

CLAIMS

1. A method of producing a peptide-MHC (pMHC)-specific binding protein, wherein said binding protein comprises a designed repeat domain with binding specificity for a target peptide-MHC complex, the method comprising the steps of:
 - (a) providing a collection of designed repeat domains;
 - (b) providing a recombinant target peptide-MHC complex; and
 - (c) screening said collection of designed repeat domains for specific binding to said target peptide-MHC complex to obtain at least one designed repeat domain with binding specificity for said target peptide-MHC complex.
2. The method of claim 1, wherein said designed repeat domain is a designed ankyrin repeat domain.
3. The method of claim 1 or 2, wherein said collection comprises designed repeat domains that comprise fixed positions and randomized positions, and wherein designed repeat domains of said collection differ from each other in at least one of the randomized positions.
4. The method of any one of the preceding claims, wherein said collection of designed repeat proteins is provided by ribosome display.
5. The method of any one of the preceding claims, wherein the method further comprises the steps of (i) providing a second recombinant peptide-MHC complex, wherein said peptide of said second peptide-MHC complex comprises an amino acid sequence that differs from said target peptide by at least one amino acid residue; and (ii) removing from said collection by negative selection designed repeat domains with binding specificity for said second recombinant peptide-MHC complex.
6. The method of any one of the preceding claims, wherein said repeat domain with binding specificity for a target peptide-MHC complex binds to said target peptide-MHC complex in PBS with a dissociation constant (K_D) below $10^{-7}M$.
7. The method of any one of the preceding claims, wherein binding of said repeat domain with binding specificity for a target peptide-MHC complex to said target peptide-MHC complex comprises interaction of said repeat domain with at least one, at least two, at least three, at least four, at least five, at least six, or at least seven amino acid residues of said target peptide.
8. The method of any one of the preceding claims, wherein binding of said repeat domain with binding specificity for a target peptide-MHC complex to said target peptide-MHC complex comprises interaction of said repeat domain with at least one amino acid residue of said MHC.

9. The method of any one of the preceding claims, wherein said target peptide is derived from an intracellular protein, preferably an intracellular protein expressed in a tumor cell.
10. The method of any one of claims 1 to 9, wherein said target peptide is derived from NY-ESO-1, preferably wherein said target peptide comprises or consists of the amino acid sequence of SEQ ID NO: 19 or SEQ ID NO: 34.
11. The method of any one of claims 1 to 9, wherein said target peptide is derived from EBNA-1, preferably wherein said target peptide comprises or consists of the amino acid sequence of SEQ ID NO: 92.
12. The method of any one of claims 1 to 9, wherein said target peptide is derived from MAGE-A3, preferably wherein said target peptide comprises or consists of the amino acid sequence of SEQ ID NO: 155.
13. The method of any one of claims 1 to 9, wherein said target peptide is derived from HBcAg, preferably wherein said target peptide comprises or consists of the amino acid sequence of SEQ ID NO: 255.
14. The method of any one of the preceding claims, wherein said MHC is MHC class I.
15. A recombinant binding protein comprising a designed repeat domain obtainable by the method of any one of the preceding claims.
16. A recombinant binding protein comprising a first designed repeat domain, wherein said first repeat domain has binding specificity for a first target peptide-MHC complex, wherein preferably said first target peptide is selected from the group consisting of (i) a peptide derived from a protein expressed in a tumor cell, (ii) a peptide derived from a protein of an infectious agent, preferably a viral infectious agent, and (iii) a peptide derived from a protein associated with an autoimmune disorder.
17. The binding protein of claim 16, wherein said first target peptide is derived from an intracellular protein, preferably an intracellular protein expressed in a tumor cell.
18. The binding protein of claim 16 or 17, wherein said first repeat domain binds to said first target peptide-MHC complex in PBS with a dissociation constant (K_D) below $10^{-7}M$.
19. The binding protein of any one of claims 16 to 18, wherein binding of said first repeat domain to said first target peptide-MHC complex comprises interaction of said first repeat domain with at least one, at least two, at least three, at least four, at least five, at least six, or at least seven amino acid residues of said first target peptide.

20. The binding protein of any one of claims 16 to 19, wherein binding of said first repeat domain to said first target peptide-MHC complex comprises interaction of said first repeat domain with at least one amino acid residue of said first MHC.
21. The binding protein of any one of claims 16 to 20, wherein said first MHC is MHC class I.
22. The binding protein of claim 21, wherein said MHC class I is HLA-A*02.
23. The binding protein of any one of claims 16 to 22, wherein said first target peptide is derived from NY-ESO-1.
24. The binding protein of any one of claims 16 to 23, wherein said first target peptide has the amino acid sequence of SEQ ID NO: 19 or SEQ ID NO: 34.
25. The binding protein of any one of claims 16 to 24, wherein said first repeat domain is a designed ankyrin repeat domain.
26. The binding protein of claim 25, wherein said first ankyrin repeat domain comprises an ankyrin repeat module comprising an amino acid sequence selected from the group consisting of (1) SEQ ID NOs: 37 to 72 and (2) sequences in which up to 9 amino acids in any of SEQ ID NOs: 37 to 72 are substituted by another amino acid.
27. The binding protein of claim 25 or 26, wherein said first ankyrin repeat domain comprises an amino acid sequence with at least 80% amino acid sequence identity with any one of SEQ ID NOs: 20 to 33, wherein G at position 1 and/or S at position 2 of SEQ ID NOs: 20 to 33 are optionally missing, and wherein A at the second last position of SEQ ID NOs: 20 to 33 is optionally substituted by L and/or A at the last position of SEQ ID NOs: 20 to 33 is optionally substituted by N.
28. The binding protein of any one of claims 15 to 27, wherein said binding protein further comprises a second designed repeat domain, wherein said second repeat domain has binding specificity for a second target peptide-MHC complex, wherein preferably said second target peptide is selected from the group consisting of (i) a peptide derived from a protein expressed in a tumor cell, (ii) a peptide derived from a protein of an infectious agent, preferably a viral infectious agent, and (iii) a peptide derived from a protein associated with an autoimmune disorder.
29. The binding protein of claim 28, wherein said second target peptide is derived from an intracellular protein, preferably an intracellular protein expressed in a tumor cell.

30. The binding protein of claim 28 or 29, wherein said second repeat domain with binding specificity for a second target peptide-MHC complex binds to said second target peptide-MHC complex in PBS with a dissociation constant (K_D) below $10^{-7}M$.
- 5 31. The binding protein of any one of claims 28 to 30, wherein binding of said second repeat domain to said second target peptide-MHC complex comprises interaction of said second repeat domain with at least one, at least two, at least three, at least four, at least five, at least six, or at least seven amino acid residues of said second target peptide.
- 10 32. The binding protein of any one of claims 28 to 31, wherein binding of said second repeat domain to said second target peptide-MHC complex comprises interaction of said second repeat domain with at least one amino acid residue of said second MHC.
- 15 33. The binding protein of any one of claims 28 to 32, wherein said second MHC is MHC class I.
34. The binding protein of any one of claims 28 to 33, wherein said second target peptide is derived from the same protein as said first target peptide.
- 20 35. The binding protein of any one of claims 28 to 34, wherein said second target peptide has the same amino acid sequence as said first target peptide.
36. The binding protein of any one of claims 28 to 35, wherein said second repeat domain has the same amino acid sequence as said first repeat domain.
- 25 37. The binding protein of any one of claims 28 to 35, wherein said second repeat domain has a different amino acid sequence as compared to said first repeat domain.
38. The binding protein of any one of claims 28 to 34, wherein said second target peptide has a different amino acid sequence as compared to said first target peptide.
- 30 39. The binding protein of any one of claims 28 to 33, wherein said second target peptide is derived from a protein that is different from the protein, from which said first target peptide is derived.
40. The binding protein of any one of claims 28 to 39, wherein said second MHC is HLA-A*02.
- 35 41. The binding protein of any one of claims 28 to 39, wherein said second MHC is not HLA-A*02.
42. The binding protein of any one of claims 28 to 41, wherein said second repeat domain is a designed ankyrin repeat domain.
- 40

43. The binding protein of claim 42, wherein said second ankyrin repeat domain comprises an ankyrin repeat module comprising an amino acid sequence selected from the group consisting of (1) SEQ ID NOs: 37 to 72 and (2) sequences in which up to 9 amino acids in any of SEQ ID NOs: 37 to 72 are substituted by another amino acid.
- 5
44. The binding protein of claim 42 or 43, wherein said second ankyrin repeat domain comprises an amino acid sequence with at least 80% amino acid sequence identity with any one of SEQ ID NOs: 20 to 33, wherein G at position 1 and/or S at position 2 of SEQ ID NOs: 20 to 33 are optionally missing, and wherein A at the second last position of SEQ ID NOs: 20 to 33 is optionally substituted by L and/or A at the last position of SEQ ID NOs: 20 to 33 is optionally substituted by N.
- 10
45. The binding protein of any one of claims 28 to 34, 40, and 42 to 44, wherein said binding protein comprises a polypeptide comprising an amino acid sequence with at least 80% amino acid sequence identity with any one of SEQ ID NOs: 16 to 18.
- 15
46. The binding protein of any one of claims 15 to 45, wherein said binding protein further comprises a binding agent with binding specificity for a protein expressed on the surface of an immune cell.
47. The binding protein of claim 46, wherein said immune cell is a T-cell.
- 20
48. The binding protein of claim 47, wherein said T-cell is a CD8+ cytotoxic T-cell.
49. The binding protein of any one of claims 46 to 47, wherein said protein expressed on the surface of an immune cell is a protein that is part of the T-cell receptor complex.
- 25
50. The binding protein of claim 49, wherein said protein that is part of the T-cell receptor complex is cluster of differentiation 3 (CD3).
51. The binding protein of any one of claims 46 to 50, wherein said binding agent is an antibody, an antibody mimetic, a scaffold protein, a repeat protein, or a designed repeat domain.
- 30
52. The binding protein of any one of claims 46 to 51, wherein said binding agent is a designed ankyrin repeat domain.
- 35
53. The binding protein of any one of claims 46 to 52, wherein said binding agent is covalently linked to said first repeat domain and/or, if present, said second repeat domain with a peptide linker.
54. The binding protein of claim 53, wherein said peptide linker is a proline-threonine-rich peptide linker.

55. The binding protein of any one of claims 53 to 54, wherein the amino acid sequence of said peptide linker has a length from 1 to 17 amino acids.
56. The binding protein of any one of claims 15 to 55, wherein said binding protein is capable of inhibiting recognition of said first target peptide-MHC complex and/or, if said binding protein comprises said second repeat domain, of said second target peptide-MHC complex by T-cell receptors.
57. A nucleic acid encoding the designed repeat domain with binding specificity for a first or a second target peptide-MHC complex as defined in any one of claims 15 to 56 or encoding the binding protein of any one of claims 15 to 56.
58. A pharmaceutical composition comprising the binding protein of any one of claims 15 to 56 or the nucleic acid of claim 57, and a pharmaceutically acceptable carrier and/or diluent.
- ~~59. A method of tumor-localized activation of T-cells in a mammal, preferably a human, the method comprising the step of administering to said mammal the binding protein of any one of claims 15 to 55 or the nucleic acid of claim 57, wherein said first target peptide and/or, if said binding protein comprises said second repeat domain, said second target peptide is derived from a protein expressed in a tumor cell.~~
- ~~60. The binding protein of any one of claims 15 to 55 or the nucleic acid of claim 57 for use in a method of tumor-localized activation of T-cells in a mammal, preferably a human, wherein said first target peptide and/or, if said binding protein comprises said second repeat domain, said second target peptide is derived from a protein expressed in a tumor cell.~~
- ~~61. A method of infection-localized activation of T-cells in a mammal, preferably a human, the method comprising the step of administering to said mammal the binding protein of any one of claims 15 to 55 or the nucleic acid of claim 57, wherein said first target peptide and/or, if said binding protein comprises said second repeat domain, said second target peptide is derived from a protein of an infectious agent.~~
- ~~62. The binding protein of any one of claims 15 to 55 or the nucleic acid of claim 57 for use in a method of infection-localized activation of T-cells in a mammal, preferably a human, wherein said first target peptide and/or, if said binding protein comprises said second repeat domain, said second target peptide is derived from a protein of an infectious agent.~~

63. ~~A method of treating a medical condition, the method comprising the step of administering to a patient in need thereof a therapeutically effective amount of the binding protein of any one of claims 15 to 56, the nucleic acid of claim 57, or the pharmaceutical composition of claim 58.~~
- 5 64. ~~The binding protein of any one of claims 15 to 56, the nucleic acid of claim 57, or the pharmaceutical composition of claim 58 for use in a method of treating a medical condition.~~
65. ~~A method of diagnosing a medical condition in a mammal, preferably a human, the method comprising the steps of:~~
- 10 (i) ~~contacting a cell or tissue sample obtained from said mammal with the binding protein of any one of claims 15 to 56; and~~
- (ii) ~~detecting specific binding of said binding protein to said cell or tissue sample.~~
66. ~~The method of claim 63 or 65 or the binding protein for use according to claim 64, wherein said medical condition is a cancer, an infectious disease, preferably a viral infectious disease, or an autoimmune disease.~~
- 15 67. ~~The method of claim 63 or 65 or the binding protein for use according to claim 64, wherein said medical condition is a cancer.~~
- 20 68. ~~The method of claim 63 or 65 or the binding protein for use according to claim 64, wherein said medical condition is an infectious disease, preferably a viral infectious disease.~~
69. ~~The method of claim 63 or 65 or the binding protein for use according to claim 64, wherein said medical condition is an autoimmune disease.~~
- 25 70. ~~A method of targeting tumor cells in a patient having a tumor for destruction of the tumor cells, the method comprising the step of administering to the patient a therapeutically effective amount of the binding protein of any one of claims 15 to 56, the nucleic acid of claim 57, or the pharmaceutical composition of claim 58, wherein said first target peptide and/or, if said binding protein comprises said second repeat domain, said second target peptide is derived from a protein expressed in the tumor cells, preferably an intracellular protein expressed in the tumor cells.~~
- 30 71. ~~The method of targeting tumor cells of claim 70, wherein said binding protein further comprises a toxic agent capable of killing a tumor cell.~~
- 35 72. ~~The binding protein of any one of claims 15 to 56, the nucleic acid of claim 57, or the pharmaceutical composition of claim 58 for use in a method of targeting tumor cells in a patient having a tumor for destruction of the tumor cells, wherein said first target peptide and/or, if said binding protein~~

- comprises said second repeat domain, said second target peptide is derived from a protein expressed in the tumor cells, preferably an intracellular protein expressed in the tumor cells.
- 5 73. ~~The binding protein, nucleic acid or pharmaceutical composition for use according to claim 72, wherein the binding protein further comprises a toxic agent capable of killing a tumor cell.~~
- 10 74. ~~A method of targeting infected cells in a patient having a viral infectious disease for destruction of the infected cells, the method comprising the step of administering to the patient a therapeutically effective amount of the binding protein of any one of claims 15 to 56, the nucleic acid of claim 57, or the pharmaceutical composition of claim 58, wherein said first target peptide and/or, if said binding protein comprises said second repeat domain, said second target peptide is derived from a protein expressed in the infected cells, preferably a virus-specific protein expressed in the infected cells.~~
- 15 75. ~~The method of targeting tumor cells of claim 74, wherein said binding protein further comprises a toxic agent capable of killing an infected cell.~~
- 20 76. ~~The binding protein of any one of claims 15 to 56, the nucleic acid of claim 57, or the pharmaceutical composition of claim 58 for use in a method of targeting infected cells in a patient having a viral infectious disease for destruction of the infected cells, wherein said first target peptide and/or, if said binding protein comprises said second repeat domain, said second target peptide is derived from a protein expressed in the infected cells, preferably a virus-specific protein expressed in the infected cells.~~
- 25 77. ~~The binding protein, nucleic acid or pharmaceutical composition for use according to claim 76, wherein the binding protein further comprises a toxic agent capable of killing an infected cell.~~
- 30 78. ~~The method of any one of claims 63, 66 to 67, 69 and 70 or the binding protein, nucleic acid or pharmaceutical composition of any of claims 64, 72 and 73, wherein said medical condition or tumor is selected from the group consisting of liposarcoma, neuroblastoma, synovial sarcoma, melanoma and ovarian cancer.~~