

## CLAIMS

~~What is claimed is:~~

1. A method for preparing T cells, comprising  
contacting CD8+ T cells with a first multimer comprising a target peptide in complex with an MHC molecule and with a second multimer comprising an irrelevant peptide in complex with an MHC molecule,  
wherein the first multimer is labelled with a first detectable agent and  
the second multimer is labelled with a second detectable agent,  
wherein the first detectable agent is different from the second detectable agent,  
wherein the irrelevant peptide has less than 50% sequence identity to the target peptide,  
sorting the contacted CD8+ T cells to collect sorted CD8+ T cells that are detected positive for the first detectable agent and detected negative for the second detectable agent, and  
expanding the collected CD8+ T cells.
2. The method of claim 1, wherein the contacting is performed in the presence of a first binding agent binding to the first detectable agent and/or a second binding agent binding to the second detectable agent.
3. The method of claim 2, wherein the first binding agent and the second binding agent are antibodies.
4. The method of any one of claims 1-3, wherein the contacting is performed at from about 4°C to about 37°C.
5. The method of any one of claims 1-4, wherein the contacting is performed in the presence of an protein kinase inhibitor (PKI).
6. The method of claim 5, wherein the PKI is at least one selected from the group consisting of afatinib, axitinib, bosutinib, cetuximab, cobimetinib, crizotinib, cabozantinib, dasatinib, entrectinib, erdafitinib, erlotinib, fostamatinib, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, mubritinib, nilotinib, pazopanib, pegaptanib, ruxolitinib, sorafenib, sunitinib, SU6656, vandetanib, and vemurafenib.

7. The method of any one of claims 1-6, wherein the sorting, the collecting, and the expanding are performed in a closed system.
8. The method of any one of claims 1-7, wherein the target peptide consists of the amino acid sequence of SEQ ID NO: 141.
9. The method of any one of claims 1-8, wherein the irrelevant peptide is at least one selected from the group consisting of SEQ ID NOS: 1-140 and 142-161.
10. The method of any one of claims 1-8, wherein the irrelevant peptide is at least one selected from SEQ ID NOS: 16, 18, 105, 118, and 161.
11. A method for preparing T cells, comprising
  - culturing CD8<sup>+</sup> T cells in the presence of at least one cytokine,
  - contacting the cultured CD8<sup>+</sup> T cells with a first multimer comprising a target peptide in a complex with an MHC molecule and a second multimer comprising an irrelevant peptide in a complex with an MHC molecule, wherein the first multimer is labelled with a first detectable agent and the second multimer is labelled with a second detectable agent, wherein the first detectable agent is detectably different from the second detectable agent,
  - sorting the contacted CD8<sup>+</sup> T cells to collect sorted CD8<sup>+</sup> T cells that are detected positive for the first detectable agent and detected negative for the second detectable agent, and
  - expanding the collected CD8<sup>+</sup> T cells.
12. The method of claim 11, wherein the blood sample is peripheral blood mononuclear cell (PBMC) or a product of leukapheresis.
13. The method of claim 12 or 13, wherein the blood sample is obtained from a patient.
14. The method of any one of claims 11-13, wherein the blood sample is obtained from a donor.
15. The method of any one of claims 11-14, wherein the at least one cytokine is selected from interleukin (IL)-1, IL-2, IL-7, IL-10, IL-15, IL-17, IL-21, and IL-23.

16. The method of any one of claims 11-15, wherein the multimer is a tetramer.
17. The method of any one of claims 11-16, wherein the MHC molecule is a class I MHC molecule.
18. The method of any one of claims 11-17, wherein the first and the second detectable agents each comprise a fluorescent compound.
19. The method of any one of claims 11-18, wherein the sorting and the collecting are performed by using a microchip-based cell sorter.
20. The method of any one of claims 11-19, wherein the microchip-based cell sorter is a single-use, disposable, and fully closed microfluidic chip cell sorter.
21. The method of any one of claims 11-20, wherein the expanding is performed in the presence of at least one cytokine.
22. The method of claim 21, wherein the at least one cytokine is selected from IL-1, IL-2, IL-7, IL-10, IL-15, IL-17, IL-21, and IL-23.
23. The method of any one of claims 11-22, wherein the sorting, the collecting, and the expanding are performed in a closed system.
24. The method of any one of claims 11-23, wherein the contacting is performed in the presence of a first binding agent binding to the first detectable agent and/or a second binding agent binding to the second detectable agent.
25. The method of claim 24, wherein the first binding agent and the second binding agent are antibodies.
26. The method of any one of claims 11-25, wherein the contacting is performed at from about C to about 37°C.
27. The method of any one of claims 11-26, wherein the irrelevant peptide is at least one selected from the group consisting of SEQ ID NO: 1-161.
28. The method of any one of claims 11-27, wherein the irrelevant peptide in a complex with an MHC molecule does not bind to the same T cells as does the target peptide in a complex with an MHC molecule.

29. The method of any one of claims 11-28, wherein the contacting is performed in the presence of a protein kinase inhibitor (PKI).

30. The method of claim 29, wherein the PKI is at least one selected from the group consisting of afatinib, axitinib, bosutinib, cetuximab, cobimetinib, crizotinib, cabozantinib, dasatinib, entrectinib, erdafitinib, erlotinib, fostamatinib, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, mubritinib, nilotinib, pazopanib, pegaptanib, ruxolitinib, sorafenib, sunitinib, SU6656, vandetanib, and vemurafenib.

31. The method of any one of claims 11-30, wherein the contacting is performed in the presence of a third multimer comprising a target peptide in a complex with an MHC molecule, wherein the third multimer is labelled with a third detectable agent, wherein the third detectable agent is detectably different from the first and the second detectable agents, and wherein the sorting comprises collecting the sorted CD8<sup>+</sup> T cells that are detected positive for the first and the third detectable agents and detected negative for the second detectable agents.

32. The method of any one of claims 11-31, wherein the multimers are filtered prior to use in the contacting.

33. The method of any one of claims 11-32, wherein the blood sample comprises cells at a concentration from about  $0.1 \times 10^6$  cells/ml to about  $1000 \times 10^6$  cells/ml, from about  $1 \times 10^6$  cells/ml to about  $900 \times 10^6$  cells/ml, from about  $5 \times 10^6$  cells/ml to about  $800 \times 10^6$  cells/ml, from about  $10 \times 10^6$  cells/ml to about  $700 \times 10^6$  cells/ml, from about  $20 \times 10^6$  cells/ml to about  $600 \times 10^6$  cells/ml, from about  $25 \times 10^6$  cells/ml to about  $500 \times 10^6$  cells/ml, from about  $30 \times 10^6$  cells/ml to about  $400 \times 10^6$  cells/ml, from about  $35 \times 10^6$  cells/ml to about  $300 \times 10^6$  cells/ml, from about  $40 \times 10^6$  cells/ml to about  $200 \times 10^6$  cells/ml, from about  $45 \times 10^6$  cells/ml to about  $150 \times 10^6$  cells/ml, from about  $50 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $55 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $60 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $65 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $70 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $75 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $80 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $85 \times 10^6$  cells/ml

to about  $100 \times 10^6$  cells/ml, from about  $90 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, or from about  $95 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml.

34. The method of any one of claims 11-33, wherein said method for preparing T cells does not include use of an antigen presenting cell or agonists for CD3, CD28, CD134, CD278, or CD137 for activating the T cells prior to or during the step of culturing the isolated T cells.

35. The method of any one of claims 11-34, further comprising obtaining the expanded T cells, wherein the period of time between the isolating and the obtaining of the expanded T cells is from about 7 days to about 14 days, from about 7 days to about 21 days, from about 7 days to about 28 days, from about 14 days to about 21 days, from about 14 days to about 28 days, or from about 21 days to about 28 days.

36. The method of any one of claims 11-35, wherein said method comprises isolating CD4<sup>+</sup> T cells rather than CD8<sup>+</sup> T cells, or isolating both CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells.

37. The method of any one of claims 11-36, wherein the culturing the isolated CD8<sup>+</sup> T cells is performed in the absence of a T cell activation agent so that the isolated CD8<sup>+</sup> T cells are not activated.

38. A method for preparing T cells, comprising  
    contacting a blood sample with  
        a first multimer comprising a target peptide in a complex with an MHC molecule, wherein the first multimer is labelled with a first detectable agent, and  
        a second multimer comprising an irrelevant peptide, which is different from the target peptide, in a complex with an MHC molecule, wherein the second multimer is labelled with a second detectable agent,  
    wherein the first and the second detectable agents are detectably different detectable agents,  
    sorting the contacted cells to collect the sorted cells that are detected positive for the first detectable agent and are detected negative for the second detectable agent, and

expanding the collected T cells.

39. The method of claim 38, wherein the blood sample is peripheral blood mononuclear cell (PBMC) or a product of leukapheresis.

40. The method of claim 38 or 39, wherein the blood sample is obtained from a patient.

41. The method of any one of claims 38-40, wherein the blood sample is obtained from a donor.

42. The method of any one of claims 38-41, wherein the contacting is performed in the presence of a first binding agent binding to the first detectable agent and/or a second binding agent binding to the second detectable agent.

43. The method of claim 42, wherein the first binding agent and the second binding agent are antibodies.

44. The method of any one of claims 38-43, wherein the contacting is performed at from about 4°C to about 37°C.

45. The method of any one of claims 38-44, wherein the irrelevant peptide is at least one selected from the group consisting of SEQ ID NO: 1-161.

46. The method of any one of claims 38-45, wherein the irrelevant peptide in a complex with an MHC molecule does not bind to the same T cells as does the target peptide in a complex with an MHC molecule.

47. The method of any one of claims 38-46, wherein the contacting is performed in the presence of an protein kinase inhibitor (PKI).

48. The method of claim 47, wherein the PKI is at least one selected from the group consisting of afatinib, axitinib, bosutinib, cetuximab, cobimetinib, crizotinib, cabozantinib, dasatinib, entrectinib, erdafitinib, erlotinib, fostamatinib, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, mubritinib, nilotinib, pazopanib, pegaptanib, ruxolitinib, sorafenib, sunitinib, SU6656, vandetanib, and vemurafenib.

49. The method of any one of claims 38-48, wherein the contacting is performed in the presence of a third multimer comprising a target peptide in a complex with an MHC molecule, wherein the third multimer is labelled with a third detectable agent, wherein the third detectable agent is detectably different from the first and the second detectable agents, and wherein the sorting comprises collecting the sorted CD8<sup>+</sup> T cells that are detected positive for the first and the third detectable agents and detected negative for the second detectable agents.

50. The method of any one of claims 38-49, wherein the multimers are filtered through a filter prior to use in the contacting.

51. The method of any one of claims 38-50, wherein said method for preparing T cells does not include use of an antigen presenting cell or agonists for CD3, CD28, CD134, CD278, or CD137 for activating the T cells prior to or during the step of culturing the isolated T cells.

52. The method of any one of claims 38-51, further comprising obtaining the expanded T cells, wherein the period of time between the isolating and the obtaining of the expanded T cells is from about 7 days to about 14 days, from about 7 days to about 21 days, from about 7 days to about 28 days, from about 14 days to about 21 days, from about 14 days to about 28 days, or from about 21 days to about 28 days.

53. The method of any one of claims 38-52, wherein the blood sample comprises cells at a concentration from about  $0.1 \times 10^6$  cells/ml to about  $1000 \times 10^6$  cells/ml, from about  $1 \times 10^6$  cells/ml to about  $900 \times 10^6$  cells/ml, from about  $5 \times 10^6$  cells/ml to about  $800 \times 10^6$  cells/ml, from about  $10 \times 10^6$  cells/ml to about  $700 \times 10^6$  cells/ml, from about  $20 \times 10^6$  cells/ml to about  $600 \times 10^6$  cells/ml, from about  $25 \times 10^6$  cells/ml to about  $500 \times 10^6$  cells/ml, from about  $30 \times 10^6$  cells/ml to about  $400 \times 10^6$  cells/ml, from about  $35 \times 10^6$  cells/ml to about  $300 \times 10^6$  cells/ml, from about  $40 \times 10^6$  cells/ml to about  $200 \times 10^6$  cells/ml, from about  $45 \times 10^6$  cells/ml to about  $150 \times 10^6$  cells/ml, from about  $50 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $55 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $60 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $65 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $70 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $75 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $80 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, from about  $85 \times 10^6$  cells/ml

to about  $100 \times 10^6$  cells/ml, from about  $90 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml, or from about  $95 \times 10^6$  cells/ml to about  $100 \times 10^6$  cells/ml.

54. A composition comprising the peptide-specific T cells prepared by the method of any one of claims 1-53.

55. The composition of claim 54, further comprising an adjuvant.

56. The composition of claim 55, wherein the adjuvant is selected from anti-CD40 antibody, imiquimod, resiquimod, GM-CSF, cyclophosphamide, interferon-alpha, interferon-beta, CpG oligonucleotides, poly-(I:C), RNA, sildenafil, particulate formulations with poly(lactide co-glycolide) (PLG), virosomes, IL-1, IL-2, IL-4, IL-7, IL-12, IL-13, IL-15, IL-21, and IL-23.

57. ~~The composition of any one of claims 54-56 for use in the treatment of~~  
~~A method of treating a patient who has cancer, comprising administering to said patient the~~  
~~composition of any one of claims 54-56,~~ wherein said cancer is selected from hepatocellular carcinoma (HCC), colorectal carcinoma (CRC), glioblastoma (GB), gastric cancer (GC), esophageal cancer, non-small cell lung cancer (NSCLC), pancreatic cancer (PC), renal cell carcinoma (RCC), benign prostate hyperplasia (BPH), prostate cancer (PCA), ovarian cancer (OC), melanoma, breast cancer (BRCA), chronic lymphocytic leukemia (CLL), Merkel cell carcinoma (MCC), small cell lung cancer (SCLC), Non-Hodgkin lymphoma (NHL), acute myeloid leukemia (AML), gallbladder cancer and cholangiocarcinoma (GBC, CCC), urinary bladder cancer (UBC), and uterine cancer (UEC).  
41. The composition according to any one of claims 37-39 for use in the treatment of cancer in a patient, wherein the composition is administered to said patient and wherein said cancer is selected from hepatocellular carcinoma (HCC), colorectal carcinoma (CRC), glioblastoma (GB), gastric cancer (GC), esophageal cancer, non-small cell lung cancer (NSCLC), pancreatic cancer (PC), renal cell carcinoma (RCC), benign prostate hyperplasia (BPH), prostate cancer (PCA), ovarian cancer (OC), melanoma, breast cancer (BRCA), chronic lymphocytic leukemia (CLL), Merkel cell carcinoma (MCC), small cell lung cancer (SCLC), Non-Hodgkin lymphoma (NHL), acute myeloid leukemia (AML), gallbladder cancer and cholangiocarcinoma (GBC, CCC), urinary bladder cancer (UBC), and uterine cancer (UEC).