CLAIMS:

1. A recombinant adenovirus comprising a nucleotide sequence inserted in an insertion site, wherein the insertion site is located between the stop codon of a first viral transcription unit and the stop codon of a second viral transcription unit,
wherein the stop codon of the first viral transcription unit is nearer to the stop codon of the second viral transcription unit than the start site of the first viral transcription unit is to the stop codon of the second viral transcription unit,
wherein the stop codon of the second viral transcription unit is nearer to the stop codon of the first viral transcription unit than the start site of the second viral transcription unit is to the stop codon of the first viral transcription unit, and
wherein there is no viral transcription unit between the first viral transcription unit and the second viral transcription unit before the nucleotide sequence is inserted.

2. The recombinant adenovirus of claim 1, wherein the first viral transcription unit is adenovirus IX gene and the second viral transcription unit is adenovirus IVa2 gene.

3. The recombinant adenovirus of claim 1, wherein the first viral transcription unit is adenovirus fiber gene and the second viral transcription unit is ORF6 or ORF6/7 of adenovirus E4 gene.

4. A recombinant adenovirus comprising a nucleotide sequence inserted in an IX-E2 insertion site, wherein the IX-E2 insertion site is located between the stop codon of adenovirus IX gene and the stop codon of adenovirus IVa2 gene.

5. The recombinant adenovirus of any one of claims 1, 2, and 4, wherein the nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4093 of the Ad5 genome (SEQ ID NO: 1).

6. The recombinant adenovirus of any one of claims 1, 2, and 4, wherein the nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3970 of the Ad35 genome (SEQ ID NO: 41).

7. The recombinant adenovirus of any one of claims 1, 2, 4, and 5, wherein the nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4050, nucleotides corresponding to 4050 and 4070, or nucleotides corresponding to 4070 and 4093 of the Ad5 genome (SEQ ID NO: 1).

8. The recombinant adenovirus of any one of claims 1, 2, 4, and 6, wherein the nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3920, nucleotides corresponding to 3920 and 3940, or nucleotides corresponding to 3940 and 3970 of the Ad35 genome (SEQ ID NO: 41).

9. A recombinant adenovirus comprising a nucleotide sequence inserted in an L5-E4 insertion site, wherein the L5-E4 insertion site is located between the stop codon of adenovirus fiber gene and the stop codon of ORF6 or ORF6/7 of adenovirus E4 gene.

10. The recombinant adenovirus of any one of claims 1, 3, and 9, wherein the nucleotide sequence is inserted between nucleotides corresponding to 32785 to 32916 of the Ad5 genome (SEQ ID NO: 1).

11. The recombinant adenovirus of any one of claims 1, 3, 9, and 10, wherein the nucleotide sequence is inserted between nucleotides corresponding to 32785 and 32800, nucleotides corresponding to 32800 and 32820, nucleotides corresponding to 32820 and 32840, nucleotides corresponding to 32840 and 32860, nucleotides corresponding to 32860 and 32880, nucleotides corresponding to 32880 and 32900, or nucleotides corresponding to 32900 and 32916 of the Ad5 genome (SEQ ID NO: 1).

12. The recombinant adenovirus of any one of claims 1, 3, and 9, wherein the nucleotide sequence is inserted between nucleotides corresponding to 31799 and 31821 of the Ad35 genome (SEQ ID NO: 41).

13. The recombinant adenovirus of any one of claims 1, 3, 9, and 12, wherein the nucleotide sequence is inserted between nucleotides corresponding to 31799 and

~~32810, or nucleotides corresponding to 32810 and 31821 of the Ad35 genome (SEQ ID NO: 41).~~

~~14. A recombinant adenovirus comprising a first nucleotide sequence inserted in an IX-E2 insertion site and a second nucleotide sequence inserted in an L5-E4 insertion site, wherein the IX-E2 insertion site is located between the stop codon of adenovirus IX gene and the stop codon of adenovirus IVa2 gene, and wherein the L5-E4 insertion site is located between the stop codon of adenovirus fiber gene and the stop codon of ORF6 or ORF6/7 of adenovirus E4 gene.~~

~~15. The recombinant adenovirus of claim 14, wherein the first nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4093 of the Ad5 genome (SEQ ID NO: 1).~~

~~16. The recombinant adenovirus of claim 14 or claim 15, wherein the first nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4050, nucleotides corresponding to 4050 and 4070, or nucleotides corresponding to 4070 and 4093 of the Ad5 genome (SEQ ID NO: 1).~~

~~17. The recombinant adenovirus of claim 14, wherein the first nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3970 of the Ad35 genome (SEQ ID NO: 41).~~

~~18. The recombinant adenovirus of claim 14 or claim 15, wherein the first nucleotide sequence is inserted between nucleotides corresponding to 3899 and 3920, nucleotides corresponding to 3920 and 3940, or nucleotides corresponding to 3940 and 3970 of the Ad35 genome (SEQ ID NO: 41).~~

~~19. The recombinant adenovirus of any one of claims 14-18, wherein the second nucleotide sequence is inserted between nucleotides corresponding to 32785 to 32916 of the Ad5 genome (SEQ ID NO: 1).~~

20. The recombinant adenovirus of any one of claims 14-19, wherein the second nucleotide sequence is inserted between nucleotides corresponding to 32785 and 32800, nucleotides corresponding to 32801 and 32820, nucleotides corresponding to 32821 and 32840, nucleotides corresponding to 32841 and 32860, nucleotides corresponding to 32861 and 32880, nucleotides corresponding to 32881 and 32900, or nucleotides corresponding to 32901 and 32916 of the Ad5 genome (SEQ ID NO: 1).

21. The recombinant adenovirus of any one of claims 14-18, wherein the second nucleotide sequence is inserted between nucleotides corresponding to 31799 and 31821 of the Ad5 genome (SEQ ID NO: 41).

22. The recombinant adenovirus of any one of claims 14-18 and 21, wherein the second nucleotide sequence is inserted between nucleotides corresponding to 31799 and 32810, or nucleotides corresponding to 32810 and 31821 of the Ad35 genome (SEQ ID NO: 41).

23. The recombinant adenovirus of any one of claims 4-8 and 14-22, wherein the IX-E2 insertion site comprises a deletion of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 nucleotides.

24. The recombinant adenovirus of any one of claims 9-23, wherein the L5-E4 insertion site comprises a deletion of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or 130 nucleotides.

25. The recombinant adenovirus of any one of claims 1-24, wherein the nucleotide sequence comprises at least one transgene.

26. The recombinant adenovirus of claim 25, wherein the nucleotide sequence further comprises a promoter, wherein the transgene is operably linked to the promoter.

27. The recombinant adenovirus of any one of claims 1-26 comprises, in a 5' to 3' orientation:

(i) a first polyadenylation signal;

(ii) a promoter;

(iii) a transgene;

(iv) a second polyadenylation signal; and

(v) a third polyadenylation signal;

wherein the transgene is operably linked to the promoter, wherein the transgene is inserted between the first polyadenylation signal and the third polyadenylation signal.

28. The recombinant adenovirus of claim 27, wherein the first polyadenylation signal is the polyadenylation signal of the fiber (L5) gene, the second polyadenylation signal is the polyadenylation signal of the transgene, and the third polyadenylation signal is the polyadenylation signal of the ORF6 or ORF6/7 of the adenovirus E4 gene, or the first polyadenylation signal is the polyadenylation signal of the IX gene, the second polyadenylation signal is the polyadenylation signal of the transgene, and the third polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene.

29. The recombinant adenovirus of any one of claims 1-26 comprises, in a 5' to 3' orientation:

(i) a first polyadenylation signal;

(ii) a second polyadenylation signal;

(iii) a promoter;

(iv) a transgene;

(v) a third polyadenylation signal; and

(vi) a fourth polyadenylation signal;

wherein the transgene is operably linked to the promoter, wherein the transgene is inserted between the first polyadenylation signal and the fourth polyadenylation signal, and wherein the second polyadenylation signal is in the opposite transcriptional direction of the first polyadenylation signal.

30. The recombinant adenovirus of claim 29, wherein the first polyadenylation signal is the polyadenylation signal of the fiber (L5) gene, the third polyadenylation signal is the polyadenylation signal of the transgene, and the fourth polyadenylation signal is the polyadenylation signal of the ORF6 or ORF6/7 of the adenovirus E4 gene, or the first polyadenylation signal is the polyadenylation signal of the IX gene, the third

polyadenylation signal is the polyadenylation signal of the transgene, and the fourth polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene.

31. The recombinant adenovirus of any one of claims 26-30, wherein the promoter is a ubiquitous promoter, a tissue-specific promoter, or tumor-specific promoter.

32. The recombinant adenovirus of any one of claims 1-31, wherein the recombinant adenovirus further comprises a nucleotide sequence inserted in an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site.

33. The recombinant adenovirus of any one of claims 1-32, wherein the nucleotide sequence comprises a first nucleotide sequence comprising a first transgene and a second nucleotide sequence comprising a second transgene, wherein the first nucleotide sequence and the second nucleotide sequence are separated by a linker.

34. The recombinant adenovirus of claim 33, wherein the linker encodes a peptide cleavable by a protease or proteases.

35. The recombinant adenovirus of claim 33 or claim 34, wherein the linker encodes an internal ribosome entry site (IRES) or a self-cleaving 2A peptide.

36. The recombinant adenovirus of claim 32, wherein the E1b-19K insertion site is located between the start site of Elb-19K and the stop codon of Elb-19K.

37. The recombinant adenovirus of claim 32 or claim 36, wherein the E1b-19K insertion site comprises a deletion of from about 100 to about 305, about 100 to about 300, about 100 to about 250, about 100 to about 200, about 100 to about 150, about 150 to about 305, about 150 to about 300, about 150 to about 250, or about 150 to about 200 nucleotides adjacent the start site of E1b-19K.

38. The recombinant adenovirus of any one of claims 32, 36, and 37, wherein the E1b-19K insertion site comprises a deletion of about 200 nucleotides adjacent the start site of E1b-19K.

39. The recombinant adenovirus of any one of claims 32, 36, and 37, wherein the E1b-19K insertion site comprises a deletion corresponding to nucleotides 1714-1917 of the Ad5 genome (SEQ ID NO: 1).

40. The recombinant adenovirus of any one of claims 32, 36, and 37, wherein the E1b-19K insertion site comprises a deletion corresponding to nucleotides 1611-2153 of the Ad35 genome (SEQ ID NO: 41).

41. The recombinant adenovirus of claim 32, wherein the E3 insertion site is located between the stop codon of adenovirus pVIII gene and the start site of adenovirus fiber gene.

42. The recombinant adenovirus of claim 32 or claim 41, wherein the E3 insertion site wherein the E3 insertion site is located between the stop codon of E3-10.5K and the stop codon of E3-14.7K.

43. The recombinant adenovirus any one of claims 32, 41, and 42, wherein the E3 insertion site comprises a deletion of from about 500 to about 3185, from about 500 to about 3000, from about 500 to about 2500, from about 500 to about 2000, from about 500 to about 1500, from about 500 to about 1000, from about 1000 to about 3185, from about 1000 to about 3000, from about 1000 to about 2500, from about 1000 to about 2000, from about 1000 to about 1500, from about 1500 to about 3185, from about 1500 to about 3000, from about 1500 to about 2000, from about 2000 to about 3185, from about 2000 to about 3000, from about 2000 to about 2500, from about 2500 to about 3185, from about 2500 to about 3000, or about 3000 to about 3185 nucleotides.

44. The recombinant adenovirus of any one of claims 32 and 41-43, wherein the E3 insertion site comprises a deletion of from about 500 to about 1551, from about 500 to about 1500, from about 500 to about 1000, from about 1000 to about 1551, from about 1000 to about 1500, or from about 1500 to about 1551 nucleotides adjacent the stop codon of E3-10.5K.

45. The recombinant adenovirus of any one of claims 32 and 41-44, wherein the E3 insertion site comprises a deletion of about 1,050 nucleotides adjacent the stop codon of E3-10.5K.

46. The recombinant adenovirus any one of claims 32 and 41-44, wherein the E3 insertion site comprises a deletion of 1,063 nucleotides following the stop codon of E3-10.5K.

47. The recombinant adenovirus any one of claims 32 and 41-43, wherein the E3 insertion site comprises a deletion corresponding to the Ad5 dl309 E3 deletion.

48. The recombinant adenovirus any one of claims 32 and 41-47, wherein the E3 insertion site comprises a deletion corresponding to nucleotides 29773-30836 of the Ad5 genome (SEQ ID NO: 1).

49. The recombinant adenovirus any one of claims 32 and 41-46, wherein the E3 insertion site comprises a deletion corresponding to nucleotides 29119-30622 of the Ad35 genome (SEQ ID NO: 41).

50. The recombinant adenovirus of any one of claims 1-49, wherein the recombinant adenovirus comprises an E1a promoter having one or more deletions of a functional Pea3 binding site.

51. The recombinant adenovirus of claim 50, wherein the deletion comprises a deletion of nucleotides corresponding to about -300 to about -250 upstream of the initiation site of Ela.

52. The recombinant adenovirus of claim 50, wherein the deletion comprises a deletion of nucleotides corresponding to -305 to -255 upstream of the initiation site of Ela.

53. The recombinant adenovirus of claim 50, wherein the deletion comprises a deletion of nucleotides corresponding to 195-244 of the Ad5 genome (SEQ ID NO: 1).

54. The recombinant adenovirus of claim 50, wherein the E1a promoter comprises the sequence GGTGTTTTGG (SEQ ID NO: 4).

55. The recombinant adenovirus of any one of claims 1-54, wherein the recombinant adenovirus comprises one or more Pea3 transcription binding site deletions without one or more E2F transcription binding site deletions in the E1A region.

56. The recombinant adenovirus of any one of claims 1-54, wherein the recombinant adenovirus comprises one or more E2F transcription binding site deletions without one or more Pea3 transcription binding site deletions in the E1A region.

57. The recombinant adenovirus of any one of claims 1-48, wherein the recombinant adenovirus comprises a modified TATA box-based promoter operably linked to a gene, wherein the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative cell and/or a modified CAAT box-based promoter operably linked to a gene, wherein the modified CAAT box-based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative cell.

58. The recombinant adenovirus of claim 57, wherein the modified TATA box-based promoter is an early gene promoter.

59. The recombinant adenovirus of claim 58, wherein the modified TATA box-based promoter is an E1a promoter, E1b promoter, or E4 promoter.

60. The recombinant adenovirus of claim 59, wherein the modified TATA box-based promoter is an E1a promoter.

61. The recombinant adenovirus of any one of claims 57-60, wherein the modification included in the modified TATA box-based promoter comprises a deletion of the entire TATA box.

~~62. The recombinant adenovirus of any one of claims 57-60, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to -27 to -24 of the E1a promoter.~~

~~63. The recombinant adenovirus of any one of claims 57-62, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to -31 to -24 of the E1a promoter.~~

~~64. The recombinant adenovirus of any one of claims 57-63, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to -44 to +54 of the E1a promoter.~~

~~65. The recombinant adenovirus of any one of claims 57-64, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to -146 to +54 of the E1a promoter.~~

~~66. The recombinant adenovirus of any one of claims 57-60, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 472 to 475 of the Ad5 genome (SEQ ID NO: 1).~~

~~67. The recombinant adenovirus of claim 66, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 468 to 475 of the Ad5 genome (SEQ ID NO: 1).~~

~~68. The recombinant adenovirus of claim 67, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 455 to 552 of the Ad5 genome (SEQ ID NO: 1).~~

~~69. The recombinant adenovirus of claim 68, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 353 to 552 of the Ad5 genome (SEQ ID NO: 1).~~

70. The recombinant adenovirus of any one of claims 57-69, wherein the recombinant adenovirus comprises a polynucleotide deletion that results in a virus comprising the sequence CTAGGACTG (SEQ ID NO: 5), AGTGCCCG (SEQ ID NO: 44) and/or TATTCCCG (SEQ ID NO: 45).

71. The recombinant adenovirus of claim 57, wherein the modified CAAT box-based promoter is an early gene promoter.

72. The recombinant adenovirus of claim 71, wherein the modified CAAT box-based promoter is an E1a promoter, E1b promoter, or E4 promoter.

73. The recombinant adenovirus of claim 72, wherein the modified CAAT box-based promoter is an E1a promoter.

74. The recombinant adenovirus of any one of claims 71-73, wherein the modification included in the modified CAAT box-based promoter comprises a deletion of the entire CAAT box.

75. The recombinant adenovirus of any one of claims 71-74, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to -76 to -68 of the E1a promoter.

76. The recombinant adenovirus of any one of claims 71-74, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 423 to 431 of the Ad5 genome (SEQ ID NO: 1).

77. The recombinant adenovirus of any one of claims 71-76, wherein the recombinant adenovirus comprises a polynucleotide deletion that results in a virus comprising the sequence TTCCGTGGCG (SEQ ID NO: 46).

78. The recombinant adenovirus of any one of claims 57-80, wherein the recombinant adenovirus comprises a deletion of nucleotides corresponding to 477 to 484 of the Ad35 genome (SEQ ID NO: 41).

79. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes a monomeric, dimeric, trimeric, tetrameric, or multimeric protein.

80. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes a RNA that has a therapeutic activity.

81. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes a fusion protein comprising at least one binding domain.

82. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes an immunomodulatory molecule.

83. The recombinant adenovirus of claim 82, wherein the immunomodulatory molecule is a costimulatory ligand, a cytokine, or a cytokine receptor.

84. The recombinant adenovirus of claim 82 or claim 83, wherein the immunomodulatory molecule is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-7, IL-10, IL-10 trap, IL-10R, IL-12A/p35, IL-12B/p40, IL-15, IL-23A/p19, IL24, IL-27, IL-33, IL-35, IL-15, an IL-15 receptor fusion protein, TGF-β, a TGF-β trap, an IL-10 trap, VEGF, indoleamin-2,3-dioxygenase (IDO), inducible T-cell co-stimulator ligand (ICOS-L), CD80, CD137L, TNF-α, IFN-α, IFN- β, IFN-γ, or GM-CSF, GITR ligand (GITRL), OX40 ligand (OX40L), CD40 ligand (CD40L), drug-inducible CD40 (iCD40), CD154, CD70, CD86, CD137, CD137L, BORIS/CTCFL, bone morphogenetic protein (BMP), TNFSF9, FGF, ICAM, Podocalyxin, functional fragments thereof, and derivatives thereof.

85. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes an antigen-binding molecule.

86. The recombinant adenovirus of claim 85, wherein the antigen-binding molecule is an anti-PD-1 antibody, an anti-TGF-β antibody, an anti-PD-L1 antibody, and an anti-CTLA-4 antibody, or functional fragments thereof.

87. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes an antigen or a ligand to the antigen.

88. The recombinant adenovirus of claim 87, wherein the antigen is selected from the group consisting of CAIX, CEA, CD5, CD7, CD10, CD19, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD80, CD133, CD135 (Flt3), Flt3I, CD138, a cytomegalovirus (CMV) infected cell antigen, 4-1BB, EGP-2, EGP-40, EpCAM, erbB2, erbB3, erbB4, FBP, Fetal acetylcholine receptor, KRAS, HPV E6, E7, BING-4, EphA3, calcium activated chloride channel-2, cyclin B1, 9D7, Ep-CAM, SAP-1, PRAME, SSX-2, immature laminin receptor, folate receptor-a, telomerase, tyrosinase, melan-A, NY-ESO-1, GD2, GD3, hTERT, IL13R-a2, x-light chain, KDR, LeY, LI cell adhesion molecule, MAGE-A1, MAGE-A3, MART1, MART2, MUC1, Mesothelin, HER-2/neu, EGFRvIII, NKG2D ligands, NY-ES0-1, gp100, TRP-1/-2, TRP-1/-2, P polypeptide, MC1R, prostate specific antigen, BRAF, androgen-receptor, β-catenin, BRCA1/2, CDK4, CML66, fibronectin, p53, TGF-βRII, T cell receptor, oncofetal antigen, 5T4, PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, functional fragments thereof, and derivatives thereof.

89. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes a toxin.

90. The recombinant adenovirus of claim 89, wherein the toxin is pseudomonas exotoxin, ricin toxin, or diphtheria toxin.

91. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes an enzyme.

92. The recombinant adenovirus of claim 91, wherein the enzyme is selected from the group consisting of beta-glucuronidase, beta-galactosidase, beta-glucosidase, carboxypeptidase, beta-lactamase, esterase, metalloproteinase, relaxin, collagenase, streptokinase, arginase, NOS-2, fragments thereof, and derivatives thereof.

93. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes a cell cycle control agent, a growth factor, an anticoagulant, a pro-drug activating gene, a tumor suppressor gene, an apoptotic gene, an anti-platelet agent, a clotting factor, a cystic fibrosis transmembrane conductance regulator (CFTR) protein, fragments thereof, or derivatives thereof.

94. The recombinant adenovirus of any one of claims 25-78, wherein one or more of the transgene, the first transgene, and the second transgene encodes angiostatin, endostatin, acetylcholine, DKK1/Wnt, Ox40L, GITRL, secreted flagellin, thymidine kinase, functional fragments thereof, or derivatives thereof.

95. The recombinant adenovirus of any one of claims 1-94, wherein the recombinant adenovirus is a type 5 adenovirus (Ad5) or type 35 adenovirus (Ad35).

96. The recombinant adenovirus of any one of claims 1-95, wherein the recombinant adenovirus is oncolytic.

97. The recombinant adenovirus of any one of claims 1-96, wherein the recombinant adenovirus selectively replicates in a hyperproliferative cell.

98. The recombinant adenovirus of any one of claims 1-97, wherein the recombinant adenovirus selectively expresses a transgene in a hyperproliferative cell.

99. The recombinant adenovirus of claim 97 or claim 98, wherein the hyperproliferative cell is a tumor cell.

100. An isolated nucleotide sequence comprising the recombinant adenovirus of any one of claims 1-99, optionally wherein the nucleotide sequence is cDNA.

101. An isolated vector comprising the nucleotide sequence of claim 100.

102. An isolated cell comprising the nucleotide sequence of claim 100 or the vector of claim 101.

103. A method of inhibiting proliferation of a tumor cell comprising exposing the tumor cell to an effective amount of the recombinant adenovirus of any one of claims 1-99 to inhibit proliferation of the tumor cell.

104. A method of inhibiting tumor growth in a subject in need thereof, wherein the method comprises administering to the subject to an effective amount of the recombinant adenovirus of any one of claims 1-99 to inhibit growth of the tumor.

105. The method of claim 104, wherein the tumor is a HER2/neu positive tumor, and wherein the recombinant adenovirus comprises an E1a promoter having no more than one deletion of a functional Pea3 binding site.

106. The method of claim 105, wherein the HER2/neu positive tumor is from breast cancer, gastric cancer, ovarian cancer, bladder cancer, salivary gland cancer, endometrial cancer, pancreatic cancer, or non-small-cell lung cancer (NSCLC).

107. The method of claim 103 or claim 104, wherein the tumor is selected from the group consisting of melanoma, squamous cell carcinoma of the skin, basal cell carcinoma, head and neck tumor, breast tumor, anal cancer, cervical cancer, non-small cell lung cancer, mesothelioma, small cell lung tumor, renal cell carcinoma, prostate tumor, gastroesophageal tumor, colorectal tumor, testicular tumor, bladder tumor, ovarian tumor, hepatocellular carcinoma, cholangiocarcinoma, brain tumor, endometrial

~~tumor, neuroendocrine tumor, merkel cell carcinoma, gastrointestinal stromal tumor, a sarcoma, and pancreatic tumor.~~

~~108. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of the recombinant adenovirus of any one of claims 1-99 to treat the cancer in the subject.~~

~~109. The method of claim 108, wherein the cancer is selected from melanoma, squamous cell carcinoma of the skin, basal cell carcinoma, head and neck cancer, breast cancer, anal cancer, cervical cancer, non-small cell lung cancer, mesothelioma, small cell lung cancer, renal cell carcinoma, prostate cancer, gastroesophageal cancer, colorectal cancer, testicular cancer, bladder cancer, ovarian cancer, hepatocellular carcinoma, cholangiocarcinoma, brain cancer, endometrial cancer, neuroendocrine cancer, merkel cell carcinoma, gastrointestinal stromal tumors, a sarcoma, prostate cancer, leukemia, lymphoma and pancreatic cancer.~~

~~110. A method of treating a disease or condition in a subject in need thereof, wherein the method comprising administering to the subject to an effective amount of the recombinant adenovirus of any one of claims 1-99.~~

~~111. The method of claim 110, wherein the disease or condition is selected from the group consisting of an infection, diabetic retinopathy, psoriasis, rheumatoid arthritis, endometriosis, macular degenerative disorders and benign growth disorders such as prostate enlargement and lipomas, a vascular disorder, a cardiovascular disease, an infection, cirrhosis of the liver, a connective tissue disorder, a tumor, a vascular lesion, an ulcerative lesion, an inflammation, thrombosis, and neointima formation.~~

~~112. The method of any one of claims 104-111, wherein the subject is a mammal.~~

~~113. The method of any one of claims 104-112, wherein the subject is a human.~~

~~114. The method of claim 113, wherein the subject is a pediatric human.~~

~~115. The method of claim 113, wherein the subject is an adult human.~~

~~116. The method of any one of claims 104-115, wherein the recombinant adenovirus is administered by intramuscular, intravenous, intraarterial, intratumoral, intradermal, inhalation, transdermal, topical, eye drops, intranasal, transmucosal, or rectal administration.~~

~~117. The method of any one of claims 104-116, further comprising administering to the subject one or more therapies selected from the group consisting of surgery, radiation, chemotherapy, immunotherapy, hormone therapy, and virotherapy.~~

~~118. The method of any one of claims 104-117, further comprising administering to the subject one or more immune checkpoint modulators.~~

~~119. The method of claim 117, wherein the immune checkpoint modulator is an inhibitor, an antagonist, or an agonist of one or more molecules selected from the group consisting of PD-1, PD-L1, PD-L2, 2B4, TIGIT, LAG3, Tim3, BTLA, CD160, GITR, KIR, 4-1BB, and CTLA4.~~

~~120. A pharmaceutical composition comprising the recombinant adenovirus of any one of claims 1-99 and at least one pharmaceutically acceptable carrier or diluent.~~

~~121. A formulation for adenoviruses comprising:~~

~~(a) the recombinant adenovirus any one of claims 1-99;~~

~~(b) at least one buffer;~~

~~(c) at least one tonicity modifier;~~

~~(d) at least one sugar or at least one stabilizing agent, or both; and~~

~~wherein the formulation has a pH ranging between about 7.0 and about 9.0.~~

~~122. The formulation of claim 121, wherein the stabilizing agent is glycerol.~~

~~123. The formulation of claim 121 or claim 122, wherein the stabilizing agent is at about 2% to about 5% (v/v).~~

~~124. The formulation of any one of claims 121-123, wherein the buffer is Tris.~~

~~125. The formulation of any one of claims 121-123, wherein the buffer is at concentration of about 1mM to about 30 mM.~~

~~126. The formulation of any one of claims 119-123, wherein the tonicity modifier is NaCl.~~

~~127. The formulation of any one of claims 119-124, wherein the tonicity modifier is at concentration of about 10 mM to about 250 mM.~~

~~128. The formulation of any one of claims 119-125, wherein the formulation comprises a first tonicity modifier and a second tonicity modifier, wherein the first tonicity modifier is a monovalent cation, and wherein the second tonicity modifier is a divalent cation.~~

~~129. The formulation of any one of claims 119-128, wherein the tonicity modifier or the divalent cation is $MgCl_2$.~~

~~130. The formulation of any one of claims 119-129, wherein the tonicity modifier or the divalent cation is at a concentration of about 0.1 mM to about 5 mM.~~

~~131. The formulation of any one of claims 119-130, wherein the sugar is sucrose.~~

~~132. The formulation of any one of claims 119-131, wherein the sugar is at weight to volume percentage from about 2% to about 8%.~~

~~133. The formulation of any one of claims 119-132, further comprising at least one non-ionic surfactant.~~

~~134. The formulation of claim 133, wherein the non-ionic surfactant is polysorbate-80 or polysorbate-40.~~

135. The formulation of claim 133 or claim 134, wherein the non-ionic surfactant is at a concentration of about 0.001% to about 1%.

136. The formulation of any one of claims 119-135, further comprising at least one inhibitor of free radical oxidation.

137. The formulation of claim 136, wherein the inhibitor of free radical oxidation is EDTA.

138. The formulation of claim 136 or claim 137, wherein the inhibitor of free radical oxidation is at a concentration of about 0.01 mM to about 5 mM.

139. The formulation of any one of claims 119-138, further comprising at least one cryoprotectant.

140. The formulation of claim 139, wherein the cryoprotectant is EtOH.

141. The formulation of claim 139 or claim 140, wherein the cryoprotectant is at a concentration of about 0.5%.

142. The formulation of any one of claims 119-141, wherein the formulation has an osmolarity of about 200 mOs/L to about 800 mOs/L.

143. The formulation of any one of claims 119-142, wherein the recombinant adenovirus is at concentration from about 1×10^{7} vp/mL to 1×10^{13} vp/mL.

144. The formulation of any one of claims 119-143, wherein the formulation comprises about 20 mM Tris, about 25 mM NaCl, about 2.5% glycerol, and wherein the formulation has a pH of about 8.0.

145. The formulation of any one of claims 119-144, wherein the formulation comprises about 20 mM Tris, about 25 mM NaCl, about 3-5% sucrose, and wherein the formulation has a pH of about 8.0.

146. The formulation of any one of claims 119-145, wherein the formulation comprises about 10 mM Tris, about 75 mM NaCl, about 5% sucrose, about 0.02% polysorbate-80, about 1 mM $MgCl_2$, about 0.1 mM EDTA, about 0.5% EtOH, and wherein the formulation has a pH of about 8.0.

147. The formulation of any one of claims 119-146, wherein the formulation further comprises at least one immunoadjuvant.

148. The formulation of claim 147, wherein the immunoadjuvant is selected from 1) Alum, 2) Saponins, 3) non-ionic polymer surfactants, 4) monophosphoryl lipid A, 5) muramyl dipeptides, and 6) cytokines.

149. The formulation of any one of claims 119-148, wherein the formulation further comprises at least one dye.

150. The formulation of any one of claims 119-149, wherein the formulation further comprises at least one reversible protease inhibitor.

151. The formulation of claim 150, wherein the reversible protease inhibitor is an inhibitor of an L3/p23 cysteine protease.

152. The formulation of any one of claims 119-151, wherein the formulation further comprises at least one antioxidant.

153. The formulation of claim 152, the antioxidant is vitamin A, vitamin C, vitamin E, vitamin B6, vitamin B12, folic acid, or folate.

Claims

1. A recombinant adenovirus comprising

a first nucleotide sequence inserted in an IX-E2 insertion site, wherein the IX-E2 insertion site is located between the stop codon of adenovirus IX gene and the stop codon of adenovirus IVa2 gene;

a second nucleotide sequence inserted in an L5-E4 insertion site, wherein the L5-E4 insertion site is located between the stop codon of adenovirus fiber gene and the stop codon of ORF6 or ORF6/7 of adenovirus E4 gene;

or both the first nucleotide sequence inserted in the IX-E2 insertion site and the second nucleotide sequence inserted in the L5-E4 insertion site,

wherein the first nucleotide sequence inserted in the IX-E2 insertion site comprises, in a 5' to 3' orientation:

(i) a first IX-E2 polyadenylation signal;

(iii) a IX-E2 promoter;

(iv) a first transgene;

(v) a third IX-E2polyadenylation signal; and

(vi) a fourth IX-E2 polyadenylation signal;

wherein the first IX-E2 polyadenylation signal is the polyadenylation signal of the IX gene, the third IX-E2 polyadenylation signal is the polyadenylation signal of the first transgene, and the fourth IX-E2 polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene; and

wherein the second nucleotide sequence inserted in an L5-E4 insertion site comprises, in a 5' to 3' orientation:

(i) a first L5-E4 polyadenylation signal;

(ii) an second L5-E4 polyadenylation signal;

(iii) the L5-E4 promoter;

(iv) the second transgene;

(v) a third L5-E4 polyadenylation signal; and

(vi) a fourth L5-E4 polyadenylation signal;

wherein the second L5-E4 polyadenylation signal is in the opposite transcriptional direction of the first L5-E4 polyadenylation signal, and wherein the first L5-E4 polyadenylation signal is the polyadenylation signal of the fiber (L5) gene, the third L5-E4 polyadenylation

signal is the polyadenylation signal of the second transgene, and the fourth L5-E4 polyadenylation signal is the polyadenylation signal of the ORF6 or ORF6/7 of the adenovirus E4 gene.

2. The recombinant adenovirus of claim 1, wherein the first nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4093 of the Ad5 genome (SEQ ID NO: 1) or between nucleotides corresponding to 3899 and 3970 of the Ad35 genome (SEQ ID NO: 41).

3. The recombinant adenovirus of claim 2, wherein the second nucleotide sequence is inserted between nucleotides corresponding to 32785 to 32916 of the Ad5 genome (SEQ ID NO: 1) or between nucleotides corresponding to 31799 and 31821 of the Ad35 genome (SEQ ID NO: 41).

4. The recombinant adenovirus of claim 1, wherein the first nucleotide sequence further comprises a second IX-E2 polyadenylation signal between the first IX-E2 polyadenylation signal and the IX-E2 promoter and the second polyadenylation signal is in the opposite transcriptional direction of the first polyadenylation signal.

5. The recombinant adenovirus of any one of claims 1-4, wherein the recombinant adenovirus further comprises a nucleotide sequence inserted in an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site.

6. The recombinant adenovirus of any one of claims 1-5, wherein the recombinant adenovirus selectively replicates in a hyperproliferative cell.

7. The recombinant adenovirus of any one of claims 1-6, wherein the recombinant adenovirus selectively expresses the first transgene, the second transgene, or both the first transgene and the second transgene in a hyperproliferative cell.

8. Use of the recombinant adenovirus of any one of claims 1-7 in the manufacture of a medicament for inhibiting tumor growth.

9. A pharmaceutical composition comprising the recombinant adenovirus of any one of claims 1-7 and at least one pharmaceutically acceptable carrier or diluent.

10. A formulation for adenoviruses comprising:

(a) the recombinant adenovirus of any one of claims 1-7;

(b) at least one buffer;

(c) at least one tonicity modifier;

(d) at least one sugar or at least one stabilizing agent, or both; and

wherein the formulation has a pH ranging between about 7.0 and about 9.0.