

CLAIMS

1. A method for predicting immunogenic amino acid modifications, the method comprising the steps:
 - 5 a) ascertaining a score for binding of a modified peptide to one or more MHC molecules,
 - b) ascertaining a score for binding of a non-modified peptide to one or more MHC molecules, and
 - c) ascertaining a score for binding of the modified peptide when present in a MHC-peptide complex to one or more T cell receptors, comprising ascertaining a score for chemical and
 - 10 physical similarities between the non-modified and modified amino acids, wherein,
if the non-modified peptide has a score for binding to one or more MHC molecules satisfying a threshold indicating binding to one or more MHC molecules and the modified peptide has a score for binding to one or more MHC molecules satisfying a threshold indicating binding to
 - 15 one or more MHC molecules, the modification or modified peptide is predicted as immunogenic if the non-modified and modified amino acids have a score for the chemical and physical similarities satisfying a threshold indicating chemical and physical dissimilarity.
2. The method of claim 1 wherein the modified peptide comprises a fragment of a
20 modified protein, said fragment comprising the modification(s) present in the protein.
3. The method of claim 1 or 2 wherein the non-modified peptide has the germline amino acid at the position(s) corresponding to the position(s) of the modification(s) in the modified peptide.
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4. The method of any one of claims 1 to 3 wherein the non-modified peptide and modified peptide are identical but for the modification(s).
5. The method of any one of claims 1 to 4 wherein the non-modified peptide and
30 modified peptide are 8 to 15, preferably 8 to 12 amino acids in length.
6. The method of any one of claims 1 to 5 wherein the one or more MHC molecules comprise different MHC molecule types, in particular different MHC alleles.

7. The method of any one of claims 1 to 6 wherein the one or more MHC molecules are MHC class I molecules and/or MHC class II molecules.

8. The method of any one of claims 1 to 7 wherein the score for binding to one or more
5 MHC molecules is ascertained by a process comprising a sequence comparison with a database of MHC-binding motifs.

9. The method of any one of claims 1 to 8 wherein step a) comprises ascertaining whether said score satisfies a pre-determined threshold for binding to one or more MHC
10 molecules.

10. The method of any one of claims 1 to 9 wherein step b) comprises ascertaining whether said score satisfies a pre-determined threshold for binding to one or more MHC
15 molecules.

11. The method of any one of claims 1 to 10 wherein the threshold applied in step a) is different to the threshold applied in step b).

12. The method of any one of claims 1 to 11 wherein the pre-determined threshold for
20 binding to one or more MHC molecules reflects a probability for binding to one or more MHC molecules.

13. The method of any one of claims 1 to 12 wherein step c) comprises ascertaining whether said score satisfies a pre-determined threshold for the chemical and physical
25 similarities between amino acids.

14. The method of any one of claims 1 to 13 wherein the score for the chemical and physical similarities is ascertained on the basis of the probability of amino acids being
interchanged in nature.

30 15. The method of any one of claims 1 to 14 wherein the more frequently amino acids are interchanged in nature the more similar the amino acids are considered.

16. The method of any one of claims 1 to 15 wherein the chemical and physical similarities are determined using evolutionary based log-odds matrices.

17. The method of any one of claims 1 to 16 wherein if the non-modified peptide binds to one or more MHC molecules or has a probability for binding to one or more MHC molecules and the modified peptide binds to one or more MHC molecules or has a probability for binding to one or more MHC molecules, the modification or modified peptide is predicted as immunogenic if the non-modified and modified amino acids are chemically and physically dissimilar or have a probability of being chemically and physically dissimilar.

18. The method of any one of claims 1 to 17 wherein the modification is not in an anchor position for binding to one or more MHC molecules.

19. The method of any one of claims 1 to 18 wherein, if the non-modified peptide does not bind to one or more MHC molecules or has a probability for not binding to one or more MHC molecules and the modified peptide binds to one or more MHC molecules or has a probability for binding to one or more MHC molecules, the modification or modified peptide is predicted as immunogenic.

20. The method of claim 19 wherein the modification is in an anchor position for binding to one or more MHC molecules.

21. The method of any one of claims 1 to 20 which comprises performing step a) on two or more different modified peptides, said two or more different modified peptides comprising the same modification(s).

22. The method of claim 21 wherein the two or more different modified peptides comprising the same modification(s) comprise different fragments of a modified protein, said different fragments comprising the same modification(s) present in the protein.

23. The method of claim 21 or 22 wherein the two or more different modified peptides comprising the same modification(s) comprise all potential MHC binding fragments of a modified protein, said fragments comprising the same modification(s) present in the protein.

24. The method of any one of claims 21 to 23 further comprising selecting (the) modified peptide(s) from the two or more different modified peptides comprising the same modification(s) having a probability or having the highest probability for binding to one or more MHC molecules.

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25. The method of any one of claims 21 to 24 wherein the two or more different modified peptides comprising the same modification(s) differ in length and/or position of the modification(s).

10 26. The method of any one of claims 1 to 25 which comprises performing step a) and optionally one or both of steps b) and c) on two or more different modified peptides.

27. The method of claim 26 wherein said two or more different modified peptides comprise the same modification(s) and/or comprise different modifications.

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28. The method of claim 27 wherein the different modifications are present in the same and/or in different proteins.

20 29. The method of any one of claims 21 to 28 which comprises comparing the scores of two or more of said different modified peptides.

30. The method of any one of claims 1 to 29 further comprising identifying non-synonymous mutations in one or more protein-coding regions.

25 31. The method of any one of claims 1 to 30 wherein modifications are identified by partially or completely sequencing the genome or transcriptome of one or more cells such as one or more cancer cells and optionally one or more non-cancerous cells and identifying mutations in one or more protein-coding regions.

30 32. The method of claim 30 or 31 wherein said mutations are somatic mutations.

33. The method of claim 30 to 32 wherein said mutations are cancer mutations.