

What is claimed is

1. A method for characterizing the binding of a polypeptide molecule comprising at least one defined peptide binding domain ("pMHC binding polypeptide") to a peptide ("target peptide") of a peptide:MHC complex, comprising

- a) providing a sample comprising at least one peptide:MHC complex to be analyzed,
- b) contacting said sample with said polypeptide molecule ("pMHC binding polypeptide"), and allowing said at least one peptide binding domain of said polypeptide molecule to bind to said at least one peptide:MHC complex,
- c) isolating said at least one peptide:MHC complex bound to said at least one peptide binding domain, and
- d) identifying said peptide of said at least one peptide:MHC complex as isolated in step c), and thereby identifying the binding of said polypeptide molecule to said peptide of said at least one peptide:MHC complex.

2. The method according to claim 1, wherein said polypeptide molecule is optionally attached to a matrix material, and/or wherein said polypeptide molecule further comprises at least one attachment site binding to or being attached to a matrix material.

3. The method according to claim 1 or 2, wherein characterizing the binding of a polypeptide molecule to a peptide of a peptide:MHC complex, comprises identifying the binding of the polypeptide molecule to said peptide of a peptide:MHC complex.

4. The method according to any one of claims 1 to 3, wherein said sample is selected from natural or artificial samples comprising at least one peptide:MHC complex, such as a cellular lysate, or a sample comprising purified or enriched peptide:MHC complexes.

5. The method according to any one of claims 1 to 4, wherein said polypeptide molecule comprising said at least one peptide binding domain is selected from bispecific, trispecific, tetraspecific or multispecific molecules.

6. The method according to any one of claims 1 to 5, wherein said polypeptide molecule comprising said at least one peptide binding domain is a molecule or is derived from a molecule selected from an antibody, a simultaneous multiple interaction T-cell engaging (SMITE) bispecific, a bispecific T-cell engager (BiTE), an scFV, a diabody, a dual-affinity re-targeting antibody (DART), a tandem antibody (TandAb), a soluble TCR, an scTCR, a mutated TCR, for example comprising S-bridges, a truncated TCR, and a bispecific T-cell receptor (TCR)-antibody fusion molecule.
7. The method according to any one of claims 1 to 6, wherein said polypeptide molecule comprises at least one second binding domain that is selected from a domain binding to a cell surface molecule known to induce the activation of immune cells, and immune response-related molecules, preferably a second binding domain binding to CD3 or one of its chains, like the CD3 γ , CD3 δ , and CD3 ϵ chains, CD4, CD7, CD8, CD10, CD11b, CD11c, CD14, CD16, CD18, CD22, CD25, CD28, CD32a, CD32b, CD33, CD41, CD41b, CD42a, CD42b, CD44, CD45RA, CD49, CD55, CD56, CD61, CD64, CD68, CD94, CD90, CD117, CD123, CD125, CD134, CD137, CD152, CD163, CD193, CD203c, CD235a, CD278, CD279, CD287, Nkp46, NKG2D, GITR, Fc ϵ RI, TCR α/β , TCR γ/δ , and/or HLA-DR.
8. The method according to any one of claims 1 to 7, wherein said polypeptide molecule comprising said at least one peptide binding domain is a bispecific molecule comprising a peptide binding domain that is derived from a T-cell receptor (TCR).
9. The method according to any one of claims 1 – 8, wherein said polypeptide molecule comprising said at least one peptide binding domain is a soluble molecule.
10. The method according to any one of claims 2 to 9, wherein the attachment site is positioned in the at least one binding domain, the at least one second domain or is a separate attachment group, and does not essentially interfere with the binding of said molecule.
11. The method according to any one of claims 1 to 10, wherein said identifying in step d) comprises a method selected from mass spectrometry and peptide sequencing.

12. The method according to any one of claims 1 to 11, wherein characterizing the binding of a polypeptide molecule to a peptide of a peptide:MHC complex comprises identifying a superordinate binding motif for said peptide:MHC binding domain.

13. The method according to any one of claims 1 to 12, wherein characterizing the binding of a polypeptide molecule to a peptide of a peptide:MHC complex comprises.

a) Identifying a position-based and/or superordinate binding motif of the polypeptide molecule for said peptide:MHC binding domain, and/or

b) Identifying off-target peptides of the polypeptide molecule for peptide:MHC binding domains using the peptides identified by the presented application.

14. The method according to any one of claims 1 to 13, wherein characterizing the binding of a polypeptide molecule to a peptide of a peptide:MHC complex comprises exploring and/or identifying the cross-reactivity of said peptide for other peptide:MHC binding domains.

15. The method according to any one of claims 1 to 14, further comprising the step of identifying the presentation of said peptide motif or peptide motifs on cancerous and/or non-cancerous cells or tissues.

16. The method according to any one of claims 1 to 15, wherein said method further comprises adding to said sample in step a) at least

- one peptide having a known sequence and/or
- one defined and/or preselected peptide:MHC complex the peptide of which has a known sequence,

preferably in a predetermined amount ("spiking").

17. The method according to claim 16, wherein the sequence of said peptide is altered or mutated relative to the sequence of the peptide ("target peptide") of a peptide:MHC complex to which the polypeptide molecule comprising at least one defined peptide binding domain ("pMHC binding polypeptide") binds.

18. The method according to claim 16 or 17, wherein a series of mutants of the peptide ("target peptide") of a peptide:MHC complex to which the polypeptide molecule comprising at least one defined peptide binding domain ("pMHC binding polypeptide") binds is created and added to the sample in step a), each mutant having, over its entire length or over at least a subfraction thereof, the amino acid residue at one position substituted for an alternative amino acid,

19. The method according to any one of claims 16 to 18, wherein each mutant has, over its entire length or over at least a subfraction thereof, the amino acid residue at one position substituted for alanine or glycine.

20. The method according to any one of claims 16 to 19, wherein the anchoring positions of the peptide ("target peptide") of a peptide:MHC complex to which the polypeptide molecule comprising at least one defined peptide binding domain ("pMHC binding polypeptide") are not altered/mutated.

21. The method according to any one of claims 2 to 20, wherein said matrix material is selected from Sepharose® or agarose.

22. The method according to any one of claims 1 to 21, wherein said method further comprises contacting said sample in step b) and/or c) with one or more other binding domain molecules, such as, for example, broad specific TCRs and/or antibodies.

23. The method according to claim 22, wherein at least one broad specific binding domain molecule is an MHC pan-specific binding molecule which has binding specificity against one or more MHC molecules alone, irrespective of the peptide bound thereto. 23. The method according to claim 21 or 22, wherein

- (i) a first fraction of the sample is contacted with the pMHC binding polypeptide, and
- (ii) a second fraction of the sample is contacted with another pMHC binding polypeptide or the MHC pan-specific binding molecule

and wherein further, after isolating the peptide:MHC complexes bound to the pMHC binding polypeptide and the other pMHC binding polypeptide or the MHC pan-specific binding molecule, the isolation efficiencies achieved in the two fractions are compared.

24. The method according to any one of claims 1 to 23, wherein, in step b) or c), a large excess of pMHC binding polypeptides and optionally MHC pan-specific binding molecules is applied.

25. The method according to any one of claims 23 to 24, wherein the binding affinities of the binders (binding polypeptides or binding molecules) used in the first and second fraction to the different peptides in the sample are determined based on the comparison of the isolation efficiencies.

26. The method according to any one of claims 1 to 25, wherein at least one molecule in said method comprises a detectable marker or label.

27. The method according to any one of claims 1 to 26, further comprising a computational analysis of said identification and/or off target binding.

28. The method according to any one of claims 14 - 22, wherein the cross-reactivity for said peptide:MHC binding domain is further explored by at least one cytotoxicity experiment.

29. A kit, comprising materials for performing the method according to any one of claims 1 to 28, such as, for example, a) a matrix material, and b) polypeptide molecules comprising said at least one peptide binding domain binding to at least one peptide:MHC complex.

30. A polypeptide molecule comprising at least one defined peptide binding domain ("pMHC binding polypeptide"), wherein the binding of which to a peptide ("target peptide") of a peptide:MHC complex was characterized with a method according to any one of claims 1 – 28.