

CLAIMS

1. A method for preparing an antigen-specific T-cell population, comprising:
providing a sample comprising T-cells from a patient or a suitable donor;
5 contacting said sample with a population of first particles which are paramagnetic
and comprise on their surfaces an MHC-peptide antigen-presenting complex, wherein the
particles are from about 10 to about 500 nm in diameter, and
 placing a magnetic field in proximity to the paramagnetic particles, and
 recovering antigen-specific T-cells associated with the magnetic particles.
- 10 2. The method of claim 1, wherein the antigen-specific T cells are cytotoxic T
lymphocytes, helper T cells, or regulatory T cells.
3. The method of claim 1, wherein the antigen-specific T cells are CD8+ cytotoxic T
lymphocytes.
4. The method of claim 3, wherein the T-cells are from a patient and the patient is a
15 cancer patient.
5. The method of claim 4, wherein the cancer is a solid tumor.
6. The method of claim 4, wherein the cancer is melanoma, colon cancer, duodenal
cancer, prostate cancer, breast cancer, ovarian cancer, ductal cancer, hepatic cancer,
pancreatic cancer, renal cancer, endometrial cancer, testicular cancer, stomach
20 cancer, dysplastic oral mucosa, polyposis, head and neck cancer, invasive oral
cancer, nonsmall cell lung carcinoma, small-cell lung cancer, mesothelioma,
transitional and squamous cell urinary carcinoma, brain cancer, neuroblastoma, and
glioma.
7. The method of claim 4, wherein the cancer is melanoma.
- 25 8. The method of claim 4, wherein the cancer is a hematological malignancy.
9. The method of claim 1, wherein the antigen-specific T cells are CD8+ cytotoxic T
lymphocytes and/or CD4+ helper T cells.
10. The method of claim 9, wherein the T cells are from a patient and the patient has an
infectious disease.
- 30 11. The method of claim 1, wherein the T cells are regulatory T cells, and wherein the
patient has an autoimmune disease.
12. The method of any one of claims 1 to 11, wherein the sample comprising T cells is
from a suitable donor who is HLA-matched to an adoptive transfer recipient,

wherein the recovered antigen-specific T-cells are adoptively transferred to the recipient.

13. The method of claim 1, wherein the sample comprising T cells is selected from a group consisting of: a peripheral blood mononuclear cell (PBMC) sample, memory
5 T cells, naïve T cells, previously activated T cells, and tumor infiltrating lymphocytes.
14. The method of claim 1, wherein the sample comprising T cells is from bone marrow, lymph node tissue, spleen tissue, or a tumor.
15. The method of claim 1, wherein the sample comprising T cell is isolated by
10 leukapheresis.
16. The method of any one of claims 1, wherein the sample is enriched for CD8+ cells, CD4+ cells, or T regulatory cells.
17. The method of claim 16, wherein the sample is enriched for CD8+antigen-specific T cells by placing the cells in a magnetic field.
18. The method of claim 16, wherein at least 10^6 CD8-enriched cells are isolated for
15 antigen-specific T cell enrichment by the steps of contacting, placing, and recovering.
19. The method of any one of claims 1 to 18, wherein the paramagnetic particles comprising on their surface an MHC-peptide antigen-presenting complex comprise
20 iron dextran beads.
20. The method of any one of claims 1 to 19, wherein the paramagnetic particles comprising on their surface an MHC-peptide antigen-presenting complex further comprise a lymphocyte costimulatory ligand.
21. The method of any one of claims 1 to 20, wherein the antigen presenting complex is
25 a MHC class I or MHC class II complex.
22. The method of claim 21, wherein the antigen presenting complex is monomeric or dimeric.
23. The method of claim 22, wherein the antigen presenting complex is dimeric,
wherein the complex comprises a fusion of MHC with immunoglobulin heavy chain
30 sequences associated by disulfide bonds.
24. The method of claim 23, wherein the immunoglobulin sequences are IgG sequences.

25. The method of any one of claims 21 to 24, wherein the MHC is an MHC class I complex comprising HLA-A, HLA-B, HLA-C, or HLA-E.
26. The method of claim 25, wherein the MHC is an HLA-A2.
27. The method of any one of claims 23 to 26, wherein the immunoglobulin sequences
5 are humanized monoclonal antibody sequences.
28. The method of claim 20, wherein the lymphocyte costimulatory ligand is one or more selected from the group consisting of: an antibody or antigen-binding fragment thereof that specifically binds to CD28, CD80 (B7-1), CD86 (B7-2), B7-H3, 4-1BBL, 4-1BB, CD27, CD30, CD134 (OX-40L), B7h (B7RP-1), CD40,
10 LIGHT, an antibody or antigen-binding fragment thereof that specifically binds to HVEM, an antibody or antigen-binding fragment thereof that specifically binds to CD40L, an antibody or antigen binding fragment thereof that specifically binds to OX40, and an antibody or antigen-binding fragment thereof that specifically binds to 4-1BB.
29. The method of claim 28, wherein the costimulatory ligand is selected from the group consisting of a monoclonal antibody, a F(ab')₂, a Fab, scFv, and a single chain antibody.
30. The method of claim 29, wherein the monoclonal antibody or fragment is humanized.
31. The method of any one of claims 20 to 30, wherein the co-stimulatory ligand is a humanized murine monoclonal antibody or fully human antibody against CD28.
32. The method of claim 31, wherein the co-stimulatory ligand is a humanized monoclonal antibody 9.3 or antigen-binding fragment thereof.
33. The method of any one of claims 1 to 32, wherein the magnetic particles have a size
25 within about 20 to about 200 nm.
34. The method of any one of claims 20 to 33, wherein the antigen presenting complex and the costimulatory ligand are covalently bound to the surface of the paramagnetic particle.
35. The method of any one of claims 1 to 34, wherein the particles are substantially
30 spherical.
36. The method of any one of claims 1 to 34, wherein the particles are non-spherical.

37. The method of any one of claims 1 to 36, wherein from about 3 to about 10 different antigens are presented by the antigen presenting complexes in the population of paramagnetic particles.
38. The method of claim 4, wherein the antigen-presenting complex presents a tumor associated antigen
39. The method of any one of claims 1 to 38, wherein the recovered antigen-specific T-cells are enriched for antigen-specific, naïve T cells.
40. The method of any of claims 20 to 39, wherein the recovered antigen-specific T cells are expanded in culture for about 2 days to about 9 weeks.
41. The method of claim 40, wherein the cells are expanded in culture in the presence of a magnetic field.
42. The method of claim 40, wherein the antigen-specific T cells are expanded in culture for about 5 days to about 2 weeks, or about 5 days to about 10 days.
43. The method of claim 42, wherein the antigen-specific enriched T cells are expanded in culture for about 1 week, after which time a second round of enrichment and expansion is optionally performed.
44. The method of any one of claims 40 to 43, wherein 2, 3, 4, or 5 enrichment and expansion rounds are performed.
45. The method of claim 1 or 44, wherein the method results in at least about 5% antigen-specific T cells.
46. The method of claim 45, wherein the method results in at least about 10% antigen-specific T cells, or at least about 15% antigen-specific T cells, or at least about 20% antigen-specific T cells, or at least about 25% antigen-specific T cells.
47. The method of claim 46, wherein at least about 10^6 , or at least about 10^7 antigen specific T cells are obtained.
48. The method of claim 47, wherein said at least about 10^6 , or at least about 10^7 antigen-specific T cells are administered to a patient recipient as adoptive immunotherapy.
49. A method for identifying a T cell antigen or component of a T cell repertoire, comprising:
- providing a sample comprising T cells from a subject,
- screening the isolated T cells against a library of paramagnetic particles each comprising on their surface an MHC-peptide antigen-presenting complex, wherein the

particles are from about 10 to about 500 nm in diameter, wherein each particle presents a candidate peptide antigen, and

identifying one or more peptide antigens that cause activation of the subject's T cells.

- 5 50. The method of claim 49, wherein the subject is a cancer patient.
51. The method of claim 50, wherein the candidate peptides comprise peptide antigen's predicted from genetic analysis of the patient's cancer.
52. The method of any one of claims 49 to 51, wherein the step of screening comprises incubating the subject's T cells and the library of paramagnetic particles in the
- 10 presence of a magnetic field.
53. The method of any one of claims 49 to 52, wherein activation of the subject's T cells is identified by cytokine expression, which is optionally measured by immunochemistry or RT-PCR, or activation of the subject's T cells is identified by measuring intracellular signaling indicative of T cell activation.
- 15 54. The method of claim 52 or 53, wherein selected peptide antigens are loaded onto artificial antigen presenting cells and administered to the patient.
55. The method of any one of claims 49 to 54, wherein T cells that recognize the identified antigens are enriched and expanded *ex vivo*, and administered to the subject.
- 20 56. The method of claim 49, wherein the subject is suspected of having an immune disease, and the paramagnetic particles present a library of candidate peptides indicative of autoimmune and/or immune disease.
57. The method of claim 49, wherein the subject is suspected of having an infectious disease, and the paramagnetic particles, present a library of candidate peptides
- 25 indicative of infectious diseases.
58. The method of claim 55 wherein the T cells that recognize the identified antigens are enriched by the process of:
- providing a sample comprising T-cells from a patient or a suitable donor; and
- recovering antigen-specific T-cells by density gradient centrifugation or flow
- 30 cytometry.
59. The method of claim 1 further comprising the step of phenotypically characterizing the recovered T-cells.

60. The method of claim 20 further comprising the step of expanding the recovered T-cells in culture.
61. The method of claim 1 further comprising the step of isolating the recovered T cells from the magnetic particles.
- 5 62. The method of claim 1 or 61 further comprising the step of injecting the isolated T cells into a recipient that has a disease for treatment of the disease.
63. The method of claim 1 wherein the recovered T-cells are cultured *ex vivo*.
64. The method of claim 1 wherein the recovered T cells comprise less than 10^{-6} of the T cells in the sample.
- 10 65. The method of claim 48 wherein the sample comprises T cells from a suitable donor who is HLA-mismatched to an adoptive transfer recipient, wherein the recovered antigen-specific T-cells are adoptively transferred to the recipient.
66. The method of any one of claims 20 to 33, wherein the antigen presenting complex and the costimulatory ligand are bound to the surface of the paramagnetic particle
- 15 via an antibody.
67. The method of claim 4 wherein the antigen-presenting complex presents a neoantigen predicted from genetic analysis of the patient's tumor, wherein the neoantigen is formed by a mutation selected from the group consisting of a passenger mutation, a driver mutation, an oncogene forming mutation, and a tumor
- 20 suppressor destroying mutation.
68. The method of claim 4 wherein the antigen-presenting complex presents an antigen predicted from genetic analysis of the patient's tumor.
69. The method of claim 68 further comprising the step of vaccinating the patient with the antigen.
- 25 70. The method of claim 68 further comprising the step of stimulating T-cells of the patient with the antigen.
71. The method of claim 20 wherein the antigen-specific T-cells express one or more activation markers.
72. The method of claim 20 wherein the paramagnetic particles comprise at least 5
- 30 MHC-peptide antigen-presenting complexes on their surface.
73. The method of claim 20 wherein the paramagnetic particles comprise at least 10 MHC-peptide antigen-presenting complexes on their surface.

74. The method of claim 20 wherein the paramagnetic particles comprise at least 5 lymphocyte costimulatory ligand molecules on their surface.
75. The method of claim 20 wherein the paramagnetic particles comprise at least 10 lymphocyte costimulatory ligand molecules on their surface.
- 5 76. The method of any one of claims 1 to 48, wherein the paramagnetic particles comprise on their surface an MHC-peptide antigen-presenting complex, and further contacting the sample with second particles comprising a lymphocyte costimulatory ligand.
77. The method of claim 62 wherein the disease is a chronic infectious disease.
- 10 78. The method of claim 67 wherein the step of contacting employs limiting dilutions of said population.
79. The method of claim 1 further comprising isolating mRNA from said antigen-specific T-cells and performing a sequence determination reaction on said mRNA or cDNA derived from said mRNA.
- 15 80. The method of claim 71 wherein the paramagnetic particles are detectably labeled and the method further comprises assaying the antigen-specific T-cells for expression of T-cell activation markers.
81. The method of any one of claims 20 to 33, wherein the antigen presenting complex and the costimulatory ligand are bound to the surface of the paramagnetic particles via a biotin-avidin or biotin-streptavidin binding pair.
- 20 82. The method of claim 59 wherein the phenotypic characterization comprises assaying for a marker selected from the group consisting of CD62L, CD45RA, PD-1, CTLA-4, Tim-3, Lag-3, and ICS.
83. The method of claim 49, further comprising the step of enriching for T cells in the sample that are specific for an antigen, prior to the step of screening.
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