

What is claimed is:

1. A Melanoma-Associated Antigen A4 (MAGE-A4)-specific chimeric antigen receptor (CAR), wherein said MAGE-A4-specific chimeric antigen receptor interacts with amino acids 286-294, or a portion thereof, of SEQ ID NO: 32, and wherein said MAGE-A4-specific CAR specifically binds to an HLA bound MAGE-A4 polypeptide.
2. A Melanoma-Associated Antigen A4 (MAGE-A4)-specific chimeric antigen receptor (CAR), wherein said MAGE-A4-specific chimeric antigen receptor interacts with amino acids 230-239, or a portion thereof, of SEQ ID NO: 32, and wherein said MAGE-A4-specific CAR specifically binds to an HLA bound MAGE-A4 polypeptide.
3. The MAGE-A4-specific CAR of claim 1 or 2, wherein said MAGE-A4-specific CAR comprises, from N-terminus to C-terminus: (a) an extracellular ligand-binding domain comprising an anti-MAGE-A4 antigen-binding domain; (b) a hinge; (c) a transmembrane domain; and (d) a cytoplasmic domain comprising a costimulatory domain and a signaling domain.
4. The MAGE-A4-specific CAR of claim 3, wherein the extracellular ligand-binding domain comprises an anti-MAGE-A4 single chain variable fragment (scFv) domain comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR).
5. The MAGE-A4-specific CAR of claim 4, wherein the anti-MAGE-A4 scFv domain comprises a first linker between the LCVR and the HCVR.
6. The MAGE-A4-specific CAR of any one of claims 1-5, further comprising a second linker between the extracellular ligand-binding domain and the hinge.
7. The MAGE-A4-specific CAR of claim 6, wherein the first linker and the second linker comprise amino acid sequences selected from the group consisting of SEQ ID NOs: 23-26.
8. The MAGE-A4-specific CAR of claim 7, wherein the first linker comprises the amino acid sequence of SEQ ID NO: 25 and the second linker comprises the amino acid sequence of SEQ ID NO: 23.
9. The MAGE-A4-specific CAR of any one of claims 1-8, wherein the hinge, the transmembrane domain, or both, are from a CD8 α polypeptide.

10. The MAGE-A4-specific CAR of any one of claims 1-9, wherein the costimulatory domain comprises a 4-1BB costimulatory domain.

11. The MAGE-A4-specific CAR of any one of claims 1-9, wherein the hinge, the transmembrane domain, or both, are from a CD28 polypeptide.

12. The MAGE-A4-specific CAR of claim 11, wherein the costimulatory domain comprises a CD28 costimulatory domain.

13. The MAGE-A4-specific CAR of any one of claims 1-12, wherein the signaling domain comprises a CD3zeta signaling domain.

14. The MAGE-A4-specific CAR of any one of claims 1 or 3-13, wherein the LCVR comprises the complementarity determining regions (CDRs) of a LCVR comprising an amino acid sequence of SEQ ID NO: 10.

15. The MAGE-A4-specific CAR of any one of claims 1 or 3-13, wherein the LCVR comprises the complementarity determining regions (CDRs) of a LCVR comprising an amino acid sequence of SEQ ID NO: 37.

16. The MAGE-A4-specific CAR of claim 14 or 15, wherein the LCVR comprises LCDR1-LCDR2-LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 12-14-16.

17. The MAGE-A4-specific CAR of any one of claims 1 or 3-16, wherein the HCVR comprises the CDRs of a HCVR comprising an amino acid sequence of SEQ ID NO: 2.

18. The MAGE-A4-specific CAR of claim 17, wherein the HCVR comprises HCDR1-HCDR2-HCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 4-6-8.

19. The MAGE-A4-specific CAR of any one of claims 1 or 3-18, wherein the LCVR comprises an amino acid sequence of SEQ ID NO: 10, or an amino acid sequence having 95%-99% sequence identity to an amino acid sequence of SEQ ID NO: 10; and the HCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, or an amino acid sequence

having 95%-99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 2.

20. The MAGE-A4-specific CAR of any one of claims 1 or 3-18, wherein the LCVR comprises an amino acid sequence of SEQ ID NO: 37, or an amino acid sequence having 95%-99% sequence identity to an amino acid sequence of SEQ ID NO: 37; and the HCVR comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, or an amino acid sequence having 95%-99% sequence identity to an amino acid sequence of SEQ ID NO: 2.

21. The MAGE-A4-specific CAR of claim 19 or 20, wherein the LCVR comprises an amino acid sequence of SEQ ID NO: 10, and the HCVR comprises an amino acid sequence of SEQ ID NO: 2.

22. The MAGE-A4-specific CAR of claim 19 or 20, wherein the LCVR comprises an amino acid sequence of SEQ ID NO: 37, and the HCVR comprises an amino acid sequence of SEQ ID NO: 2.

23. The MAGE-A4-specific CAR of any one of claims 2-13, wherein the LCVR comprises the complementarity determining regions (CDRs) of a LCVR comprising an amino acid sequence of SEQ ID NO: 59.

24. The MAGE-A4-specific CAR of claim 23, wherein the LCVR comprises LCDR1-LCDR2-LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 61-63-65.

25. The MAGE-A4-specific CAR of any one of claims 2-13 or 23-24, wherein the HCVR comprises the CDRs of a HCVR comprising an amino acid sequence of SEQ ID NO: 51.

26. The MAGE-A4-specific CAR of claim 25, wherein the HCVR comprises HCDR1-HCDR2-HCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 53-55-57.

27. The MAGE-A4-specific CAR of any one of claims 2-13 or 23-26, wherein the LCVR comprises an amino acid sequence of SEQ ID NO: 59, or an amino acid sequence having 95%-99% sequence identity to an amino acid sequence of SEQ ID NO: 59; and the HCVR

comprises an of SEQ ID NO: 51, or an amino acid sequence having 95%-99% sequence identity to an amino acid sequence of SEQ ID NO: 51.

28. The MAGE-A4-specific CAR of claim 27, wherein the LCVR comprises an amino acid sequence of SEQ ID NO: 59, and the HCVR comprises an amino acid sequence of SEQ ID NO: 51.

29. The MAGE-A4-specific CAR of any one of claims 1-10 and 13-28, wherein the hinge comprises the amino acid sequence of SEQ ID NO: 27.

30. The MAGE-A4-specific CAR of any one of claims 1-10 and 13-29, wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 28.

31. The MAGE-A4-specific CAR of any one of claims 1-10 and 13-30, wherein the 4-1BB costimulatory domain comprises the amino acid sequence of SEQ ID NO: 29.

32. The MAGE-A4-specific CAR of any one of claims 1-8 and 11-28, wherein the hinge comprises the amino acid sequence of SEQ ID NO: 41.

33. The MAGE-A4-specific CAR of any one of claims 1-8, 11-28, and 32, wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 43.

34. The MAGE-A4-specific CAR of any one of claims 1-8, and 11-28, and 32-33, wherein the CD28 costimulatory domain comprises the amino acid sequence of SEQ ID NO: 45.

35. The MAGE-A4-specific CAR of any one of claims 1-34, wherein the CD3zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 30.

36. The MAGE-A4-specific CAR of claim 1, comprising the amino acid sequence of SEQ ID NO: 22.

37. The MAGE-A4-specific CAR of claim 1, comprising the amino acid sequence of SEQ ID NO: 39.

38. The MAGE-A4-specific CAR of claim 1, comprising the amino acid sequence of SEQ ID NO: 47.

39. The MAGE-A4-specific CAR of claim 2, comprising the amino acid sequence of SEQ ID NO: 71.
40. The MAGE-A4-specific CAR of claim 2, comprising the amino acid sequence of SEQ ID NO: 73.
41. The MAGE-A4-specific CAR of any one of claims 1 and 3-22, wherein said chimeric antigen receptor specifically binds to one or more amino acids at positions 286-294 of SEQ ID NO: 32.
42. The MAGE-A4-specific CAR of any one of claims 2-13 and 23-28, wherein said chimeric antigen receptor specifically binds to one or more amino acids at positions 230-239 of SEQ ID NO: 32.
43. The MAGE-A4-specific CAR of any one of claims 1-26, wherein said HLA is HLA-A2.
44. An isolated nucleic acid molecule encoding the MAGE-A4-specific CAR of any one of claims 1-43.
45. The isolated nucleic acid molecule of claim 44, comprising a nucleotide sequence of SEQ ID NO: 21.
46. The isolated nucleic acid molecule of claim 44, comprising a nucleotide sequence of SEQ ID NO: 38.
47. The isolated nucleic acid molecule of claim 44, comprising a nucleotide sequence of SEQ ID NO: 48.
48. The isolated nucleic acid molecule of claim 44, comprising a nucleotide sequence of SEQ ID NO: 70.
49. The isolated nucleic acid molecule of claim 44, comprising a nucleotide sequence of SEQ ID NO: 72.
50. A vector comprising the nucleic acid molecule of any one of claims 44-49.

51. The vector of claim 50, wherein the vector is a DNA vector, an RNA vector, a plasmid, a lentivirus vector, an adenovirus vector, or a retroviral vector.
52. The vector of claim 51, wherein the vector is a lentivirus vector.
53. A cell comprising the nucleic acid molecule of any one of claims 44-49, or a vector of any one of claims 50-52.
54. The cell of claim 53, wherein the cell is a human T cell.
55. An engineered cell comprising a chimeric antigen receptor of any one of claims 1-43.
56. The engineered cell of claim 55 that is an immune cell.
57. The engineered cell of claim 56, wherein the immune cell is an immune effector cell.
58. The engineered cell of claim 57, wherein the immune effector cell is a T lymphocyte.
59. The engineered cell of claim 58, wherein the T lymphocyte is an inflammatory T lymphocyte, a cytotoxic T lymphocyte, a regulatory T lymphocyte, or a helper T lymphocyte.
60. The engineered cell of claim 59 that is a CD8⁺ cytotoxic T lymphocyte.
61. The engineered cell of any one of claims 55-60 for use in the treatment of a MAGE-A4-expressing cancer.
62. The engineered cell of claim 61, wherein the MAGE-A4-expressing cancer is multiple myeloma.
63. The engineered cell of claim 61, wherein the MAGE-A4-expressing cancer is melanoma.
64. An engineered human T cell comprising a chimeric antigen receptor comprising, from N-terminus to C-terminus: (a) an extracellular ligand-binding domain comprising an anti-

MAGE-A4 single chain variable fragment (scFv) domain comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR); (b) a hinge; (c) a transmembrane domain; and (d) a cytoplasmic domain comprising a 4-1BB costimulatory domain or a CD28 costimulatory domain and a CD3zeta signaling domain.

65. The engineered human T cell of claim 64, wherein said anti-MAGE-A4 scFv specifically binds to one or more amino acid residues of positions 286-294 of SEQ ID NO: 32.

66. The engineered human T cell of claim 64 or 65, wherein the scFv domain comprises a HCVR/LCVR amino acid sequence pair comprising the amino acid sequences of SEQ ID NOs: 2/10.

67. The engineered human T cell of claim 64 or 65, wherein the scFv domain comprises a HCVR/LCVR amino acid sequence pair comprising the amino acid sequences of SEQ ID NOs: 2/37.

68. The engineered human T cell of claim 64, wherein said anti-MAGE-A4 scFv domain specifically binds to one or more amino acid residues of positions 230-239 of SEQ ID NO: 32.

69. The engineered human T cell of claim 64 or 65, wherein the scFv domain comprises a HCVR/LCVR amino acid sequence pair comprising the amino acid sequences of SEQ ID NOs: 51/59.

70. The engineered human T cell of any one of claims 64-69, wherein the hinge comprises the amino acid sequence of SEQ ID NO: 27.

71. The engineered human T cell of any one of claims 64-70, wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 28.

72. The engineered human T cell of any one of claims 64-71, wherein the 4-1BB costimulatory domain comprises the amino acid sequence of SEQ ID NO: 29.

73. The engineered human T cell of any one of claims 64-69, wherein the hinge comprises the amino acid sequence of SEQ ID NO: 41.

74. The engineered human T cell of any one of claims 64-69 and 73, wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 43.

75. The engineered human T cell of any one of claims 64-69 and 73-74, wherein the CD28 costimulatory domain comprises the amino acid sequence of SEQ ID NO: 45.

76. The engineered human T cell of any one of claims 64-75, wherein the CD3zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 30.

77. The engineered human T cell of claim 64, comprising a chimeric antigen receptor comprising the amino acid sequence of SEQ ID NO: 22.

78. The engineered human T cell of claim 65, comprising a chimeric antigen receptor comprising the amino acid sequence of SEQ ID NO: 39.

79. The engineered human T cell of claim 65, comprising a chimeric antigen receptor comprising the amino acid sequence of SEQ ID NO: 47.

80. The engineered human T cell of claim 65, comprising a chimeric antigen receptor comprising the amino acid sequence of SEQ ID NO: 71.

81. The engineered human T cell of claim 65, comprising a chimeric antigen receptor comprising the amino acid sequence of SEQ ID NO: 73.

82. A pharmaceutical composition comprising a genetically-modified human T cell and a pharmaceutically acceptable carrier, wherein the genetically-modified human T cell comprises a chimeric antigen receptor according to any one of claims 1-43.

83. A pharmaceutical composition comprising the engineered cell of any one of claims 55-63 and a pharmaceutically acceptable carrier.

84. A pharmaceutical composition comprising the engineered human T cell of any one of claims 64-81 and a pharmaceutically acceptable carrier.

85. The pharmaceutical composition of any one of claims 82-84 for use in the treatment of a MAGE-A4-expressing cancer.

86. The pharmaceutical composition of claim 85, wherein the MAGE-A4-expressing cancer is multiple myeloma.

87. The pharmaceutical composition of claim 85, wherein the MAGE-A4-expressing cancer is melanoma.

88. Use of the chimeric antigen receptor of any one of claims 1-43, the nucleic acid molecule of any one of claims 44-49, the vector of any one of claims 50-52, the cell of claim 53 or 54, the engineered cell of any one of claims 55-63, or the engineered human T cell of any one of claims 64-81 in the manufacture of a medicament for the treatment of a MAGE-A4-expressing cancer.

89. The use of claim 88, wherein the MAGE-A4-expressing cancer is multiple myeloma.

90. The use of claim 88, wherein the MAGE-A4-expressing cancer is melanoma.

91. A method of enhancing T lymphocyte activity in a subject comprising, introducing into the subject a T lymphocyte comprising a chimeric antigen receptor of any one of claims 1-43.

92. A method for treating a subject suffering from cancer comprising, introducing into the subject a therapeutically effective amount of a T lymphocyte comprising a chimeric antigen receptor of any one of claims 1-43.

93. A method for stimulating a T cell-mediated immune response to a target cell population or tissue in a subject comprising, administering to the subject an effective amount of a cell genetically modified to express a chimeric antigen receptor of any one of claims 1-43.

94. A method of providing anti-tumor immunity in a subject, the method comprising administering to the subject an effective amount of a cell genetically modified to express a chimeric antigen receptor of any one of claims 1-43.

95. The method of any one of claims 91-94, wherein the subject is a human.

96. The method of any one of claims 91-95, wherein the subject has multiple myeloma, synovial sarcoma, esophageal cancer, head and neck cancer, lung cancer, bladder cancer, ovarian cancer, uterine cancer, stomach cancer, cervical cancer, breast cancer, or melanoma.

97. The method of claim 96, wherein the subject has multiple myeloma.

98. A method of engineering a population of cells to express a chimeric antigen receptor, comprising:

- (a) introducing into a population of immune cells nucleic acid molecules encoding a chimeric antigen receptor of any one of claims 1-43;
- (b) culturing said population of immune cells under conditions to express said nucleic acid molecules; and
- (c) isolating said immune cells expressing said chimeric antigen receptor at the cells' surface.

99. The method of claim 98, further comprising obtaining said population of immune cells from a subject prior to introducing said nucleic acid molecule.

100. A method of treating a MAGE-A4-expressing cancer in a subject, comprising:

- (a) engineering a population of cells according to claim 99; and
- (b) reintroducing said population of cells expressing said chimeric antigen receptor into said subject.

101. The method of claim 100, wherein said MAGE-A4-expressing cancer is multiple myeloma.

102. An isolated antigen-binding protein that competes for binding with a MAGE-A4-specific CAR of any one of claims 1-43.

103. The isolated antigen-binding protein of claim 102, wherein said isolated antigen-binding protein is a CAR.

104. An isolated antigen-binding protein that binds to the same epitope as a MAGE-A4-specific CAR of any one of claims 1-43.

105. The isolated antigen-binding protein of claim 104, wherein said isolated antigen-binding protein is a CAR.

106. An isolated recombinant antibody or antigen-binding fragment thereof that specifically binds to a Melanoma-Associated Antigen A4 (MAGE-A4) polypeptide, wherein the antibody has one or more of the following characteristics:

- (a) binds to the MAGE-A4 polypeptide with an EC50 of less than about 10^{-9} M;
- (b) demonstrates an increase in survival in an animal with cancer after administration to said animal, as compared to a comparable animal without said administration; and/or
- (c) comprises (i) three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within a heavy chain variable region (HCVR) comprising an amino acid sequence having at least about 90% sequence identity to an HCVR set forth in Table 1; and (ii) three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence having at least about 90% sequence identity to an LCVR set forth in Table 1.

107. The antibody or antigen-binding fragment of claim 106, wherein the MAGE-A4 polypeptide is an HLA-A2 bound MAGE-A4 polypeptide.

108. The isolated antibody or antigen-binding fragment thereof of any one of claims 106 and 107, comprising an HCVR having an amino acid sequence of SEQ ID NO: 2.

109. The isolated antibody or antigen-binding fragment thereof of any one of claims 107-108, comprising an LCVR having an amino acid sequence of SEQ ID NO: 10.

110. The isolated antibody or antigen-binding fragment thereof of any one of claims 106-109, comprising an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 2/10.

111. The isolated antibody or antigen-binding fragment thereof of any one of claims 107-108, comprising an LCVR having an amino acid sequence of SEQ ID NO: 37.

112. The isolated antibody or antigen-binding fragment thereof of any one of claims 106-108 and 111, comprising an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 2/37.

113. An isolated antibody or antigen-binding fragment thereof of any one of claims 106-112, comprising:

- (a) an HCDR1 domain having an amino acid sequence of SEQ ID NO: 4;
- (b) an HCDR2 domain having an amino acid sequence of SEQ ID NO: 6;
- (c) an HCDR3 domain having an amino acid sequence of SEQ ID NO: 8;
- (d) an LCDR1 domain having an amino acid sequence of SEQ ID NO: 12;
- (e) an LCDR2 domain having an amino acid 14; and
- (f) an LCDR3 domain having an amino acid sequence of SEQ ID NO: 16.

114. The isolated antibody or antigen-binding fragment thereof of any one of claims 106 and 107, comprising an HCVR having an amino acid sequence of SEQ ID NO: 51.

115. The isolated antibody or antigen-binding fragment thereof of any one of claims 106, 107, and 114, comprising an LCVR having an amino acid sequence of SEQ ID NO: 59.

116. An isolated antibody or antigen-binding fragment thereof of any one of claims 106, 107, 114, and 115, comprising:

- (a) an HCDR1 domain having an amino acid sequence of SEQ ID NO: 53;
- (b) an HCDR2 domain having an amino acid sequence of SEQ ID NO: 55;
- (c) an HCDR3 domain having an amino acid sequence of SEQ ID NO: 57;
- (d) an LCDR1 domain having an amino acid sequence of SEQ ID NO: 61;
- (e) an LCDR2 domain having an amino acid sequence of SEQ ID NO: 63; and
- (f) an LCDR3 domain having an amino acid sequence of SEQ ID NO: 65.

117. The isolated antibody or antigen-binding fragment thereof of any one of claims 106, 107, and 114-116, comprising an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 51/59.

118. The isolated antibody or antigen-binding fragment thereof of any one of claims 106-117 which is an IgG1 antibody.

119. The isolated antibody or antigen-binding fragment thereof of any one of claims 106-117 which is an IgG4 antibody.

120. The isolated antibody or antigen-binding fragment thereof of any one of claims 106-119 which is a bispecific antibody.

121. A pharmaceutical composition comprising the isolated antibody or antigen-binding fragment thereof of any one of claims 106-120 and a pharmaceutically acceptable carrier or diluent.

122. The pharmaceutical composition of claim 121, wherein said pharmaceutical composition further comprises a second therapeutic agent.

123. The pharmaceutical composition of claim 122, wherein said second therapeutic agent is selected from the group consisting of: an anti-tumor agent, steroids, and targeted therapies.

124. A polynucleotide molecule comprising a polynucleotide sequence that encodes an HCVR or an LCVR of an antibody as set forth in any one of claims 106-120.

125. A vector comprising the polynucleotide of claim 124.

126. A cell comprising the vector of claim 125.

127. A method of treating a MAGE-A4 expressing cancer, the method comprising administering an antibody or antigen-binding fragment of any one of claims 106-120, or the pharmaceutical composition of any one of claims 121-123, to a subject.

128. The method of claim 127, wherein said pharmaceutical composition is administered in combination with a second therapeutic agent.

129. The method of claim 128, wherein said second therapeutic agent is selected from the group consisting of: an anti-tumor agent, steroids, and targeted therapies.