

CLAIMS

We claim:

1. A T cell receptor (TCR) that binds specifically to an HLA-A2 presented cancer testis antigen melanoma-associated antigen 4 (MAGE-A4) peptide comprising the amino acid sequence of KVLEHVVRV (SEQ ID NO:609) (MAGE-A4 286-294), wherein the TCR comprises an alpha chain variable domain comprising a complementary determining region (CDR)3, wherein the CDR3 comprises the amino acid sequence of Formula I:

N₁-N₂-N₃-N₄-N₅-N₆-N₇-N₈-N₉-N₁₀-N₁₁-N₁₂-N₁₃-N₁₄-N₁₅ (Formula I), wherein

N₁ is a non-polar amino acid;

N₂, which may or may not be present, is Val;

N₃ is Tyr, Gly, Leu, Val, Glu, Met, Ala, or Phe;

N₄, which may or may not be present, is Arg, Glu, Ser, Asn, Gln, Lys, Asp, Gly, or Met;

N₅, which may or may not be present, is Ser, Arg, Glu, Leu, Ala, Asp, Pro, Met, Gly or Lys;

N₆, which may or may not be present, is Ala, Asp, Gly, Ser, Val, Pro, Leu, Tyr, or Thr;

N₇ is Thr, Pro, Ser, Glu, Asp, Trp, Arg, Asn, Ile, Gln, or Leu;

N₈ is His, Trp, Thr, Lys, Tyr, or Ala;

N₉ is Asn, Gly, Lys, Ile, Ser, or Arg;

N₁₀, which may or may not be present, is Gln, Lys, Gly, Thr, Leu, Asp, or Ser;

N₁₁, which may or may not be present, is Phe, Asn, Thr, Tyr, Ala, Leu, Met or Glu;

N₁₂, which may or may not be present, is Lys, Phe, Tyr, or Asp;

N₁₃, which may or may not be present, is Lys or Gly;

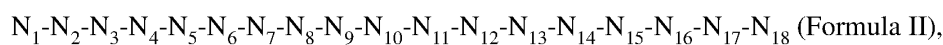
N₁₄, which may or may not be present, is Thr, Leu, or Tyr; and

N₁₅ is Tyr, Gln, Ile, Thr, Val, or Arg.

2. The TCR of claim 1, wherein N₁ is Ala, Ile, or Gly.

3. A T cell receptor (TCR) that binds specifically to an HLA-A2 presented cancer testis antigen melanoma-associated antigen 4 (MAGE-A4) peptide comprising the amino acid sequence of KVLEHVVRV (SEQ ID NO:609) (MAGE-A4 286-294), wherein

the TCR comprises a beta chain variable domain comprising complementary determining region (CDR)3, wherein the CDR3 comprises the amino acid sequence of Formula II:



wherein

N₁ is Ala or Ser;

N₂ is Ala, Ser, or Thr;

N₃ is Ser, Gly, or Trp;

N₄ is Leu, Tyr, Trp, Asp, Phe, Gly, Pro, or His;

N₅, which may or may not be present, is Gly or Asp;

N₆, which may or may not be present, is Phe or Arg;

N₇, which may or may not be present, is Trp, Phe, Asp, Pro, Tyr, Gly, Thr, Ser, or Val;

N₈, which may or may not be present, is Pro, Arg, Asp, Tyr, Gln, Asn, or Gly;

N₉, which may or may not be present, is Asp;

N₁₀, which may or may not be present, is Arg;

N₁₁, which may or may not be present, is Gly, Ala, or Thr;

N₁₂ is Ser, Trp, Thr, Gly, Val, Leu, Arg, Met, Tyr, or Gln;

N₁₃, which may or may not be present, is Gly;

N₁₄, which may or may not be present, is Asn, Asp, Gly, Thr, Pro, Gln, or His;

N₁₅, which may or may not be present, is Thr, Ser, Glu, Asn, Tyr, Gln, Asp, or Pro;

N₁₆, which may or may not be present, is Glu, Pro, Lys, Thr, Ala, Gly, or Gln;

N₁₇, which may or may not be present, is Ala, Leu, Ile, Tyr, or Gln; and

N₁₈ is Phe, His, Tyr, or Thr.

4. The TCR of claim 1 or 2, wherein the alpha chain variable domain further comprises a CDR1 and a CDR2, wherein the CDR1 comprises any one of the alpha chain variable domain CDR1 amino acid sequences set forth in Table 2 and the CDR2 independently comprises any one of the alpha chain variable domain CDR2 amino acid sequences set forth in Table 2.

5. The TCR of claim 3, wherein the beta chain variable domain further comprises a CDR1 and a CDR2, wherein the CDR1 comprises any one of the beta chain variable CDR1 amino acid sequences set forth in Table 2 and the CDR2 independently comprises any one of the beta chain variable domain CDR2 amino acid sequences set forth in Table 2.

6. The TCR of any one of claims 1-5, wherein the TCR comprises at least one TCR alpha chain variable domain and/or at least one beta chain variable domain.
7. The TCR of claim 6, wherein the TCR comprises a TCR alpha chain variable domain and a TCR beta chain variable domain.
8. The TCR of any one of claims 3-7, comprising alpha chain variable domain CDR1, CDR2 and CDR3 contained within any one of the alpha chain variable domain sequences listed in Table 4; and beta chain variable domain CDR1, CDR2 and CDR3 contained within any one of the beta chain variable domain sequences listed in Table 4.
9. The TCR of any one of claims 1-8, comprising an alpha chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the alpha chain variable domain amino acid sequences listed in Table 4.
10. The TCR of any one of claims 1-9, comprising a beta chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the beta chain variable domain amino acid sequences listed in Table 4.
11. The TCR of any one of claims 1-10, comprising: (a) an alpha chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the alpha chain variable domain amino acid sequences listed in Table 4; and (b) a beta chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the beta chain variable domain amino acid sequences listed in Table 4.
12. The TCR of any one of claims 3-11, comprising:
 - (a) an alpha chain variable domain CDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 17, 33, 49, 65, 81, 97, 113, 129, 145,

161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369, 385, 401, 417, 433, 449, 465, 481, 497, 513, 529, 545, 561, 577, and 593;

(b) an alpha chain variable domain CDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, 578, and 594;

(c) an alpha chain variable domain CDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 403, 419, 435, 451, 467, 483, 499, 515, 531, 547, 563, 579, and 595;

(d) a beta chain variable domain CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, 217, 233, 249, 265, 281, 297, 313, 329, 345, 361, 377, 393, 409, 425, 441, 457, 473, 489, 505, 521, 537, 553, 569, 585, and 601;

(e) a beta chain variable domain CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, 586, and 602; and

(f) a beta chain variable domain CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 11, 27, 43, 59, 75, 91, 107, 123, 139, 155, 171, 187, 203, 219, 235, 251, 267, 283, 299, 315, 331, 347, 363, 379, 395, 411, 427, 443, 459, 475, 491, 507, 523, 539, 555, 571, 587, and 603.

13. The TCR of claim 12, comprising an alpha chain variable domain/beta chain variable domain amino acid sequence pair selected from the group consisting of SEQ ID NOs: 7/15, 23/31, 39/47, 55/63, 71/79, 87/95, 103/111, 119/127, 135/143, 151/159, 167/175, 183/191, 199/207, 215/223, 231/239, 247/255, 263/271, 279/287, 295/303, 311/319, 327/335, 343/351, 359/367, 375/383, 391/399, 407/415, 423/431, 439/447, 455/463, 471/479, 487/495, 503/511, 519/527, 535/543, 551/559, 567/575, 583/591, and 599/607.

14. The TCR of claim 12, comprising an alpha chain variable domain/beta chain variable domain amino acid sequence pair selected from the group consisting of SEQ ID NOs: 87/31, 23/95, 231/607, 231/223, 231/591, 231/255, 231/271, 231/79, 231/47, 231/399,

599/239, 599/223, 599/591, 599/255, 599/271, 599/79, 599/47, 599/399, 215/239, 215/607, 215/591, 215/255, 215/271, 215/79, 215/47, 215/399, 583/239, 583/607, 583/223, 583/255, 583/271, 583/79, 583/47, 583/399, 247/239, 247/607, 247/223, 247/591, 247/271, 247/79, 247/47, 247/399, 263/239, 263/607, 263/223, 263/591, 263/255, 263/79, 263/47, 263/399, 71/239, 71/607, 71/223, 71/591, 71/255, 71/271, 71/47, 71/399, 39/239, 39/607, 39/223, 39/591, 39/255, 39/271, 39/79, 39/399, 391/239, 391/607, 391/223, 391/591, 391/255, 391/271, 391/79, 391/47, 439/127, 439/319, 439/287, 439/15, 439/111, 439/383, 439/191, 439/511, 439/527, 439/559, 439/207, 119/447, 119/319, 119/287, 119/15, 119/111, 119/383, 119/191, 119/511, 119/527, 119/559, 119/207, 311/447, 311/127, 311/287, 311/15, 311/111, 311/383, 311/191, 311/511, 311/527, 311/559, 311/207, 279/447, 279/127, 279/319, 279/15, 279/111, 279/383, 279/191, 279/511, 279/527, 279/559, 279/207, 7/447, 7/127, 7/319, 7/287, 7/111, 7/383, 7/191, 7/511, 7/527, 7/559, 7/207, 103/447, 103/127, 103/319, 103/287, 103/15, 103/383, 103/191, 103/511, 103/527, 103/559, 103/207, 375/447, 375/127, 375/319, 375/287, 375/15, 375/111, 375/191, 375/511, 375/527, 375/559, 375/207, 183/447, 183/127, 183/319, 183/287, 183/15, 183/111, 183/383, 183/511, 183/527, 183/559, 183/207, 503/447, 503/127, 503/319, 503/287, 503/15, 503/111, 503/383, 503/191, 503/527, 503/559, 503/207, 519/447, 519/127, 519/319, 519/287, 519/15, 519/111, 519/383, 519/191, 519/511, 519/559, 519/207, 551/447, 551/127, 551/319, 551/287, 551/15, 551/111, 551/383, 551/191, 551/511, 551/527, 551/207, 199/447, 199/127, 199/319, 199/287, 199/15, 199/111, 199/383, 199/191, 199/511, 199/527, and 199/559.

15. An TCR that competes for binding to the TCR of any one of claims 1-14.
16. The TCR of any one of claims 1-15, further comprising a detectable moiety.
17. A pharmaceutical composition comprising the TCR of any one of claims 1-16 and a pharmaceutically acceptable carrier or diluent.
18. An isolated cell presenting the TCR of any one of claims 1-16.
19. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes an alpha chain variable domain of the TCR as set forth in any one of claims 1-16.

20. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a beta chain variable domain of the TCR as set forth in any one of claims 1-16.

21. A vector comprising the polynucleotide molecule of claim 19 or 20.

22. An isolated cell expressing the vector of claim 21.

23. A method of treating a subject having a MAGE-A4-associated disease or disorder, comprising administering to the subject a therapeutically effective amount of the TCR as set forth in any one of claims 1-16, the pharmaceutical composition of claim 17, or a plurality of the isolated cells of claim 18, thereby treating the subject.

24. The method of claim 23, wherein the MAGE-A4-associated disease or disorder is MAGE-A4-associated cancer.

25. The method of claim 24, wherein the MAGE-A4-associated cancer is a liposarcoma, a neuroblastoma, a myeloma, a melanoma, a metastatic melanoma, a synovial sarcoma, a bladder cancer, an esophageal cancer, an esophageal squamous cell carcinoma, a hepatocellular cancer, a head and neck cancer, a non-small cell lung cancer, an ovarian cancer, an ovarian epithelial cancer, a prostate cancer, a breast cancer, an astrocytic tumor, a glioblastoma multiforme, an anaplastic astrocytoma, a brain tumor, a fallopian tube cancer, primary peritoneal cavity cancer, advanced solid tumors, soft tissue sarcoma, a sarcoma, a myelodysplastic syndrome, an acute myeloid leukemia, a Hodgkin lymphoma, a non-Hodgkin lymphoma, a Hodgkin disease, a multiple myeloma, a metastatic solid tumors, a colorectal carcinoma, a stomach cancer, a gastric cancer, a rhabdomyosarcoma, a myxoid round cell liposarcoma, or a recurrent non-small cell lung cancer.

26. The method of any one of claims 23-25, wherein the TCR, the pharmaceutical composition, or the plurality of cells is administered to the subject in combination with a second therapeutic agent.

27. The method of any one of claims 23-26, wherein the TCR, the pharmaceutical composition, or the plurality of cells is administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, intramuscularly or intracranially to the subject.

28. A polynucleotide molecule encoding a T cell receptor (TCR), wherein the TCR binds specifically to an HLA-A2 presented cancer testis antigen melanoma-associated antigen 4 (MAGE-A4) peptide comprising the amino acid sequence of KVLEHVVRV (SEQ ID NO:609) (MAGE-A4 286-294), wherein the TCR has a property selected from the group consisting of: (a) does not bind to cells expressing predicted off-target peptides as determined by luminescence assay; (b) activates a T cell response about two times greater than a patient-derived MAGE-A4-specific TCR as determined by a TCR-mediated T cell signaling luminescent bioassay; and (c) activates a T cell response about two times greater than an affinity-matured (e.g., by phage display) MAGE-A4-specific TCR as determined by a TCR-mediated T cell signaling luminescent bioassay.

29. The polynucleotide molecule of claim 28, encoding at least one TCR alpha chain variable domain and/or at least one beta chain variable domain.

30. The polynucleotide molecule of claim 28, wherein the TCR comprises alpha chain variable domain complementary determining regions (CDR) 1, CDR2, and CDR3 contained within any one of the alpha chain variable domain sequences listed in Table 4; and beta chain variable domain CDR1, CDR2 and CDR3 contained within any one of the beta chain variable domain sequences listed in Table 4.

31. The polynucleotide molecule of claim 29 or 30, wherein the TCR comprises alpha chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the alpha chain variable domain amino acid sequences listed in Table 4.

32. The polynucleotide molecule of any one of claims 28-31, wherein the TCR comprises beta chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the beta chain variable domain amino acid sequences listed in Table 4.

33. The polynucleotide molecule of any one of claims 28-32, wherein the TCR comprises (a) an alpha chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the alpha chain variable domain amino acid sequences listed in Table 4; and (b) a beta chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the beta chain variable domain amino acid sequences listed in Table 4.

34. The polynucleotide molecule of any one of claims 28-33, wherein the TCR comprises

(a) an alpha chain variable domain CDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 17, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369, 385, 401, 417, 433, 449, 465, 481, 497, 513, 529, 545, 561, 577, and 593;

(b) an alpha chain variable domain CDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, 578, and 594;

(c) an alpha chain variable domain CDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 403, 419, 435, 451, 467, 483, 499, 515, 531, 547, 563, 579, and 595;

(d) a beta chain variable domain CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 25, 41, 57, 73, 89, 105, 121, 137, 153, 169, 185, 201, 217, 233, 249, 265, 281, 297, 313, 329, 345, 361, 377, 393, 409, 425, 441, 457, 473, 489, 505, 521, 537, 553, 569, 585, and 601;

(e) a beta chain variable domain CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, 586, and 602; and

(f) a beta chain variable domain CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 11, 27, 43, 59, 75, 91, 107, 123, 139, 155, 171, 187,

203, 219, 235, 251, 267, 283, 299, 315, 331, 347, 363, 379, 395, 411, 427, 443, 459, 475, 491, 507, 523, 539, 555, 571, 587, and 603.

35. The polynucleotide molecule of claim 34, wherein the TCR comprises an alpha chain variable domain/beta chain variable domain amino acid sequence pair selected from the group consisting of SEQ ID NOs: 7/15, 23/31, 39/47, 55/63, 71/79, 87/95, 103/111, 119/127, 135/143, 151/159, 167/175, 183/191, 199/207, 215/223, 231/239, 247/255, 263/271, 279/287, 295/303, 311/319, 327/335, 343/351, 359/367, 375/383, 391/399, 407/415, 423/431, 439/447, 455/463, 471/479, 487/495, 503/511, 519/527, 535/543, 551/559, 567/575, 583/591, and 599/607.

36. The polynucleotide molecule of claim 34, wherein the TCR comprises an alpha chain variable domain/beta chain variable domain amino acid sequence pair selected from the group consisting of SEQ ID NOs: 87/31, 23/95, 231/607, 231/223, 231/591, 231/255, 231/271, 231/79, 231/47, 231/399, 599/239, 599/223, 599/591, 599/255, 599/271, 599/79, 599/47, 599/399, 215/239, 215/607, 215/591, 215/255, 215/271, 215/79, 215/47, 215/399, 583/239, 583/607, 583/223, 583/255, 583/271, 583/79, 583/47, 583/399, 247/239, 247/607, 247/223, 247/591, 247/271, 247/79, 247/47, 247/399, 263/239, 263/607, 263/223, 263/591, 263/255, 263/79, 263/47, 263/399, 71/239, 71/607, 71/223, 71/591, 71/255, 71/271, 71/47, 71/399, 39/239, 39/607, 39/223, 39/591, 39/255, 39/271, 39/79, 39/399, 391/239, 391/607, 391/223, 391/591, 391/255, 391/271, 391/79, 391/47, 439/127, 439/319, 439/287, 439/15, 439/111, 439/383, 439/191, 439/511, 439/527, 439/559, 439/207, 119/447, 119/319, 119/287, 119/15, 119/111, 119/383, 119/191, 119/511, 119/527, 119/559, 119/207, 311/447, 311/127, 311/287, 311/15, 311/111, 311/383, 311/191, 311/511, 311/527, 311/559, 311/207, 279/447, 279/127, 279/319, 279/15, 279/111, 279/383, 279/191, 279/511, 279/527, 279/559, 279/207, 7/447, 7/127, 7/319, 7/287, 7/111, 7/383, 7/191, 7/511, 7/527, 7/559, 7/207, 103/447, 103/127, 103/319, 103/287, 103/15, 103/383, 103/191, 103/511, 103/527, 103/559, 103/207, 375/447, 375/127, 375/319, 375/287, 375/15, 375/111, 375/191, 375/511, 375/527, 375/559, 375/207, 183/447, 183/127, 183/319, 183/287, 183/15, 183/111, 183/383, 183/511, 183/527, 183/559, 183/207, 503/447, 503/127, 503/319, 503/287, 503/15, 503/111, 503/383, 503/191, 503/527, 503/559, 503/207, 519/447, 519/127, 519/319, 519/287, 519/15, 519/111, 519/383, 519/191, 519/511, 510/559, 519/207, 551/447, 551/127, 551/319, 551/287, 551/15,

551/111, 551/383, 551/191, 551/511, 551/527, 551/207, 199/447, 199/127, 199/319, 199/287, 199/15, 199/111, 199/383, 199/191, 199/511, 199/527, and 199/559.

37. The polynucleotide molecule of claim 33, wherein the TCR comprises

(a) an alpha chain variable domain CDR1 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340, 356, 372, 388, 404, 420, 436, 452, 468, 484, 500, 516, 532, 548, 564, 580, and 596;

(b) an alpha chain variable domain CDR2 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 5, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, 181, 197, 213, 229, 245, 261, 277, 293, 309, 325, 341, 357, 373, 389, 405, 421, 437, 453, 469, 485, 501, 517, 533, 549, 565, 581, and 597;

(c) an alpha chain variable domain CDR3 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342, 358, 374, 390, 406, 422, 438, 454, 470, 486, 502, 518, 534, 550, 566, 582, and 598;

(d) a beta chain variable domain CDR1 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572, 588, and 604;

(e) a beta chain variable domain CDR2 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 13, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365, 381, 397, 413, 429, 445, 461, 477, 493, 509, 525, 541, 557, 573, 589, and 605; and

(f) a beta chain variable domain CDR3 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, 574, 590, and 606.

38. The polynucleotide molecule of claim 37, wherein the TCR comprises an alpha chain variable domain/beta chain variable domain nucleic acid sequence pair selected from the group consisting of SEQ ID NOs: 8/16, 24/32, 40/48, 56/64, 72/80, 88/96, 104/112, 120/128, 136/144, 152/160, 168/176, 184/192, 200/208, 216/224, 232/240, 248/256, 264/272,

280/288, 296/304, 312/320, 328/336, 344/352, 360/368, 376/384, 392/400, 408/416, 424/432, 440/448, 456/464, 472/480, 488/496, 504/512, 520/528, 536/544, 552/560, 568/576, 584/592, 600/608.

39. A vector comprising the polynucleotide molecule of any one of claims 28-38.

40. An isolated cell comprising the vector of claim 39.

41. A method of treating a subject having a MAGE-A4-associated disease or disorder, comprising administering to the subject a plurality of the cells of claim 40, thereby treating the subject.

42. The method of claim 41, wherein the MAGE-A4-associated disease or disorder is MAGE-A4-associated cancer.

43. The method of claim 43, wherein the MAGE-A4-associated cancer is a liposarcoma, a neuroblastoma, a myeloma, a melanoma, a metastatic melanoma, a synovial sarcoma, a bladder cancer, an esophageal cancer, an esophageal squamous cell carcinoma, a hepatocellular cancer, a head and neck cancer, a non-small cell lung cancer, an ovarian cancer, an ovarian epithelial cancer, a prostate cancer, a breast cancer, an astrocytic tumor, a glioblastoma multiforme, an anaplastic astrocytoma, a brain tumor, a fallopian tube cancer, primary peritoneal cavity cancer, advanced solid tumors, soft tissue sarcoma, a sarcoma, a myelodysplastic syndrome, an acute myeloid leukemia, a Hodgkin lymphoma, a non-Hodgkin lymphoma, a Hodgkin disease, a multiple myeloma, a metastatic solid tumors, a colorectal carcinoma, a stomach cancer, a gastric cancer, a rhabdomyosarcoma, a myxoid round cell liposarcoma, or a recurrent non-small cell lung cancer.

44. The method of any one of claims 41-43, wherein the plurality of cells is administered to the subject in combination with a second therapeutic agent.

45. A T cell receptor (TCR) that binds specifically to an HLA-A2 presented cancer testis antigen melanoma-associated antigen 4 (MAGE-A4) peptide comprising the amino acid sequence of GGYDGREHTV (SEQ ID NO:612) (MAGE-A4 230-239), wherein

the TCR comprises a complementary determining region 3 (CDR3) contained within an alpha chain variable domain of any one of SEQ ID NOs: 620, 636, 652, 668, 684, 700, 716, 732, 748, 764, 780, 796, 812, 828, 844, and 860.

46. A T cell receptor (TCR) that binds specifically to an HLA-A2 presented cancer testis antigen melanoma-associated antigen 4 (MAGE-A4) peptide comprising the amino acid sequence of GVDGREHTV (SEQ ID NO:612) (MAGE-A4 230-239), wherein the TCR comprises a complementary determining region 3 (CDR3) contained within a beta chain variable domain of any one of SEQ ID NOs: 628, 644, 660, 676, 692, 708, 724, 740, 756, 772, 788, 804, 820, 836, 852, and 868.

47. The TCR of claim 45, wherein the alpha chain variable domain further comprises a CDR1 and a CDR2, wherein the CDR1 comprises any one of the alpha chain variable domain CDR1 amino acid sequences set forth in Table 6 and the CDR2 independently comprises any one of the alpha chain variable domain CDR2 amino acid sequences set forth in Table 6.

48. The TCR of claim 46, wherein the beta chain variable domain further comprises a CDR1 and a CDR2, wherein the CDR1 comprises any one of the beta chain variable CDR1 amino acid sequences set forth in Table 6 and the CDR2 independently comprises any one of the beta chain variable domain CDR2 amino acid sequences set forth in Table 6.

49. The TCR of any one of claims 45-48, wherein the TCR comprises at least one TCR alpha chain variable domain and/or at least one beta chain variable domain.

50. The TCR of claim 49, wherein the TCR comprises a TCR alpha chain variable domain and a TCR beta chain variable domain.

51. The TCR of any one of claims 46-50, comprising alpha chain variable domain CDR1, CDR2 and CDR3 contained within any one of the alpha chain variable domain sequences listed in Table 8; and beta chain variable domain CDR1, CDR2 and CDR3 contained within any one of the beta chain variable domain sequences listed in Table 8.

52. The TCR of any one of claims 45-51, comprising an alpha chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the alpha chain variable domain amino acid sequences listed in Table 8.

53. The TCR of any one of claims 45-52, comprising a beta chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the beta chain variable domain amino acid sequences listed in Table 8.

54. The TCR of any one of claims 45-53, comprising: (a) an alpha chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the alpha chain variable domain amino acid sequences listed in Table 8; and (b) a beta chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the beta chain variable domain amino acid sequences listed in Table 8.

55. The TCR of any one of claims 46-54, comprising:

(a) an alpha chain variable domain CDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 614, 630, 646, 662, 678, 694, 710, 726, 742, 758, 774, 790, 806, 822, 838, and 854;

(b) an alpha chain variable domain CDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 615, 631, 647, 663, 679, 695, 711, 727, 743, 759, 775, 791, 807, 823, 839, and 855;

(c) an alpha chain variable domain CDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 616, 632, 648, 664, 680, 696, 712, 728, 744, 760, 776, 792, 808, 824, 840, and 856;

(d) a beta chain variable domain CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 622, 638, 654, 670, 686, 702, 718, 734, 750, 766, 782, 798, 814, 830, 846, and 862;

(e) a beta chain variable domain CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 623, 639, 655, 671, 687, 703, 719, 735, 751, 767, 783, 799, 815, 831, 847, and 863; and

(f) a beta chain variable domain CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 624, 640, 656, 672, 688, 704, 720, 736, 752, 768, 784, 800, 816, 832, 848, and 864.

56. The TCR of claim 55, comprising an alpha chain variable domain/beta chain variable domain amino acid sequence pair selected from the group consisting of SEQ ID NOs: 620/628, 636/644, 652/660, 668/676, 684/692, 700/708, 716/724, 732/740, 748/756, 764/772, 780/788, 796/804, 812/820, 828/836, 844/852, and 860/868.

57. The TCR of claim 55, comprising an alpha chain variable domain/beta chain variable domain amino acid sequence pair selected from the group consisting of SEQ ID NOs: 620/628, 620/644, 620/660, 620/676, 620/692, 620/708, 620/724, 620/740, 620/756, 620/772, 620/788, 620/804, 620/820, 620/836, 620/852, 620/868, 636/628, 636/644, 636/660, 636/676, 636/692, 636/708, 636/724, 636/740, 636/756, 636/772, 636/788, 636/804, 636/820, 636/836, 636/852, 636/868, 652/628, 652/644, 652/660, 652/676, 652/692, 652/708, 652/724, 652/740, 652/756, 652/772, 652/788, 652/804, 652/820, 652/836, 652/852, 652/868, 668/628, 668/644, 668/660, 668/676, 668/692, 668/708, 668/724, 668/740, 668/756, 668/772, 668/788, 668/804, 668/820, 668/836, 668/852, 668/868, 684/628, 684/644, 684/660, 684/676, 684/692, 684/708, 684/724, 684/740, 684/756, 684/772, 684/788, 684/804, 684/820, 684/836, 684/852, 684/868, 700/628, 700/644, 700/660, 700/676, 700/692, 700/708, 700/724, 700/740, 700/756, 700/772, 700/788, 700/804, 700/820, 700/836, 700/852, 700/868, 716/628, 716/644, 716/660, 716/676, 716/692, 716/708, 716/724, 716/740, 716/756, 716/772, 716/788, 716/804, 716/820, 716/836, 716/852, 716/868, 732/628, 732/644, 732/660, 732/676, 732/692, 732/708, 732/724, 732/740, 732/756, 732/772, 732/788, 732/804, 732/820, 732/836, 732/852, 732/868, 748/628, 748/644, 748/660, 748/676, 748/692, 748/708, 748/724, 748/740, 748/756, 748/772, 748/788, 748/804, 748/820, 748/836, 748/852, 748/868, 764/628, 764/644, 764/660, 764/676, 764/692, 764/708, 764/724, 764/740, 764/756, 764/772, 764/788, 764/804, 764/820, 764/836, 764/852, 764/868, 780/628, 780/644, 780/660, 780/676, 780/692, 780/708, 780/724, 780/740, 780/756, 780/772, 780/788, 780/804, 780/820, 780/836, 780/852, 780/868, 796/628, 796/644, 796/660, 796/676, 796/692, 796/708, 796/724, 796/740, 796/756, 796/772, 796/788, 796/804,

796/820, 796/836, 796/852, 796/868, 812/628, 812/644, 812/660, 812/676, 812/692, 812/708, 812/724, 812/740, 812/756, 812/772, 812/788, 812/804, 812/820, 812/836, 812/852, 812/868, 828/628, 828/644, 828/660, 828/676, 828/692, 828/708, 828/724, 828/740, 828/756, 828/772, 828/788, 828/804, 828/820, 828/836, 828/852, 828/868, 844/628, 844/644, 844/660, 844/676, 844/692, 844/708, 844/724, 844/740, 844/756, 844/772, 844/788, 844/804, 844/820, 844/836, 844/852, 844/868, 860/628, 860/644, 860/660, 860/676, 860/692, 860/708, 860/724, 860/740, 860/756, 860/772, 860/788, 860/804, 860/820, 860/836, 860/852, and 860/868.

58. The TCR of any one of claims 45-57, further comprising a detectable moiety.

59. The TCR of any one of claims 45-57, wherein said isolated TCR has an on-target binding/off-target binding value of greater than 5, greater than 10, greater than 15, greater than 20, greater than 50, greater than 100, greater than 200, greater than 300, greater than 400, greater than 500, greater than 600, greater than 700, greater than 800, greater than 900, or greater than 1000.

60. The TCR of claim 61, wherein said isolated TCR has an on-target binding/off-target binding value of greater than 10.

61. The TCR of claim 61, wherein said isolated TCR has an on-target binding/off-target binding value of greater than 500.

62. A TCR that competes for binding to the isolated TCR of any one of claims 45-61.

63. A pharmaceutical composition comprising the TCR of any one of claims 45-61 and a pharmaceutically acceptable carrier or diluent.

64. An isolated cell presenting the TCR of any one of claims 45-61.

65. A polynucleotide molecule comprising a polynucleotide sequence that encodes an alpha chain variable domain of the TCR as set forth in any one of claims 45, 47, and 49-61.

66. A isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a beta chain variable domain of the TCR as set forth in any one of claims 46, 48, and 49-61.

67. A vector comprising the polynucleotide sequence of claim 65 or 66.

68. An isolated cell expressing the vector of claim 67.

69. A method of treating a subject having a MAGE-A4-associated disease or disorder, comprising administering to the subject a therapeutically effective amount of the TCR of any one of claims 45-61, the pharmaceutical composition of claim 63, or the isolated cell of claim 64, thereby treating the subject.

70. The method of claim 69, wherein the MAGE-A4-associated disease or disorder is MAGE-A4-associated cancer.

71. The method of claim 70, wherein the MAGE-A4-associated cancer is a liposarcoma, a neuroblastoma, a myeloma, a melanoma, a metastatic melanoma, a synovial sarcoma, a bladder cancer, an esophageal cancer, an esophageal squamous cell carcinoma, a hepatocellular cancer, a head and neck cancer, a non-small cell lung cancer, an ovarian cancer, an ovarian epithelial cancer, a prostate cancer, a breast cancer, an astrocytic tumor, a glioblastoma multiforme, an anaplastic astrocytoma, a brain tumor, a fallopian tube cancer, primary peritoneal cavity cancer, advanced solid tumors, soft tissue sarcoma, a sarcoma, a myelodysplastic syndrome, an acute myeloid leukemia, a Hodgkin lymphoma, a non-Hodgkin lymphoma, a Hodgkin disease, a multiple myeloma, a metastatic solid tumors, a colorectal carcinoma, a stomach cancer, a gastric cancer, a rhabdomyosarcoma, a myxoid round cell liposarcoma, or a recurrent non-small cell lung cancer.

72. The method of any one of claims 69-71, wherein the TCR, the pharmaceutical composition, or the cell is administered to the subject in combination with a second therapeutic agent.

73. The method of any one of claims 69-71, wherein said administering is parenteral.

74. A polynucleotide molecule encoding a T cell receptor (TCR), wherein the TCR binds specifically to an HLA-A2 presented cancer testis antigen melanoma-associated antigen 4 (MAGE-A4) peptide comprising the amino acid sequence of GGYDGREHTV (SEQ ID NO:612) (MAGE-A4 230-239), wherein the TCR has a property selected from the group consisting of: (a) does not bind to cells expressing predicted off-target peptides as determined by a luminescence assay; (b) does not bind to cells expressing predicted off-target peptides as determined by a flow cytometry assay; (c) activates a T cell response about two times greater than a patient-derived MAGE-A4-specific TCR as determined by a TCR-mediated T cell signaling luminescent bioassay; and (d) activates a T cell response about two times greater than an affinity-matured (e.g., by phage display) MAGE-A4-specific TCR as determined by a TCR-mediated T cell signaling luminescent bioassay.

75. The polynucleotide molecule of claim 74, encoding at least one TCR alpha chain variable domain and/or at least one beta chain variable domain.

76. The polynucleotide molecule of claim 74, wherein the TCR comprises alpha chain variable domain complementary determining regions (CDR) 1, CDR2, and CDR3 contained within any one of the alpha chain variable domain sequences listed in Table 8; and beta chain variable domain CDR1, CDR2 and CDR3 contained within any one of the beta chain variable domain sequences listed in Table 8.

77. The polynucleotide molecule of claim 75 or 76, wherein the TCR comprises alpha chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the alpha chain variable domain amino acid sequences listed in Table 8.

78. The polynucleotide molecule of any one of claims 74-77, wherein the TCR comprises a beta chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the beta chain variable domain amino acid sequences listed in Table 8.

79. The polynucleotide molecule of any one of claims 74-78, wherein the TCR comprises (a) an alpha chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the alpha chain variable domain amino acid sequences listed in Table 8; and (b) a beta chain variable domain having an amino acid sequence that has at least 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences of the beta chain variable domain amino acid sequences listed in Table 8.

80. The polynucleotide molecule of any one of claims 74-79, wherein the TCR comprises

(a) an alpha chain variable domain CDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 614, 630, 646, 662, 678, 694, 710, 726, 742, 758, 774, 790, 806, 822, 838, and 854;

(b) an alpha chain variable domain CDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 615, 631, 647, 663, 679, 695, 711, 727, 743, 759, 775, 791, 807, 823, 839, and 855;

(c) an alpha chain variable domain CDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 616, 632, 648, 664, 680, 696, 712, 728, 744, 760, 776, 792, 808, 824, 840, and 856;

(d) a beta chain variable domain CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 622, 638, 654, 670, 686, 702, 718, 734, 750, 766, 782, 798, 814, 830, 846, and 862;

(e) a beta chain variable domain CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 623, 639, 655, 671, 687, 703, 719, 735, 751, 767, 783, 799, 815, 831, 847, and 863; and

(f) a beta chain variable domain CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 624, 640, 656, 672, 688, 704, 720, 736, 752, 768, 784, 800, 816, 832, 848, and 864.

81. The polynucleotide molecule of claim 80, wherein the TCR comprises an alpha chain variable domain/beta chain variable domain amino acid sequence pair selected from the group consisting of SEQ ID NOs: 620/628, 636/644, 652/660, 668/676, 684/692,

700/708, 716/724, 732/740, 748/756, 764/772, 780/788, 796/804, 812/820, 828/836, 844/852, and 860/868.

82. The polynucleotide molecule of claim 80, wherein the TCR comprises an alpha chain variable domain/beta chain variable domain amino acid sequence pair selected from the group consisting of SEQ ID NOs: 620/628, 620/644, 620/660, 620/676, 620/692, 620/708, 620/724, 620/740, 620/756, 620/772, 620/788, 620/804, 620/820, 620/836, 620/852, 620/868, 636/628, 636/644, 636/660, 636/676, 636/692, 636/708, 636/724, 636/740, 636/756, 636/772, 636/788, 636/804, 636/820, 636/836, 636/852, 636/868, 652/628, 652/644, 652/660, 652/676, 652/692, 652/708, 652/724, 652/740, 652/756, 652/772, 652/788, 652/804, 652/820, 652/836, 652/852, 652/868, 668/628, 668/644, 668/660, 668/676, 668/692, 668/708, 668/724, 668/740, 668/756, 668/772, 668/788, 668/804, 668/820, 668/836, 668/852, 668/868, 684/628, 684/644, 684/660, 684/676, 684/692, 684/708, 684/724, 684/740, 684/756, 684/772, 684/788, 684/804, 684/820, 684/836, 684/852, 684/868, 700/628, 700/644, 700/660, 700/676, 700/692, 700/708, 700/724, 700/740, 700/756, 700/772, 700/788, 700/804, 700/820, 700/836, 700/852, 700/868, 716/628, 716/644, 716/660, 716/676, 716/692, 716/708, 716/724, 716/740, 716/756, 716/772, 716/788, 716/804, 716/820, 716/836, 716/852, 716/868, 732/628, 732/644, 732/660, 732/676, 732/692, 732/708, 732/724, 732/740, 732/756, 732/772, 732/788, 732/804, 732/820, 732/836, 732/852, 732/868, 748/628, 748/644, 748/660, 748/676, 748/692, 748/708, 748/724, 748/740, 748/756, 748/772, 748/788, 748/804, 748/820, 748/836, 748/852, 748/868, 764/628, 764/644, 764/660, 764/676, 764/692, 764/708, 764/724, 764/740, 764/756, 764/772, 764/788, 764/804, 764/820, 764/836, 764/852, 764/868, 780/628, 780/644, 780/660, 780/676, 780/692, 780/708, 780/724, 780/740, 780/756, 780/772, 780/788, 780/804, 780/820, 780/836, 780/852, 780/868, 796/628, 796/644, 796/660, 796/676, 796/692, 796/708, 796/724, 796/740, 796/756, 796/772, 796/788, 796/804, 796/820, 796/836, 796/852, 796/868, 812/628, 812/644, 812/660, 812/676, 812/692, 812/708, 812/724, 812/740, 812/756, 812/772, 812/788, 812/804, 812/820, 812/836, 812/852, 812/868, 828/628, 828/644, 828/660, 828/676, 828/692, 828/708, 828/724, 828/740, 828/756, 828/772, 828/788, 828/804, 828/820, 828/836, 828/852, 828/868, 844/628, 844/644, 844/660, 844/676, 844/692, 844/708, 844/724, 844/740, 844/756, 844/772, 844/788, 844/804, 844/820, 844/836, 844/852, 844/868, 860/628, 860/644, 860/660, 860/676, 860/692, 860/708, 860/724, 860/740, 860/756, 860/772, 860/788, 860/804, 860/820, 860/836, 860/852, and 860/868.

83. The polynucleotide molecule of claim 33, wherein the TCR comprises

(a) an alpha chain variable domain CDR1 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 617, 633, 649, 665, 681, 697, 713, 729, 745, 761, 777, 793, 809, 825, 841, and 857;

(b) an alpha chain variable domain CDR2 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 618, 634, 650, 666, 682, 698, 714, 730, 746, 762, 778, 794, 810, 826, 842, and 858;

(c) an alpha chain variable domain CDR3 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 619, 635, 651, 667, 683, 699, 715, 731, 747, 763, 779, 795, 811, 827, 843, and 859;

(d) a beta chain variable domain CDR1 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 625, 641, 657, 673, 689, 705, 721, 737, 753, 769, 785, 801, 817, 833, 849, and 865;

(e) a beta chain variable domain CDR2 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 626, 642, 658, 674, 690, 706, 722, 738, 754, 770, 786, 802, 818, 834, 850, and 866; and

(f) a beta chain variable domain CDR3 encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 627, 643, 659, 675, 691, 707, 723, 739, 755, 771, 787, 803, 819, 835, 851, and 867.

84. The isolated nucleic acid molecule of claim 83, wherein the TCR comprises an alpha chain variable domain/beta chain variable domain nucleic acid sequence pair selected from the group consisting of SEQ ID NOs: 621/629, 637/645, 653/661, 669/677, 685/693, 701/709, 717/725, 733/741, 749/757, 765/773, 781/789, 797/805, 813/821, 829/837, 845/853, and 861/869.

85. A vector comprising the polynucleotide sequence of the isolated nucleic acid molecule of any one of claims 74-84.

86. An isolated cell comprising the vector of claim 85.

87. A method of treating a subject having a MAGE-A4-associated disease or disorder, comprising administering to the subject the cell of claim 86, thereby treating the subject.

88. The method of claim 87, wherein the MAGE-A4-associated disease or disorder is MAGE-A4-associated cancer.

89. The method of claim 88, wherein the MAGE-A4-associated cancer is a liposarcoma, a neuroblastoma, a myeloma, a melanoma, a metastatic melanoma, a synovial sarcoma, a bladder cancer, an esophageal cancer, an esophageal squamous cell carcinoma, a hepatocellular cancer, a head and neck cancer, a non-small cell lung cancer, an ovarian cancer, an ovarian epithelial cancer, a prostate cancer, a breast cancer, an astrocytic tumor, a glioblastoma multiforme, an anaplastic astrocytoma, a brain tumor, a fallopian tube cancer, primary peritoneal cavity cancer, advanced solid tumors, soft tissue sarcoma, a sarcoma, a myelodysplastic syndrome, an acute myeloid leukemia, a Hodgkin lymphoma, a non-Hodgkin lymphoma, a Hodgkin disease, a multiple myeloma, a metastatic solid tumors, a colorectal carcinoma, a stomach cancer, a gastric cancer, a rhabdomyosarcoma, a myxoid round cell liposarcoma, or a recurrent non-small cell lung cancer.

90. The method of any one of claims 87-89, wherein the cell is administered to the subject in combination with a second therapeutic agent.