

CLAIMS

What is claimed is:

1. A method of generating recombinant expression constructs for use in immune therapy in a mammal, comprising:
 - generating a first recombinant nucleic acid having a sequence that encodes a polytope, wherein the polytope comprises a plurality of filtered neoepitope sequences; wherein the polytope further comprises a trafficking element that directs the polytope to a sub-cellular location selected from the group consisting of a recycling endosome, a sorting endosome, and a lysosome; wherein the first recombinant nucleic acid comprises a first promoter operably linked to the sequence that encodes the polytope to drive expression of the polytope in the mammal; and
 - generating a second recombinant nucleic acid having the sequence that encodes the polytope, wherein the second recombinant nucleic acid comprises a second promoter operably linked to the sequence that encodes the polytope to drive expression of the polytope in a non-mammalian cell.
2. The method of claim 1 wherein the first promoter is a constitutive promoter or wherein the first promoter is inducible by hypoxia, IFN-gamma, or IL-8.
3. The method of any one of the preceding claims wherein the trafficking element is selected from the group consisting of a CD1b leader sequence, a CD1a tail, a CD1c tail, and a LAMP1-transmembrane sequence.
4. The method of any one of the preceding claims wherein the filtered neoepitope sequences are filtered by comparing tumor versus matched normal of the same patient.
5. The method of any one of the preceding claims wherein the filtered neoepitope sequences are filtered to have binding affinity to an MHC complex of equal or less than 200 nM.
6. The method of any one of claims 1-5 wherein the filtered neoepitope sequences bind to MHC-I and wherein the trafficking element directs the polytope to the recycling endosome, sorting endosome, or lysosome.

7. The method of any one of claims 1-5 wherein the filtered neoepitope sequences bind to MHC-II and wherein the trafficking element directs the polytope to the recycling endosome, sorting endosome, or lysosome.
8. The method of any one of the preceding claims wherein the first recombinant nucleic acid further comprises an additional sequence that encodes a second polytope, wherein the second polytope comprises a second trafficking element that directs the second polytope to a different sub-cellular location and wherein the second polytope comprises a second plurality of filtered neoepitope sequences.
9. The method of claim 8 wherein at least one of the filtered neoepitope sequences and at least one of the second filtered neoepitope sequences are identical.
10. The method of any one of the preceding claims wherein the first recombinant nucleic acid further comprises a sequence that encodes at least one of a co-stimulatory molecule, an immune stimulatory cytokine, and a protein that interferes with or down-regulates checkpoint inhibition.
11. The method of claim 10 wherein the co-stimulatory molecule is selected from the group consisting of CD80, CD86, CD30, CD40, CD30L, CD40L, ICOS-L, B7-H3, B7-H4, CD70, OX40L, 4-1BBL, GITR-L, TIM-3, TIM-4, CD48, CD58, TL1A, ICAM-1, and LFA3.
12. The method of claim 10 wherein the immune stimulatory cytokine is selected from the group consisting of IL-2, IL-12, IL-15, IL-15 super agonist (ALT803), IL-21, IPS1, and LMP1.
13. The method of claim 10 wherein the protein that interferes is an antibody or an antagonist of CTLA-4, PD-1, TIM1 receptor, 2B4, or CD160.
14. The method of any one of the preceding claims wherein the first recombinant nucleic acid is replicated in a bacterial cell or a yeast cell.
15. The method of any one of the preceding claims wherein the first recombinant nucleic acid is a shuttle vector for generation of a recombinant virus.

16. The method of claim 15 wherein the recombinant virus is an adenovirus, optionally with at least one of an E1 and an E2b gene deleted.
17. The method of any one of the preceding claims further comprising a step of formulating the first recombinant nucleic acid into a pharmaceutical formulation for injection.
18. The method of any one of the preceding claims wherein the second promoter is a constitutive bacterial or a yeast promoter.
19. The method of any one of the preceding claims wherein the non-mammalian cell is an *E. coli* cell or a *Saccharomyces cerevisiae* cell.
20. The method of any one of the preceding claims further comprising the steps of:
 - transfecting the second recombinant nucleic acid into a bacterial cell or a yeast cell;
 - expressing the polytope in the bacterial cell or the yeast cell; and
 - formulating the bacterial cell or the yeast cell into a pharmaceutical formulation for injection.
21. The method of claim 1 wherein the trafficking element is selected from the group consisting of a CD1b leader sequence, a CD1a tail, a CD1c tail, and a LAMP1-transmembrane sequence.
22. The method of claim 1 wherein the filtered neoepitope sequences are filtered by comparing tumor versus matched normal of the same patient.
23. The method of claim 1 wherein the filtered neoepitope sequences are filtered to have binding affinity to an MHC complex of equal or less than 200 nM.
24. The method of claim 1 wherein the filtered neoepitope sequences bind to MHC-I and wherein the trafficking element directs the polytope to the recycling endosome, sorting endosome, or lysosome.
25. The method of claim 1 wherein the filtered neoepitope sequences bind to MHC-II and wherein the trafficking element directs the polytope to the recycling endosome, sorting endosome, or lysosome.

26. The method of claim 1 wherein the first recombinant nucleic acid further comprises an additional sequence that encodes a second polytope, wherein the second polytope comprises a second trafficking element that directs the second polytope to a different sub-cellular location and wherein the second polytope comprises a second plurality of filtered neoepitope sequences.
27. The method of claim 26 wherein at least one of the filtered neoepitope sequences and at least one of the second filtered neoepitope sequences are identical.
28. The method of claim 1 wherein the first recombinant nucleic acid further comprises a sequence that encodes at least one of a co-stimulatory molecule, an immune stimulatory cytokine, and a protein that interferes with or down-regulates checkpoint inhibition.
29. The method of claim 28 wherein the co-stimulatory molecule is selected from the group consisting of CD80, CD86, CD30, CD40, CD30L, CD40L, ICOS-L, B7-H3, B7-H4, CD70, OX40L, 4-1BBL, GITR-L, TIM-3, TIM-4, CD48, CD58, TL1A, ICAM-1, and LFA3.
30. The method of claim 28 wherein the immune stimulatory cytokine is selected from the group consisting of IL-2, IL-12, IL-15, IL-15 super agonist (ALT803), IL-21, IPS1, and LMP1.
31. The method of claim 28 wherein the protein that interferes is an antibody or an antagonist of CTLA-4, PD-1, TIM1 receptor, 2B4, or CD160.
32. The method of claim 1 wherein the first recombinant nucleic acid is replicated in a bacterial cell or a yeast cell.
33. The method of claim 1 wherein the first recombinant nucleic acid is a shuttle vector for generation of a recombinant virus.
34. The method of claim 33 wherein the recombinant virus is an adenovirus, optionally with at least one of an E1 and an E2b gene deleted.
35. The method of claim 1 further comprising a step of formulating the first recombinant nucleic acid into a pharmaceutical formulation for injection.

36. The method of claim 1 wherein the second promoter is a constitutive bacterial or a yeast promoter.
37. The method of claim 1 wherein the non-mammalian cell is an *E. coli* cell or a *Saccharomyces cerevisiae* cell.
38. The method of claim 1 further comprising the steps of:
transfecting the second recombinant nucleic acid into a bacterial cell or a yeast cell;
expressing the polytope in the bacterial cell or the yeast cell; and
formulating the bacterial cell or the yeast cell into a pharmaceutical formulation for injection.
39. A recombinant bacterial or yeast expression vector for immune therapy of a mammal, comprising:
a recombinant nucleic acid having a sequence that encodes a polytope operably linked to a bacterial or yeast promoter to drive expression of the polytope;
wherein the polytope comprises a trafficking element that directs the polytope to a sub-cellular location of a mammalian immune competent cell selected from the group consisting of recycling endosome, sorting endosome, and lysosome;
and
wherein the polytope comprises a plurality of filtered neoepitope sequences.
40. The vector of claim 39 wherein the promoter is a constitutive promoter.
41. The vector of any one of claims 39-40 wherein the trafficking element is selected from the group consisting of a CD1b leader sequence, a CD1a tail, a CD1c tail, and a LAMP1-transmembrane sequence.
42. The vector of any one of claims 39-41 wherein the filtered neoepitope sequences are filtered by comparing tumor versus matched normal of the same patient.
43. The vector of any one of claims 39-42 wherein the filtered neoepitope sequences bind to MHC-I and wherein the trafficking element directs the polytope to the recycling endosome, sorting endosome, or lysosome.

44. The vector of any one of claims 39-42 wherein the filtered neoepitope sequences bind to MHC-II and wherein the trafficking element directs the polytope to the recycling endosome, sorting endosome, or lysosome.
45. The vector of any one of claims 39-45 wherein the recombinant nucleic acid further comprises an additional sequence that encodes a second polytope, wherein the second polytope comprises a second trafficking element that directs the second polytope to a different sub-cellular location and wherein the second polytope comprises a second plurality of filtered neoepitope sequences.
46. The vector of claim 45 wherein at least one of the filtered neoepitope sequences and at least one of the second filtered neoepitope sequences are identical.
47. The vector of any one of claims 39-46 wherein the expression vector is a bacterial expression vector.
48. The vector of any one of claims 39-46 wherein the expression vector is a yeast expression vector.
49. The vector of claim 39 wherein the trafficking element is selected from the group consisting of a CD1b leader sequence, a CD1a tail, a CD1c tail, and a LAMP1-transmembrane sequence.
50. The vector of claim 39 wherein the filtered neoepitope sequences are filtered by comparing tumor versus matched normal of the same patient.
51. The vector of claim 39 wherein the filtered neoepitope sequences bind to MHC-I and wherein the trafficking element directs the polytope to the recycling endosome, sorting endosome, or lysosome.
52. The vector of claim 39 wherein the filtered neoepitope sequences bind to MHC-II and wherein the trafficking element directs the polytope to the recycling endosome, sorting endosome, or lysosome.
53. The vector of claim 39 wherein the recombinant nucleic acid further comprises an additional sequence that encodes a second polytope, wherein the second polytope comprises a second trafficking element that directs the second polytope to a different sub-

cellular location and wherein the second polytope comprises a second plurality of filtered neoepitope sequences.

54. The vector of claim 53 wherein at least one of the filtered neoepitope sequences and at least one of the second filtered neoepitope sequences are identical.
55. The vector of claim 39 wherein the expression vector is a bacterial expression vector.
56. The vector of claim 39 wherein the expression vector is a yeast expression vector.
57. A recombinant yeast cell transfected with the vector of any one of claims 40-48.
58. A recombinant yeast cell transfected with the vector of any one of claims 49-56.
59. A recombinant bacterial cell transfected with the vector of any one of claims 40-48.
60. A recombinant bacterial cell transfected with the vector of any one of claims 49-56.
61. A pharmaceutical composition comprising the recombinant yeast cell of claim 57.
62. A pharmaceutical composition comprising the recombinant yeast cell of claim 58.
63. A pharmaceutical composition comprising the recombinant bacterial cell of claim 59.
64. A pharmaceutical composition comprising the recombinant bacterial cell of claim 60.
65. (DNA/cell-based) A method of preparing first and second treatment compositions for an individual having a tumor, comprising:
- identifying a plurality of expressed neoepitope sequences from omics data of the tumor, wherein each of the expressed neoepitope sequences have a calculated binding affinity of equal or less than 500 nM to at least one of MHC-I and MHC-II of the individual;
 - generating a first recombinant nucleic acid having a sequence that encodes a polytope, wherein the polytope comprises the plurality of expressed neoepitope sequences;
 - wherein the polytope further comprises a trafficking element that directs the polytope to a sub-cellular location selected from the group consisting of a recycling endosome, a sorting endosome, and a lysosome;

wherein the first recombinant nucleic acid comprises a first promoter operably linked to the sequence that encodes the polytope to drive expression of the polytope in a cell of the individual;

formulating the first recombinant nucleic acid into a DNA vaccine formulation to so obtain the first treatment composition;

generating a second recombinant nucleic acid that includes the sequence that encodes the polytope, wherein the second recombinant nucleic acid comprises a second promoter operably linked to the sequence that encodes the polytope to drive expression of the polytope in a bacterial cell or a yeast cell;

transfecting the bacterial cell or the yeast cell with the second recombinant nucleic acid and expressing the polytope in the bacterial cell or the yeast cell; and

formulating the transfected bacterial cell or the yeast cell into a cell-based vaccine formulation to so obtain the second treatment composition.

66. The method of claim 65 wherein the plurality of expressed neoepitope sequences are identified using incremental synchronous alignment of omics data from the tumor and omics data from a non-tumor sample of the same individual.
67. The method of any one of claims 65-66 wherein the first recombinant nucleic acid is an expression vector.
68. The method of any one of claims 65-67 wherein the trafficking element is selected from the group consisting of a CD1b leader sequence, a CD1a tail, a CD1c tail, and a LAMP1-transmembrane sequence.
69. The method of any one of claims 65-68 wherein the second promoter is a constitutive bacterial or a yeast promoter.
70. The method of any one of claims 65-69 wherein the bacterial cell or the yeast cell is an *E. coli* cell or a *Saccharomyces cerevisiae* cell.
71. The method of any one of claims 65-70 wherein the cell-based vaccine formulation is formulated for injection.
72. The method of any one of claims 65-71 further comprising a step of generating a third recombinant nucleic acid that is a viral expression vector that includes the sequence that

encodes the polytope, and wherein the third recombinant nucleic acid comprises a third promoter operably linked to the sequence that encodes the polytope to drive expression of the polytope in a cell of the individual.

73. The method of claim 72 wherein the third promoter is a constitutive promoter or wherein the third promoter is inducible by hypoxia, IFN-gamma, or IL-8.

74. The method of claim 65 wherein the first recombinant nucleic acid is an expression vector.

75. The method of claim 65 wherein the trafficking element is selected from the group consisting of a CD1b leader sequence, a CD1a tail, a CD1c tail, and a LAMP1-transmembrane sequence.

76. The method of claim 65 wherein the second promoter is a constitutive bacterial or a yeast promoter.

77. The method of claim 65 wherein the bacterial cell or the yeast cell is an *E. coli* cell or a *Saccharomyces cerevisiae* cell.

78. The method of claim 65 wherein the cell-based vaccine formulation is formulated for injection.

79. The method of claim 65 further comprising a step of generating a third recombinant nucleic acid that is a viral expression vector that includes the sequence that encodes the polytope, and wherein the third recombinant nucleic acid comprises a third promoter operably linked to the sequence that encodes the polytope to drive expression of the polytope in a cell of the individual.

80. The method of claim 79 wherein the third promoter is a constitutive promoter or wherein the third promoter is inducible by hypoxia, IFN-gamma, or IL-8.

81. (AdV/cell based) A method of preparing first and second treatment compositions for an individual having a tumor, comprising:

identifying a plurality of expressed neoepitope sequences from omics data of the tumor, wherein the expressed neoepitope sequences have a calculated binding

affinity of equal or less than 500 nM to at least one of MHC-I and MHC-II of the individual;

generating a first recombinant nucleic acid having a sequence that encodes a polytope, wherein the polytope comprises the plurality of expressed neoepitope sequences, wherein the first recombinant nucleic acid is a viral expression vector;

wherein the polytope further comprises a trafficking element that directs the polytope to a sub-cellular location selected from the group consisting of a recycling endosome, a sorting endosome, and a lysosome;

wherein the first recombinant nucleic acid comprises a first promoter operably linked to the sequence that encodes the polytope to drive expression of the polytope in a cell of the individual;

forming viral particles from the viral expression vector and formulating the viral particles into a viral vaccine formulation to so obtain the first treatment composition;

generating a second recombinant nucleic acid having the sequence that encodes the polytope, wherein the second recombinant nucleic acid comprises a second promoter operably linked to the sequence that encodes the polytope to drive expression of the polytope in a non-mammalian cell;

transfecting a bacterial cell or a yeast cell with the second recombinant nucleic acid and expressing the polytope in the bacterial cell or the yeast cell; and

formulating the transfected bacterial cell or the yeast cell into a cell-based vaccine formulation to so obtain the second treatment composition.

82. The method of claim 81 wherein the plurality of expressed neoepitope sequences are identified using incremental synchronous alignment of omics data from the tumor and omics data from a non-tumor sample of the same individual.
83. The method of any one of claims 81-82 wherein the trafficking element is selected from the group consisting of a CD1b leader sequence, a CD1a tail, a CD1c tail, and a LAMP1-transmembrane sequence.
84. The method of any one of claims 81-83 wherein the first promoter is a constitutive promoter or wherein the first promoter is inducible by hypoxia, IFN-gamma, or IL-8.

85. The method of any one of claims 81-84 wherein the viral expression vector is an adenoviral expression vector, optionally having E1 and E2b genes deleted.
86. The method of any one of claims 81-85 wherein the second promoter is a constitutive bacterial or a yeast promoter.
87. The method of any one of claims 81-86 wherein the non-mammalian cell or the yeast cell is an *E. coli* cell or a *Saccharomyces cerevisiae* cell.
88. The method of any one of claims 81-87 wherein the viral vaccine formulation and the cell-based vaccine formulation are formulated for injection.
89. The method of claim 81 wherein the trafficking element is selected from the group consisting of a CD1b leader sequence, a CD1a tail, a CD1c tail, and a LAMP1-transmembrane sequence.
90. The method of claim 81 wherein the first promoter is a constitutive promoter or wherein the first promoter is inducible by hypoxia, IFN-gamma, or IL-8.
91. The method of claim 81 wherein the viral expression vector is an adenoviral expression vector, optionally having E1 and E2b genes deleted.
92. The method of claim 81 wherein the second promoter is a constitutive bacterial or a yeast promoter.
93. The method of claim 81 wherein the non-mammalian cell or the yeast cell is an *E. coli* cell or a *Saccharomyces cerevisiae* cell.
94. The method of claim 81 wherein the viral vaccine formulation and the cell-based vaccine formulation are formulated for injection.