

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), optionally wherein the anti-MAGE-A4 scFv domain comprises a first linker between the LCVR and the HCVR, and optionally wherein the MAGE-A4-specific CAR comprises a second linker between the extracellular ligand-binding domain and the hinge;

optionally wherein the first linker and the second linker comprise amino acid sequences selected from the group consisting of SEQ ID NOs: 23-26, optionally 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.

5. The MAGE-A4-specific CAR of any one of claims 1-4, wherein:
- (a) the hinge, the transmembrane domain, or both, are from a CD8 α polypeptide;
 - (b) the costimulatory domain comprises a 4-1BB costimulatory domain;
 - (c) the hinge, the transmembrane domain, or both, are from a CD28 polypeptide;

- (d) the costimulatory domain comprises a CD28 costimulatory domain; or
 - (e) the signaling domain comprises a CD3zeta signaling domain.
6. The MAGE-A4-specific CAR of any one of claims 1 or 3-5, wherein:
- (a) the LCVR comprises the complementarity determining regions (CDRs) of a LCVR comprising an amino acid sequence of SEQ ID NO: 10;
 - (b) the LCVR comprises the complementarity determining regions (CDRs) of a LCVR comprising an amino acid sequence of SEQ ID NO: 37;
 - (c) the LCVR comprises LCDR1-LCDR2-LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 12-14-16;
 - (d) the HCVR comprises the CDRs of a HCVR comprising an amino acid sequence of SEQ ID NO: 2;
 - (e) the HCVR comprises HCDR1-HCDR2-HCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 4-6-8;
 - (f) 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;
 - (g) 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;
 - (h) 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; or
 - (i) 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.
7. The MAGE-A4-specific CAR of any one of claims 2-5, wherein:
- (a) the LCVR comprises the complementarity determining regions (CDRs) of a LCVR comprising an amino acid sequence of SEQ ID NO: 59;

- (b) the LCVR comprises LCDR1-LCDR2-LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 61-63-65;
- (c) the HCVR comprises the CDRs of a HCVR comprising an amino acid sequence of SEQ ID NO: 51;
- (d) the HCVR comprises HCDR1-HCDR2-HCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 53-55-57;
- (e) 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; or
- (f) 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.

- 8. The MAGE-A4-specific CAR of any one of claims 1-7, wherein:
 - (a) the hinge comprises the amino acid sequence of SEQ ID NO: 27;
 - (b) the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 28;
 - (c) the 4-1BB costimulatory domain comprises the amino acid sequence of SEQ ID NO: 29;
 - (d) the hinge comprises the amino acid sequence of SEQ ID NO: 41;
 - (e) the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 43;
 - (f) the CD28 costimulatory domain comprises the amino acid sequence of SEQ ID NO: 45; or
 - (g) the CD3zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 30.

- 9. The MAGE-A4-specific CAR of claim 1, comprising the amino acid sequence of SEQ ID NO: 22, the amino acid sequence of SEQ ID NO: 39, or the amino acid sequence of SEQ ID NO: 47.

- 10. The MAGE-A4-specific CAR of claim 2, comprising the amino acid sequence of SEQ ID NO: 71, or the amino acid sequence of SEQ ID NO: 73.

11. The MAGE-A4-specific CAR of any one of claims 1-10, wherein said HLA is HLA-A2.

12. An isolated nucleic acid molecule encoding the MAGE-A4-specific CAR of any one of claims 1-11, optionally wherein the nucleic acid molecule comprises a nucleotide sequence of SEQ ID NO: 21, a nucleotide sequence of SEQ ID NO: 38, a nucleotide sequence of SEQ ID NO: 48, a nucleotide sequence of SEQ ID NO: 70, or a nucleotide sequence of SEQ ID NO: 72; or a vector comprising the nucleic acid molecule, optionally wherein the vector is a DNA vector, an RNA vector, a plasmid, a lentivirus vector, an adenovirus vector, or a retroviral vector.

13. A cell comprising the nucleic acid molecule, or a vector of claim 12, optionally wherein the cell is a human T cell.

14. An engineered cell comprising a chimeric antigen receptor of any one of claims 1-11, optionally wherein:

- (a) the engineered cell is an immune cell;
- (b) the engineered cell is an immune cell, and the immune cell is an immune effector cell;
- (c) the engineered cell is a T lymphocyte;
- (d) the engineered cell is a T lymphocyte and the T lymphocyte is an inflammatory T lymphocyte, a cytotoxic T lymphocyte, a regulatory T lymphocyte, or a helper T lymphocyte, optionally a CD8⁺ cytotoxic T lymphocyte.

15. Use of the engineered cell of claim 14 in the manufacture of a medicament for treating a MAGE-A4-expressing cancer, optionally wherein the MAGE-A4-expressing cancer is multiple myeloma or melanoma.

16. 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, optionally wherein:

- (a) the anti-MAGE-A4 scFv specifically binds to one or more amino acid residues of positions 286-294 of SEQ ID NO: 32;
- (b) the scFv domain comprises a HCVR/LCVR amino acid sequence pair comprising the amino acid sequences of SEQ ID NOs: 2/10;
- (c) the scFv domain comprises a HCVR/LCVR amino acid sequence pair comprising the amino acid sequences of SEQ ID NOs: 2/37;
- (d) the anti-MAGE-A4 scFv domain specifically binds to one or more amino acid residues of positions 230-239 of SEQ ID NO: 32;
- (e) the scFv domain comprises a HCVR/LCVR amino acid sequence pair comprising the amino acid sequences of SEQ ID NOs: 51/59;
- (f) the hinge comprises the amino acid sequence of SEQ ID NO: 27.;
- (g) the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 28;
- (h) the 4-1BB costimulatory domain comprises the amino acid sequence of SEQ ID NO: 29;
- (i) the hinge comprises the amino acid sequence of SEQ ID NO: 41;
- (j) the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 43;
- (k) the CD28 costimulatory domain comprises the amino acid sequence of SEQ ID NO: 45;
- (l) the CD3zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 30;
- (m) the chimeric antigen receptor comprises the amino acid sequence of SEQ ID NO: 22, the amino acid sequence of SEQ ID NO: 39, the amino acid sequence of SEQ ID NO: 47, the amino acid sequence of SEQ ID NO: 71, or the amino acid sequence of SEQ ID NO: 73.

17. A pharmaceutical composition comprising:

- (a) 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 1-11;

- (b) the engineered cell of claim 14 and a pharmaceutically acceptable carrier; or
- (c) the engineered human T cell of claim 16 and a pharmaceutically acceptable carrier.

18. Use of the pharmaceutical composition of claim 17 in the manufacture of a medicament for treating a MAGE-A4-expressing cancer, optionally wherein the MAGE-A4-expressing cancer is multiple myeloma or melanoma.

19. Use of the chimeric antigen receptor of any one of claims 1-11, the nucleic acid molecule or the vector of claim 12, the cell of claim 13, the engineered cell of claim 14, or the engineered human T cell of claim 16 in the manufacture of a medicament for the treatment of a MAGE-A4-expressing cancer, optionally wherein the MAGE-A4-expressing cancer is multiple myeloma or melanoma.

20. Use of:

- (a) a T lymphocyte comprising a chimeric antigen receptor of any one of claims 1-11 in the manufacture of a medicament for enhancing T lymphocyte activity in a subject;
- (b) a T lymphocyte comprising a chimeric antigen receptor of any one of claims 1-11 in the manufacture of a medicament for treating a subject suffering from cancer;
- (c) a cell genetically modified to express a chimeric antigen receptor of any one of claims 1-11 in the manufacture of a medicament for stimulating a T cell-mediated immune response to a target cell population or tissue in a subject; or
- (d) a cell genetically modified to express a chimeric antigen receptor of any one of claims 1-11 in the manufacture of a medicament for providing anti-tumor immunity in a subject.

21. The use of claim 20, wherein the subject is a human; and/or 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, optionally wherein the subject has multiple myeloma.

22. 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 1-11, which have optionally been obtained from a subject;

(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.

23. Use of a population of cells engineered according to claim 22 in the manufacture of a medicament for treating a MAGE-A4-expressing cancer in a subject by reintroducing said population of cells expressing said chimeric antigen receptor into said subject, optionally wherein the MAGE-A4-expressing cancer is multiple myeloma.

24. An isolated antigen-binding protein that competes for binding with, or that binds to the same epitope as, a MAGE-A4-specific CAR of any one of claims 1-11, optionally wherein said isolated antigen-binding protein is a CAR.

25. 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.

26. The antibody or antigen-binding fragment of claim 25, wherein:

(a) the MAGE-A4 polypeptide is an HLA-A2 bound MAGE-A4 polypeptide;

(b) the isolated antibody or antigen-binding fragment thereof comprises an HCVR having an amino acid sequence of SEQ ID NO: 2;

(c) the isolated antibody or antigen-binding fragment thereof comprises an LCVR having an amino acid sequence of SEQ ID NO: 10;

(d) the isolated antibody or antigen-binding fragment thereof comprises an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 2/10;

(e) the isolated antibody or antigen-binding fragment thereof comprises an LCVR having an amino acid sequence of SEQ ID NO: 37; or

(f) the isolated antibody or antigen-binding fragment thereof comprises an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 2/37.

27. An isolated antibody or antigen-binding fragment thereof of claim 25 or 26:

(A) 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.; or

(B) comprising an HCVR having an amino acid sequence of SEQ ID NO: 51

and/or comprising an LCVR having an amino acid sequence of SEQ ID NO: 59; or

(C) 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; or

(D) comprising an HCVR/LCVR amino acid sequence pair of SEQ ID NOs:

51/59; or

(E) which is an IgG1 antibody; or

- (F) which is an IgG4 antibody; or
- (G) which is a bispecific antibody.

28. A pharmaceutical composition comprising the isolated antibody or antigen-binding fragment thereof of any one of claims 25-27 and a pharmaceutically acceptable carrier or diluent, optionally wherein said pharmaceutical composition further comprises a second therapeutic agent, which is optionally selected from the group consisting of: an anti-tumor agent, steroids, and targeted therapies.

29. 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 25-27; or a vector comprising the polynucleotide; or a cell comprising the vector.

30. Use of an antibody or antigen-binding fragment of any one of claims 25-27, or the pharmaceutical composition of claim 28 in the manufacture of a medicament for treating a MAGE-A4 expressing cancer in a subject, optionally wherein said pharmaceutical composition is for use in combination with a second therapeutic agent, which is optionally selected from the group consisting of: an anti-tumor agent, steroids, and targeted therapies.