

CLAIMS

1. A nucleic acid molecule comprising (i) a first nucleotide sequence encoding a recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human melanoma-associated antigen 2 (MAGE-A2) ("anti-MAGE-A2 TCR"); and (ii) a second nucleotide sequence, wherein the second nucleotide sequence or the polypeptide encoded by the second nucleotide sequence inhibits the expression of an endogenous TCR,

wherein the anti-MAGE-A2 TCR cross competes for binding to human MAGE-A2 with a reference TCR, which comprises an alpha chain and a beta chain, and wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2.

2. The nucleic acid molecule of claim 1, wherein the anti-MAGE-A2 TCR binds to an epitope of MAGE-A2 consisting of an amino acid sequence as set forth in SEQ ID NO: 13.
3. The nucleic acid molecule of claim 2, wherein the epitope is complexed with an HLA class II molecule selected from an HLA-DP, HLA-DQ, or HLA-DR allele, or any combination thereof.
4. The nucleic acid molecule of claim 3, wherein the HLA class II molecule is an HLA-DP allele, optionally an HLA-DP4 allele.
5. The nucleic acid molecule of any one of claims 1 to 4, wherein the anti-MAGE-A2 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a variable region comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and

wherein the beta chain comprises a variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3;

wherein:

- (i) the beta chain CDR3 of the anti-MAGE-A2 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10;
- (ii) the beta chain CDR2 of the anti-MAGE-A2 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 9;
- (iii) the beta chain CDR1 of the anti-MAGE-A2 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 8;
- (iv) the alpha chain CDR3 of the anti-MAGE-A2 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 7;
- (v) the alpha chain CDR2 of the anti-MAGE-A2 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 6;
- (vi) the alpha chain CDR1 of the anti-MAGE-A2 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 5; or
- (vii) any combination of (i) to (vi).

6. The nucleic acid molecule of claim 5, wherein

- (i) the alpha chain variable domain of the anti-MAGE-A2 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth SEQ ID NO: 1;
- (ii) the beta chain variable domain of the anti-MAGE-A2 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth SEQ ID NO: 2; or
- (iii) both (i) and (ii).

7. The nucleic acid molecule of claim 5 or 6, wherein:

- (a) the alpha chain of the anti-MAGE-A2 TCR further comprises a constant region, wherein the constant region is different from endogenous constant region of the alpha chain, and wherein
 - (i) the alpha chain of the anti-MAGE-A2 TCR further comprises a constant region, wherein the alpha chain constant region comprises an amino acid sequence having at least about 85% sequence identity to a constant region present in the amino acid sequence set forth SEQ ID NO: 1; or
 - (ii) the alpha chain constant region comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth SEQ ID NO: 1;
 - (b) the beta chain of the anti-MAGE-A2 TCR further comprises a constant region, wherein the constant region is different from endogenous constant regions of the beta chain, and wherein
 - (i) the beta chain of the anti-MAGE-A2 TCR further comprises a constant region, wherein the beta chain constant region comprises an amino acid sequence having at least about 85% sequence identity to a constant region present in the amino acid sequence set forth SEQ ID NO: 2; or
 - (ii) the beta chain constant region comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth SEQ ID NO: 2; or
 - (c) both (a) and (b).
8. The nucleic acid molecule of any one of claims 5 to 7, wherein
- (i) the alpha chain of the anti-MAGE-A2 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 1;

- (ii) the beta chain of the anti-MAGE-A2 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 2;
 - (iii) both (i) and (ii).
- 9. The nucleic acid molecule of any one of claims 1 to 8, wherein the second nucleotide sequence is one or more siRNAs that reduce the expression of endogenous TCRs, wherein the one or more siRNAs are complementary to a target sequence within a nucleotide sequence encoding a constant region of the endogenous TCRs.
- 10. The nucleic acid molecule of any one of claims 1 to 9, wherein the anti-MAGE-A2 TCR comprises an alpha chain constant region, a beta chain constant region, or both; and wherein the alpha chain constant region, the beta chain constant region, or both comprises an amino acid sequence having at least 1, at least 2, at least 3, at least 4, or at least 5 substitutions within the target sequence relative to the corresponding amino acid sequence of an endogenous TCR.
- 11. A vector comprising the nucleic acid molecule of any one of claims 1 to 10.
- 12. A T cell receptor (TCR) or an antigen binding portion thereof comprising the alpha chain variable domain of the anti-MAGE-A2 TCR and the beta chain variable domain of the anti-MAGE-A2 TCR of any one of claims 5 to 11.
- 13. A bispecific TCR comprising a first antigen-binding domain and a second antigen-binding domain, wherein the first antigen-binding domain comprises the TCR or an antigen-binding portion thereof of claim 12.
- 14. A cell comprising the nucleic acid molecule of any one of claims 1 to 10, the vector of claim 11, the TCR of claim 12, or the bispecific TCR of claim 13.
- 15. Use of the cell of claim 14 in the manufacture of a medicament for treating a cancer in a subject in need thereof.

16. A method of engineering an antigen-targeting cell, comprising transducing a cell collected from a subject in need of a T cell therapy with the nucleic acid molecule of any one of claims 1 to 10 or the vector of claim 11.
17. An HLA class II molecule complexed to a peptide, wherein the HLA class II molecule comprises an alpha chain and a beta chain; and wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 13.
18. An antigen presenting cell (APC), comprising the HLA class II molecule of claim 17.
19. A method of enriching a target population of T cells obtained from a human subject, comprising contacting the T cells with the HLA class II molecule of claim 17 or the APC of claim 18, wherein following the contacting, the enriched population of T cells comprises a higher number of T cells capable of binding the HLA class II molecule relative to the number of T cells capable of binding the HLA class II molecule prior to the contacting.
20. A cancer vaccine comprising a peptide having an amino acid sequence as set forth in SEQ ID NO: 13.