragments of the polypeptide comprising the disease-specific amino acid modification when presented in the context of MHC molecules are reactive with T cells restricted to different MHC classes.

5. A method for predicting whether a disease-specific amino acid modification within a polypeptide expressed in a diseased cell is useful for immunotherapy, the method comprising determining whether the same or different fragments of the polypeptide comprising the disease-specific amino acid modification when presented in the context of MHC molecules are reactive with T cells restricted to different MHC classes.

6. The method of item 4 or 5, wherein the T cells restricted to different MHC classes are CD4+ and CD8+ T cells.

7. The method of any one of items 4 to 6, wherein T cell reactivity to the same or different fragments of the 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.

8. A method for predicting whether a disease-specific amino acid modification within a polypeptide expressed in a diseased cell is useful for immunotherapy, the method comprising determining whether a fragment of the 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.

9. The method of item 8, wherein the different T cell receptors are of different clonotypes.

10. The method of item 8 or 9, wherein T cell reactivity to a fragment of the 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.

11. A method for predicting whether a disease-specific amino acid modification within a polypeptide expressed in a diseased cell is useful for immunotherapy, the method comprising determining whether the same or different fragments of the polypeptide comprising the disease-

specific amino acid modification are presented in the context of different MHC molecules of the same class.

12. The method of item 11, wherein the different MHC molecules of the same class are different MHC class I molecules.

13. The method of item 11 or 12, wherein presentation of the same or different fragments of the polypeptide comprising the disease-specific amino acid modification in the context of different MHC molecules of the same class indicates that the disease-specific amino acid modification is useful for immunotherapy.

14. The method of any one of items 11 to 13 further comprising determining whether the same or different fragments of the polypeptide comprising the disease-specific amino acid modification 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.

15. A method for predicting whether a disease-specific amino acid modification within a polypeptide expressed in a diseased cell is useful for immunotherapy, the method comprising determining whether the same or different fragments of the polypeptide comprising the disease-specific amino acid modification 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.

16. The method of item 14 or 15, wherein the different MHC molecules of the same class are different MHC class I molecules.

17. The method of any one of items 14 to 16, wherein the different T cells restricted to the same MHC class are different CD8+ T cells.

18. The method of any one of items 14 to 17, wherein T cell reactivity to the same or different fragments of the 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.

19. A method for predicting whether a disease-specific amino acid modification within a polypeptide expressed in a diseased cell is useful for immunotherapy, the method comprising determining one or more of the following:

(i) determining whether the same or different fragments of the polypeptide comprising the disease-specific amino acid modification are presented in the context of MHC molecules of different classes,

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

(iii) determining whether a fragment of the 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,

(iv) determining whether the same or different fragments of the polypeptide comprising the disease-specific amino acid modification are presented in the context of different MHC molecules of the same class, and

(v) determining whether the same or different fragments of the polypeptide comprising the disease-specific amino acid modification 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.

20. The method of item 19 comprising determining whether the same or different fragments of the polypeptide comprising the disease-specific amino acid modification are presented in the context of MHC molecules of different classes and when presented in the context of MHC molecules are reactive with T cells restricted to different MHC classes.

21. The method of item 19 or 20 comprising determining whether the same or different fragments of the polypeptide comprising the disease-specific amino acid modification are presented in the context of different MHC molecules of the same class and 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.

22. The method of any one of items 19 to 21, wherein the MHC molecules of different classes are MHC class I molecules and MHC class II molecules.

23. The method of any one of items 19 to 22, wherein the T cells restricted to different MHC classes are CD4+ and CD8+ T cells.

24. The method of any one of items 19 to 23, wherein presentation of the same or different fragments of the polypeptide comprising the disease-specific amino acid modification in the context of MHC molecules of different classes indicates that the disease-specific amino acid modification is useful for immunotherapy.

25. The method of any one of items 19 to 24, wherein T cell reactivity to the same or different fragments of the 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.

26. The method of any one of items 19 to 25, wherein the different T cell receptors are of different clonotypes.

27. The method of any one of items 19 to 26, wherein T cell reactivity to a fragment of the 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.

28. The method of any one of items 19 to 27, wherein the different MHC molecules of the same class are different MHC class I molecules.

29. The method of any one of items 19 to 28, wherein the different T cells restricted to the same MHC class are different CD8+ T cells.

30. The method of any one of items 19 to 29, wherein presentation of the same or different fragments of the polypeptide comprising the disease-specific amino acid modification in the context of different MHC molecules of the same class indicates that the disease-specific amino acid modification is useful for immunotherapy.

31. The method of any one of items 19 to 30, wherein T cell reactivity to the same or different fragments of the 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.

32. 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 polypeptides expressed in diseased cells each polypeptide comprising at least one disease-specific amino acid modification, and
(ii) determining whether the same or different fragments of a polypeptide comprising the same disease-specific amino acid modification are presented in the context of MHC molecules of different classes, and
(iii) repeating step (ii) for at least one further amino acid modification identified under (i).

33. The method of item 32, wherein the MHC molecules of different classes are MHC class I molecules and MHC class II molecules.

34. The method of item 32 or 33, wherein presentation of the same or different fragments of the polypeptide comprising the disease-specific amino acid modification in the context of MHC molecules of different classes indicates that the disease-specific amino acid modification is useful for immunotherapy.

35. The method of any one of items 32 to 34 wherein step (ii) further comprises determining whether the same or different fragments of the polypeptide comprising the same disease-specific amino acid modification when presented in the context of MHC molecules are reactive with T cells restricted to different MHC classes.

36. 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 polypeptides expressed in diseased cells each polypeptide comprising at least one disease-specific amino acid modification, and
(ii) determining whether the same or different fragments of a polypeptide comprising the same disease-specific amino acid modification when presented in the context of MHC molecules 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).

37. The method of item 35 or 36, wherein the T cells restricted to different MHC classes are CD4+ and CD8+ T cells.

38. The method of any one of items 35 to 37, wherein T cell reactivity to the same or different fragments of the 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.

39. 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 polypeptides expressed in diseased cells each polypeptide comprising at least one disease-specific amino acid modification, and
(ii) determining whether a fragment of a 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).

40. The method of item 39, wherein the different T cell receptors are of different clonotypes.

41. The method of item 39 or 40, wherein T cell reactivity to a fragment of the 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.

42. 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 polypeptides expressed in diseased cells each polypeptide comprising at least one disease-specific amino acid modification, and
(ii) determining whether the same or different fragments of a polypeptide comprising the same disease-specific amino acid modification are presented in the context of different MHC molecules of the same class, and
(iii) repeating step (ii) for at least one further amino acid modification identified under (i).

43. The method of item 42, wherein the different MHC molecules of the same class are different MHC class I molecules.

44. The method of item 42 or 43, wherein presentation of the same or different fragments of the polypeptide comprising the disease-specific amino acid modification in the context of different MHC molecules of the same class indicates that the disease-specific amino acid modification is useful for immunotherapy.

45. The method of any one of items 42 to 44 wherein step (ii) further comprises determining whether the same or different fragments of the polypeptide comprising the same disease-specific amino acid modification 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.

46. 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 polypeptides expressed in diseased cells each polypeptide comprising at least one disease-specific amino acid modification, and

(ii) determining whether the same or different fragments of a polypeptide comprising the same disease-specific amino acid modification 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).

47. The method of item 46, wherein the different MHC molecules of the same class are different MHC class I molecules.

48. The method of any one of items 45 to 47, wherein the different T cells restricted to the same MHC class are different CD8+ T cells.

49. The method of any one of items 45 to 48, wherein T cell reactivity to the same or different fragments of the 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.

50. 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 polypeptides expressed in diseased cells each polypeptide comprising at least one disease-specific amino acid modification, and

(ii) determining one or more of the following:

(1) determining whether the same or different fragments of a polypeptide comprising the same disease-specific amino acid modification are presented in the context of MHC molecules of different classes,

(2) determining whether the same or different fragments of a polypeptide comprising the same disease-specific amino acid modification when presented in the context of MHC molecules are reactive with T cells restricted to different MHC classes,

(3) determining whether a fragment of a 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,

(4) determining whether the same or different fragments of a polypeptide comprising the same disease-specific amino acid modification are presented in the context of different MHC molecules of the same class, and

(5) determining whether the same or different fragments of a polypeptide comprising the same disease-specific amino acid modification 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).

51. The method of item 50 wherein step (ii) comprises determining whether the same or different fragments of a polypeptide comprising the same disease-specific amino acid modification are presented in the context of MHC molecules of different classes and when presented in the context of MHC molecules are reactive with T cells restricted to different MHC classes.

52. The method of item 50 or 51 wherein step (ii) comprises determining whether the same or different fragments of a polypeptide comprising the same disease-specific amino acid modification are presented in the context of different MHC molecules of the same class and 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.

53. The method of any one of items 50 to 52, wherein the MHC molecules of different classes are MHC class I molecules and MHC class II molecules.

54. The method of any one of items 50 to 53, wherein the T cells restricted to different MHC classes are CD4+ and CD8+ T cells.

55. The method of any one of items 50 to 54, wherein presentation of the same or different fragments of the polypeptide comprising the disease-specific amino acid modification in the context of MHC molecules of different classes indicates that the disease-specific amino acid modification is useful for immunotherapy.

56. The method of any one of items 50 to 55, wherein T cell reactivity to the same or different fragments of the 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.

57. The method of any one of items 50 to 56, wherein the different T cell receptors are of different clonotypes.

58. The method of any one of items 50 to 57, wherein T cell reactivity to a fragment of the 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.

59. The method of any one of items 50 to 58, wherein the different MHC molecules of the same class are different MHC class I molecules.

60. The method of any one of items 50 to 59, wherein the different T cells restricted to the same MHC class are different CD8+ T cells.

61. The method of any one of items 50 to 60, wherein presentation of the same or different fragments of the polypeptide comprising the disease-specific amino acid modification in the context of different MHC molecules of the same class indicates that the disease-specific amino acid modification is useful for immunotherapy.

62. The method of any one of items 50 to 61, wherein T cell reactivity to the same or different fragments of the 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.

63. The method of any one of items 32 to 62, wherein amino acid modifications tested in step (ii) are present in the same polypeptide.

64. The method of any one of items 32 to 63, wherein amino acid modifications tested in step (ii) are present in different polypeptides.

65. The method of any one of items 32 to 64, which comprises comparing the scores obtained for the different amino acid modifications tested in step (ii).

66. The method of any one of items 1 to 65, wherein the disease-specific amino acid modification(s) is (are) due to (a) disease-specific somatic mutation(s).

67. The method of any one of items 1 to 66, wherein the disease is cancer and the immunotherapy is anti-cancer immunotherapy.

68. The method of any one of items 1 to 67, wherein the immunotherapy comprises administration of one or more of the following:
(i) a polypeptide expressed in diseased cells, the polypeptide comprising at least one disease-specific amino acid modification,
(ii) a polypeptide comprising a fragment of the polypeptide under (i), the fragment comprising at least one disease-specific amino acid modification, and
(iii) a nucleic acid encoding the polypeptide under (i) or (ii).

69. The method of any one of items 1 to 68, which is useful in providing a vaccine.

70. 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 items 1 to 69,
(ii) providing a vaccine comprising one or more of the following:
(1) a polypeptide expressed in diseased cells, the polypeptide comprising at least one of the disease-specific amino acid modifications which are predicted to be useful for immunotherapy,
(2) a polypeptide comprising a fragment of the 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 polypeptide under (i) or (ii).

71. The method of any one of items 1 to 70, 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.

72. A vaccine produced according to the method of any one of items 69 to 71.

73. A method of treating cancer, said method comprising administering an immunogenic composition comprising a polypeptide comprising a disease-specific amino acid modification identified according to the method of any one of items 1 to 68 or a nucleic acid encoding the polypeptide.

74. The method of item 73, wherein said immunogenic composition is a vaccine.

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

DETAILED DESCRIPTION OF THE INVENTION

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 will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", *H.G.W. Leuenberger, B. Nagel, and H. Kölbl, Eds., (1995) Helvetica Chimica Acta, CH-4010 Basel, Switzerland.*