construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

EXAMPLES

[0380] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

Ad5 [E1-, E2b-]-PSA (ETBX-071) Vaccine in Mice

[0381] This example describes pre-clinical testing of the Ad5 [E1-, E2b-]-PSA vaccine in a mouse model. Studies were performed to assess the use of Ad5 [E1-, E2b-]-PSA as a cancer vaccine in a BALB/c mouse model. Ad5 [E1-, E2b-]-PSA induced potent CMI against PSA in mice. Studies were also performed to show anti-tumor activity of the vaccine in a murine model of PSA expressing cancer. These data indicate that *in vivo* delivery of Ad5 [E1-, E2b-]-PSA can induce PSA directed anti-tumor immunity against PSA expressing cancers.

[0382] Pre-clinical studies were performed in a BALB/c murine model to demonstrate the immunogenicity of the Ad5 [E1-, E2b-]-PSA vaccine.

Induction of CMI responses after Ad5 [E1-, E2b-]-PSA immunization

[0383] To assess CMI induction by flow cytometry following multiple homologous immunizations with Ad5 [E1-, E2b-]-PSA, groups of Ad5 immune BALB/c mice (n=5/group) were immunized three times SC at 1-week intervals with 10^{10} VP of Ad5 [E1-, E2b-]-PSA. Control mice were injected with buffer solution only. Two weeks following the last immunization, splenocytes were harvested and exposed to PSA protein and assessed for CMI responses by ELISpot for IFN-γ or IL-2 secreting splenocytes.

[0384] PSA directed CMI responses were induced in vaccinated but not control mice (**FIGS. 1A and 1B**). Specificity of the CMI responses was demonstrated in ELISpot assays using irrelevant HIV-gag or cytomegalovirus virus (CMV) antigens (**FIGS. 2A and 2B**). Antibody

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising a replication-defective virus vector comprising a nucleic acid sequence encoding a prostate specific antigen (PSA) having an amino acid sequence at least 85% identical with SEQ ID NO: 1 or SEQ ID NO: 34, wherein the replication-defective virus vector is an adenovirus vector having deletions in the E1 gene region and the E2b gene region.

2. The composition of claim 1, wherein the vector comprises a nucleic acid sequence encoding a PSA having a nucleic acid sequence at least 85% identical with SEQ ID NO: 35 or the nucleic acid sequence encoding PSA has at least 99% identical with SEQ ID NO: 2.

3. The composition of claim 1, further comprising a second replication-defective virus vector comprising a second nucleic acid sequence encoding a Brachyury antigen, a third replication-defective virus vector comprising a third nucleic acid sequence encoding a MUC1 antigen, or a combination thereof.

4. The composition of claim 3, wherein the Brachyury antigen binds to HLA-A2, HLA-A3, HLA-A24, or a combination thereof.

5. The composition of claim 3 or 4, wherein the Brachyury antigen is a modified Brachyury antigen comprising an amino acid sequence set forth in WLLPGTSTV (SEQ ID NO: 7).

6. The composition of any of claims 3-5, wherein the Brachyury antigen is a modified Brachyury antigen comprising an amino acid sequence at least 85% identical to SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 42.

7. The composition of any of claims 3-6, wherein the second replication-defective vector comprises a nucleotide sequence at least 99% identical with SEQ ID NO: 3, SEQ ID NO: 4, positions 13 to 1242 of SEQ ID NO: 4, SEQ ID NO: 42.

8. The composition of any of claims 3-7, wherein the second replication-defective vector comprises a nucleotide sequence at least 99% to SEQ ID NO: 12 (Ad vector with sequence encoding a modified Brachyury antigen), positions 1033-2083 of SEQ ID NO: 12, or SEQ ID NO: 42.

9. The composition of any of claims 3-8, wherein the MUC1 antigen comprises a sequence at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, or at least 99% identical to SEQ ID NO: 10 or SEQ ID NO: 41.

10. The composition of any of claims 3-9, wherein the third nucleic acid sequence encoding a MUC1 antigen comprises at least 99% identity to SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 41.

11. The composition of any of claims 3-10, wherein the MUC-1 antigen binds to HLA-A2, HLA-A3, HLA-A24, or a combination thereof.

12. The composition of any of claims 3-11, wherein the replication-defective virus vector, the second replication-defective virus vector, and the third replication-defective virus vector are adenovirus vectors.

13. The composition of claim 12, wherein each of the adenovirus vectors also comprises a deletion in an E3 region, an E4 region, or a combination thereof.

14. The composition of claim 12 or 13, wherein each of the adenovirus vectors comprises a deletion in an E4 region.

15. The composition of any of claims 12-14, wherein each of the adenovirus vectors comprises a deletion in an E1 region, an E2b region, and an E3 region.

16. The composition of any one of claims 1-15, wherein the composition comprises from at least $1x10^9$ virus particles to at least $5x10^{12}$ virus particles.

17. The composition of any of claims 1-16, wherein the composition comprises at least $5x10^9$ virus particles.

18. The composition of any of claims 1-17, wherein the composition comprises at least $5x10^{10}$ virus particles.

19. The composition of any of claims 1-18, wherein the composition comprises at least $5x10^{11}$ virus particles.

20. The composition of any one of claims 1-19, wherein the composition comprises at least $5x10^{12}$ virus particles.

21. The composition of any of claims 1-20, wherein the composition or the replication-defective virus vector further comprises a nucleic acid sequences encoding a costimulatory molecule.

22. The composition of claim 21, wherein the costimulatory molecule comprises B7, ICAM-1, LFA-3, or a combination thereof.

23. The composition of claim 21 or 22, wherein the costimulatory molecule comprises a combination of B7, ICAM-1, and LFA-3.

24. The composition of any one of claims 1-23, wherein the composition further comprises a plurality of nucleic acid sequences encoding a plurality of costimulatory molecules positioned in the same replication-defective virus vector.

25. The composition of any one of claims 1-24, wherein the composition further comprises a plurality of nucleic acid sequences encoding a plurality of costimulatory molecules positioned in separate replication-defective virus vectors.

26. The composition of any one of claims 1-25, wherein the composition further comprises a nucleic acid sequence encoding one or more additional target antigens or immunological epitopes thereof.

27. The composition of any one of claims 1-26, wherein the replication-defective virus vector further comprises a nucleic acid sequence encoding one or more additional target antigens or immunological epitopes thereof.

28. The composition of claim 26 or 27, wherein the one or more additional target antigens is a tumor neo-antigen, tumor neo-epitope, tumor-specific antigen, tumor-associated antigen, tissue-specific antigen, bacterial antigen, viral antigen, yeast antigen, fungal antigen, protozoan antigen, parasite antigen, mitogen, or a combination thereof.

29. The composition of any one of claims 26-28, wherein the one or more additional target antigens is CEA, folate receptor alpha, WT1, HPV E6, HPV E7, p53, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A10, MAGE-A12, BAGE, DAM-6, -10, GAGE-1, -2, -8, GAGE-3, -4, -5, -6, -7B, NA88-A, NY-ESO-1, MART-1, MC1R, Gp100, PSCA, PSMA, PAP, Tyrosinase, TRP-1, TRP-2, ART-4, CAMEL, Cyp-B, Her2/neu, BRCA1, BRACHYURY, BRACHYURY(TIVS7-2, polymorphism), BRACHYURY (IVS7 T/C polymorphism), T BRACHYURY, T, hTERT, hTRT, iCE, MUC1, MUC1 (VNTR polymorphism), MUC1c, MUC1n, MUC2, PRAME, P15, RU1, RU2, SART-1, SART-3, WT1, AFP, β-catenin/m, Caspase-8/m, CDK-4/m, Her2/neu, Her3, ELF2M, GnT-V, G250, HSP70-2M, HST-2, KIAA0205, MUM-1, MUM-2, MUM-3, Myosin/m, RAGE, SART-2, TRP-2/INT2, 707-AP, Annexin II, CDC27/m,

TPI/mbcr-abl, ETV6/AML, LDLR/FUT, Pml/RARα, or TEL/AML1, a splice variant, a functional epitope, or a combination thereof.

30. The composition of any one of claims 26-29, wherein the one or more additional target antigens is CEA.

31. The composition of any one of claims 26-29, wherein the one or more additional target antigens is CEA, Brachyury, and MUC1.

32. The composition of claim 31, wherein CEA is at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, or at least 99% identical to SEQ ID NO: 37 or SEQ ID NO: 38.

33. The composition of any one of claims 26-29, wherein the one or more additional target antigens is HER3.

34. The composition of any one of claims 26-29, wherein the one or more additional target antigens is HPV E6 or HPV E7.

35. The composition of any one of claims 1-34, wherein the replication-defective virus vector further comprises a selectable marker.

36. The composition of claim 35, wherein the selectable marker is a lacZ gene, thymidine kinase, gpt, GUS, or a vaccinia K1L host range gene, or a combination thereof.

37. The composition of any one of claims 1-36, wherein the replication-defective virus vector further comprises a nucleic acid sequence encoding an immunological fusion partner.

38. The composition of claim 1, wherein the PSA has an amino acid sequence at least 90%, at least 92%, at least 95%, at least 97%, or at least 99% identical with SEQ ID NO: 1 or SEQ ID NO: 34.

39. The composition of claim 2, wherein the PSA has an amino acid sequence at least 90%, at least 92%, at least 95%, at least 97%, or at least 99% identical with SEQ ID NO: 35.

40. The composition of claims 6, wherein the modified Brachyury antigen comprises an amino acid sequence at least 90%, at least 92%, at least 95%, at least 97%, or at least 99% identical to SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 42.

41. A pharmaceutical composition comprising the composition according to any one of claims 1-40 and a pharmaceutically acceptable carrier.

42. A host cell comprising the composition according to any one of claims 1-40.

43. A method of preparing a tumor vaccine, the method comprising preparing a pharmaceutical composition according to claim 41.

44. The composition of any of claims 1-40 for use in therapy.

45. Use of the composition according to any one of claims 1-40 in the manufacture of a pharmaceutical composition for treating a PSA-expressing cancer in a subject in need thereof.