CLAIMS

1. A method of generating recombinant expression constructs, the method comprising:
   generating a first recombinant nucleic acid having a sequence that encodes a polytope, wherein the polytope comprises a plurality of filtered neoepitope sequences, the filtered neoepitope sequences are filtered to have binding affinity to an MHC complex of equal or less than 200 nM;
   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 mammalian cell; 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 claims 1-4 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.

6. The method of any one of claims 1-4 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.

7. 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.

8. The method of claim 7 wherein at least one of the filtered neoepitope sequences and at least one of the second filtered neoepitope sequences are identical.

9. 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.

10. The method of claim 9 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.

11. The method of claim 9 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.

12. The method of claim 9 wherein the protein that interferes is an antibody or an antagonist of CTLA-4, PD-1, TIM1 receptor, 2B4, or CD160.

13. 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.

14. 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.

15. The method of claim 14 wherein the recombinant virus is an adenovirus, optionally with at least one of an E1 and an E2b gene deleted.

16. 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.

17. The method of claim 1 further comprising a step of using the generated recombinant expression constructs in immune therapy in a mammal.

18. 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 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.