CLAIMS

What is claimed is:

1. A computer-implemented method comprising:

a) receiving, as an input, a computational representation of a target peptide presented in a complex with a major histocompatibility complex (MHC) molecule (MHC-target peptide complex);

b) determining binding affinity of the target peptide to the MHC molecule;

c) generating sequences of a plurality of mutated peptides each associated with a mutation at a respective amino acid position of the target peptide;

d) determining binding affinity of each mutated peptide of the plurality of mutated peptides to the MHC molecule;

e) predicting the amino acid position(s) of the target peptide involved in binding to the MHC molecule to form said MHC-target peptide complex based at least in part on a comparison of the binding affinity for each mutated peptide to the binding affinity of the target peptide; and

f) providing, as an output, an indication of the amino acid position(s) available to interact with the antigen-recognition molecule such that the amino acid position(s) available to interact with the antigen-recognition molecule exclude the positions predicted to be involved in binding to the MHC molecule at step e).

2. A computer-implemented method comprising:

a) receiving, as an input, a computational representation of a target peptide presented in a complex with a major histocompatibility complex (MHC) molecule (MHC-target peptide complex);

b) predicting all amino acid positions within the target peptide that are available to interact with an antigen-recognition molecule that recognizes the MHC-target peptide complex by:

i) generating sequences of a plurality of mutated peptides each associated with a mutation at a respective amino acid position of the target peptide,

ii) determining binding affinity of each mutated peptide of the plurality of mutated peptides to the MHC molecule, and

iii) predicting the amino acid position(s) of the target peptide involved in binding to the MHC molecule to form said MHC-target peptide complex based at least in part on a comparison of the binding affinity for each mutated peptide to the binding affinity of the target peptide, wherein all amino acid positions within the target peptide that are available to interact with the antigen-recognition molecule exclude the positions predicted to be involved in binding to the MHC molecule;

c) generating a working list of peptides, within a total pool of predicted or detected peptides of suitable length, such that peptides listed in the working list each include at least two amino acids that (i) are located at positions corresponding to positions within the target peptide that are available to interact with the antigen-recognition molecule and (ii) are identical to the corresponding amino acids of the target peptide;

d) determining binding affinity, of each of the peptides listed in the working list, to the MHC molecule;

e) filtering the working list to include only peptide(s) having a calculated binding affinity to the MHC molecule greater than a first threshold value, thereby generating a working list of off-target peptide(s); and

f) providing, as an output, the working list of off-target peptide(s) and/or the number of the off-target peptide(s) in the working list.

3. The computer-implemented method of claim 2, comprising:

providing, as an input, a computational representation of the antigen-recognition molecule, the antigen-recognition molecule being capable of binding to the MHC-target peptide complex;

determining binding affinity of the antigen-recognition molecule to a plurality of MHC-peptide complexes each including a respective off-target peptide from the working list and the MHC molecule; and

filtering the working list to include only off-target peptides which comprise a binding motif for the antigen-recognition molecule.

4. The computer-implemented method of any one of claims 2-3, comprising:

providing, as an input, off-target peptide expression in essential, normal tissues of a specific patient; and

providing, as an output, an indication of the off-target effects for said patient.

5. A method for predicting amino acid position(s) within a target peptide presented in a complex with a major histocompatibility complex (MHC) molecule (MHC-target peptide complex), the amino acid position(s) being available to interact with an antigen-recognition molecule that recognizes said MHC-target peptide complex, the method comprising:

a) determining binding affinity of the target peptide to the MHC molecule;

b) generating a plurality of mutated peptides each associated with a mutation at a respective amino acid position of the target peptide;

c) determining binding affinity of each mutated peptide of the plurality of mutated peptides to the MHC molecule; and

d) predicting the amino acid position(s) of the target peptide involved in binding to the MHC molecule to form said MHC-target peptide complex based at least in part on a comparison of the binding affinity for each mutated peptide to the binding affinity of the target peptide such that the amino acid position(s) available to interact with the antigen-recognition molecule exclude the positions predicted to be involved in binding to the MHC molecule.

6. The method of claim 5, wherein the binding affinity of the target peptide to the MHC molecule is determined using a computer-based model, or wherein the binding affinity of the target peptide to the MHC molecule is determined through an experimental measurement;
optionally wherein the binding affinity of the target peptide to the MHC molecule is determined using the half maximal inhibitory concentration (IC_{50}) value or a percentile rank value.

7. The method of any one of claims 1-6, wherein generating a plurality of mutated peptides or generating sequences of a plurality of mutated peptides comprises mutating the respective amino acid to a glycine amino acid or an alanine amino acid.

8. The method of any one of claims 1-7, further comprising predicting that a respective position of the plurality of positions is not involved in binding to the MHC molecule to form said MHC-target peptide complex when the binding affinity of a mutated peptide with a mutation associated with the respective position is approximately equal to the binding affinity of the target

peptide, or when both of the following conditions are met: (i) the binding affinity of a mutated peptide with a mutation associated with the respective position is approximately equal to the binding affinity of the target peptide, and (ii) the amino acid of the target peptide at the respective position is a non-glycine residue.

9. The method of claim 8, comprising predicting that a respective position of the plurality of positions is not involved in binding to the MHC molecule to form said MHC-target peptide complex when:

a) the percentile rank of a mutated peptide with a mutation associated with the respective position is less than 1.0 if the percentile rank of the target peptide is less than or equal to 0.5,

b) the percentile rank of a mutated peptide with a mutation associated with the respective position is less than 2.0 if the percentile rank of the target peptide is between 0.5 and 2.0, or

c) the percentile rank of a mutated peptide with a mutation associated with the respective position is less than 4.0 if the percentile rank of the target peptide is greater than or equal to 2.0, as determined by the percentile rank value.

10. The method of any one of claims 5-9, further comprising verifying that the respective position of the plurality of positions within the target peptide is not involved in binding to the MHC molecule to form said MHC target-peptide complex based on a known structure of the MHC-target peptide complex.

11. A method of identifying off-target peptide(s) associated with a target peptide presented in a complex with a major histocompatibility complex (MHC) molecule (MHC-target peptide complex), the method comprising:

a) predicting all amino acid positions within the target peptide that may are available to interact with an antigen-recognition molecule that recognizes the MHC-target peptide complex by:

i) generating sequences of a plurality of mutated peptides each associated with a mutation at a respective amino acid position of the target peptide,

ii) determining binding affinity of each mutated peptide of the plurality of mutated peptides to the MHC molecule, and

iii) predicting the amino acid position(s) of the target peptide involved in binding to the MHC molecule to form said MHC-target peptide complex based at least in part on a comparison of the binding affinity for each mutated peptide to the binding affinity of the target peptide, wherein all amino acid positions within the target peptide that are available to interact with the antigen-recognition molecule exclude the positions predicted to be involved in binding to the MHC molecule;

b) identifying within a total pool of predicted or detected peptides of suitable length similar peptides that include at least two amino acids that (i) are located at positions corresponding to positions within the target peptide that are available to interact with the antigen-recognition molecule and (ii) are identical to the corresponding amino acids of the target peptide;

c) determining binding affinity of each of the identified similar peptide(s) to the MHC molecule; and

d) identifying off-target peptide(s) based at least in part on identifying similar peptide(s) having a calculated binding affinity to the MHC molecule stronger than a first threshold value and/or estimating the number of off-target peptide(s) based at least in part on counting similar peptide(s) having a calculated binding affinity to the MHC molecule stronger than a first threshold value.

12. The method of claim 11, wherein step (b) comprises identifying within a total pool of predicted or detected peptides of suitable length similar peptides that include at least three, at least four or at least five amino acids that (i) are located at positions corresponding to positions within the target peptide that are available to interact with the antigen-recognition molecule and (ii) are identical to the corresponding amino acids of the target peptide.

13. The method of any one of claims 11-12, wherein the binding affinity in step (c) is determined by determining the percentile rank value, and wherein the number of off-target peptide(s) is estimated in step (d) based at least in part on counting similar peptide(s) having percentile rank value lower than 2.0; and/or wherein the total pool of detected peptides in step (b) is based on mass-spectrometry data.

14. The method of any one of claims 11-13 or 2-4, comprising:

(i) including in the off-target peptide(s) or working list only peptide(s) which are expressed in essential, normal tissues,
(ii) including in the off-target peptide(s) or working list only similar peptide(s) having the calculated binding affinity to the MHC molecule stronger than the first threshold value and detectable expression in essential, normal tissues, or
(iii) for each of the identified off-target peptide(s), determining whether such peptide is expressed in essential, normal tissues, and optionally generating a list of potential secondary target peptides comprising peptides having a calculated binding affinity to the MHC molecule greater than the first threshold value and having a low expression in essential, normal tissues.

15. The method of claim 14, wherein the peptide expression is determined based on the level of the corresponding mRNA or protein expression, or wherein the peptide expression is determined based on mass-spectrometry data.

16. The method of any one of claims 11-15, or 2-4, further comprising calculating a Degree of Similarity (DoS) score for the similar peptide(s), the DoS score being based at least in part on a number of amino acids of a respective similar peptide identical to amino acids at corresponding positions of the target peptide, which amino acids of the target peptide are available to interact with the antigen-recognition molecule,
optionally wherein only positions of the target peptide identified as being unbound to the MHC molecule are considered in calculating the DoS score, and/or wherein the method further comprises including in the off-target peptide(s) or working list only similar peptide(s) having a DoS score greater than a second threshold value.

17. The method of any one of claims 1-16, wherein the MHC molecule is a class I MHC molecule, and the target peptide, predicted peptides or detected peptides are 8-12 amino acids in length, and/or wherein the antigen-recognition molecule is a T cell Receptor (TCR), a chimeric antigen receptor (CAR), an antibody, or an antigen-binding fragment thereof.