Claims

1. A recombinant multigenome retroviral vector preparation comprising:

   a. a first retroviral particle comprising two copies of a first retroviral vector genome comprising a first sequence of interest; wherein the first sequence of interest encodes a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain;

   b. a second retroviral particle comprising two copies of a second retroviral vector genome comprising a second sequence of interest; wherein the second sequence of interest encodes a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain; and

   c. a third retroviral particle comprising one copy of the first retroviral vector genome comprising the first sequence of interest and one copy of the second retroviral vector genome comprising the second sequence of interest;

   wherein the first and second sequences of interest are different; and

   wherein the retroviral vector preparation is pseudotyped with a heterologous envelope glycoprotein.

2. The recombinant multigenome retroviral vector preparation of claim 1, wherein the heterologous envelope glycoprotein is selected from the group consisting of VSVg, measles envelope glycoprotein, and a Sindbis envelope glycoprotein.

3. The recombinant multigenome retroviral vector preparation of any one of claims 1-2, wherein the retroviral vector preparation is a lentiviral vector preparation.

4. The recombinant multigenome retroviral vector preparation of any one of claims 1-3, wherein the first sequence of interest encodes a tumor-associated antigen or one or more neoantigens and the second sequence of interest encodes an immunomodulatory molecule.

5. The recombinant multigenome retroviral vector preparation of any one of claims 1-3, wherein the first sequence of interest encodes a tumor-associated antigen and the second sequence of interest encodes a different tumor-associated antigen.

6. A retroviral vector packaging system for producing a pseudotyped multigenome retroviral vector preparation, comprising:

a. a first nucleic acid molecule encoding a heterologous viral envelope protein;

b. a second nucleic acid molecule encoding gag and pol proteins;

c. a third nucleic acid molecule encoding rev;

d. a fourth nucleic acid molecule comprising a first retroviral vector genome comprising a first sequence of interest; wherein the first sequence of interest encodes a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain;

e. a fifth nucleic acid molecule comprising a second retroviral vector genome comprising a second sequence of interest; wherein the second sequence of interest encodes a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain;

f. a sixth nucleic acid molecule encoding vpx; and

g. a packaging cell or cell line.

7. The retroviral vector packaging system of claim 6 wherein the retroviral vector preparation is a lentiviral vector preparation.

8. The retroviral vector packaging system of claim 6 or claim 7 wherein the first sequence of interest encodes a tumor-associated antigen or one or more neoantigens and the second sequence of interest encodes an immunomodulatory molecule.

9. The retroviral vector packaging system of claim 6 wherein the first sequence of interest encodes a tumor-associated antigen and the second sequence of interest encodes a different tumor-associated antigen.

10. The retroviral vector packaging system of any one of claims 6-9 wherein the viral envelope protein is selected from the group consisting of VSVg, measles envelope glycoprotein, and an alphavirus envelope glycoprotein.

11. The retroviral vector packaging system of any one of claims 6-10 wherein equal amounts of the fourth nucleic acid molecule and the fifth nucleic acid molecule are used.

12. The retroviral vector packaging system of any one of claims 6-10 wherein the relative input ratio of the fourth nucleic acid molecule to the fifth nucleic acid molecule used in the packaging system is 3:2.

13. The retroviral vector packaging system of any one of claims 7-12 wherein the first lentiviral vector genome and the second lentiviral vector genome comprise a palindromic dimer initiation site (DIS) sequence.

14. The retroviral vector packaging system of any one of claims 7-12 wherein the first lentiviral vector genome comprises a first palindromic DIS sequence and the second lentiviral vector genome comprise a second palindromic DIS sequence.

15. The retroviral vector packaging system of any one of claims 7-12 wherein the a palindromic DIS sequence in the first lentiviral vector genome and the second lentiviral vector genome is the same.

16. The retroviral vector packaging system of any one of claims 6-15 wherein the pol protein has a non-functional integrase.

17. The recombinant multigenome retroviral vector preparation of any one of claims 1-5, further comprising:

d. a fourth retroviral particle comprising two copies of a third retroviral vector genome comprising a third sequence of interest;

e. a fifth retroviral particle comprising one copy of the first retroviral vector genome comprising a first sequence of interest and one copy of the third retroviral vector genome comprising the third sequence of interest; and

f. a sixth retroviral particle comprising one copy of the second retroviral vector genome comprising a second sequence of interest and one copy of the third retroviral vector genome comprising the third sequence of interest.

18. The recombinant multigenome retroviral vector preparation of any one of claims 1-5 or claim 17 wherein the first retroviral vector genome comprises about 50% of the vector genomes in the multigenome vector preparation.

19. A packaging cell line, comprising:

a. at least two nucleic acid molecules each comprising a retroviral vector genome, a first nucleic acid molecule comprising a first sequence of interest and a second nucleic acid molecule comprising a second sequence of interest; wherein each of the first and second sequences of interest are different and encode a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain; and

b. one or more nucleic acid molecules encoding the components necessary to generate pseudotyped retroviral vector particles; wherein the components necessary to generate pseudotyped retroviral vector particles are selected from the group consisting of gag, pol, heterologous envelope, rev, and vpx proteins.

20. The packaging cell line of claim 19, consisting of 2 to 8 nucleic acid molecules each comprising a retroviral vector genome comprising a different sequence of interest.

21. The packaging cell line of claim 20, consisting of 5 nucleic acid molecules each comprising a retroviral vector genome comprising a different sequence of interest.

22. The packaging cell line of claim 21, wherein the relative input ratio of the 5 nucleic acid molecules each comprising a retroviral vector genome comprising a different sequence of interest is selected from one of the input ratios shown in Table 3.

23. The packaging cell line of claim 21, wherein the relative input ratio of the 5 nucleic acid molecules each comprising a retroviral vector genome comprising a different sequence of interest is 4:1:1:1:1.

24. The packaging cell line of any one of claims 19-23, wherein the different sequences of interest are selected from the group consisting of the sequences encoding MAGEA1, MAGEA4, NYESO1, MAGEA3, MAGEA10, ScFvanti-PD1, IL12, IL23, CD40, ScFvanti-PDL1, and ScFvanti-CTLA4, or an immunogenic variant of any of the foregoing having at least 80%-90% identity to the wild type sequence.

25. The packaging cell line of any one of claims 19-24 wherein the packaging cell line stably expresses one or more of the components necessary to generate retroviral vector particles.

26. A method for producing a pseudotyped multigenome retroviral vector preparation comprising culturing a packaging cell line transfected with

a. at least two nucleic acid molecules each comprising a retroviral vector genome, a first nucleic acid molecule comprising a first sequence of interest and a second nucleic acid molecule comprising a second

sequence of interest; wherein each of the first and second sequences of interest are different and encode a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain; and

b. one or more nucleic acid molecules encoding the components necessary to generate pseudotyped retroviral vector particles; wherein the components necessary to generate pseudotyped retroviral vector particles are selected from the group consisting of gag, pol, heterologous envelope, rev, and vpx proteins.

27. A recombinant multigenome retroviral vector preparation of any one of claims 1-5, 17, or 18 for use in therapy.

28. A recombinant multigenome retroviral vector preparation of any one of claims 1-5, 17, or 18 for use in a method of treatment of a patient.

29. A recombinant multigenome retroviral vector preparation of one of claims 1-5, 17, or 18 for use in a method of treating cancer in a patient.

30. A recombinant multigenome retroviral vector preparation of any one of claims 1-5, 17, or 18 for use in a method of stimulating an immune response in a patient.