

What is claimed is:

1. A Melanoma Associated Antigen A4 (MAGE-A4)-specific chimeric antigen receptor comprising, 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, wherein the extracellular ligand-binding domain comprises an anti-MAGE-A4 single chain variable fragment (scFv) domain comprising a light chain variable region (LCVR) comprising complementarity determining regions LCDR1, LCDR2, and LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 12, 14, and 16, and a heavy chain variable region (HCVR) comprising complementarity determining regions HCDR1, HCDR2, and HCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 4, 6, and 8.
2. The chimeric antigen receptor of claim 1, wherein the anti-MAGE-A4 scFv domain comprises a linker between the LCVR and the HCVR.
3. The chimeric antigen receptor of claim 2, wherein the linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 23-26.
4. The chimeric antigen receptor of claim 3, wherein the linker comprises the amino acid sequence of SEQ ID NO: 25.
5. The chimeric antigen receptor of any one of claims 1-4, further comprising a linker between the extracellular ligand-binding domain and the hinge.
6. The chimeric antigen receptor of any one of claims 1-5, wherein the LCVR comprises the amino acid sequence of SEQ ID NO: 10, and the HCVR comprises the amino acid sequence of SEQ ID NO: 2.
7. The chimeric antigen receptor of any one of claims 1-5, wherein the LCVR comprises the amino acid sequence of SEQ ID NO: 10, and the HCVR comprises the amino acid sequence of SEQ ID NO: 83.
8. The chimeric antigen receptor of any one of claims 1-7:

(a) wherein the hinge, the transmembrane domain, or both, are from a CD8 α polypeptide;

(b) wherein the costimulatory domain comprises a 4-1BB costimulatory domain;
and

(c) wherein the signaling domain comprises a CD3zeta signaling domain.

9. The chimeric antigen receptor of any one of claims 1-7:

(a) wherein one or more of the hinge, the transmembrane domain, and the costimulatory domain are from a CD28 polypeptide; and

(b) wherein the signaling domain comprises a CD3zeta signaling domain.

10. The chimeric antigen receptor of any one of claims 1-7:

(a) wherein the hinge comprises the amino acid sequence of SEQ ID NO: 27;

(b) wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 28;

(c) wherein the costimulatory domain comprises the amino acid sequence of SEQ ID NO: 29; or

(d) wherein the signaling domain comprises the amino acid sequence of SEQ ID NO: 30.

11. The chimeric antigen receptor of any one of claims 1-7:

(a) wherein the hinge comprises the amino acid sequence of SEQ ID NO: 34;

(b) wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 36;

(c) wherein the costimulatory domain comprises the amino acid sequence of SEQ ID NO: 38; or

(d) wherein the signaling domain comprises the amino acid sequence of SEQ ID NO: 30.

12. The chimeric antigen receptor of claim 1, comprising the amino acid sequence of SEQ ID NO: 22, the amino acid sequence of SEQ ID NO: 105, the amino acid sequence of sin 120, or the amino acid sequence of SEQ ID NO: 121.

13. An isolated nucleic acid molecule encoding the chimeric antigen receptor of any one of claims 1-12.
14. A vector comprising the nucleic acid molecule of claim 13.
15. The vector of claim 14, wherein the vector is a DNA vector, an RNA vector, a plasmid, a lentivirus vector, an adenovirus vector, or a retroviral vector.
16. An isolated cell comprising the isolated nucleic acid molecule of claim 13.
17. The isolated cell of claim 16, wherein the cell is a human T cell.
18. An engineered cell comprising a chimeric antigen receptor of any one of claims 1-12.
19. The engineered cell of claim 18 that is an immune cell.
20. The engineered cell of claim 19, wherein the immune cell is an immune effector cell, a T lymphocyte, an inflammatory T lymphocyte, a cytotoxic T lymphocyte, a regulatory T lymphocyte, a helper T lymphocyte, or a CD8⁺ cytotoxic T lymphocyte.
21. 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 costimulatory domain and a signaling domain, wherein the LCVR of the anti-MAGE-A4 scFv domain comprises complementarity determining regions LCDR1, LCDR2, and LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 12, 14, and 16, and the HCVR of the anti-MAGE-A4 scFv domain comprises complementarity determining regions HCDR1, HCDR2, and HCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 4, 6, and 8.
22. The engineered human T cell of claim 21, wherein the scFv domain comprises a LCVR/HCVR amino acid sequence pair comprising the amino acid sequences of SEQ ID NOs: 10/2, or SEQ ID NOs: 10/83.

23. The engineered human T cell of claim 21:

- (a) wherein the hinge comprises the amino acid sequence of SEQ ID NO: 27;
- (b) wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 28;
- (c) wherein the costimulatory domain comprises the amino acid sequence of SEQ ID NO: 29; or
- (d) wherein the signaling domain comprises the amino acid sequence of SEQ ID NO: 30.

24. The engineered human T cell of claim 21:

- (a) wherein the hinge comprises the amino acid sequence of SEQ ID NO: 34;
- (b) wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 36;
- (c) wherein the costimulatory domain comprises the amino acid sequence of SEQ ID NO: 38; or
- (d) wherein the signaling domain comprises the amino acid sequence of SEQ ID NO: 30.

25. The engineered human T cell of claim 21, comprising the amino acid sequence of SEQ ID NO: 22, the amino acid sequence of SEQ ID NO: 105, the amino acid sequence of sin 120, or the amino acid sequence of SEQ ID NO: 121.

26. 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 1-12.

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

- (a) providing a population of immune cells;
- (b) introducing into the immune cells a nucleic acid molecule encoding a chimeric antigen receptor of any one of claims 1-12;
- (c) culturing the immune cells under conditions to express the nucleic acid molecules; and

(d) isolating the immune cells expressing the chimeric antigen receptor at the cells' surface.

28. The method of claim 27, further comprising obtaining the population of immune cells from a subject prior to introducing the nucleic acid molecule.

29. Use of a cell comprising a chimeric antigen receptor of any one of claims 1-12 in the manufacture of a medicament for (i) enhancing T lymphocyte activity in a subject, (ii) treating a subject with cancer, (iii), stimulating a T-cell mediated immune response to a target cell population or tissue in a subject, or (iv) providing anti-tumor immunity in a subject.

30. An antigen-binding protein that specifically binds an HLA-bound Melanoma-Associated Antigen A4 (MAGE-A4), wherein the antigen-binding protein comprises a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises complementarity determining regions LCDR1, LCDR2, and LCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 12, 14, and 16, and wherein the HCVR comprises complementarity determining regions HCDR1, HCDR2, and HCDR3 comprising the amino acid sequences, respectively, of SEQ ID NOs: 4, 6, and 8.

31. The antigen-binding protein of claim 30, wherein said antigen-binding protein is a bispecific antibody.

32. The antigen-binding protein of 31, wherein said antigen-binding protein interacts with CD3.

33. A pharmaceutical composition comprising the antigen-binding protein of any one of claims 30-32, and a pharmaceutically acceptable carrier or diluent.

34. A polynucleotide molecule comprising a polynucleotide sequence that encodes the HCVR of the antigen-binding protein of any one of claims 30-32.

35. A polynucleotide molecule comprising a polynucleotide sequence that encodes the LCVR of the antigen-binding protein of any one of claims 30-32.

36. A polynucleotide molecule comprising a polynucleotide sequence that encodes the HCVR and the LCVR of the antigen-binding protein of any one of claims 30-32.

37. Use of an antigen-binding protein of any one of claims 30-32 in the manufacture of a medicament for treating a MAGE-A4 expressing cancer in a subject.