Claims

1. A method for selecting a number A of neoepitopes for an individual suffering from or suspected of suffering from cancer, said method comprising the steps of:

a. Receiving one or more neoepitopes, each neoepitope comprising at least one minimal epitope, wherein each neoepitope comprises at least one mutation compared to a reference sequence, wherein the minimal epitope consists of a number of amino acids equal to or smaller than the number of amino acids of the neoepitope and comprises said at least one mutation; wherein obtaining the neoepitopes comprises the step of identifying mutations in nucleic acid sequences which are specific for the tumor;

b. Determining MHC I and/or MHC II binding affinity for at least one minimal epitope and a corresponding reference peptide, wherein the binding affinity is determined by *in silico* prediction by calculating the %Rank score, wherein a low %Rank score indicates a strong binding affinity and a high %Rank score indicates a weak binding affinity, and wherein the corresponding reference peptide is obtained from body fluid or healthy tissue of the same individual or in a normal, healthy population;

c. Selecting neoepitopes comprising at least one minimal epitope predicted to bind to MHC I and/or to MHC II, preferably wherein the at least one minimal epitope is a minimal epitope predicted to bind to MHC I, thereby obtaining MHC binding neoepitopes;

d. Ranking the MHC binding neoepitopes with respect to the likelihood of clinical utility, wherein said ranking comprises:

I. determining the number of minimal epitopes comprised in the MHC binding neoepitopes selected in step c, wherein neoepitopes comprising a high number of minimal epitopes are ranked higher than neoepitopes comprising a lower number of minimal epitopes;

II. determining the MHC I binding differential of the MHC I binding neoepitopes selected in step c, wherein the MHC I binding differential is given by the formula (%Rank score (MHC I) for reference) / (%Rank score (MHC I) for neoepitope) wherein neoepitopes with a high MHC I binding differential are ranked higher than neoepitopes with a lower MHC I binding differential, and wherein amongst the neoepitopes with a high MHC I

binding differential those having the mutation in an anchoring position are prioritized;

III. prioritizing the MHC I binding neoepitopes selected in step c with respect to the MHC I rank, wherein neoepitopes with a low %Rank score (MHC I) are ranked higher than neoepitopes with a higher %Rank score, wherein the %Rank score (MHC I) of a neoepitope comprising one or more minimal epitopes predicted to bind to MHC I is equal to the lowest %Rank score (MHC I) of said one or more minimal epitopes; and

IV. determining the BLOSUM score, wherein the MHC I binding neoepitopes selected in step c are prioritized with respect to their BLOSUM score in a descending order, wherein the BLOSUM score of a neoepitope is equal to the BLOSUM score of the best ranking minimal epitope it comprises, wherein a minimal epitope with a BLOSUM score < 2 is prioritized ; and

e. Selecting A neoepitopes among the highest ranking MHC binding neoepitopes of step d, thereby selecting A neoepitopes likely to have clinical utility.

2. The method according to claim 1, wherein in step b MHC I binding affinity and MHC II binding affinity are determined for at least one minimal epitope, such as at least two, three or four minimal epitopes, within each of said neoepitopes.

3. The method according to any one of the preceding claims, wherein A is an integer of from 1 to 100, such as from 5 to 50, such as from 5 to 30, such as from 10 to 20, such as 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25.

4. The method according to any one of the preceding claims, wherein step a comprises identifying mutations in nucleic acid sequences in one or more sample derived from the individual compared to a reference sample, preferably wherein the reference sample is obtained from a healthy tissue of the individual in need of treatment or from a normal, healthy population, wherein the one or more sample is a sample derived from tumor cells of the individual.

5. The method according to claim 4, wherein step c further comprises selecting MHC I binding and/or MHC II binding neoepitopes which are found in at least two samples derived from tumor cells of the individual.

6. The method according to any one of the preceding claims, wherein the neoepitope comprises 1 to 50 minimal epitopes, such as 1 to 40 minimal epitopes, such as 1 to 30 minimal epitopes, such as 1 to 20 minimal epitopes.

7. The method according to any one of the preceding claims, wherein step c comprises selecting neoepitopes comprising at least one minimal epitope predicted to bind to MHC I and at least one minimal epitope predicted to bind to MHC II.

8. The method according to any one of the preceding claims, wherein step d IV comprises prioritizing neoepitopes having a %Rank score (MHC I) below 2.0, such as below 1.5, such as below 1, preferably equal to or below 0.5.

9. The method according to any one of the preceding claims, wherein MHC I binding neoepitopes having a %Rank score (MHC I) above 10 are excluded or down-prioritized, preferably wherein MHC I binding neoepitopes having a %Rank score (MHC I) above 2 are excluded or down-prioritized.

10. The method according to any one of the preceding claims, wherein neoepitopes found in more than one sample derived from tumor cells are ranked higher than neoepitopes found in only one sample derived from tumor cells, preferably wherein the mutations are identified by at least two different variant callers.

11. The method according to any one of the preceding claims, further comprising a step of determining the RNA expression levels of said neoepitopes, and selecting neoepitopes with a detectable normalised transcript-level expression, such as a transcript-level expression above 0 transcript per million (TPM).

12. The method according to any one of the preceding claims, further comprising a step of determining the allele frequency of mutations present in the MHC binding neoepitopes, and selecting neoepitopes with an allele frequency greater than 0.05.

13. The method according to any one of the preceding claims, wherein step d further comprises a step of down-prioritizing or excluding neoepitopes which do not comprise any minimal epitopes predicted to bind to MHC I.

14. The method according to any one of the preceding claims, wherein neoepitopes comprising at least 5 MHC binding minimal epitopes are ranked higher than neoepitopes comprising less than 5 MHC binding minimal epitopes.

15. The method according to any one of the preceding claims, wherein the neoepitopes of step d, II are ranked by assigning to each neoepitope the highest score of the minimal epitopes they comprise, and wherein the ranking of the neoepitopes is performed in a descending order 1) to 7) as follows:
    1) if the mutation is in an anchoring position and the binding differential of the minimal epitope is equal to or greater than 20, the minimal epitope has the highest score;
    2) if the mutation is in a non-anchoring position and the binding differential of the minimal epitope is equal to or greater than 20, the score of the minimal epitope is lower than the score of the minimal epitopes of 1);
    3) if the mutation is in an anchoring position and the binding differential of the minimal epitope is lower than 20 and equal to or greater than 3, the score of the minimal epitope is lower than the score of the minimal epitopes of 2);
    4) if the mutation is in a non-anchoring position and the binding differential of the minimal epitope is lower than 20 and equal to or greater than 3, the score of the minimal epitope is lower than the score of the minimal epitopes of 3);
    5) if the mutation is in an anchoring position and the binding differential of the minimal epitope is less than 3 and equal to or greater than 1, the score of the minimal epitope is lower than the score of the minimal epitopes of 4);
    6) if the mutation is in a non-anchoring position and the binding differential of the minimal epitope is less than 3 and equal to or greater than 1, the score of the minimal epitope is lower than the score of the minimal epitopes of 5;

7) if the minimal epitope has a mutation with a binding differential lower than 1, regardless of the position of the mutation, the minimal epitope has the lowest score.

16. A method of preparing a cancer vaccine comprising A neoepitopes, said method comprising a step of selecting said A neoepitopes using the method according to any one of claims 1 to 15.

17. The method of claim 16, wherein said cancer vaccine comprises a polynucleotide encoding a polypeptide comprising a) a targeting unit, b) a dimerization unit, c) a first linker and d) an antigenic unit comprising said A neoepitopes.