

MAGE-A4 T CELL RECEPTORS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 62/862,726, filed on June 18, 2019, and U.S. Provisional Patent Application No.: 62/871,793, filed on July 9, 2019. The entire contents of each of the foregoing applications are incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 15, 2020, is named 118003_00320_SL.txt and is 344,832 bytes in size.

BACKGROUND

[0003] T cell receptors (TCRs) are membrane bound heterodimers comprising an α and β chain resembling an immunoglobulin variable (V) and constant (C) region. The TCR α chain includes a V- α chain covalently linked to a C- α chain, whereas the β chain includes a V- β chain covalently linked to a C- β chain. The V- α and V- β chains form a pocket or cleft that can bind an antigen in the context of a major histocompatibility complex (MHC) (known in humans as an HLA complex). (Davis *Ann. Rev. of Immunology* 3: 537 (1985); *Fundamental Immunology* 3rd Ed., W. Paul Ed. New York (1993)).

[0004] TCRs are primary effectors of the immune system that have unique advantages as a platform for developing therapeutics. While antibody therapeutics are limited to recognition of pathogens in the blood and extracellular spaces, or to protein targets on the cell surface, T cell receptors can recognize antigens displayed with MHC molecules on the surface of cells, including antigens derived from intracellular proteins. Depending on the subtype of T cells that recognize displayed antigen and become activated, TCRs can participate in controlling various immune responses. For instance, T cells are involved in regulation of the humoral immune response through induction of differentiation of B cells into antibody producing cells. In addition, activated T cells act to initiate cell-mediated immune responses. In addition, TCRs have been reported to mediate cell killing, increase B cell proliferation, and impact the development and severity of various disorders including cancer, allergies, viral infections and autoimmune disorders.

[0005] In view of the function of TCRs, antigen-specific TCRs have been evaluated for use in immunotherapy for their ability to redirect T cells to tumors expressing the antigen. TCRs will bind to a small peptide, only 8-12 amino acids in length, which are bound on the surface of a target cell by the Major Histocompatibility Complex (MHC). TCRs can therefore recognize intracellular antigens derived from cancer or viral proteins because these antigens are processed and displayed as peptides in the context of the surface MHC. Hence, TCRs can recognize additional internal cell targets not available to antibodies or therapies that cannot penetrate the cell.

[0006] However, the challenge of the industry is to engineer TCRs that lack immunogenicity when administered to a patient and have fine specificity to the particular peptide antigen of interest, without cross-reacting to other peptides on MHC or similar epitopes found in the natural protein repertoire.

[0007] MAGE-A4, or Melanoma-Associated Antigen A4, is a well-known cancer-testis antigen (CTAs) on the X chromosome. The function of MAGE-A4 is unknown, but it may be involved in cell cycle progression/regulation, transcriptional control, cell survival and/or apoptosis. For example, overexpression of MAGE-A4 has been shown to promote growth of spontaneously transformed oral keratinocytes; and inhibit growth arrest of cells in G1 (Bhan, *et al.* (2012) *Oncol Rep* 28(4):1496).

[0008] MAGE-A4 is abundantly expressed by many tumors of different histological types, such as head and neck squamous cell carcinoma, lung carcinoma, such as non-small cell lung carcinoma, esophageal squamous cell carcinoma, colon carcinoma, bladder cancer, mucosal and cutaneous melanomas, ovarian carcinoma, *e.g.*, serous carcinoma, and uterine carcinoma but, in normal healthy adult tissues, MAGE-A4 expression is restricted to the testes.

[0009] The ability of MAGE-A4 antigens to elicit immune responses together with its restricted expression pattern have rendered MAGE-A4 a good candidate for cancer immunotherapy.

[0010] There is an unmet need in the art for new targeting agents based on T cell receptors that specifically bind to MAGE-A4 antigens, as well as methods for producing and using such agents in therapeutic and diagnostic settings.

SUMMARY

[0011] The present invention provides T cell receptors (TCRs) that were generated against a MAGE-A4 peptide antigen in the context of MHC (HLA-A2). The unique TCR sequences identified have shown specific binding to the small peptide MAGE-A4 presented in the groove of an HLA molecule and exhibited activation of T cells in a reporter assay. Furthermore, the TCRs of the invention do not cross-react with other “like” peptides.

[0012] Accordingly, in one aspect, the present invention provides a T cell receptor (TCR) (e.g., an isolated TCR or a TCR expressed on an isolated cell) 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.

[0013] In one embodiment, N₁ is Ala, Ile, or Gly.

[0014] In another aspect, the present invention provides isolated 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:

$N_1-N_2-N_3-N_4-N_5-N_6-N_7-N_8-N_9-N_{10}-N_{11}-N_{12}-N_{13}-N_{14}-N_{15}-N_{16}-N_{17}-N_{18}$ (Formula

II),

wherein

N_1 is Ala or Ser;

N_2 is Ala, Ser, or Thr;

N_3 is Ser, Gly, or Trp;

N_4 is Leu, Tyr, Trp, Asp, Phe, Gly, Pro, or His;

N_5 , which may or may not be present, is Gly or Asp;

N_6 , which may or may not be present, is Phe or Arg;

N_7 , which may or may not be present, is Trp, Phe, Asp, Pro, Tyr, Gly, Thr, Ser,

or Val;

N_8 , which may or may not be present, is Pro, Arg, Asp, Tyr, Gln, Asn, or Gly;

N_9 , which may or may not be present, is Asp;

N_{10} , which may or may not be present, is Arg;

N_{11} , which may or may not be present, is Gly, Ala, or Thr;

N_{12} is Ser, Trp, Thr, Gly, Val, Leu, Arg, Met, Tyr, or Gln;

N_{13} , which may or may not be present, is Gly;

N_{14} , which may or may not be present, is Asn, Asp, Gly, Thr, Pro, Gln, or His;

N_{15} , which may or may not be present, is Thr, Ser, Glu, Asn, Tyr, Gln, Asp, or

Pro;

N_{16} , which may or may not be present, is Glu, Pro, Lys, Thr, Ala, Gly, or Gln;

N_{17} , which may or may not be present, is Ala, Leu, Ile, Tyr, or Gln; and

N_{18} is Phe, His, Tyr, or Thr.

[0015] In one embodiment, 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.

[0016] In another embodiment, 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.

[0017] The TCR may include at least one TCR alpha chain variable domain and/or at least one beta chain variable domain; or the TCR may include a TCR alpha chain variable domain and a TCR beta chain variable domain.

[0018] In one embodiment, the TCR comprises 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.

[0019] In another embodiment, the TCR comprises 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.

[0020] In yet another embodiment, 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 4.

[0021] In one embodiment, 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.

[0022] In one embodiment, 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.

[0023] In one embodiment, 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.

[0024] In one embodiment, the TCR comprises CDR sequences contained within 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.

[0025] In another embodiment, 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, 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.

[0026] In another embodiment, the TCR comprises the CDR sequences contained within 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.

[0027] In some embodiments, the present invention provides a TCR comprising the CDRs contained within an alpha chain variable domain/beta chain variable domain amino acid sequence pair selected from the group consisting of SEQ ID NOs: 668/676, 103/111, 439/447, and 503/511. In some embodiments, a TCR of the present disclosure comprises alpha chain variable domain complementarity determining regions (CDRs) CDR1, CDR2, and CDR3, and beta chain variable domain CDRs CDR1, CDR2, and CDR3 comprising the respective amino acid sequences of : a) SEQ ID NOs: 662, 663, 664, 670, 671, and 672; b) SEQ ID NOs: 97, 98, 99, 105, 106, and 107; c) SEQ ID NOs: 433, 434, 435, 441, 442, and 443; and d) SEQ ID NOs: 497, 498, 499, 505, 506, and 507.

[0028] The present invention also provides a TCR (e.g., an isolated TCR or a TCR expressed on an isolated cell) that complete for binding to any one or more of the TCRs of the invention.

[0029] In some embodiment, the TCRs of the invention further comprise a detectable moiety.

[0030] The present invention further provides pharmaceutical compositions comprising any of the TCRs of the invention, and a pharmaceutically acceptable carrier or diluent; as well as isolated cells presenting any of the TCRs of the invention.

[0031] In one aspect, the present invention provides isolated polynucleotide molecules comprising a polynucleotide sequence that encodes an alpha chain variable domain of any of the TCRs of the invention.

[0032] In another aspect, the present invention provides isolated polynucleotide molecules comprising a polynucleotide sequence that encodes a beta chain variable domain of any of the TCRs of the invention.

[0033] The present invention also provides vectors comprising the polynucleotide molecule of the invention; cells expressing the vectors of the invention.

[0034] In one aspect, the present invention provides a method of treating a subject having a MAGE-A4-associated disease or disorder. The methods include administering to the subject a therapeutically effective amount of a TCR (e.g., an isolated TCR or a TCR expressed on an isolated cell), pharmaceutical composition, or a plurality of the cells of the invention, thereby treating the subject.

[0035] In one embodiment, the MAGE-A4-associated disease or disorder is MAGE-A4-associated cancer.

[0036] In one embodiment, 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.

[0037] In some embodiments of the invention, a TCR (e.g., an isolated TCR or a TCR expressed on an isolated cell), pharmaceutical composition, or a plurality of the cells of the invention is administered to the subject in combination with a second therapeutic agent.

[0038] The TCR, the pharmaceutical composition, or the plurality of cells may be administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, intramuscularly or intracranially to the subject.

[0039] In one aspect, the present invention provides an isolated nucleic acid 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.

[0040] In one embodiment, the isolated nucleic acid molecule encodes at least one TCR alpha chain variable domain and/or at least one beta chain variable domain.

[0041] In one embodiment, 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.

[0042] In another embodiment, the TCR (e.g., isolated TCR or TCR expressed on an isolated cell) 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.

[0043] In yet another embodiment, 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.

[0044] In one embodiment, 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.

[0045] In one embodiment, the isolated antigen-binding protein 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.

[0046] In one embodiment, 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.

[0047] In another embodiment, 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, 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.

[0048] In one embodiment, the isolated antigen-binding protein 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.

[0049] In one embodiment, 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.

[0050] The present invention also provides vectors comprising an isolated nucleic acid molecule of the invention and isolated cells comprising a vector of the invention.

[0051] In one aspect, the present invention provides 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 42, thereby treating the subject.

[0052] In one embodiment, the MAGE-A4-associated disease or disorder is MAGE-A4-associated cancer.

[0053] In one embodiment, 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.

[0054] In one embodiment, a plurality of cells is administered to the subject in combination with a second therapeutic agent.

[0055] In one aspect, the present invention provides a T cell receptor (TCR) (e.g., an isolated TCR or a TCR expressed on an isolated cell) 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 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 having a signal to noise ratio greater than or equal to 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.

[0056] In some embodiments, the TCR activates a T cell response about two times greater, or about three times greater, or about four times greater than a patient-derived MAGE-A4-specific TCR as determined by a TCR-mediated T cell signaling luminescent bioassay. In some embodiments, the TCR activates a T cell response about two times greater, or about three times greater, or about four 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

[0057] In one aspect, the present disclosure provides a T cell receptor (TCR) (e.g., an isolated TCR or a TCR expressed on an isolated cell) 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 with 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.

[0058] In one aspect, the present disclosure provides a T cell receptor (TCR) (e.g., an isolated TCR or a TCR expressed on an isolated cell) 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 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.

[0059] In some embodiments, 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. In some embodiments, 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. In some embodiments, the TCR comprises at least one TCR alpha chain variable domain and/or at least one beta chain variable domain. In some embodiments, the TCR comprises a TCR alpha chain variable domain and a TCR beta chain variable domain.

[0060] In some embodiments, the TCR comprises 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. In some embodiments, the TCR comprises 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. In some embodiments, 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. In some embodiments, 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.

[0061] In some embodiments, 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.

[0062] In some embodiments, 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. In some embodiments, 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.

[0063] In some embodiments, the TCR comprises a detectable moiety. In some embodiments, the TCR has an on-target binding/off-target binding value of greater than 2.5, greater than 3, greater than 3.5, greater than 4, greater than 4.5, 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. In some embodiments, the TCR has an on-target binding/off-target binding value of greater than 10. In some embodiments, the TCR has an on-target binding/off-target binding value of greater than 500. In some embodiments, the TCR competes for binding to a TCR (e.g., an isolated TCR or a TCR expressed on an isolated cell) as described herein.

[0064] In one aspect, the present disclosure provides a pharmaceutical composition comprising a TCR (e.g., an isolated TCR or a TCR expressed on an isolated cell) as described herein and a pharmaceutically acceptable carrier or diluent. In one aspect, the present disclosure provides an isolated cell presenting a TCR as described herein. In one aspect, the present disclosure provides an isolated polynucleic acid comprising a polynucleotide sequence that encodes an alpha chain variable domain of a TCR (e.g., an isolated TCR or a TCR expressed on an isolated cell) as described herein. In one aspect, the present disclosure provides an isolated polynucleic acid comprising a polynucleotide sequence that encodes a beta chain variable domain of a TCR (e.g., an isolated TCR or a TCR expressed on an isolated cell) as described herein. In one aspect, the present disclosure provides a vector comprising a polynucleotide sequence as described herein. In one aspect, the present disclosure provides an isolated cell expressing that vector.

[0065] In one aspect, the present disclosure provides a method of treating a subject having a MAGE-A4-associated disease or disorder, comprising administering to the subject a therapeutically effective amount of a TCR (e.g., an isolated TCR or a TCR expressed on an isolated cell) as described herein, a pharmaceutical composition as described herein, or an isolated cell as described herein, thereby treating the subject. In some embodiments, the MAGE-A4-associated disease or disorder is MAGE-A4-associated cancer. In some embodiments, 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. In some embodiments, the TCR, the pharmaceutical composition, or the cell is administered to the subject in combination with a second therapeutic agent. In some embodiments, the administering is parenteral.

[0066] In one aspect, the present disclosure provides an isolated nucleic acid 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. In some embodiments, the isolated nucleic acid molecule encodes at least one TCR alpha chain variable domain and/or at least one beta chain variable domain.

[0067] In some embodiments, 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. In some embodiments, 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. In some embodiments, 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.

[0068] In some embodiments, 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. In some embodiments, 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.

[0069] In some embodiments, 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. In some embodiments, 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.

[0070] In some embodiments, 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. In some embodiments, 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.

[0071] In one aspect, the present disclosure provides a vector comprising the polynucleotide sequence of an isolated nucleic acid molecule as described herein. In one aspect, the present disclosure provides an isolated cell comprising that vector. In one aspect, the present disclosure provides a method of treating a subject having a MAGE-A4-associated disease or disorder, comprising administering to the subject that cell, thereby treating the subject. In some embodiments, the MAGE-A4-associated disease or disorder is MAGE-A4-associated cancer. In some embodiments, 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. In some embodiments, the cell is administered to the subject in combination with a second therapeutic agent.

[0072] The present invention is further illustrated by the following detailed description and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0073] **FIG. 1** depict FACS analysis showing the expression of TRACtargeted MAGE-A4 TCRs in primary human T cells, quantified by pMHC tetramer, analysis Day 14 post-activation. MAGE-A4 targets for these TCRs were either the MAGE-A4 (286-294) peptide (KVLEHVVRV, SEQ ID NO: 609; “KVLE”) or the MAGE-A4 (230-239) peptide (GVYDGREHTV, SEQ ID NO: 612; “GVY”) Transgenic TCR sequences were introduced to primary human T cells by site-directed targeting of adeno-associated virus (AAV) vector insertion at the human TRAC locus. Cells were stained with the indicated peptide MHC

tetramer reagents (y axes) and antibodies for surface antigen markers, and analyzed by flow cytometry. Plots were gated on live, single CD8+ T cells.

[0074] **FIG. 2A** and **FIG. 2B** depict the cytotoxic activity of MAGE-A4 TCRs against MAGE-A4 expressing A375 melanoma cells (Donor 1, 2 hour assay). Primary human T cells expressing TCRs against two different HLA-A2 restricted MAGE-A4 derived peptide antigens were tested for cytotoxic activity against HLA-A2*01 MAGE-A4 expressing A375 melanoma cells in a Calcein AM dye release assay. MAGE-A4 targets for these TCRs were either the MAGE-A4 (286-294) peptide (KVLEHVVRV, SEQ ID NO: 609; "KVLE") (**FIG. 2A**) or the MAGE-A4 (230-239) peptide (GVYDGREHTV, SEQ ID NO: 612; "GVY") (**FIG. 2B**). Control cells transduced with a TCR against an irrelevant antigen were included as a control for TCR target specificity.

[0075] **FIG. 3A** and **FIG. 3B** depicts the cytotoxic activity of MAGE-A4 TCRs against MAGE-A4 expressing A375 melanoma cells (Donor 2, 2.5 hour assay). MAGE-A4 targets for these TCRs were either the MAGE-A4 (286-294) peptide (KVLEHVVRV, SEQ ID NO: 609; "KVLE") (**FIG. 3A**) or the MAGE-A4 (230-239) peptide (GVYDGREHTV, SEQ ID NO: 612; "GVY") (**FIG. 3B**). Primary human T cells expressing TCRs against two different HLA-A2 restricted MAGE-A4 derived peptide antigens were tested for cytotoxic activity against HLA-A2*01 MAGE-A4 expressing A375 melanoma cells in a Calcein AM dye release assay. Untransduced (UTD) T cells were included as a control for TCR target specificity.

[0076] **FIG. 4** depicts TCR signaling measured by activation of AP1-RE-luciferase reporter activation.

[0077] **FIG. 5** depicts the average interferon gamma signal +/- standard error of the mean (SEM) of n=3 donors, and shows that MAGE-A4 (230-239)-specific TCRs of the present disclosure exhibit specific activity against T2 cells pulsed with the MAGE-A4 (230-239) peptide.

[0078] **FIG. 6** depicts the average interferon gamma signal +/- standard error of the mean (SEM) of n=3 donors, and shows that MAGE-A4 (230-239)-specific TCRs of the present disclosure exhibit specific activity against tumor cells expressing HLA-A2 and MAGE-A4.

[0079] **FIG. 7** depicts the average interferon gamma signal +/- standard error of the mean (SEM) of n=3 donors, and shows that MAGE-A4 (230-239)-specific TCRs of the present disclosure exhibit specific activity against tumor cells expressing HLA-A2 and MAGE-A4.

[0080] FIG. 8 depicts the average interferon gamma signal +/- standard error of the mean (SEM) of n=3 donors, and shows that MAGE-A4 (230-239)-specific TCRs of the present disclosure exhibit specific activity against HLA-A2-expressing tumor cells expressing even just low or medium endogenous levels of MAGE-A4.

DETAILED DESCRIPTION

[0081] The present invention provides T cell receptors (TCRs) that were generated against a MAGE-A4 peptide antigen in the context of MHC (HLA-A2). The unique TCR sequences identified have shown specific binding to the small peptide MAGE-A4 presented in the groove of an HLA molecule and exhibited activation of T cells in a reporter assay. Furthermore, the TCRs of the invention do not cross-react with other “like” peptides.

I. Definitions

[0082] In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also part of this invention.

[0083] In the following description, for purposes of explanation, specific numbers, materials and configurations are set forth in order to provide a thorough understanding of the invention. It will be apparent, however, to one having ordinary skill in the art that the invention may be practiced without these specific details. In some instances, well-known features may be omitted or simplified so as not to obscure the present invention. Furthermore, reference in the specification to phrases such as “one embodiment” or “an embodiment” means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the invention. The appearances of phrases such as “in one embodiment” in various places in the specification are not necessarily all referring to the same embodiment.

[0084] The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0085] The term “comprising” or “comprises” is used herein in reference to compositions, methods, and respective component(s) thereof, that are essential to the disclosure, yet open to the inclusion of unspecified elements, whether essential or not.

[0086] The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[0087] The term “T cell receptor” (TCR), as used herein, refers to an immunoglobulin superfamily member having a variable binding domain, a constant domain, a transmembrane region, and a short cytoplasmic tail; see, *e.g.*, Janeway *et al.*, *Immunobiology: The Immune System in Health and Disease*, 3rd Ed., Current Biology Publications, p. 4:33, 1997) capable of specifically binding to an antigen peptide bound to a MHC receptor. A TCR can be found on the surface of a cell and generally is comprised of a heterodimer having α and β chains (also known as TCR α and TCR β , respectively), or γ and δ chains (also known as TCR γ and TCR δ , respectively). Like immunoglobulins, the extracellular portion of TCR chains (*e.g.*, α -chain, β -chain) contain two immunoglobulin regions, a variable region (*e.g.*, TCR variable α region or V α and TCR variable β region or V β ; typically amino acids 1 to 116 based on Kabat numbering at the N-terminus), and one constant region (*e.g.*, TCR constant domain α or C α and typically amino acids 117 to 259 based on Kabat, TCR constant domain β or C β , typically amino acids 117 to 295 based on Kabat) adjacent to the cell membrane. Also, like immunoglobulins, the variable domains contain complementary determining regions (CDRs) separated by framework regions (FRs). In certain embodiments, a TCR is found on the surface of T cells (or T lymphocytes) and associates with the CD3 complex. The source of a TCR of the present disclosure may be from various animal species, such as a human, mouse, rat, rabbit or other mammal. In preferred embodiments, the source of a TCR of the present invention is a mouse genetically engineered to produce TCRs comprising human alpha and beta chains (see, *e.g.*, PCT Publication No. WO 2016/164492, the entire contents of which are incorporated herein by reference).

[0088] The term “variable region” (variable region of an alpha chain (V α), variable region of a beta chain (V β)) as used herein denotes each of the alpha and beta chains which is involved directly in binding the TCR to the antigen.

[0089] The “constant region” of the alpha chain and of the beta chain are not involved directly in binding of a TCR to an antigen, but exhibit various effector functions.

[0090] The term “antigen” as used herein is meant any substance that causes the immune system to produce antibodies or specific cell-mediated immune responses against it. A disease-associated antigen is any substance that is associated with any disease that causes the immune system to produce antibodies or a specific-cell mediated response against it.

[0091] The term “MAGE-A4,” “MAGEA4” or “Melanoma-Associated Antigen A4” refers to the well-known cancer-testis antigen (CTAs) that is re-expressed in numerous cancer types.

[0092] The nucleotide and amino acid sequence of full-length MAGE-A4 is provided in GenBank as accession number NM_001011548 (DNA sequence: SEQ ID NO: 870; RNA sequence: SEQ ID NO: 610; amino acid sequence: SEQ ID NO: 611). The term “MAGE-A4” includes recombinant MAGE-A4 or a fragment thereof. The term also encompasses MAGE-A4 or a fragment thereof coupled to, for example, histidine tag, mouse or human Fc, or a signal sequence such as ROR1. In certain embodiments, the term comprises MAGE-A4 or a fragment thereof in the context of HLA-A2, linked to HLA-A2 or as displayed by HLA-A2. As used herein, the numbering of certain MAGE-A4 amino acid residues within the full-length MAGE-A4 sequence is with respect to SEQ ID NO: 611.

[0093] The term “HLA” refers to the human leukocyte antigen (HLA) system or complex, which is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans. These cell-surface proteins are responsible for the regulation of the immune system in humans. HLAs corresponding to MHC class I (A, B, and C) present peptides from inside the cell.

[0094] The term “HLA-A” refers to the group of human leukocyte antigens (HLA) that are coded for by the HLA-A locus. HLA-A is one of three major types of human MHC class I cell surface receptors. The receptor is a heterodimer, and is composed of a heavy α chain and smaller β chain. The α chain is encoded by a variant HLA-A gene, and the β chain (β 2-microglobulin) is an invariant β 2 microglobulin molecule.

[0095] The term “HLA-A2” (also referred to as “HLA-A2*01”) is one particular class I major histocompatibility complex (MHC) allele group at the HLA-A locus; the α chain is encoded by the HLA-A*02 gene and the β chain is encoded by the β 2-microglobulin or B2M locus.

[0096] The term “specifically binds,” or “binds specifically to”, or the like, means that TCR forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about 1×10^{-8} M or less (*e.g.*, a smaller KD denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, the TCRs of the invention bind specifically to an HLA-A2 presented cancer testis antigen Melanoma-Associated Antigen A4 (MAGE-A4) peptide, *e.g.*, a peptide comprising

amino acid residues 230-239 or 286-294 of MAGE-A4 (e.g., of the full-length MAGE-A4 sequence of SEQ ID NO: 611).

[0097] The term “off-target peptide” refers to a peptide that differs by 1, 2, 3, 4, 5 or more amino acids from a target peptide (e.g., a MAGE-A4 230-239 peptide or a MAGE-A4 286-294 peptide). In certain embodiments, the term includes a peptide that differs by less than or equal to 3 amino acids than the target peptide. For example, for a 9-mer peptide, if 1, 2, or 3 amino acids are not identical to the target peptide, it is considered an “off-target” peptide. In certain embodiments, amino acid identity is expressed in terms of ‘degree of similarity’ (DoS). If 6 or more amino acids within a 9-mer peptide are identical, the DoS is 6. In certain embodiments, a peptide with $\text{DoS} \leq 6$ is considered an “off-target” peptide. The term “off-target” peptide also refers to a peptide that is similar to the target peptide based on sequence homology, is predicted to bind to HLA-A2 and is comprised in a protein that is expressed in essential, normal tissues.

[0098] The term “isolated” refers to a composition, compound, substance, or molecule altered by the hand of man from the natural state. For example, a composition or substance that occurs in nature is isolated if it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not isolated, but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is isolated, as the term is employed herein. More particularly, an isolated TCR can refer to a TCR that has been removed from a cell, for example, a TCR that has been purified. TCRs can also be expressed by an isolated cell, e.g., a cell that has been isolated from an animal or a cell from cell culture. In this context, the isolated cell can express the TCR on its surface (i.e., the cell can “present” the TCR).

[0099] The term “recombinant”, as used herein, refers to TCRs of the invention created, expressed, isolated or obtained by technologies or methods known in the art as recombinant DNA technology which include, e.g., DNA splicing and transgenic expression. The term refers to TCRs expressed in a non-human mammal (including transgenic non-human mammals, e.g., transgenic mice), or a cell (e.g., CHO cells) expression system or isolated from a recombinant combinatorial human antibody library.

[00100] As used herein, the terms “polynucleotide” and “nucleic acid molecule” are used interchangeably to refer to polymeric forms of nucleotides of any length. The polynucleotides may contain deoxyribonucleotides, ribonucleotides, and/or their analogs. Nucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The term “polynucleotide” includes, for example, single-, double-

stranded and triple helical molecules, a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, ribozymes, antisense molecules, cDNA, recombinant polynucleotides, branched polynucleotides, aptamers, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A nucleic acid molecule may also comprise modified nucleic acid molecules (*e.g.*, comprising modified bases, sugars, and/or internucleotide linkers).

[00101] The term “polypeptide” is meant to refer to any polymer preferably consisting essentially of any of the 20 natural amino acids regardless of its size. Although the term “protein” is often used in reference to relatively large proteins, and “peptide” is often used in reference to small polypeptides, use of these terms in the field often overlaps. The term “polypeptide” refers generally to proteins, polypeptides, and peptides unless otherwise noted. Peptides useful in accordance with the present disclosure in general will be generally between about 0.1 to 100 KD or greater up to about 1000 KD, preferably between about 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 30 and 50 KD as judged by standard molecule sizing techniques such as centrifugation or SDS-polyacrylamide gel electrophoresis.

[00102] The term “vector” is a nucleic acid molecule that is able to replicate autonomously in a host cell and can accept foreign DNA. A vector carries its own origin of replication, one or more unique recognition sites for restriction endonucleases which can be used for the insertion of foreign DNA, and usually selectable markers such as genes coding for antibiotic resistance, and often recognition sequences (*e.g.*, promoter) for the expression of the inserted DNA. Common vectors include plasmid vectors and phage vectors.

[00103] In some embodiments, TCRs of the invention may be conjugated to a moiety such as a ligand, a detectable moiety, or a therapeutic moiety (“immunoconjugate”), such as a cytotoxin, an anti-cancer drug, or any other therapeutic moiety useful for treating a disease or condition including MAGE-A4-associated disease or disorder, such as a MAGE-A4-associated cancer.

[00104] The term “surface plasmon resonance”, as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[00105] The term “KD”, also known as K_D or K_d , is intended to refer to the equilibrium dissociation constant of a particular biomolecule and its binding partner. KD measurements are particularly useful for assessing protein-protein interactions, *e.g.* as in an antigen-binding protein-antigen interaction. The smaller the value of the KD, the greater (or

e.g. stronger) the binding interaction or affinity between the antigen-binding protein and antigen (*e.g.* target). The larger the value of the KD, the weaker the binding interaction or affinity between the antigen-binding protein and antigen.

[00106] The term “substantial identity” or “substantially identical,” when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[00107] Sequence identity can be calculated using an algorithm, for example, the Needleman Wunsch algorithm (Needleman and Wunsch 1970, *J. Mol. Biol.* 48: 443-453) for global alignment, or the Smith Waterman algorithm (Smith and Waterman 1981, *J. Mol. Biol.* 147: 195-197) for local alignment. Another preferred algorithm is described by Dufresne *et al* in *Nature Biotechnology* in 2002 (vol. 20, pp. 1269-71) and is used in the software GenePAST (GQ Life Sciences, Inc. Boston, MA).

[00108] As applied to polypeptides, the term “substantial similarity” or “substantially similar” means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 96%, 97%, 98% or 99% sequence identity. Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions. A “conservative amino acid substitution” is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) *Methods Mol. Biol.* 24: 307-331, which is herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-

containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) *Science* 256: 1443-45, herein incorporated by reference. A “moderately conservative” replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[00109] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Sequences also can be compared using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.*, Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-410 and (1997) *Nucleic Acids Res.* 25:3389-3402, each of which is herein incorporated by reference.

[00110] A “patient-derived TCR” is a TCR that is produced by isolating the alpha and beta chains of a MAGE-A4 reactive TCR isolated from the T-lymphocytes that mediated *in vivo* regression of a tumor in a subject having a MAGE-A4-associated cancer.

[00111] An “affinity-matured TCR” is a TCR that is produced by mutagenesis and selection *in vitro*. For example, untargeted or targeted (*e.g.*, oligonucleotide-directed) mutagenesis can be performed to introduce variation in TCR sequences, and the subsequent TCRs can then be screened for affinity against a target, *e.g.*, by use of phage display.

[00112] The term “activates a T cell response having a signal to noise ratio stronger or equal to a patient-derived MAGE-A4-specific TCR” or “activates a T cell response having a signal to noise ratio stronger or equal to an affinity-matured MAGE-A4-specific TCR” is meant to refer to an increase, *i.e.*, about 2-fold or more, an amplification, *i.e.*, about 2-fold, an augmentation, *i.e.*, about 2-fold, or a boost of a physiological activity, *i.e.*, about 2-fold, *i.e.*, T cell signaling, as measured by, for example, a luminescent bioassay. Reference to a greater T cell response, or a stronger T cell response or an activation signal, may be used interchangeably. Various measurements and assays of T cell response or T cell activation are well known to the skilled artisan.

[00113] By the phrase “therapeutically effective amount” is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) *The Art, Science and Technology of Pharmaceutical Compounding*). The term “effective amount” is intended to encompass contexts such as a pharmaceutically effective amount or therapeutically effective amount. For example, in certain embodiments, the effective amount is capable of achieving a beneficial state, beneficial outcome, functional activity in a screening assay, or improvement of a clinical condition.

[00114] As used herein, the term “subject” refers to an animal, preferably a mammal, in need of amelioration, prevention and/or treatment of a MAGE-A4-associated disease or disorder, such as a MAGE-A4-associated cancer (*e.g.*, a MAGE-A4-positive cancer). The term includes human subjects who have or are at risk of having a MAGE-A4-associated disease or disorder, such as an n MAGE-A4-associated cancer.

[00115] As used herein, “anti-cancer drug” means any agent useful to treat or ameliorate or inhibit cancer including, but not limited to, cytotoxins and agents such as antimetabolites, alkylating agents, anthracyclines, antibiotics, antimitotic agents, procarbazine, hydroxyurea, asparaginase, corticosteroids, cyclophosphamide, mytotane (O,P'-(DDD)), biologics (*e.g.*, antibodies and interferons) and radioactive agents. As used herein, “a cytotoxin or cytotoxic agent”, also refers to a chemotherapeutic agent and means any agent that is detrimental to cells. Examples include Taxol® (paclitaxel), temozolamide, cytochalasin B, gramicidin D, ethidium bromide, emetine, cisplatin, mitomycin, etoposide, tenoposide, vincristine, vinblastine, coichicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone,

glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

[00116] The terms “prevent”, “preventing”, “prevention”, “prophylactic treatment” and the like are meant to refer to reducing the probability of developing a disorder or condition in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition. Prevention and the like do not mean preventing a subject from ever getting the specific disease or disorder. Prevention may require the administration of multiple doses. Prevention can include the prevention of a recurrence of a disease in a subject for whom all disease symptoms were eliminated, or prevention of recurrence in a relapsing-remitting disease.

II. MAGE-A4 T Cell (TCRs) and Compositions Comprising MAGE-A4 TCRS

[00117] T cells are a subgroup of cells which, together with other immune cell types (polymorphonuclear, eosinophils, basophils, mast cells, B-cells, NK cells), constitute the cellular component of the immune system. Under physiological conditions T cells function in immune surveillance and in the elimination of foreign antigen. However, under pathological conditions there is compelling evidence that T cells play a major role in the causation and propagation of disease. In these disorders, breakdown of T cell immunological tolerance, either central or peripheral is a fundamental process in the causation of autoimmune disease.

[00118] T cells bind epitopes on small antigenic determinants on the surface of antigen-presenting cells that are associated with a major histocompatibility complex (MHC; in mice) or human leukocyte antigen (HLA; in humans) complex. T cells bind these epitopes through a T cell receptor (TCR) complex on the surface of the T cell. T cell receptors are heterodimeric structures composed of two types of chains: an α (alpha) and β (beta) chain, or a γ (gamma) and δ (delta) chain. The α chain is encoded by the nucleic acid sequence located within the α locus (on human or mouse chromosome 14), which also encompasses the entire δ locus, and the β chain is encoded by the nucleic acid sequence located within the β locus (on mouse chromosome 6 or human chromosome 7). The majority of T cells have an $\alpha\beta$ TCR; while a minority of T cells bears a $\gamma\delta$ TCR.

[00119] T cell receptor α and β polypeptides (and similarly γ and δ polypeptides) are linked to each other *via* a disulfide bond. Each of the two polypeptides that make up the TCR contains an extracellular domain comprising constant and variable regions, a transmembrane domain, and a cytoplasmic tail (the transmembrane domain and the

cytoplasmic tail also being a part of the constant region). The variable region of the TCR determines its antigen specificity, and similar to immunoglobulins, comprises three complementary determining regions (CDRs). The TCR is expressed on most T cells in the body and is known to be involved in the recognition of MHC-restricted antigens. The TCR α chain includes a covalently linked $V\alpha$ and $C\alpha$ region, whereas the β chain includes a $V\beta$ region covalently linked to a $C\beta$ region. The $V\alpha$ and $V\beta$ regions form a pocket or cleft that can bind an antigen in the context of a major histocompatibility complex (MHC) (or HLA in humans). TCRs are detection molecules with exquisite specificity, and exhibit, like antibodies, an enormous diversity.

[00120] The general structure of TCR molecules and methods of making and using, including binding to a peptide:Major Histocompatibility Complex have been disclosed. See, for example PCT/US98/04274; PCT/US98/20263; WO99/60120.

[00121] Non-human animals (*e.g.*, rodents, *e.g.*, mice or rats) can be genetically engineered to express a human or humanized T cell receptor (TCR) comprising a variable domain encoded by at least one human TCR variable region gene segment, as described in, for example, PCT Publication No. WO 2016/164492, the entire contents of which are hereby incorporated herein by reference. For example, the Veloci-T® mouse technology (Regeneron), a genetically modified mouse that allows for the production of fully human therapeutic TCRs against tumor and/or viral antigens, can be used to produce the TCRs of the invention. Those of skill in the art, through standard mutagenesis techniques, in conjunction with the assays described herein, can obtain altered TCR sequences and test them for particular binding affinity and/or specificity. Useful mutagenesis techniques known in the art include, without limitation, *de novo* gene synthesis, oligonucleotide-directed mutagenesis, region-specific mutagenesis, linker-scanning mutagenesis, and site-directed mutagenesis by PCR (see, *e.g.*, Sambrook et al. (1989) and Ausubel *et al.* (1999)).

[00122] Briefly, in one embodiment, methods for generating a TCR to a MAGE-A4 230-239 peptide or a MAGE-A4 286-294 peptide may include immunizing a non-human animal (*e.g.*, a rodent, *e.g.*, a mouse or a rat), such as a genetically engineered non-human animal that comprises in its genome an un-rearranged human TCR variable gene locus, with a MAGE-A4 230-239 peptide or a MAGE-A4 286-294 peptide; allowing the animal to mount an immune response to the peptide; isolating from the animal a T cell reactive to the peptide; determining a nucleic acid sequence of a human TCR variable region expressed by the T cell; cloning the human TCR variable region into a nucleotide construct comprising a nucleic acid sequence of a human TCR constant region such that the human TCR variable

region is operably linked to the human TCR constant region; and expressing from the construct a human T cell receptor specific for the MAGE-A4 230-239 peptide or the MAGE-A4 286-294 peptide, respectively. In one embodiment, the steps of isolating a T cell, determining a nucleic acid sequence of a human TCR variable region expressed by the T cell, cloning the human TCR variable region into a nucleotide construct comprising a nucleic acid sequence of a human TCR constant region, and expressing a human T cell receptor are performed using standard techniques known to those of skill the art.

[00123] In one embodiment, the nucleotide sequence encoding a T cell receptor specific for an antigen of interest is expressed in a cell. In one embodiment, the cell expressing the TCR is selected from a CHO, COS, 293, HeLa, PERC.6™ cell, *etc.*

[00124] In obtaining variant TCR coding sequences, those of ordinary skill in the art will recognize that TCR-derived proteins may be modified by certain amino acid substitutions, additions, deletions, and post-translational modifications, without loss or reduction of biological activity. In particular, it is well known that conservative amino acid substitutions, that is, substitution of one amino acid for another amino acid of similar size, charge, polarity and conformation, are unlikely to significantly alter protein function. The 20 standard amino acids that are the constituents of proteins can be broadly categorized into four groups of conservative amino acids as follows: the nonpolar (hydrophobic) group includes alanine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan and valine; the polar (uncharged, neutral) group includes asparagine, cysteine, glutamine, glycine, serine, threonine and tyrosine; the positively charged (basic) group contains arginine, histidine and lysine; and the negatively charged (acidic) group contains aspartic acid and glutamic acid. Substitution in a protein of one amino acid for another within the same group is unlikely to have an adverse effect on the biological activity of the protein.

[00125] In some embodiments, a TCR of the present disclosure can comprise a CDR sequence (e.g., a CDR3 sequence such as a V α CDR3 or a V β CDR3) with 1 or more substitutions as compared to a CDR sequence (e.g., a CDR3 sequence such as a V α CDR3 or a V β CDR3) of Table 6. For example, a TCR of the present disclosure can comprise a CDR sequence with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more substitutions as compared to a CDR sequence of Table 6. In general, the TCRs of the present invention function by binding to an HLA-A2 presented MAGE-A4 230-239 peptide or a HLA-A2 presented MAGE-A4 286-294 peptide. As used herein, an HLA presented peptide (such as an HLA-A2 presented peptide) can refer to a peptide that is bound to a human leukocyte antigen (HLA) protein, for example, an HLA protein expressed on the surface of a cell. Thus, a TCR that binds to an

HLA presented peptide binds to the peptide that is bound by the HLA, and optionally also binds to the HLA itself. Interaction with the HLA can confer specificity for binding to a peptide presented by a particular HLA. In some embodiments, the TCR binds to an isolated HLA presented peptide. In some embodiments, the TCR binds to an HLA presented peptide on the surface of a cell.

[00126] The present invention includes MAGE-A4 TCRs that bind a MAGE-A4 230-239 peptide or a MAGE-A4 286-294 peptide in the context of HLA-A2 with high specificity. In some embodiments, the MAGE-A4 TCRs do not bind to the MAGE-A4 230-239 peptide or the MAGE-A4 286-294 peptide in the absence of HLA-A2, or such binding is minimal. Further, in some embodiments, the MAGE-A4 TCRs do not bind to an off-target peptide in the context of HLA-A2, or such binding is minimal. As used herein, an off-target peptide can refer to a peptide that differs from a target peptide by 1, 2, 3, 4, 5, or more amino acids. In some embodiments, binding specificity can be determined by a) measuring on-target binding (e.g., binding to the HLA-A2 presented MAGE-A4 (230-239) peptide or the HLA-A2 presented MAGE-A4 (230-239) peptide), b) measuring off-target binding, and c) quantifying the difference between the two, e.g., by calculating a ratio. This ratio can be calculated, for example, by dividing the values obtained in a) and b). Measurement of on-target and off-target binding can be achieved, for example, by measuring % binding to a peptide/HLA tetramer reagent (e.g., a MAGE-A4/HLA tetramer reagent or a MAGE-A8/HLA tetramer reagent), or by other techniques known in the art. In some embodiments, an on-target binding/off-target binding value (e.g., a value obtained by dividing the values obtained in a) and b) described above) of a TCR of the present disclosure can be greater than 5, greater than 6, greater than 7, greater than 8, greater than 9, greater than 10, greater than 11, greater than 12, greater than 13, greater than 14, greater than 15, greater than 16, greater than 17, greater than 18, greater than 19, greater than 20, greater than 21, greater than 22, greater than 23, greater than 24, greater than 25, greater than 26, greater than 27, greater than 28, greater than 29, greater than 30, greater than 35, greater than 40, greater than 45, greater than 50, greater than 55, greater than 60, greater than 65, greater than 70, greater than 75, greater than 80, greater than 85, greater than 90, greater than 95, greater than 100, greater than 110, greater than 120, greater than 130, greater than 140, greater than 150, greater than 160, greater than 170, greater than 180, greater than 190, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 550, greater than 600, greater than 650, greater than 700,

greater than 750, greater than 800, greater than 850, greater than 900, greater than 950, greater than 1000, greater than 1100, greater than 1200, greater than 1300, greater than 1400, greater than 1500, greater than 1600, greater than 1700, greater than 1800, greater than 1900, or greater than 2000. In some embodiments, an on-target binding/off-target binding value (e.g., a value obtained by dividing the values obtained in a) and b) described above) can be about 5 to about 20, about 10 to about 30, about 20 to about 80, about 30 to about 70, about 40 to about 60, about 50 to about 250, about 100 to about 200, about 100 to about 1000, about 300 to about 700, about 500 to about 1500, about 800 to about 1200, about 900 to about 1100, about 800 to about 1500, about 1000 to about 1400, or about 1100 to about 1300.

[00127] In one embodiment, the invention provides a recombinant antigen-binding protein (e.g., an isolated antigen-binding protein) that binds specifically to a conformational epitope of an HLA-A2 presented human MAGE-A4 (286-294) peptide or to a conformational epitope of an HLA-A2 presented human MAGE-A4 (230-239) peptide, wherein the antigen-binding protein has a property selected from the group consisting of: (a) binds monomeric HLA-A2: MAGE-A4 (286-294) peptide or monomeric MAGE-A4 (230-239) peptide with a binding dissociation equilibrium constant (K_D) of less than about 20nM as measured in a surface plasmon resonance assay at 25°C; (b) binds monomeric HLA-A2: MAGE-A4 (286-294) peptide or monomeric MAGE-A4 (230-239) peptide with a binding dissociation equilibrium constant (K_D) of less than about 25nM as measured in a surface plasmon resonance assay at 25°C; (c) binds to HLA-A2: MAGE-A4 (286-294) peptide-expressing cells or MAGE-A4 (230-239) peptide-expressing cells with an EC_{50} less than about 6 nM and does not bind to cells expressing predicted off-target peptides as determined by luminescence assay; (d) binds to HLA-A2: MAGE-A4 (286-294) peptide-expressing cells or MAGE-A4 (230-239) peptide-expressing cells with an EC_{50} less than about 1 nM and do not substantially bind to cells expressing predicted off-target peptides as determined by luminescence assay; (e) binds to HLA-A2: MAGE-A4 (286-294) peptide-expressing cells or MAGE-A4 (230-239) peptide-expressing cells with an EC_{50} less than about 30 nM as determined by flow cytometry assay; (f) binds to HLA-A2: MAGE-A4 (286-294) peptide-expressing cells or MAGE-A4 (230-239) peptide-expressing cells with an EC_{50} less than about 75nM as determined by flow cytometry assay; (g) mediates the killing of cancer cells (e.g., melanoma cells) in a dose dependent manner as determined by Calcein AM dye release assay, and (h) the conformational epitope comprises one or more amino acids of SEQ ID NO: 611 or of SEQ ID NO: 612.

[00128] In some embodiments, the MAGE-A4 TCRs of the present disclosure have specific activity or affinity for MAGE-A4 (230-239) or for MAGE-A4 (286-294) as measured by an *in vitro* assay. For example, cells (such as T2 cells) expressing an HLA can be pulsed with a MAGE-A4 (230-239) or a MAGE-A4 (286-294) polypeptide, or an off-target polypeptide thereby inducing the cells to present the polypeptide bound to the HLA. Alternatively or in addition to using an off-target polypeptide as a control, an off-target HLA (an HLA other than the HLA that is recognized by the TCR of interest) can be used. For example, an off-target HLA can be used to present the MAGE-A4 peptide to test for specificity of binding to the HLA-A2-presented MAGE-A4 peptide. In addition, a control can be a cell line that expresses neither MAGE-A4 nor the target HLA (e.g., HLA-A2). Cells can be co-cultured with a T-cell population expressing the TCR of interest, and activity measured as a function of the amount of a cytokine (such as interferon gamma) produced by the cells. In certain embodiments, the assay can comprise *in vitro* co-cultures of a TCR-expressing T cell population with 10^{-10} M peptide-loaded T2 cells at an effector cell:target cell ratio of 1:1 (1×10^5 effector cells/96 well), and interferon gamma measurement 24 hours after co-culture (e.g., by a Meso Scale Discovery (MSD®) Sector Imager). In certain embodiments, the assay can comprise *in vitro* co-cultures of a TCR-expressing T cell population and effector cell at an effector cell:target cell ratio of 5:1 (2.5×10^5 effector cells: 5×10^4 target cells), and interferon gamma measurement 24 hours after co-culture (e.g., by a Meso Scale Discovery (MSD®) Sector Imager). In certain embodiments, TCRs are expressed in primary T cells (e.g., cytotoxic T cells, regulatory T cells, helper T cells, or any combination thereof) by replacing an endogenous locus, e.g., an endogenous TRAC locus (KO/KI), and disrupting an endogenous locus, e.g., a TRBC locus (KO), using, e.g., CRISPR, TALEN, zinc finger, or other other targeted disruption systems. *In vitro* assays comprising such cells can include cytotoxicity activity against HLA-A2*01 MAGE-A4 expressing A375 melanoma cells in a Calcein AM dye release assay.

[00129] Increasing amounts of cytokine detected can serve as an indicator of activity. The activity or specificity of a TCR of interest to its target peptide in comparison to a control (off-target) polypeptide, or the activity or specificity of a TCR of interest to its on-target HLA-bound target peptide in comparison to an off-target HLA-bound target peptide can be 2-fold or greater, 3-fold or greater, 4-fold or greater, 5-fold or greater, 6-fold or greater, 7-fold or greater, 8-fold or greater, 9-fold or greater, 10-fold or greater, 15-fold or greater, 20-fold or greater, 30-fold or greater, 40-fold or greater, 50-fold or greater, 100-fold or greater, 200-fold or greater, 300-fold or greater, 400-fold or greater, 500-fold or greater, 600-fold or

greater, 700-fold or greater, 800-fold or greater, 900-fold or greater, 1,000-fold or greater, 1,500-fold or greater, 2,000-fold or greater, 2,500-fold or greater, 3,000-fold or greater, 4,000-fold or greater, 5,000-fold or greater, 10,000-fold or greater, 20,000-fold or greater, 30,000-fold or greater, 40,000-fold or greater, 50,000-fold or greater, 60,000-fold or greater, 70,000-fold or greater, 80,000-fold or greater, 90,000-fold or greater, or 100,000-fold or greater.

[00130] In certain embodiments, the MAGE-A4 TCRs of the present disclosure are useful in inhibiting the growth of a tumor or delaying the progression of cancer when administered prophylactically to a subject in need thereof and may increase survival of the subject. For example, the administration of a MAGE-A4 TCR of the present invention may lead to shrinking of a primary tumor and may prevent metastasis or development of secondary tumors. In certain embodiments, the MAGE-A4 TCRs of the present invention are useful in inhibiting the growth of a tumor when administered therapeutically to a subject in need thereof and may increase survival of the subject. For example, the administration of a therapeutically effective amount of a MAGE-A4 TCR of the invention to a subject may lead to shrinking and disappearance of an established tumor in the subject.

[00131] In one embodiment, the invention provides a TCR (e.g., an isolated TCR or a TCR expressed on an isolated cell) that specifically binds to an HLA-A2 presented MAGE-A4 286-294 peptide, wherein the antigen-binding protein exhibits one or more of the following characteristics: (i) comprises an alpha chain variable domain comprising complementary determining regions (CDR) 1, CDR2, and CDR3, wherein the CDR3 region 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 Ala, Ile, or Gly; N₂, which may or may not be present, is Val; N₃ is Tyr, Gly, Leu, Val, Glu, Met, Ala, or Phe; N₄ 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 which may or may not be present, is Thr, Pro, Ser, Glu, Asp, Trp, Arg, Asn, Ile, Gln, or Leu; N₈ is Gly, 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; (ii) comprises a beta chain variable domain comprising beta chain variable domain comprises complementary determining regions (CDR) 1, CDR2, and

CDR3, wherein the CDR3 region comprises amino acid sequence of Formula II: N₁-N₂-N₃-N₄-N₅-N₆-N₇-N₈-N₉-N₁₀-N₁₁-N₁₂-N₁₃-N₁₄-N₁₅-N₁₆-N₁₇-N₁₈ (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; and (iii) comprises a CDR1 of the alpha chain variable domain comprising any one of the CDR1 amino acid sequences set forth in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity, and a CDR2 of the alpha chain variable domain independently comprising any one of the CDR2 amino acid sequences set forth in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (iv) comprises a CDR1 of a beta chain variable domain comprising any one of the CDR1 amino acid sequences set forth in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity, and a CDR2 of a beta chain variable domain independently comprising any one of the CDR2 amino acid sequences set forth in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (v) comprises an alpha chain variable domain CDR1, CDR2 and CDR3 contained within any one of the alpha chain variable domain sequences listed in Table 4, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; and beta chain variable domain CDR1, CDR2 and CDR3 contained within any one of the beta chain variable domain sequences listed in Table 4, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (vi) comprises an alpha chain variable domain having an amino acid sequence that

has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or about 100% 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; (vii) comprises a beta chain variable domain having an amino acid sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or about 100% 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; (viii) comprises (a) an alpha chain variable domain having an amino acid sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or about 100% 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%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or about 100% 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; (ix) 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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; 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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (x) 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, 599/607, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (xi) 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, 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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; and/or (xii) does not bind to cells expressing predicted off-target peptides.

[00132] In one embodiment, the invention provides a TCR (e.g., an isolated TCR or a TCR expressed on an isolated cell) that specifically binds to an HLA-A2 presented MAGE-A4 230-239 peptide, wherein the antigen-binding protein exhibits one or more of the following characteristics: (i) comprises a CDR1 of the alpha chain variable domain comprising any one of the CDR1 amino acid sequences set forth in Table 6, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity, and a CDR2 of the alpha chain variable domain independently comprising any one of the CDR2 amino acid sequences set forth in Table 6, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (iv) comprises a CDR1 of a beta chain variable domain comprising any one of the CDR1 amino acid sequences set forth in Table 6, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity, and a CDR2 of a beta chain variable domain independently comprising any one of the CDR2 amino acid sequences set forth in Table 6, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (v) comprises an alpha chain variable domain CDR1, CDR2 and CDR3 contained within any one of the alpha chain variable domain sequences listed in Table 8, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; and beta chain variable domain CDR1, CDR2 and CDR3 contained within any one of the beta chain variable domain sequences listed in Table 8, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (vi) comprises an alpha chain variable domain having an amino acid sequence that has at least

85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or about 100% 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;

(vii) comprises a beta chain variable domain having an amino acid sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or about 100% 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;

(viii) comprises (a) an alpha chain variable domain having an amino acid sequence that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or about 100% 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%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or about 100% 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; (ix) 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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (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, or a

substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; 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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (x) 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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (xi) 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, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; and/or (xii) does not bind to cells expressing predicted off-target peptides.

[00133] The TCRs of the present invention may possess one or more of the aforementioned biological characteristics, or any combinations thereof. Other biological characteristics of the antigen-binding proteins of the present invention will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

[00134] In certain embodiments, a polynucleotide encoding a MAGE-A4 TCR described herein is inserted into a vector. The term "vector" as used herein refers to a vehicle into which a polynucleotide encoding a protein may be covalently inserted so as to bring about the expression of that protein and/or the cloning of the polynucleotide. Such vectors may also be referred to as "expression vectors". The isolated polynucleotide may be inserted into a vector using any suitable methods known in the art, for example, without limitation, the vector may be digested using appropriate restriction enzymes and then may be ligated with the isolated polynucleotide having matching restriction ends. Expression vectors have the ability to incorporate and express heterologous or modified nucleic acid sequences coding for at least part of a gene product capable of being transcribed in a cell. In most cases, RNA molecules are then translated into a protein. Expression vectors can contain a variety of control sequences, which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operatively linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are discussed *infra*. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in human cells for expression and in a prokaryotic host for cloning and amplification.

[00135] The expression vector may have the necessary 5' upstream and 3' downstream regulatory elements such as promoter sequences such as CMV, PGK and EF1 α promoters, ribosome recognition and binding TATA box, and 3' UTR AAUAAA transcription termination sequence for the efficient gene transcription and translation in its respective host cell. Other suitable promoters include the constitutive promoter of simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), HIV LTR promoter, MoMuLV promoter, avian leukemia virus promoter, EBV immediate early promoter, and rous sarcoma virus promoter. Human gene promoters may also be used, including, but not limited to the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. In certain embodiments inducible promoters are also contemplated as part of the vectors expressing chimeric antigen receptor. This provides a molecular switch capable of turning on expression of the polynucleotide sequence of interest or turning off expression. Examples of inducible promoters include, but are not limited to a metallothionein promoter, a glucocorticoid promoter, a progesterone promoter, or a tetracycline promoter.

[00136] The expression vector may have additional sequence such as 6x-histidine (SEQ ID NO: 871), c-Myc, and FLAG tags which are incorporated into the expressed TCRs. Thus, the expression vector may be engineered to contain 5' and 3' untranslated regulatory sequences that sometimes can function as enhancer sequences, promoter regions and/or terminator sequences that can facilitate or enhance efficient transcription of the nucleic acid(s) of interest carried on the expression vector. An expression vector may also be engineered for replication and/or expression functionality (*e.g.*, transcription and translation) in a particular cell type, cell location, or tissue type. Expression vectors may include a selectable marker for maintenance of the vector in the host or recipient cell.

[00137] Examples of vectors are plasmid, autonomously replicating sequences, and transposable elements. Additional exemplary vectors include, without limitation, plasmids, phagemids, cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. Examples of categories of animal viruses useful as vectors include, without limitation, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, and papovavirus (*e.g.*, SV40). Examples of expression vectors are Lenti-XTM Bicistronic Expression System (Neo) vectors (Clontech), pCneo vectors (Promega) for expression in mammalian cells; pLenti4/V5-DESTTM, pLenti6/V5-DESTTM, and pLenti6.2N5-GW/lacZ (Invitrogen) for lentivirus-mediated gene

transfer and expression in mammalian cells. The coding sequences of the TCRs disclosed herein can be ligated into such expression vectors for the expression of the chimeric protein in mammalian cells.

[00138] In certain embodiments, the nucleic acids encoding the TCR of the present invention are provided in a viral vector. A viral vector can be those derived from retrovirus, lentivirus, or foamy virus. As used herein, the term, "viral vector," refers to a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral vector particle. The viral vector can contain the coding sequence for the various proteins described herein in place of nonessential viral genes. The vector and/or particle can be utilized for the purpose of transferring DNA, RNA or other nucleic acids into cells either *in vitro* or *in vivo*. Numerous forms of viral vectors are known in the art.

[00139] In certain embodiments, the viral vector containing the coding sequence for a TCR described herein is a retroviral vector or a lentiviral vector. The term "retroviral vector" refers to a vector containing structural and functional genetic elements that are primarily derived from a retrovirus. The term "lentiviral vector" refers to a vector containing structural and functional genetic elements outside the LTRs that are primarily derived from a lentivirus.

[00140] The retroviral vectors for use herein can be derived from any known retrovirus (*e.g.*, type c retroviruses, such as Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV)). Retroviruses of the invention also include human T cell leukemia viruses, HTLV-1 and HTLV-2, and the lentiviral family of retroviruses, such as Human Immunodeficiency Viruses, HIV-1, HIV-2, simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), equine immunodeficiency virus (EIV), and other classes of retroviruses.

[00141] A lentiviral vector for use herein refers to a vector derived from a lentivirus, a group (or genus) of retroviruses that give rise to slowly developing disease. Viruses included within this group include HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi; a caprine arthritis-encephalitis virus; equine infectious anemia virus; feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). Preparation of the recombinant lentivirus can be achieved using the methods according to Dull *et al.* and Zufferey *et al.* (Dull *et al.*, *J. Virol.*, 1998; 72: 8463-8471 and Zufferey *et al.*, *J. Virol.* 1998; 72:9873-9880).

[00142] Retroviral vectors (*i.e.*, both lentiviral and non-lentiviral) for use in the present invention can be formed using standard cloning techniques by combining the desired DNA sequences in the order and orientation described herein (*Current Protocols in Molecular Biology*, Ausubel, F. M. *et al.* (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals; Eglitis, *et al.* (1985) *Science* 230:1395-1398; Danos and Mulligan (1988) *Proc. Natl. Acad. Sci. USA* 85:6460-6464; Wilson *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:3014-3018; Armentano *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6141-6145; Huber *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:8039-8043; Ferry *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:8377-8381; Chowdhury *et al.* (1991) *Science* 254:1802-1805; van Beusechem *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:7640-7644; Kay *et al.* (1992) *Human Gene Therapy* 3:641-647; Dai *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:10892-10895; Hwu *et al.* (1993) *J. Immunol* 150:4104-4115; U.S. Pat. No. 4,868,116; U.S. Pat. No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573).

[00143] Suitable sources for obtaining retroviral (*i.e.*, both lentiviral and non-lentiviral) sequences for use in forming the vectors include, for example, genomic RNA and cDNAs available from commercially available sources, including the Type Culture Collection (ATCC), Rockville, Md. The sequences also can be synthesized chemically.

[00144] For expression of a MAGE-A4 TCR, the vector may be introduced into a host cell to allow expression of the polypeptide within the host cell. The expression vectors may contain a variety of elements for controlling expression, including without limitation, promoter sequences, transcription initiation sequences, enhancer sequences, selectable markers, and signal sequences. These elements may be selected as appropriate by a person of ordinary skill in the art, as described above. For example, the promoter sequences may be selected to promote the transcription of the polynucleotide in the vector. Suitable promoter sequences include, without limitation, T7 promoter, T3 promoter, SP6 promoter, beta-actin promoter, EF1a promoter, CMV promoter, and SV40 promoter. Enhancer sequences may be selected to enhance the transcription of the polynucleotide. Selectable markers may be selected to allow selection of the host cells inserted with the vector from those not, for example, the selectable markers may be genes that confer antibiotic resistance. Signal sequences may be selected to allow the expressed polypeptide to be transported outside of the host cell.

[00145] For cloning of the polynucleotide, the vector may be introduced into a host cell (an isolated host cell) to allow replication of the vector itself and thereby amplify the

copies of the polynucleotide contained therein. The cloning vectors may contain sequence components generally include, without limitation, an origin of replication, promoter sequences, transcription initiation sequences, enhancer sequences, and selectable markers. These elements may be selected as appropriate by a person of ordinary skill in the art. For example, the origin of replication may be selected to promote autonomous replication of the vector in the host cell.

[00146] In certain embodiments, the present disclosure provides isolated host cells containing the vectors provided herein. The host cells containing the vector may be useful in expression or cloning of the polynucleotide contained in the vector. Suitable host cells can include, without limitation, prokaryotic cells, fungal cells, yeast cells, or higher eukaryotic cells such as mammalian cells. Suitable prokaryotic cells for this purpose include, without limitation, eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis*, *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*.

[00147] The TCRs of the present invention are introduced into a host cell using transfection and/or transduction techniques known in the art. As used herein, the terms, "transfection," and, "transduction," refer to the processes by which an exogenous nucleic acid sequence is introduced into a host cell. The nucleic acid may be integrated into the host cell DNA or may be maintained extrachromosomally. The nucleic acid may be maintained transiently or may be a stable introduction. Transfection may be accomplished by a variety of means known in the art including but not limited to calcium phosphate-DNA co-precipitation, DEAE-dextran-mediated transfection, polybrene-mediated transfection, electroporation, microinjection, liposome fusion, lipofection, protoplast fusion, retroviral infection, and biolistics. Transduction refers to the delivery of a gene(s) using a viral or retroviral vector by means of viral infection rather than by transfection. In certain embodiments, retroviral vectors are transduced by packaging the vectors into virions prior to contact with a cell. For example, a nucleic acid encoding a MAGE-A4 TCR of the invention carried by a retroviral vector can be transduced into a cell through infection and pro virus integration.

[00148] As used herein, the term "genetically engineered" or "genetically modified" refers to the addition of extra genetic material in the form of DNA or RNA into the total

genetic material in a cell. The terms, "genetically modified cells," "modified cells," and, "redirected cells," are used interchangeably.

[00149] In particular, the TCRs of the present invention are introduced and expressed in immune effector cells so as to redirect their specificity to a target antigen of interest, *e.g.*, an HLA-A2 displayed MAGE-A4 peptide, *e.g.*, amino acid residues 230-239 or 286-294 of MAGE-A4.

[00150] The present invention provides methods for making the immune effector cells which express the TCRs as described herein. In one embodiment, the method comprises transfecting or transducing immune effector cells, *e.g.*, immune effector cells isolated from a subject, such as a subject having a MAGE-A4-associated disease or disorder, such that the immune effector cells express one or more TCR as described herein. In certain embodiments, the immune effector cells are isolated from an individual and genetically modified without further manipulation *in vitro*. Such cells can then be directly re-administered into the individual. In further embodiments, the immune effector cells are first activated and stimulated to proliferate *in vitro* prior to being genetically modified to express a TCR. In this regard, the immune effector cells may be cultured before or after being genetically modified (*i.e.*, transduced or transfected to express a TCR as described herein).

[00151] Prior to *in vitro* manipulation or genetic modification of the immune effector cells described herein, the source of cells may be obtained from a subject. In particular, the immune effector cells for use with the TCRs as described herein comprise T cells.

[00152] T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments, T cell can be obtained from a unit of blood collected from the subject using any number of techniques known to the skilled person, such as FICOLL separation. In one embodiment, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocyte, B cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing. In one embodiment of the invention, the cells are washed with PBS. In an alternative embodiment, the washed solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. As would be appreciated by those of ordinary skill in the art, a washing step may be accomplished by methods known to those in the art, such as by using a semiautomated

flowthrough centrifuge. After washing, the cells may be resuspended in a variety of biocompatible buffers or other saline solution with or without buffer. In certain embodiments, the undesirable components of the apheresis sample may be removed in the cell directly resuspended culture media.

[00153] In certain embodiments, T cells are isolated from peripheral blood mononuclear cells (PBMCs) by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLLTM gradient. A specific subpopulation of T cells, such as CD28+, CD4+, CD8+, CD45RA+, and CD45RO+ T cells, can be further isolated by positive or negative selection techniques. For example, enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method for use herein is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. Flow cytometry and cell sorting may also be used to isolate cell populations of interest for use in the present invention.

[00154] PBMC may be used directly for genetic modification with the TCRs using methods as described herein. In certain embodiments, after isolation of PBMC, T lymphocytes are further isolated and in certain embodiments, both cytotoxic and helper T lymphocytes can be sorted into naive, memory, and effector T cell subpopulations either before or after genetic modification and/or expansion.

[00155] The immune effector cells, such as T cells, can be genetically modified following isolation using known methods, or the immune effector cells can be activated and expanded (or differentiated in the case of progenitors) *in vitro* prior to being genetically modified. In another embodiment, the immune effector cells, such as T cells, are genetically modified with the chimeric antigen receptors described herein (*e.g.*, transduced with a viral vector comprising a nucleic acid encoding a TCR) and then are activated and expanded *in vitro*. Methods for activating and expanding T cells are known in the art and are described, for example, in U.S. Pat. No. 6,905,874; U.S. Pat. No. 6,867,041; U.S. Pat. No. 6,797,514; WO2012079000, US 2016/0175358.

[00156] The invention provides a population of modified immune effector cells for the treatment of a MAGE-A4-associated disease or disorder, *e.g.*, cancer, the modified immune effector cells comprising a MAGE-A4 TCR as disclosed herein.

[00157] TCR-expressing immune effector cells prepared as described herein can be utilized in methods and compositions for adoptive immunotherapy in accordance with known techniques, or variations thereof that will be apparent to those skilled in the art based on the instant disclosure. See, *e.g.*, US Patent Application Publication No. 2003/0170238 to Gruenberg *et al.*; see also U.S. Pat. No. 4,690,915 to Rosenberg.

III. Pharmaceutical Compositions

[00158] The invention provides therapeutic compositions comprising the MAGE-A4 TCRs of the invention or immune effector cells comprising the MAGE-A4 TCRs of the invention. Therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists:

Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) *J Pharm Sci Technol* 52:238-311.

[00159] Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted.

[00160] In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of MAGE-A4 TCRs of the invention or immune effector cells comprising the MAGE-A4 TCRs of the invention in an amount that can be approximately the same or less than that of the initial dose,

[00161] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used.

[00162] Injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous, intracranial, intraperitoneal and intramuscular injections, drip infusions, *etc.* The TCRs, pharmaceutical compositions, and cells described herein can be administered via parenteral administration. The preparations of the present disclosure may be prepared by methods publicly known. For example, the preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antigen-binding protein or its salt

described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, *etc.*, which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (*e.g.*, propylene glycol, polyethylene glycol), a nonionic surfactant [*e.g.*, polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], *etc.* As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, *etc.*, which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, *etc.* The injection thus prepared is preferably filled in an appropriate ampoule.

[00163] In some embodiments, TCR-expressing immune effector cells are formulated by first harvesting them from their culture medium, and then washing and concentrating the cells in a medium and container system suitable for administration (a "pharmaceutically acceptable" carrier) in a treatment-effective amount. Suitable infusion medium can be any isotonic medium formulation, typically normal saline, Normosol R (Abbott) or Plasma-Lyte A (Baxter), but also 5% dextrose in water or Ringer's lactate can be utilized. The infusion medium can be supplemented with human serum albumin.

[00164] A treatment-effective number of cells in the composition is typically greater than 10^2 cells, and up to 10^6 up to and including 10^8 or 10^9 cells and can be more than 10^{10} cells. The number of cells will depend upon the ultimate use for which the composition is intended as will the type of cells included therein.

[00165] The cells may be autologous or heterologous to the patient undergoing therapy. If desired, the treatment may also include administration of mitogens (*e.g.*, PHA) or lymphokines, cytokines, and/or chemokines (*e.g.*, IFN- γ , IL-2, IL-12, TNF- α , IL-18, and TNF- β , GM-CSF, IL-4, IL-13, Flt3-L, RANTES, MIP1 α , *etc.*) as described herein to enhance induction of the immune response.

[00166] The TCR expressing immune effector cell populations of the present invention may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations. Briefly, pharmaceutical compositions of the present invention may comprise a TCR-expressing immune effector cell population, such as T cells, as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine;

antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. Compositions of the present invention are preferably formulated for intravenous administration.

IV. Therapeutic Uses of MAGE-A4 TCRs or Immune Effector cells Comprising MAGE-A4 TCRs

[00167] The anti-tumor immune response induced in a subject by administering TCR expressing T cells described herein using the methods described herein, or other methods known in the art, may include cellular immune responses mediated by cytotoxic T cells capable of killing infected cells, regulatory T cells, and helper T cell responses. Humoral immune responses, mediated primarily by helper T cells capable of activating B cells thus leading to antibody production, may also be induced. A variety of techniques may be used for analyzing the type of immune responses induced by the compositions of the present invention, which are well described in the art; *e.g.*, *Current Protocols in Immunology*, Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober (2001) John Wiley & Sons, NY, N.Y.

[00168] Thus, the MAGE-A4 TCRs of the invention are useful, *inter alia*, for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by MAGE-A4. For example, the present invention provides methods for treating a MAGE-A4-associated disease or disorder, such as a MAGE-A4-associated cancer (*e.g.*, a MAGE-A4-positive cancer) (tumor growth inhibition) by administering a MAGE-A4 TCR (or pharmaceutical composition comprising a MAGE-A4 TCR or a plurality of cells comprising a MAGE-A4 TCR) as described herein to a patient in need of such treatment, and MAGE-A4 TCRs (or pharmaceutical composition comprising a MAGE-A4 TCR) for use in the treatment of a MAGE-A4-associated cancer. The antigen-binding proteins of the present invention are useful for the treatment, prevention, and/or amelioration of disease or disorder or condition such as a MAGE-A4-associated cancer and/or for ameliorating at least one symptom associated with such disease, disorder or condition. In the context of the methods of treatment described herein, the MAGE-A4 TCR (or pharmaceutical composition or plurality of cells) may be administered as a monotherapy (*i.e.*, as the only therapeutic agent) or in combination with one or more additional therapeutic agents (examples of which are described elsewhere herein).

[00169] Accordingly, the present invention provides for methods of treating an individual diagnosed with or suspected of having, or at risk of developing, a MAGE-A4-

associated disease or disorder, *e.g.*, a MAGE-A4-associated cancer, comprising administering the individual a therapeutically effective amount of the TCR-expressing immune effector cells as described herein.

[00170] In one embodiment, the invention provides a method of treating a subject diagnosed with a MAGE-A4-positive cancer comprising removing immune effector cells from a subject diagnosed with a MAGE-A4-positive cancer, genetically modifying said immune effector cells with a vector comprising a nucleic acid encoding a TCR of the instant invention, thereby producing a population of modified immune effector cells, and administering the population of modified immune effector cells to the same subject. In one embodiment, the immune effector cells comprise T cells.

[00171] The methods for administering the cell compositions described herein includes any method which is effective to result in reintroduction of *ex vivo* genetically modified immune effector cells that either directly express a TCR of the invention in the subject or on reintroduction of the genetically modified progenitors of immune effector cells that on introduction into a subject differentiate into mature immune effector cells that express the TCR. One method comprises transducing peripheral blood T cells *ex vivo* with a nucleic acid construct in accordance with the invention and returning the transduced cells into the subject.

[00172] In some embodiments of the invention, the compositions described herein are useful for treating subjects suffering from primary or recurrent cancer, including, but not limited to, MAGE-A4-associated cancer, *e.g.*, 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. In one embodiment, the MAGE-A4-associated cancer is an ovarian cancer, a melanoma, a non-small cell lung carcinoma, a hepatocellular carcinoma, a colorectal carcinoma, an esophageal squamous cell

carcinoma, an esophageal adenocarcinoma, a stomach cancer, a bladder cancer, a head and neck cancer, a gastric cancer, a synovial sarcoma, or a myxoid round cell liposarcoma.

[00173] The TCRs may be used to treat early stage or late-stage symptoms of the MAGE-A4-associated cancer. In one embodiment, a TCR of the invention may be used to treat advanced or metastatic cancer. The TCRs are useful in reducing or inhibiting or shrinking tumor growth. In certain embodiments, treatment with a TCR of the invention leads to more than 40% regression, more than 50% regression, more than 60% regression, more than 70% regression, more than 80% regression or more than 90% regression of a tumor in a subject. In certain embodiments, the TCRs may be used to prevent relapse of a tumor. In certain embodiments, the TCRs are useful in extending progression-free survival or overall survival in a subject with MAGE-A4-associated cancer. In some embodiments, the TCRs are useful in reducing toxicity due to chemotherapy or radiotherapy while maintaining long-term survival in a patient suffering from MAGE-A4-associated cancer.

[00174] One or more TCRs of the present invention may be administered to relieve or prevent or decrease the severity of one or more of the symptoms or conditions of the disease or disorder.

[00175] It is also contemplated herein to use one or more TCRs of the present invention prophylactically to patients at risk for developing a disease or disorder such as MAGE-A4-associated disease or disorder, such as a MAGE-A4-associated cancer.

[00176] In a further embodiment of the invention, the present TCRs are used for the preparation of a pharmaceutical composition for treating patients suffering from MAGE-A4-associated disease or disorder, such as a MAGE-A4-associated cancer. In another embodiment of the invention, the present TCRs are used as adjunct therapy with any other agent or any other therapy known to those skilled in the art useful for treating MAGE-A4-associated cancer.

[00177] Combination therapies may include a MAGE-A4 TCR of the invention, such as immune effector cell comprising a TCR of the invention, or a pharmaceutical composition of the invention, and any additional therapeutic agent that may be advantageously combined with a TCR of the invention. The TCRs of the present invention may be combined synergistically with one or more anti-cancer drugs or therapy used to treat or inhibit a MAGE-A4-associated disease or disorder, such as MAGE-A4-positive cancer, *e.g.*, 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.

[00178] It is contemplated herein to use the TCRs of the invention in combination with immunostimulatory and/or immunosupportive therapies to inhibit tumor growth, and/or enhance survival of cancer patients. The immunostimulatory therapies include direct immunostimulatory therapies to augment immune cell activity by either “releasing the brake” on suppressed immune cells or “stepping on the gas” to activate an immune response. Examples include targeting other checkpoint receptors, vaccination and adjuvants. The immunosupportive modalities may increase antigenicity of the tumor by promoting immunogenic cell death, inflammation or have other indirect effects that promote an anti-tumor immune response. Examples include radiation, chemotherapy, anti-angiogenic agents, and surgery.

[00179] In various embodiments, one or more TCRs of the present invention may be used in combination with a PD-1 inhibitor (*e.g.*, an anti-PD-1 antibody such as nivolumab, pembrolizumab, pidilizumab, BGB-A317 or REGN2810), a PD-L1 inhibitor (*e.g.*, an anti-PD-L1 antibody such as avelumab, atezolizumab, durvalumab, MDX-1105, or REGN3504), a CTLA-4 inhibitor (*e.g.*, ipilimumab), a TIM3 inhibitor, a BTLA inhibitor, a TIGIT inhibitor, a CD47 inhibitor, a GITR inhibitor, an antagonist of another T cell co-inhibitor or ligand (*e.g.*, an antibody to CD-28, 2B4, LY108, LAIR1, ICOS, CD160 or VISTA), an indoleamine-2,3-dioxygenase (IDO) inhibitor, a vascular endothelial growth factor (VEGF) antagonist [*e.g.*, a “VEGF-Trap” such as aflibercept or other VEGF-inhibiting fusion protein as set forth in US 7,087,411, or an anti-VEGF antibody or antigen-binding fragment thereof (*e.g.*, bevacizumab, or ranibizumab) or a small molecule kinase inhibitor of VEGF receptor (*e.g.*, sunitinib, sorafenib, or pazopanib)], an Ang2 inhibitor (*e.g.*, nesvacumab), a transforming growth factor beta (TGFβ) inhibitor, an epidermal growth factor receptor (EGFR) inhibitor (*e.g.*, erlotinib, cetuximab), a CD20 inhibitor (*e.g.*, an anti-CD20 antibody such as rituximab), an antibody to a tumor-specific antigen [*e.g.*, CA9, CA125, melanoma-associated antigen 3 (MAGE3), carcinoembryonic antigen (CEA), vimentin, tumor-M2-PK, prostate-specific antigen (PSA), mucin-1, MART-1, and CA19-9], a vaccine (*e.g.*, Bacillus

Calmette-Guerin, a cancer vaccine), an adjuvant to increase antigen presentation (*e.g.*, granulocyte-macrophage colony-stimulating factor), a bispecific antibody (*e.g.*, CD3xCD20 bispecific antibody, or PSMAxCD3 bispecific antibody), a cytotoxin, a chemotherapeutic agent (*e.g.*, dacarbazine, temozolomide, cyclophosphamide, docetaxel, doxorubicin, daunorubicin, cisplatin, carboplatin, gemcitabine, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, and vincristine), cyclophosphamide, radiotherapy, surgery, an IL-6R inhibitor (*e.g.*, sarilumab), an IL-4R inhibitor (*e.g.*, dupilumab), an IL-10 inhibitor, a cytokine such as IL-2, IL-7, IL-21, and IL-15, an antibody-drug conjugate (ADC) (*e.g.*, anti-CD19-DM4 ADC, and anti-DS6-DM4 ADC), an anti-inflammatory drug (*e.g.*, corticosteroids, and non-steroidal anti-inflammatory drugs), a dietary supplement such as anti-oxidants or any other therapy care to treat cancer. In certain embodiments, the TCRs of the present invention may be used in combination with cancer vaccines including dendritic cell vaccines, oncolytic viruses, tumor cell vaccines, etc. to augment the anti-tumor response.

[00180] Examples of cancer vaccines that can be used in combination with TCRs of the present invention include MAGE3 vaccine for melanoma and bladder cancer, MUC1 vaccine for breast cancer, EGFRv3 (*e.g.*, Rindopepimut) for brain cancer (including glioblastoma multiforme), or ALVAC-CEA (for CEA+ cancers).

[00181] In certain embodiments, the MAGE-A4 TCRs of the invention may be administered in combination with radiation therapy in methods to generate long-term durable anti-tumor responses and/or enhance survival of patients with cancer. In some embodiments, the MAGE-A4 TCRs of the invention may be administered prior to, concomitantly or after administering radiation therapy to a cancer patient. For example, radiation therapy may be administered in one or more doses to tumor lesions followed by administration of one or more doses of MAGE-A4 TCRs of the invention. In some embodiments, radiation therapy may be administered locally to a tumor lesion to enhance the local immunogenicity of a patient's tumor (adjuvating radiation) and/or to kill tumor cells (ablative radiation) followed by systemic administration of a MAGE-A4 TCRs of the invention.

[00182] The additional therapeutically active agent(s)/component(s) may be administered prior to, concurrent with, or after the administration of the MAGE-A4 TCRs of the present invention. For purposes of the present disclosure, such administration regimens are considered the administration of a MAGE-A4 TCR "in combination with" a second therapeutically active component.

[00183] The additional therapeutically active component(s) may be administered to a subject prior to administration of a MAGE-A4 TCR of the present invention. In other embodiments, the additional therapeutically active component(s) may be administered to a subject after administration of a MAGE-A4 TCR of the present invention. In yet other embodiments, the additional therapeutically active component(s) may be administered to a subject concurrent with administration of a MAGE-A4 TCR of the present invention. "Concurrent" administration, for purposes of the present invention, includes, *e.g.*, administration of a MAGE-A4 TCR and an additional therapeutically active component to a subject in a single dosage form (*e.g.*, co-formulated), or in separate dosage forms administered to the subject within about 30 minutes or less of each other. If administered in separate dosage forms, each dosage form may be administered via the same route; alternatively, each dosage form may be administered via a different route. In any event, administering the components in a single dosage form, in separate dosage forms by the same route, or in separate dosage forms by different routes are all considered "concurrent administration," for purposes of the present disclosure. For purposes of the present disclosure, administration of a MAGE-A4 TCR "prior to", "concurrent with," or "after" (as those terms are defined herein above) administration of an additional therapeutically active component is considered administration of a MAGE-A4 TCR "in combination with" an additional therapeutically active component).

[00184] The present invention is further illustrated by the following Examples, which are not intended to be limiting in any way. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the Figures, are hereby incorporated herein by reference.

EXAMPLES

Example 1. Identification of MAGE-A4 specific T cell receptors

[00185] Mice humanized for cellular immune system components, Veloci-T® mice (see, *e.g.*, PCT Publication No. WO 2016/164492, the entire contents of which are incorporated herein by reference), were immunized with MAGE-A4 (286-294) peptide (KVLEHVVRV, SEQ ID NO: 609) presented specifically by human HLA-A2, diluted in PBS and mixed with adjuvant, *e.g.* in equal volume with Complete Freund's Adjuvant (CFA; Chondrex, Inc.). Spleen suspensions from immunized mice were obtained and dissociated.

Red blood cells were lysed in ACK lysis buffer (Life Technologies), and splenocytes were suspended in RPMI complete media. Isolated splenocytes were sorted and single T cells that bind MAGE-A4 (286-294) peptide in the context of MHC were isolated by fluorescent-activated cell sorting (FACS). Isolated T cells were single well plated and mixed with TCR alpha and beta variable region-specific PCR primers. cDNAs for each single T cell were synthesized *via* a reverse transcriptase (RT) reaction. Each resulting RT product was then split and transferred into two corresponding wells for subsequent TCR beta and alpha PCRs. One set of the resulting RT products was first amplified by PCR using a 5' degenerate primer specific for TCR beta variable region leader sequence or a 5' degenerate primer specific for TCR alpha chain variable region leader sequence and a 3' primer specific for TCR constant region, to form an amplicon. The amplicons were then amplified again by PCR using a 5' degenerate primer specific for TCR beta variable region framework 1 or a 5' degenerate primer specific for TCR alpha chain variable region framework 1 and a 3' primer specific for TCR constant region, to generate amplicons for cloning. The TCR beta and alpha derived PCR products were cloned into expression vectors containing beta constant region and alpha constant region, respectively. Expression vectors expressing full-length beta and alpha chain pairs were transfected into CHO cells and tested for binding to commercial MAGE-A4/HLA tetramer reagent (HLA-A02:01 MAGE-A4 tetramer; MBL International Corporation). CHO cells were incubated with soluble HLA-A2 (KVLEHVVRV) (SEQ ID NO:609) tetramer and an antibody specific for mouse TCR constant region (clone H57-597) (Biolegend, San Diego, CA). Samples were then analyzed on an LSRFortessa X-20 (BD Biosciences, San Jose, CA). To calculate percentage of tetramer positive cells, antigen positive (Ag+) gates were set based on a negative control TCR that does not bind to the HLA-A2 (KVLEHVVRV) (SEQ ID NO:609) tetramer using FlowJo (LLC, Ashland, OR). All Ag+ TCRs had a FlowJo criteria of $\geq 1\%$ of cells in Ag+ gate with the mean fluorescence intensity (MFI) > 250. Ag+ TCRs were determined by Next Generation Sequencing and the total number of TCRs that express identical TCR alpha and beta nucleotide sequences are shown in Table 1 below.

Table 1.

TCR ID	Total Ag+ TCRs (CHOt)	% in Ag+ gate >250 MFI, >1%
PN41520	2	16.90
PN41494	1	9.24

PN41703	24	42.50
PN41573	2	85.70
PN41869	1	76.50
PN41568	8	82.90
PN41868	1	67.10
PN41577	45	80.00
PN41607	18	81.00
PN41518	19	34.90
PN41513	36	29.60
PN41702	42	8.13
PN41654	2	36.20
PN41656	5	13.80
PN41820	3	8.32
PN41746	1	90.20
PN41532	7	85.40
PN41617	11	90.60
PN41608	6	77.00
PN41475	1	65.50
PN41523	6	90.50
PN41690	3	75.30
PN41559	2	74.30
PN41822	32	63.50
PN41829	1	19.50
PN41852	1	18.00
PN41565	1	4.02
PN41805	61	30.40
PN41867	2	73.00
PN41712	16	20.70
PN41748	3	9.56
PN41557	2	8.76
PN41636	1	78.30
PN41613	1	58.70
PN41550	17	92.20
PN41839	1	85.40
PN41539	1	10.90
PN41516	1	7.83

[00186] A detailed list of the beta chain variable domain CDR1, CDR2, and CDR3 amino acid sequences, and the alpha chain variable domain CDR1, CDR2, and CDR3 amino

acid sequences of the TCRs that were determined as described above are provided in Table 2. A detailed list of the beta chain variable domain CDR1, CDR2, and CDR3 polynucleic acid sequences, and the alpha chain variable domain CDR1, CDR2, and CDR3 polynucleic acid sequences of the TCRs that were determined as described above are provided in Table 3. Table 4 provides the amino acid and nucleotide sequences of the beta chain variable and alpha chain variable regions of the TCRs.

Table 2. Amino acid CDR sequences for Veloci-T® TCRs specific for MAGE-A4 (286-294) / HLA-A2

TCR ID No.	TCR ID	V α CDR1	SEQ ID NO	V α CDR2	SEQ ID NO	V α CDR3	SEQ ID NO	V β CDR1	SEQ ID NO	V β CDR2	SEQ ID NO	V β CDR3	SEQ ID NO
1	PN41475	TSESDYY	1	QEAYKQON	2	AYRSATGNQFY	3	MDHEN	9	SYDV/KM	10	ASSLWGSNTEAF	11
2	PN41494	TSESDYY	17	QEAYKQON	18	AYRSATGNQFY	19	MDHEN	25	SYDV/KM	26	ASSLWGWNSPLH	27
3	PN41513	TSESDYY	33	QEAYKQON	34	AYRSAPGNQFY	35	MDHEN	41	SYDV/KM	42	ASSLFAATNEKLF	43
4	PN41516	SVFSS	49	VVTGGEV	50	AGERDSWGKFKQ	51	MNHNY	57	SVGAGI	58	ASSFWTGGDEKLF	59
5	PN41518	TSESDYY	65	QEAYKQON	66	AYRSAPGNQFY	67	MDHEN	73	SYDV/KM	74	ASSLWATNEKLF	75
6	PN41520	TSESDYY	81	QEAYKQON	82	AYRSARGNQFY	83	MDHEN	89	SYDV/KM	90	ASSLWGVNSPLH	91
7	PN41523	TSESDYY	97	QEAYKQON	98	AYRSAPGNQFY	99	MDHEN	105	SYDV/KM	106	ASSLWGLNTEAF	107
8	PN41532	TSESDYY	113	QEAYKQON	114	AYRSAEGNQFY	115	MDHEN	121	SYDV/KM	122	ASSLWGLNTEAF	123
9	PN41539	TRDTTTY	129	RNSFDEQN	130	ALSEGDTGGFKTI	131	MNHNY	137	SVGAGI	138	ASSYFPDRGLGNTIY	139
10	PN41550	DRGSQS	145	IYSNGD	146	AVNLSWGKFKQ	147	SGHDT	153	YEEEEE	154	ASSLDRSTEAF	155
11	PN41557	VSGLRG	161	LYSAGEE	162	AVQAVTGGGNKLT	163	DFQATT	169	SNEGSKA	170	SASWDRDYGYT	171
12	PN41559	TSESDYY	177	QEAYKQON	178	AYRSATGNQFY	179	MDHEN	185	SYDV/KM	186	ASSLWGWGTEAF	187
13	PN41565	TSESDYY	193	QEAYKQON	194	AYRSPTGNQFY	195	MDHEN	201	SYDV/KM	202	ASSLWAMNTEAF	203
14	PN41568	TSESDYY	209	QEAYKQON	210	AYRSARGNQFY	211	MDHEN	217	SYDV/KM	218	ASSLWGTNEKLF	219
15	PN41573	TSESDYY	225	QEAYKQON	226	AYRSATGNQFY	227	MDHEN	233	SYDV/KM	234	ASSLWGTNEKLF	235
16	PN41577	TSESDYY	241	QEAYKQON	242	AYRSAPGNQFY	243	MDHEN	249	SYDV/KM	250	ASSLWGTNEKLF	251
17	PN41607	TSESDYY	257	QEAYKQON	258	AYRSASGNQFY	259	MDHEN	265	SYDV/KM	266	ASSLWGTNEKLF	267
18	PN41608	TSESDYY	273	QEAYKQON	274	AYRSAPGNQFY	275	MDHEN	281	SYDV/KM	282	ASSFWGWNTAEAF	283
19	PN41613	TSESDYY	289	QEAYKQON	290	AYRSATGNQFY	291	MNHEY	297	SMNVEV	298	ASSLLYSNQPOH	299
20	PN41617	TSESDYY	305	QEAYKQON	306	AYRSASGNQFY	307	MDHEN	313	SYDV/KM	314	ASSLWGMNTEAF	315
21	PN41636	SSNFYA	321	MTLNGDE	322	ALNRDDKII	323	KGHDR	329	SFDV/KD	330	ATSDPQRNQPOH	331
22	PN41654	NSASDY	337	IRSNMDK	338	AEKRDNYGQNFV	339	MDHEN	345	SYDV/KM	346	ASSLYSYGYT	347
23	PN41656	TSDPSYG	353	QGSYDQON	354	AMRDLTTSPTYKYI	355	MDHEN	361	SYDV/KM	362	ASSFGGWGYGYT	363
24	PN41690	TSESDYY	369	QEAYKQON	370	AYRSAIGNQFY	371	MDHEN	377	SYDV/KM	378	ASSLWGVNTEAF	379
25	PN41702	TSESDYY	385	QEAYKQON	386	AYRSATGNQFY	387	MDHEN	393	SYDV/KM	394	ASSLWAVNEKLF	395
26	PN41703	TISGNEY	401	GLKNN	402	IVRPGGTYKYI	403	MDHEN	409	SYDV/KM	410	ATGGDNQPOH	411
27	PN41712	TSENYY	417	QEAYKQON	418	ALNTGNQFY	419	DFQATT	425	SNEGSKA	426	SAWDGFYGYT	427

28	PN41746	TSESDDY	433	QEAYKQON	434	AYRSATGNQFY	435	MDHEN	441	SYDV/KM	442	ASSLWGLNTEAF	443
29	PN41748	KTLYG	449	LOKGGEE	450	GADMYSGGADGLT	451	DFQATT	457	SNEGSKA	458	SAWDGFYGYT	459
30	PN41805	TSENYY	465	QEAYKQON	466	AFGMYSGGADGLT	467	PRHDT	473	FYEKMQ	474	ASSPTGTGDGYT	475
31	PN41820	SSVPPY	481	YTTGATLV	482	AVSSGSARQLT	483	MDHEN	489	SYDV/KM	490	ASSHDRWDYGYT	491
32	PN41822	TSESDDY	497	QEAYKQON	498	AYRSATGNQFY	499	MDHEN	505	SYDV/KM	506	ASSLWALNTEAF	507
33	PN41829	TSESDDY	513	QEAYKQON	514	AYRSATGNQFY	515	MDHEN	521	SYDV/KM	522	ASSLWALNTEAF	523
34	PN41839	TISGNEY	529	GLKNN	530	IYGGSGQNLJ	531	SGHDT	537	YEEEE	538	ASSFSRVQPOH	539
35	PN41852	TSESDDY	545	QEAYKQON	546	AYRSALGNDMR	547	MDHEN	553	SYDV/KM	554	ASSLWALNTEAF	555
36	PN41867	TSENYY	561	QEAYKQON	562	AFMKTOGGSEKLV	563	PRHDT	569	FYEKMQ	570	ASSLVGYGYT	571
37	PN41868	TSESDDY	577	QEAYKQON	578	AYRSATGNQFY	579	MDHEN	585	SYDV/KM	586	ASSLWGTHEKLF	587
38	PN41869	TSESDDY	593	QEAYKQON	594	AYRSSTGNQFY	595	MDHEN	601	SYDV/KM	602	ASSLWGTNEKLF	603

Table 3. Nucleic acid CDR sequences for Veloci-T® TCRs specific for MAGE-A4 (286-294) / HLA-A2

TCR No.	TCR ID	V α CDR1	SEQ ID NO	V α CDR2	SEQ ID NO	V α CDR3	SEQ ID NO	V β CDR1	SEQ ID NO	V β CDR2	SEQ ID NO	V β CDR3	SEQ ID NO
1	PN41475	ACCAGT GAGAGT GATTATT AT	4	CAAGAAAGCT TATAAGCAA CAGAAT	5	GCTTATAGGAGCGC GACCGGTAACCACT TCTAT	6	ATGGAC CATGAA AAT	12	TCATATG ATGTTAA AATG	13	GCCAGCAGTTTATG GGGCAGTAACCACTG AAGCTTTC	14
2	PN41494	ACCAGT GAGAGT GATTATT AT	20	CAAGAAAGCT TATAAGCAA CAGAAT	21	GCTTATAGGAGCGC GACCGGTAACCACT TCTAT	22	ATGGAC CATGAA AAT	28	TCATATG ATGTTAA AATG	29	GCCAGCAGTTTATG GGGGTGGAAATTCAC CCCTCCAC	30
3	PN41513	ACCAGT GAGAGT GATTATT AT	36	CAAGAAAGCT TATAAGCAA CAGAAT	37	GCTTATAGGAGCGC TCCCGGTAACCACT TCTAT	38	ATGGAC CATGAA AAT	44	TCATATG ATGTTAA AATG	45	GCCAGCAGTTTATTT GCAACTAATGAAAA ACTGTTT	46

4	PN41516	AGTGT TTTCCAG C	52	GTAGTTACG GGTGGAGA AGTG	53	GCAGGAGAGAGGG ACAGCTGGGGGAA ATTCCAG	54	ATGAAC CATAAC TAC	60	TCAGTTG GTGCTGG TATC	61	GCCAGCAGTTTTG GACAGGGGGCGATG AAAAACTGTTT	62
5	PN41518	ACCAGT GAGAGT GATTATT AT	68	CAAGAAAGCT TATAAGCAA CAGAAAT	69	GCTTATAGGAGCGC GCCCCGTAACCACT TCTAT	70	ATGGAC CATGAA AAT	76	TCATATG ATGTTAA AATG	77	GCCAGCAGTTTATG GGCAACTAATGAAA AACTGTTT	78
6	PN41520	ACCAGT GAGAGT GATTATT AT	84	CAAGAAAGCT TATAAGCAA CAGAAAT	85	GCTTATAGGAGCGC TCGGGGTAACCACT TCTAT	86	ATGGAC CATGAA AA	92	TCATATG ATGTTAA AATG	93	GCCAGCAGTTTATG GGGGTAAATTCAC CCCTCCAC	94
7	PN41523	ACCAGT GAGAGT GATTATT AT	100	CAAGAAAGCT TATAAGCAA CAGAAAT	101	GCTTATAGGAGCGC GCCCCGTAACCACT TCTAT	102	ATGGAC CATGAA AAT	108	TCATATG ATGTTAA AATG	109	GCCAGCAGTTTATG GGGGTTGAACACTG AAGCTTTC	110
8	PN41532	ACCAGT GAGAGT GATTATT AT	116	CAAGAAAGCT TATAAGCAA CAGAAAT	117	GCTTATAGGAGCGC GGAGGGTAACCACT TCTAT	118	ATGGAC CATGAA AAT	124	TCATATG ATGTTAA AATG	125	GCCAGCAGTTTATG GGGGTTGAACACTG AAGCTTTC	126
9	PN41539	ACCCGTG ATACTAC TTATTAC	132	CGGAACTCT TTTGATGAG CAAAAT	133	GCTCTGAGTGAGGG GGATACTGGAGGCT TCAAAACTATC	134	ATGAAC CATAAC TAC	140	TCAGTTG GTGCTGG TATC	141	GCCAGCAGTTACTT CCCCGACAGGGGGC TTGGAAACACCATA TAT	142
10	PN41550	GACCGA GGTTCCC AGTCC	148	ATATACTCC AATGGTGAC	149	GCCGTGAACCTTGAG CTGGGGGAAATTCC AG	150	TCTGGG CATGAC ACT	156	TATTATG AGGAGG AAGAG	157	GCCAGCAGCTTGGA CAGGAGCACTGAAG CTTTC	158
11	PN41557	GTCAGC GGTTAA GAGGG	164	CTGTATTCA GCTGGGGAA GAA	165	GCTGTGAGGCGGT CACGGGAGGAGGA AACAAACTCACC	166	GACTTT CAGGCC ACAACT	172	TCCAATG AGGGCT CCAAGG CC	173	AGTGCTAGTTGGGA CAGGACTATGGCT ACACC	174

12	PN41559	ACCAGT GAGAGT GATTATT AT	180	CAAGAAAGCT TATAAGCAA CAGAAAT	181	GCTTATAGGAGCGC CACCGGTAACCACT TCTAT	182	ATGGAC CATGAA AAT	188	TCATATG ATGTTAA AATG	189	GCCAGCAGTTTATG GGGTTGGGCACTG AAGCTTTC	190
13	PN41565	ACCAGT GAGAGT GATTATT AT	196	CAAGAAAGCT TATAAGCAA CAGAAAT	197	GCTTATAGGAGCGC CACCGGTAACCACT TCTAT	198	ATGGAC CATGAA AAT	204	TCATATG ATGTTAA AATG	205	GCCAGCAGTTTATG GGCAATGAACACTG AAGCTTTC	206
14	PN41568	ACCAGT GAGAGT GATTATT AT	212	CAAGAAAGCT TATAAGCAA CAGAAAT	213	GCTTATAGGAGCGC GCGGGTAACCACT TCTAT	214	ATGGAC CATGAA AAT	220	TCATATG ATGTTAA AATG	221	GCCAGCAGTTTATG GGGGAATAATGAAA AACTGTTT	222
15	PN41573	ACCAGT GAGAGT GATTATT AT	228	CAAGAAAGCT TATAAGCAA CAGAAAT	229	GCTTATAGGAGCGC AACCGGTAACCACT TCTAT	230	ATGGAC CATGAA AAT	236	TCATATG ATGTTAA AATG	237	GCCAGCAGTTTATG GGGAATAATGAAA AACTGTTT	238
16	PN41577	ACCAGT GAGAGT GATTATT AT	244	CAAGAAAGCT TATAAGCAA CAGAAAT	245	GCTTATAGGAGCGC GCCCCGTAACCACT TCTAT	246	ATGGAC CATGAA AAT	252	TCATATG ATGTTAA AATG	253	GCCAGCAGTTTATG GGGGAATAATGAAA AACTGTTT	254
17	PN41607	ACCAGT GAGAGT GATTATT AT	260	CAAGAAAGCT TATAAGCAA CAGAAAT	261	GCTTATAGGAGCGC GTCCGGTAACCACT TCTAT	262	ATGGAC CATGAA AAT	268	TCATATG ATGTTAA AATG	269	GCCAGCAGTTTATG GGGCACTAATGAAA AACTGTTT	270
18	PN41608	ACCAGT GAGAGT GATTATT AT	276	CAAGAAAGCT TATAAGCAA CAGAAAT	277	GCTTATAGGAGCGC GCCCCGTAACCACT TCTAT	278	ATGGAC CATGAA AAT	284	TCATATG ATGTTAA AATG	285	GCCAGCAGTTTCTG GGGATGGAAACACTG AAGCTTTC	286
19	PN41613	ACCAGT GAGAGT GATTATT AT	292	CAAGAAAGCT TATAAGCAA CAGAAAT	293	GCTTATAGGAGCGC GACCGGTAACCACT TCTAT	294	ATGAAC CATGAG TAT	300	TCAATGA ATGTTGA GGTG	301	GCCAGCAGTTTACT GTATAGCAATCAGC CCCAGCAT	302

20	PN41617	ACCAGT GAGAGT GATTATT AT	308	CAAGAAGCT TATAAGCAA CAGAAT	309	GCTTATAGGAGCGC GTCCGGTAACCACT TCTAT	310	ATGGAC CATGAA AAT	316	TCATATG ATGTTAA AATG	317	GCCAGCAGTTTATG GGGGATGAACACTG AAGCTTTC	318
21	PN41636	TCCAGCA ATTTTAA TGCC	324	ATGACTTTA AATGGGGAT GAA	325	GGCCTGAACAGAG ATGACAAAGATCATC	326	AAGGGT CATGAT AGA	332	TCCTTTG ATGTCAA AGAT	333	GCCACCCAGTGATCC TCAGAGAAATCAGC CCCAGCAT	334
22	PN41654	AACAGC GCCTCAG ACTAC	340	ATTCGTTCA AATATGGAC AAA	341	GCAGAGAAGAGGG ATAACTATGGTCAG AATTTGTTC	342	ATGGAC CATGAA AAT	348	TCATATG ATGTTAA AATG	349	GCCAGCAGTTTATA CAGCTATGGCTACA CC	350
23	PN41656	ACCAGT GATCCA AGTTATG GT	356	CAGGGGTCT TATGACCCAG CAAAAT	357	GCAATGAGAGACC TTACTACCTCAGGA ACCTACAAATACAT C	358	ATGGAC CATGAA AAT	364	TCATATG ATGTTAA AATG	365	GCCAGCAGTTTCGG GGGCTGGGGCTATG GCTACACC	366
24	PN41690	ACCAGT GAGAGT GATTATT AT	372	CAAGAAGCT TATAAGCAA CAGAAT	373	GCTTATAGGAGCGC GATCCGTAACCACT TCTAT	374	ATGGAC CATGAA AAT	380	TCATATG ATGTTAA AATG	381	GCCAGCAGTTTATG GGGTGTGAACACTG AAGCTTTC	382
25	PN41702	ACCAGT GAGAGT GATTATT AT	388	CAAGAAGCT TATAAGCAA CAGAAT	389	GCTTATAGGAGCGC GACCCGTAACCACT TCTAT	390	ATGGAC CATGAA AAT	396	TCATATG ATGTTAA AATG	397	GCCAGCAGTTTATG GGCCGTTAATGAAA AACTGTTT	398
26	PN41703	ACCATCA GTGGAA ATGAGTA T	404	GGTCTAAAA AACAAAT	405	ATCGTCAGACCCGG GGGAACCTACAAA TACATC	406	ATGGAC CATGAA AAT	412	TCATATG ATGTTAA AATG	413	GCGACCGGGGGTGA CAATCAGCCCCAGC AT	414
27	PN41712	ACCAGT GAGAAAT AATTATT AT	420	CAAGAAGCT TATAAGCAA CAGAAT	421	GGCCTCAACACCCGG TAACCAGTTCTAT	422	GACTTT CAGGCC ACAACT	428	TCCAATG AGGGCT CCAAGG CC	429	AGTGCCTGGGACGG ATTCTATGGCTACAC C	430

28	PN41746	ACCAGT GAGAGT GATTATT AT	436	CAAGAAGCT TATAAGCAA CAGAAT	437	GCTTATAGGAGCGC GACCGGTAACCACT TCTAT	438	ATGGAC CATGAA AAT	444	TCATATG ATGTTAA AATG	445	GCCAGCAGTTTATG GGGCTTAAACACTG AAGCTTTC	446
29	PN41748	AAGACG TTATATG GC	452	CTACAGAAA GGTGGGA AGAG	453	GGAGCAGACATGT ATTGAGGAGGAGG TGCTGACGGACTCA CC	454	GACTTT CAGGCC ACAAC	460	TCCAATG AGGGCT CCAAGG CC	461	AGTGCCCTGGGACGG ATTCTATGGCTACAC C	462
30	PN41805	ACCAGT GAGAA AATTATT AT	468	CAAGAAGCT TATAAGCAA CAGAAT	469	GCTTTCGGTATGTA TTCAGGAGGAGGT GCTGACGGACTCAC C	470	CCTAGA CACGAC ACT	476	TTTTATG AAAAGA TGCAG	477	GCCAGCAGCCCTAC CGGGACAGGGGATG GCTACACC	478
31	PN41820	TCGTCTG TTCCACC ATAT	484	TACACAACA GGGGCCACC CTGGTT	485	GCTGTGAGTTCTGG TTCTGCAAGGCAAC TGACC	486	ATGGAC CATGAA AAT	492	TCATATG ATGTTAA AATG	493	GCCAGCAGTTCATGA CAGGTGGGACTATG GCTACACC	494
32	PN41822	ACCAGT GAGAGT GATTATT AT	500	CAAGAAGCT TATAAGCAA CAGAAT	501	GCTTATAGGAGCGC CACCGGTAACCACT TCTAT	502	ATGGAC CATGAA AAT	508	TCATATG ATGTTAA AATG	509	GCCAGCAGTTTGTG GGCATTGAACACTG AAGCTTTC	510
33	PN41829	ACCAGT GAGAGT GATTATT AT	516	CAAGAAGCT TATAAGCAA CAGAAT	517	GCTTATAGGAGCGC GACCGGTAACCACT TCTAT	518	ATGGAC CATGAA AAT	524	TCATATG ATGTTAA AATG	525	GCCAGCAGTTTATTC GCATTGAACACTGA AGCTTTC	526
34	PN41839	ACCATCA GTGGAA ATGAGTA T	532	GGTCTAAAA AACAAAT	533	ATCGTTTATGGAGG AAGCCAAAGGAAAT CTCATC	534	TCTGGG CATGAC ACT	540	TATTATG AGGAGG AAGAG	541	GCCAGCAGCTTCAG CAGGTCCAGCCCC AGCAT	542
35	PN41852	ACCAGT GAGAGT GATTATT AT	548	CAAGAAGCT TATAAGCAA CAGAAT	549	GCTTATAGGAGCGC GTTGGGCAATGACA TGCGC	550	ATGGAC CATGAA AAT	556	TCATATG ATGTTAA AATG	557	GCCAGCAGTTTATG GGCACTGAACACTG AAGCTTTC	558

36	PN41867	564	<u>ACCAGT</u> <u>GAGAA</u> <u>AATTATT</u> <u>AT</u>	<u>CAAGAAAGCT</u> <u>TATAAGCAA</u> <u>CAGAA</u>	<u>GCTTTCATGAAAAC</u> <u>TCAGGGCGGATCTG</u> <u>AAAAGCTGGTC</u>	<u>CCTAGA</u> <u>CACGAC</u> <u>ACT</u>	<u>TTTTATG</u> <u>AAAAGA</u> <u>TGCAG</u>	<u>GCCAGCAGCTTAGT</u> <u>TGGGTATGGCTACA</u> <u>CC</u>	574
		580	<u>ACCAGT</u> <u>GAGAGT</u> <u>GATTATT</u> <u>AT</u>	<u>CAAGAAAGCT</u> <u>TATAAGCAA</u> <u>CAGAA</u>	<u>GCTTATAGGAGCGC</u> <u>AACCGTAACCACT</u> <u>TCTAT</u>	<u>ATGGAC</u> <u>CATGAA</u> <u>AAT</u>	<u>TCATATG</u> <u>ATGTTAA</u> <u>AATG</u>	<u>GCCAGCAGTTTATG</u> <u>GGGACACATGAAA</u> <u>AACTGTTT</u>	590
		596	<u>ACCAGT</u> <u>GAGAGT</u> <u>GATTATT</u> <u>AT</u>	<u>CAAGAAAGCT</u> <u>TATAAGCAA</u> <u>CAGAA</u>	<u>GCTTATAGGAGCTC</u> <u>CACCGTAACCACT</u> <u>TCTAT</u>	<u>ATGGAC</u> <u>CATGAA</u> <u>AAT</u>	<u>TCATATG</u> <u>ATGTTAA</u> <u>AATG</u>	<u>GCCAGCAGTTTATG</u> <u>GGAACTAATGAAA</u> <u>AACTGTTT</u>	606
37	PN41868	565							
		581							
		597							
38	PN41869	565							
		581							
		597							

Table 4: Amino acid and nucleic acid sequences for Veloci-T® TCRs specific for MAGE-A4 (286-294) / HLA-A2

<i>TCR name Domain name</i>	<i>Domain Sequences</i> Amino Acid Sequence (SEQ ID NO); CDR1, CDR2, and CDR3 sequences are <u>underlined</u> Nucleic Acid Sequence (SEQ ID NO) ; CDR1, CDR2, and CDR3 sequences are <u>underlined</u>
PN41475 V α	AQTVTQSQPEMSVQEAETVTL SCTYDTSES DYYLFWYKQPPSRQMILVIR <u>QEAYKQQNATVNRFSVNFQKA AKSFSLKISDSQLGDAAMYFCAYRSATG</u> <u>NQFYFGTGTSLTVIP</u> (SEQ ID NO: 7) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGAC <u>ACCAGTGAGAGTGATTAT</u> <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> <u>ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGTGAATCGTTT</u> <u>TCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTCA</u> <u>GACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAGC</u> <u>GCGACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGTC</u> <u>ATTCCA</u> (SEQ ID NO: 8)
PN41475 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP EGYSVSREKKERFSLILESASTNQTSMYLCASSLWGS</u> <u>NTEAFFGQGTRLTVV</u> (SEQ ID NO: 15) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT <u>CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT</u> <u>ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT</u> <u>CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT</u> <u>GGGGCAGTAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACA</u> <u>GTTGTA</u> (SEQ ID NO: 16)
PN41494 V α	AQTVTQSQPEMSVQEAETVTL SCTYDTSES DYYLFWYKQPPSRQMILVIR <u>QEAYKQQNATENRFSVNFQKA AKSFSLKISDSQLGDAAMYFCAYRSATG</u> <u>NQFYFGTGTSLTVIP</u> (SEQ ID NO: 23) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGAC <u>ACCAGTGAGAGTGATTAT</u> <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> <u>ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT</u> <u>CTCTGTGAACTTCCAGAAGGCAGCCAAATCCTTCAGTCTCAAGATCTC</u> <u>AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG</u> <u>CGCGACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT</u> <u>CATTCCA</u> (SEQ ID NO: 24)
PN41494 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP EGYSVSREKKERFSLILESASTNQTSMYLCASSLWGW</u> <u>NSPLHFGNGTRLTVT</u> (SEQ ID NO: 31)

	GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGGGTGGAATTCACCCCTCCACTTTGGGAACGGGACCAGGCTCACTG TGACA (SEQ ID NO: 32)
PN41513 V α	AQTVTQSQPEMSVQEAETVTLSCYDTS<u>ESDY</u>YLFWYKQPPSRQMILVIR <u>QEAYKQON</u>ATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSAPG NQFYFGTGTSLTVIP (SEQ ID NO: 39) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGAC<u>ACCAGT</u>GAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACCTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCTCCCGGTAACCAGTTCTATTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 40)
PN41513 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF SYDVKMKEKGDIPGYVSREKKERFSLILESASTNQTSMYLC<u>ASSLFATN</u> EKLFFGSGTQLSVL (SEQ ID NO: 47) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT TTGCAACTAATGAAAACTGTTTTTTGGCAGTGGAACCCAGCTCTCTG TCTTG (SEQ ID NO: 48)
PN41516 V α	TQLLEQSPQFLSIQEGENLTVYCNSSSVFSSLQWYRQEPGEGPVLLVTVVV TGGEVKKLRLTFQFGDARKDSSLHITAAQPGDTGLYLC<u>AGERDSWGKFQ</u> FGAGTQVVVTP (SEQ ID NO: 55) ACCCAGCTGCTGGAGCAGAGCCCTCAGTTTCTAAGCATCCAAGAGGG AGAAAATCTCACTGTGTACTGCAACTCCTCAAGTGTTTTTCCAGCTTA CAATGGTACAGACAGGAGCCTGGGGAAGGTCCTGTCTCCTGGTGAC AGTAGTTACGGGTGGAGAAGTGAAGAAGCTGAAGAGACTAACCTTTC AGTTTGGTGATGCAAGAAAGGACAGTTCTCTCCACATCACTGCGGCCC AGCCTGGTGATACAGGCCTCTACCTCTGTGCAGGAGAGAGGGACAGC TGGGGGAAATCCAGTTTGGAGCAGGGACCCAGGTTGTGGTCAACCCA (SEQ ID NO: 56)
PN41516 V β	NAGVTQTPKFRILKIGQSM TLQCAQDMNHNYMYWYRQDPGMGLKLIYY SVGAGITDKGEVPNGYNVSRSTTEYFPLRLELAAPSQTSVYFC<u>ASSFWTG</u> GDEKLFFGSGTQLSVL (SEQ ID NO: 63) AATGCTGGTGTCACCTCAGACCCCAAAATTCCGCATCCTGAAGATAGGA CAGAGCATGACACTGCAGTGTGCCAGGATATGAACCATAACTACAT

	GTACTGGTATCGACAAGACCCAGGCATGGGGCTGAAGCTGATTATTATTA TTCAGTTGGTGCTGGTATCACTGATAAAGGAGAAGTCCCGAATGGCTA CAACGTCTCCAGATCAACCACAGAGTATTTCCCGCTCAGGCTGGAGTT GGCTGCTCCCTCCCAGACATCTGTGTACTTCTGTGCCAGCAGTTTTTGG ACAGGGGGCGATGAAAACTGTTTTTTGGCAGTGGAACCCAGCTCTCT GTCTTG (SEQ ID NO: 64)
PN41518 V α	AQTVTQSQPEMSVQEAETVTLSTYDTSESDDYFLFWYKQPPSRQMILVIR QEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSAPG NQFYFGTGTSLTVIP (SEQ ID NO: 71) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCGCCCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 72)
PN41518 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF SYDVKMKEKGDIPGYSVSREKKERFSLILESASTNQTSMYLCASSLWAT NEKLFFGSGTQLSVL (SEQ ID NO: 79) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGCAACTAATGAAAACTGTTTTTTGGCAGTGGAACCCAGCTCTCTG TCTTG (SEQ ID NO: 80)
PN41520 V α	AQTVTQSQPEMSVQEAETVTLSTYDTSESDDYFLFWYKQPPSRQMILVIR QEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSARG NQFYFGTGTSLTVIP (SEQ ID NO: 87) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCTCGGGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 88)
PN41520 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF SYDVKMKEKGDIPGYSVSREKKERFSLILESASTNQTSMYLCASSLWGV NSPLHFGNGTRLTVT (SEQ ID NO: 95) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT

	CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGGGGTAAATTACCCCTCCACTTTGGGAACGGGACCAGGCTCACTG TGACA (SEQ ID NO: 96)
PN41523 V α	AQTVTQSQPEMSVQEAETVTLSCTYDTS <u>ESDY</u> YLFWYKQPPSRQMILVIR <u>QEAYKQON</u> ATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSAPG <u>NQFY</u> FGTGTSLTVIP (SEQ ID NO: 103) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGAC <u>ACCAGT</u> GAGAGTGATTAT <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> <u>ATTCGCCAAGAAGCTTATAAGCAACAGAAT</u> GCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGT <u>GCTTATAGGAG</u> <u>CGCGCCCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT</u> CATTCCA (SEQ ID NO: 104)
PN41523 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEK</u> GDIPEGYSVSREKKERFSLILESASTNQTSMYLC <u>ASSLWGL</u> <u>NTEAFFGQ</u> GTRLTVV (SEQ ID NO: 111) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT <u>CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT</u> ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT <u>GGGGTTGAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAG</u> TTGTA (SEQ ID NO: 112)
PN41532 V α	AQTVTQSQPEMSVQEAETVTLSCTYDTS <u>ESDY</u> YLFWYKQPPSRQMILVIR <u>QEAYKQON</u> ATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSAEG <u>NQFY</u> FGTGTSLTVIP (SEQ ID NO: 119) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGAC <u>ACCAGT</u> GAGAGTGATTAT <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> <u>ATTCGCCAAGAAGCTTATAAGCAACAGAAT</u> GCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGT <u>GCTTATAGGAG</u> <u>CGCGGAGGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT</u> CATTCCA (SEQ ID NO: 120)
PN41532 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEK</u> GDIPEGYSVSREKKERFSLILESASTNQTSMYLC <u>ASSLWGL</u> <u>NTEAFFGQ</u> GTRLTVV (SEQ ID NO: 127) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT <u>CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT</u> ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT

	<u>GGGGGTTGAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTA</u> (SEQ ID NO: 128)
PN41539 V α	<u>AQKVTQAQTEISVVEKEDVTLDCVYETRDTTYLFWYKQPPSGELVFLIR</u> <u>RNSFDEQNEISGRYSWNFQKSTSSFNFTITASQVVDSAVYFCALSEGDTGG</u> <u>EKTIFGAGTRLFVKA</u> (SEQ ID NO: 135) GCTCAGAAGGTAACCTCAAGCGCAGACTGAAATTTCTGTGGTGGAGAA GGAGGATGTGACCTTGGACTGTGTGTATGAAACCCGTGATACTACTTA TTACTTATTCTGGTACAAGCAACCACCAAGTGGAGAATTGGTTTTCTT ATTCGT<u>CGGAACTCTTTTGATGAGCAAAATGAAATAAGTGGTCGGTAT</u> TCTTGGAACCTCCAGAAATCCACCAGTTCCTTCAACTTCACCATCACA GCCTCACAAGTCGTGGACTCAGCAGTATACTTCTGTGCTCTGAGTGAG GGGGATACTGGAGGCTTCAAACTATCTTTGGAGCAGGAACAAGACT ATTTGTTAAAGCA (SEQ ID NO: 136)
PN41539 V β	<u>NAGVTQTPKFRILKIGQSMTLQCAQDMNHNYMYWYRQDPGMGLKLIYY</u> <u>SVGAGITDKGEVPNGYNVSRSTTEYFPLRLELAAPSQTSVYFCASSYFPDR</u> <u>GLGNTIYFGEGSWLTVV</u> (SEQ ID NO: 143) AATGCTGGTGTCACTCAGACCCCAAAATTCCGCATCCTGAAGATAGGA CAGAGCATGACACTGCAGTGTGCCCAGGATATGAACCATAACTACAT GTACTGGTATCGACAAGACCCAGGCATGGGGCTGAAGCTGATTTATTA TTCAGTTGGTGTCTGGTATCACTGATAAAGGAGAAGTCCCGAATGGCTA CAACGTCTCCAGATCAACCACAGAGTATTTCCCGCTCAGGCTGGAGTT GGCTGCTCCCTCCCAGACATCTGTGTACTTCTGTGCCAGCAGTTACTTC CCGGACAGGGGGCTTGGAAACACCATATATTTGGAGAGGGGAAGTTG GCTCACTGTTGTA (SEQ ID NO: 144)
PN41550 V α	<u>QKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPELIMSIY</u> <u>SNGDKEDGRFTAQLNKASQYVSLLRDSQPSDSATYLC</u><u>AVNLSWGKFQF</u> <u>GAGTQVVVTP</u> (SEQ ID NO: 151) CAGAAGGAGGTGGAGCAGAATTCTGGACCCCTCAGTGTTCAGAGGG AGCCATTGCCTCTCTCAACTGCACTTACAGTGACCGAGGTTCCAGTC CTTCTTCTGGTACAGACAATATTCTGGGAAAAGCCCTGAGTTGATAAT GTCCATATACTCCAATGGTGACAAAGAAGATGGAAGGTTTACAGCAC AGCTCAATAAAGCCAGCCAGTATGTTTCTCTGCTCATCAGAGACTCCC AGCCCAGTGATTACGCCACCTACCTCTGTGCCGTGAAGTTGAGCTGGG GGAAATTCCAGTTTGGAGCAGGGACCCAGGTTGTGGTCACCCCA (SEQ ID NO: 152)
PN41550 V β	<u>DAGVTQSPHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQY</u> <u>YEEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLC</u><u>ASSLDRST</u> <u>EAFGQGTRLTVV</u> (SEQ ID NO: 159) GACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGG ACAGCAAGTGACTCTGAGATGCTCTCCTAAGTCTGGGCATGACACTGT GTCCTGGTACCAACAGGCCCTGGGTCAGGGGCCCCAGTTTATCTTTCA GTATTATGAGGAGGAAGAGAGACAGAGAGGCAACTTCCTGATCGAT TCTCAGGTCACCAGTTCCCTAACTATAGCTCTGAGCTGAATGTGAACG CCTTGTTGCTGGGGGACTCGGCCCTCTATCTCTGTGCCAGCAGCTTGG ACAGGAGCACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTG TA (SEQ ID NO: 160)

PN41557 V α	EDQVTQSPEALRLQEGESSLNCSYTVSGLRGLFWYRQDPGKGPEFLFTL <u>YSAGEEKEKERLKATLTKKESFLHITAPKPEDSATYLC</u><u>AVQAVTG</u><u>GGNKL</u> <u>TFGTGTQLKVEL</u> (SEQ ID NO: 167) GAAGACCAGGTGACGCAGAGTCCCCGAGGCCCTGAGACTCCAGGAGGG AGAGAGTAGCAGTCTCAACTGCAGTTACACAGTCAGCGGTTTAAGAG <u>GGCTGTTCTGGTATAGGCAAGATCCTGGGAAAGGCCCTGAATTCCTCT</u> TCACCCTGTATTCAGCTGGGGAAGAAAAGGAGAAAGAAAGGCTAAAA GCCACATTAACAAAGAAGGAAAGCTTTCTGCACATCACAGCCCCTAA ACCTGAAGACTCAGCCACTTATCTCTGT<u>GCTGTGCAGGCGGTCACGGG</u> <u>AGGAGGAAACAACTCACCTTTGGGACAGGCACTCAGCTAAAAGTGG</u> AACTC (SEQ ID NO: 168)
PN41557 V β	GAVVSQHPSWVICKSGTSVKIECRSL<u>DFQATT</u>MFWYRQFPKQSLMLMAT <u>SNEGSKATYE</u>QGVKDKFLINHASLTLSTLTVTSAHPEDSSFYICS<u>SASWDR</u> <u>DYGYTFGSGTRLTVV</u> (SEQ ID NO: 175) GGTGCTGTCGTCTCTCAACATCCGAGCTGGGTTATCTGTAAGAGTGGA ACCTCTGTGAAGATCGAGTGCCGTTCCCTGG<u>ACTTT</u>CAGGCCACA<u>ACT</u> ATGTTTTGGTATCGTCAGTTCCTGAAACAGAGTCTCATGCTGATGGCA ACTTCCAATGAGGGCTCCAAGGCCACATACGAGCAAGGCGTCGAGAA GGACAAGTTTCTCATCAACCATGCAAGCCTGACCTTGCTCCACTCTGAC AGTGACCAGTGCCCATCCTGAAGACAGCAGCTTCTACATCTGC<u>AGTGC</u> TAGTTGGGACAGGGACTATGGCTACACCTTCGGTTCGGGGACCAGGTT AACCGTTGTA (SEQ ID NO: 176)
PN41559 V α	AQTVTQSQPEMSVQEAETVTLSCITYDTS<u>ESDY</u>YLFWYKQPPSRQMILVIR <u>QEAYKQQN</u>ATENRFSVNFQKAAKSFSCLKISDSQLGDAAMYFCAYRSATG <u>NQFYFGTGTSLTVIP</u> (SEQ ID NO: 183) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACCTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCCACCGGTAACCAGTTCTATTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 184)
PN41559 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMD<u>HEN</u>MFWYRQDPGLGLRLIYF <u>SYDVKMKEK</u>GDIPEGYSVSREKKERFSLILESASTNQTSMYLC<u>ASSL</u>WGW <u>GTEAFFGQGTRLTVV</u> (SEQ ID NO: 191) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGGTTGGGGCACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAG TTGTA (SEQ ID NO: 192)

PN41565 V α	AQTVTQSQPEMSVQEAETVTLSCYDT<u>SESDYYLFWYKQPPSRQMILVIR</u> <u>QEAYKQQN</u>ATENRFSVNFQKAAKSFSLSKISDSQLGDAAMYFCAYRSPTG <u>NQFYFGTGTSLTVIP</u> (SEQ ID NO: 199) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGAC<u>ACCAGTGAGAGTGATTAT</u> <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG <u>CCCCACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT</u> CATTCCA (SEQ ID NO: 200)
PN41565 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGD</u>IPeGYVSREKKERFSLILESASTNQTSMYLC<u>ASSLWAM</u> <u>NTEAFFGQGT</u>RLTVV (SEQ ID NO: 207) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT <u>GGGCAATGAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACA</u> GTTGTA (SEQ ID NO: 208)
PN41568 V α	AQTVTQSQPEMSVQEAETVTLSCYDT<u>SESDYYLFWYKQPPSRQMILVIR</u> <u>QEAYKQQN</u>ATENRFSVNFQKAAKSFSLSKISDSQLGDAAMYFCAYRSARG <u>NQFYFGTGTSLTVIP</u> (SEQ ID NO: 215) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG <u>CGCGCGGGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT</u> CATTCCA (SEQ ID NO: 216)
PN41568 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGD</u>IPeGYVSREKKERFSLILESASTNQTSMYLC<u>ASSLWGT</u> <u>NEKLFFGSGT</u>QLSVL (SEQ ID NO: 223) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT <u>GGGGGACTAATGAAAACTGTTTTTGGCAGTGGAACCCAGCTCTCTG</u> TCTTG (SEQ ID NO: 224)

PN41573 V α	AQTVTQSQPEMSVQEAETVTL SCTYDTSESDYYLFWYKQPPSRQMILVIR <u>QEAYKQQN</u>ATENRFSVNFQKAAKSFSLSKISDSQLGDAAMYFCAYRSATG <u>NQFY</u>FGTGTSLTVIP (SEQ ID NO: 231) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGAC<u>ACCAGT</u>GAGAGTGATTAT <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCAACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 232)
PN41573 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEK</u>GDIPEGYSVSREKKERFSLILESASTNQTSMYLC<u>ASSLWGT</u> <u>NEKLFF</u>SGTQLSVL (SEQ ID NO: 239) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTGT GGGGAACTAATGAAAACTGTTTTTTGGCAGTGGAACCCAGCTCTCTG TCTTG (SEQ ID NO: 240)
PN41577 V α	AQTVTQSQPEMSVQEAETVTL SCTYDTSESDYYLFWYKQPPSRQMILVIR <u>QEAYKQQN</u>ATENRFSVNFQKAAKSFSLSKISDSQLGDAAMYFCAYRSAPG <u>NQFY</u>FGTGTSLTVIP (SEQ ID NO: 247) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTTAGCTGCACATATGAC<u>ACCAGT</u>GAGAGTGATTAT <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCGCCCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 248)
PN41577 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEK</u>GDIPEGYSVSREKKERFSLILESASTNQTSMYLC<u>ASSLWGT</u> <u>NEKLFF</u>SGTQLSVL (SEQ ID NO: 255) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGGGACTAATGAAAACTGTTTTTTGGCAGTGGAACCCAGCTCTCTG TCTTG (SEQ ID NO: 256)
PN41607 V α	AQTVTQSQPEMSVQEAETVTL SCTYDTSESDYYLFWYKQPPSRQMILVIR <u>QEAYKQQN</u>ATENRFSVNFQKAAKSFSLSKISDSQLGDAAMYFCAYRSASG <u>NQFY</u>FGTGTSLTVIP (SEQ ID NO: 263)

	GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGAC<u>ACCAGTGAGAGTGATTAT</u> <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCGTCCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 264)
PN41607 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP</u>EGYSVSREKKERFSLILESASTNQTSMYLC<u>ASSLWGT</u> NEKLFFGSGTQLSVL (SEQ ID NO: 271) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGGCACTAATGAAAACTGTTTTTTGGCAGTGGAACCCAGCTCTCTG TCTTG (SEQ ID NO: 272)
PN41608 V α	AQTVTQSQPEMSVQEAETVTLSTYDTSSESDYYLFWYKQPPSRQMILVIR QEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSAPG NQFYFGTGTS�TVIP (SEQ ID NO: 279) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCGCCCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 280)
PN41608 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP</u>EGYSVSREKKERFSLILESASTNQTSMYLC<u>ASSFWGW</u> NTEAFFGQGTRLTVV (SEQ ID NO: 287) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTCT GGGGATGGAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACA GTTGTA (SEQ ID NO: 288)
PN41613 V α	AQTVTQSQPEMSVQEAETVTLSTYDTSSESDYYLFWYKQPPSRQMILVIR QEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSATG NQFYFGTGTS�TVIP (SEQ ID NO: 295)

	GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGAC<u>ACCAGTGAGAGTGATTAT</u> <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCGACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 296)
PN41613 V β	EAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYY <u>MNVEVTDKGDVPEGYKVS</u> <u>RKEKRN</u> <u>FPLILESP</u> <u>PNQTS</u> <u>LYFC</u> <u>ASSLLYSN</u> <u>QPQHFGDGTRLSIL</u> (SEQ ID NO: 303) GAAGCCCAAGTGACCCAGAACCCAAGATACCTCATCACAGTGAAGTGG AAAGAAGTTAACAGTGACTTGTCTCAGAATATGAACCATGAGTATAT GTCCTGGTATCGACAAGACCCAGGGCTGGGCTTAAGGCAGATCTACTA TTCATGAATGTTGAGGTGACTGATAAGGGAGATGTTCTGAAGGGTA CAAAGTCTCTCGAAAAGAGAAGAGGAATTTCCCCCTGATCCTGGAGTC GCCCAGCCCCAACCAGACCTCTCTGTACTTCTGTGCCAGCAGTTTACT GTATAGCAATCAGCCCCAGCATTTTGGTGATGGGACTCGACTCTCCAT CCTA (SEQ ID NO: 304)
PN41617 V α	AQVTQSQPEMSVQEAETVTLSCITYDTSESDDYFLFWYKQPPSRQMILVIR QEAYKQQNATENRFSVNFQKAAKSFSCLKISDSQLGDAAMYFCAYRSASG NQFYFGTGTSITVIP (SEQ ID NO: 311) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCGTCCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 312)
PN41617 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF SYDVKMKEKGDIPYGYSVSREKKERFSLILESASTNQTSMYLCASSLWGM NTEAFFGQGTRLTVV (SEQ ID NO: 319) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGGGATGAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACA GTTGTA (SEQ ID NO: 320)
PN41636 V α	ILNVEQSPQSLHVQEGDSTNFTCSFPSSNFYALHWYRWETAKSPEALFVM TLNGDEKKKGRISATLNTKEGYSYLYIKGSQPEDSATYLCALNRDDKIIIFG KGTRLHILP (SEQ ID NO: 327) ATACTGAACGTGGAACAAAGTCCTCAGTCACTGCATGTTTCAGGAGGG AGACAGCACCAATTTACCTGCAGCTTCCCTTCCAGCAATTTTTATGCC

	TTCACTGGTACAGATGGGAAACTGCAAAAAGCCCCGAGGCCTTGTTT GTAATGACTTTAAATGGGGATGAAAAGAAGAAAGGACGAATAAGTGC CACTCTTAATACCAAGGAGGGTTACAGCTATTTGTACATCAAAGGATC CCAGCCTGAAGACTCAGCCACATACCTCTGTGCCCTGAACAGAGATGA <u>CAAGATCATCTTTGGAAAAGGGACACGACTTCATATTCTCCCC</u> (SEQ ID NO: 328)
PN41636 V β	DADVQTTPRNRITKTGKRIMLECSQTKGHDRMYWYRQDPGLGLRLIYYS <u>FDVKDINKGEISDGYSVSRQAQAKFSLSLESAIPNQATALYFCATSDPQRNQ</u> <u>PQHFGDGTRLSIL</u> (SEQ ID NO: 335) GATGCTGATGTTACCCAGACCCCAAGGAATAGGATCACAAAGACAGG AAAGAGGATTATGCTGGAATGTTCTCAGACTAAGGGTCATGATAGAAT GTACTGGTATCGACAAGACCCAGGACTGGGCCTACGGTTGATCTATTA <u>CTCCTTTGATGTCAAAGATATAAACAAAGGAGAGATCTCTGATGGATA</u> CAGTGTCTCTCGACAGGCACAGGCTAAATTCTCCCTGTCCCTAGAGTC TGCCATCCCCAACCCAGACAGCTCTTTACTTCTGTGCCACCAGTGATCCT <u>CAGAGAAATCAGCCCCAGCATTTTGGTGATGGGACTCGACTCTCCATC</u> <u>CTA</u> (SEQ ID NO: 336)
PN41654 V α	GESVGLHLPTLSVQEGDNSIINCAYSNSASDYFIWYKQESGKGPQFIIDIRS <u>NMDKRQGQRVTVLLNKTVKHLSLQIAATQPGDSAVYFCAEKRDNYGQN</u> <u>FVFGPGTRLSVLP</u> (SEQ ID NO: 343) GGAGAGAGTGTGGGGCTGCATCTTCCTACCCTGAGTGTCCAGGAGGGT GACAACTCTATTATCAACTGTGCTTATTCAAACAGCGCCTCAGACTAC TTCATTTGGTACAAGCAAGAATCTGGAAAAGGTCTCAATTCATTATA GACATTCGTTCAAATATGGACAAAAGGCAAGGCCAAAGAGTCACCGT TTTATTGAATAAGACAGTGAAACATCTCTCTCTGCAAATTGCAGCTAC TCAACCTGGAGACTCAGCTGTCTACTTTTGTGCAGAGAAGAGGGATAA <u>CTATGGTCAGAAATTTGTCTTTTGGTCCCGGAACCAGATTGTCCGTGCTG</u> <u>CCC</u> (SEQ ID NO: 344)
PN41654 V β	DVKVTQSSRYLVKRTGEKVFLQVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIPGYSVSREKKERFSLILESASTNQTSMYLCASSLYSY</u> <u>GYTFGSGTRLTVV</u> (SEQ ID NO: 351) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT <u>CTCATATGATGTTAAATGAAAAGAAAAGGAGATATTCCTGAGGGGT</u> ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT <u>ACAGCTATGGCTACACCTTCGGTTCGGGGACCAGGTAAACCGTTGTA</u> (SEQ ID NO: 352)
PN41656 V α	AQKITQTQPGMFVQEKEAVTLDCTYDTS ~~SD~~ PSYGLFWYKQPSSGEMIFLIY <u>QGSYDQQNATEGRYSLNFQKARKSANLVISASQLGDSAMYFCAMRDLTT</u> <u>SGTYKYIFGTGTRLKVLA</u> (SEQ ID NO: 359) GCCCAGAAGATAACTCAAACCCAACCAGGAATGTTTCGTGCAGGAAAA GGAGGCTGTGACTCTGGACTGCACATATGACACCAGTGATCCAAGTTA <u>TGGTCTATTCTGGTACAAGCAGCCCAGCAGTGGGGAAATGATTTTCT</u> <u>TATTTATCAGGGGTCTTATGACCAGCAAAATGCAACAGAAGGTCGCTA</u>

	CTCATTGAATTTCCAGAAGGCAAGAAAATCCGCCAACCTTGTGCATCTC CGCTTCACAACCTGGGGGACTCAGCAATGTACTTCTGTGCAATGAGAGA CCTTACTACCTCAGGAACCTACAAATACATCTTTGGAACAGGCACCAG GCTGAAGGTTTTAGCA (SEQ ID NO: 360)
PN41656 V β	DVKVTQSSRYLVKRTGEKVFLVCVQDMDHENMFWYRQDPGLGLRLIYF SYDVKMKEKGDIPGYSVSREKKERFSLILESASTNQTSMYLCASSFGGW GYGYTFGSGTRLTVV (SEQ ID NO: 367) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTCG GGGGCTGGGGCTATGGCTACACCTTCGGTTCGGGGACCAGGTAAACCG TTGTA (SEQ ID NO: 368)
PN41690 V α	AQTVTQSQPEMSVQEAETVTLSTYDTSSESDYYLFWYKQPPSRQMILVIR QEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSAIG NQFYFGTGTSLTVIP (SEQ ID NO: 375) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCGATCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 376)
PN41690 V β	DVKVTQSSRYLVKRTGEKVFLVCVQDMDHENMFWYRQDPGLGLRLIYF SYDVKMKEKGDIPGYSVSREKKERFSLILESASTNQTSMYLCASSLWGV NTEAFFGQGTSLTVV (SEQ ID NO: 383) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGGTGTGAACACTGAAGCTTTCTTTTGGACAAGGCACCAGACTCACAG TTGTA (SEQ ID NO: 384)
PN41702 V α	AQTVTQSQPEMSVQEAETVTLSTYDTSSESDYYLFWYKQPPSRQMILVIR QEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSATG NQFYFGTGTSLTVIP (SEQ ID NO: 391) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG

	<u>CGCGACCGGTAACCCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT</u> <u>CATTCCA</u> (SEQ ID NO: 392)
PN41702 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP</u> EGYSVSREKKERFSLILESASTNQTSMYLC <u>ASSLWAV</u> <u>NEKLFFGSGTQLSVL</u> (SEQ ID NO: 399) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT <u>CTCATATGATGTTAA</u> AAATGAAAGAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT <u>GGGCCGTTAATGAAAACTGTTT</u> TTTGGCAGTGGAACCCAGCTCTCTG TCTTG (SEQ ID NO: 400)
PN41703 V α	DAKTTQPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIHG <u>LKNNETNEMASLI</u> ITEDRKSSTLILPHATLRDТАVYYCIVRPGGTYYKYIFGT GTRLKVLA (SEQ ID NO: 407) GATGCTAAGACCACCCAGCCACCCTCCATGGATTGCGCTGAAGGAAG AGCTGCAAACCTGCCTTGTAATCACTCTACCATCAGTGGAATGAGTA <u>TGTGTATTGGTATCGACAGATTCACTCCCAGGGGCCACAGTATATCAT</u> <u>TCATGGTCTAAAAACAATGAAACCAATGAAATGGCCTCTCTGATCAT</u> CACAGAAGACAGAAAGTCCAGCACCTTGATCCTGCCCCACGCTACGCT GAGAGACACTGCTGTGTACTATTGCATCGTCAGACCGGGGGGAACCTA <u>CAAATACATCTTTGGAACAGGCACCAGGCTGAAGGTTTTAGCA</u> (SEQ ID NO: 408)
PN41703 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP</u> EGYSVSREKKERFSLILESASTNQTSMYLCATGGDNQ <u>PQHFGDGTRLSIL</u> (SEQ ID NO: 415) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT <u>CTCATATGATGTTAA</u> AAATGAAAGAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCGGGGGTG <u>ACAATCAGCCCCAGCATTTT</u> TGGTGATGGGACTCGACTCTCCATCCTA (SEQ ID NO: 416)
PN41712 V α	AQTVTQSQPEMSVQEAETVTLSCITYDTSENNYYLFWYKQPPSRQMILVIR <u>QEAYKQQNATENRFSVNFQKA</u> AKSFSLKISDSQLGDTAMYFCALNTGNQ <u>FYFGTGTSLTVIP</u> (SEQ ID NO: 423) GCCCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGC AGAGACTGTGACCCTGAGTTGCACATATGACACCAGTGAGAATAATTA <u>TTATTTGTTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT</u> <u>ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACGGAGAATCGTTT</u> CTCTGTGAACCTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGACACTGCGATGTATTTCTGTGCCCTCAACAC <u>CGGTAACCAAGTTCTATTTT</u> GGGACAGGGACAAGTTTGACGGTCATTCC A (SEQ ID NO: 424)

PN41712 V β	GAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFYRQFPKQSLMLMAT <u>SNEGSKATYEQGV</u>EKDKFLINHASLTLSTLTVTSAHPEDSSFYICSAWDGF <u>YGYTFGSGTRLTVV</u> (SEQ ID NO: 431) GGTGCTGTCGTCTCTCAACATCCGAGCTGGGTTATCTGTAAGAGTGGA ACCTCTGTGAAGATCGAGTGCCGTTCCCTGGACTTTCAGGCCACA ACTATGTTTTGGTATCGTCAGTTCCTCGAAACAGAGTCTCATGCTGATGGCA ACTTCCAATGAGGGCTCCAAGGCCACATACGAGCAAGGCGTCGAGAA GGACAAGTTTCTCATCAACCATGCAAGCCTGACCTTGTCCTACTCTGAC AGTGACCAGTGCCCATCCTGAAGACAGCAGCTTCTACATCTGCAGTGC CTGGGACGGATTCTATGGCTACACCTTCGGTTCGGGGACCAGGTAA CGTTGTA (SEQ ID NO: 432)
PN41746 V α	AQTVTQSQPEMSVQEAETVTLSTYDTSESDYYLFWYKQPPSRQMILVIR <u>QEAYKQONATENRFSVN</u>FQKAAKSFSLKISDSQLGDAAMYFCAYRSATG <u>NQFYFGTGTSLTVIP</u> (SEQ ID NO: 439) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCGACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 440)
PN41746 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGD</u>IEPEGYSVSREKKERFSLILESASTNQTSMYLCASSLWGL <u>NTEAFFGQGTRLTVV</u> (SEQ ID NO: 447) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGGCTTAAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAG TTGTA (SEQ ID NO: 448)
PN41748 V α	SQELEQSQSLIVQEGKNLTINCTSSKTLYGLYWKQKYGEGLIFLMMLQ <u>KGGEKSHEKITAKLDEKKQ</u>SSLHITASQPSHAGIYLCGADMYSGGGAD <u>GLTFGKGTHLIQ</u>P (SEQ ID NO: 455) AGCCAAGAACTGGAGCAGAGTCAACAGTCCTTGATCGTCCAAGAGGG AAAGAATCTCACCATAAACTGCACGTCAATCAAAGACGTTATATGGCTT ATACTGGTATAAGCAAAAGTATGGTGAAGGTCTTATCTTCTTGATGAT GCTACAGAAAGGTGGGGAAGAGAAAAAGTCATGAAAAGATAACTGCCA AGTTGGATGAGAAAAAGCAGCAAAGTTCCTGCATATCACAGCCTCCC AGCCCAGCCATGCAGGCATCTACCTCTGTGGAGCAGACATGTATTCAG GAGGAGGTGCTGACGGACTCACCTTTGGCAAAGGGACTCATCTAATCA TCCAGCCC (SEQ ID NO: 456)

PN41748 V β	GAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFWYRQFPKQSLMLMAT <u>SNEGSKATYEQGV</u>EKDKFLINHASLTLSTLTVTSAHPEDSSFYICSAWDGF <u>YGYTFGSGTRLTVV</u> (SEQ ID NO: 463) GGTGCTGTCGTCTCTCAACATCCGAGCTGGGTTATCTGTAAGAGTGGA ACCTCTGTGAAGATCGAGTGCCGTTCCCTGGACTTTCAGGCCACA ACTATGTTTTGGTATCGTCAGTTCCTCGAAACAGAGTCTCATGCTGATGGCA ACTTCCAATGAGGGCTCCAAGGCCACATACGAGCAAGGCGTCGAGAA GGACAAGTTTCTCATCAACCATGCAAGCCTGACCTTGTCCTACTCTGAC AGTGACCAGTGCCCATCCTGAAGACAGCAGCTTCTACATCTGCAGTGC CTGGGACGGATTCTATGGCTACACCTTCGGTTCGGGGACCAGGTAA CGTTGTA (SEQ ID NO: 464)
PN41805 V α	AQTVTQSQPEMSVQEAETVTLSTYDTS~~EN~~NYLFWYKQPPSRQMILVIR <u>QEAYKQON</u>ATENRFSVNFQKAAKSFSCLKISDSQLGDTAMYFCAFGMYSG <u>GGADGLTFGKGTHLIQP</u> (SEQ ID NO: 471) GCCCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGC AGAGACTGTGACCCTGAGTTGCACATATGACACCAGTGAGAATAATTA TTATTTGTTCTGGTACAAGCAGCTCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACGGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGACACTGCGATGTATTTCTGTGCTTTCGGTAT GTATTCAGGAGGAGGTGCTGACGGACTCACCTTTGGCAAAGGGACTC ATCTAATCATCCAGCCC (SEQ ID NO: 472)
PN41805 V β	AAGVIQSPRHLIKEKRETATLKCYP~~IPR~~HDTVYWYQQGPGQDPQFLISFYE <u>KMQSDKGSIPDRFSAQQFSDYHSELN</u>MSSLELGDSALYFCASSPTGTGDG <u>YTFGSGTRLTVV</u> (SEQ ID NO: 479) GCTGCTGGAGTCATCCAGTCCCCAAGACATCTGATCAAAGAAAAGAG GGAAACAGCCACTCTGAAATGCTATCCTATCCCTAGACACGACACTGT CTACTGGTACCAGCAGGGTCCAGGTCAGGACCCCCAGTTCCTCATTTT GTTTTATGAAAAGATGCAGAGCGATAAAGGAAGCATCCCTGATCGATT CTCAGCTCAACAGTTCAGTGAATCATTTCTGAACTGAACATGAGCTC CTTGGAGCTGGGGGACTCAGCCCTGTACTTCTGTGCCAGCAGCCCTAC CGGGACAGGGGATGGCTACACCTTCGGTTCGGGGACCAGGTAAACCG TTGTA (SEQ ID NO: 480)
PN41820 V α	AQSVTPGSHVSVSEALVLLRCNYSSSVPPYLFWYVQYPNQGLQLLLK YT~~T~~GATLVK~~ING~~FEAEFKKSETSFHLTKPSAHMSDAAEYFC~~AV~~SSGSAR <u>QLTFGSGTQLTVLP</u> (SEQ ID NO: 487) GCCCAGTCGGTGACCCAGCCTGGCAGCCACGTCTCTGTCTCTGAGGGA GCCCTGGTTCTGCTGAGGTGCAACTACTCATCGTCTGTTCCACCATATC TCTTCTGGTATGTGCAATACCCCAACCAAGGACTCCAGCTTCTCCTGA AGTACACAACAGGGGGCCACCCTGGTTAAAGGCATCAACGGTTTTGAG GCTGAATTTAAGAAGAGTGAAACCTCCTTCCACCTGACGAAACCTCA GCCCATATGAGCGACGCGGCTGAGTACTTCTGTGCTGTGAGTTCTGGT TCTGCAAGGCAACTGACCTTTGGATCTGGGACACAATTGACTGTTTAA CCT (SEQ ID NO: 488)

PN41820 V β	DVKVTQSSRYLVKRTGEKVFLCVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP</u>EGYSVSREKKERFSLILESASTNQTSMYLC<u>ASSHDRW</u> <u>DYGYTFGSGTRLTVV</u> (SEQ ID NO: 495) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTCATG ACAGGTGGGACTATGGCTACACCTTCGGTTCGGGGACCAGGTAAACCG TTGTA (SEQ ID NO: 496)
PN41822 V α	AQTVTQSQPEMSVQEAETVTLSCYDTSESDYYLFWYKQPPSRQMILVIR <u>QEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSATG</u> <u>NQFYFGTGTSLTVIP</u> (SEQ ID NO: 503) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCCACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 504)
PN41822 V β	DVKVTQSSRYLVKRTGEKVFLCVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP</u>EGYSVSREKKERFSLILESASTNQTSMYLC<u>ASSLWAL</u> <u>NTEAFFGQGTRLTVV</u> (SEQ ID NO: 511) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTGT GGGCATTGAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAG TTGTA (SEQ ID NO: 512)
PN41829 V α	AQTVTQSQPEMSVQEAETVTLSCYDTSESDYYLFWYKQPPSRQMILVIR <u>QEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSATG</u> <u>NQFYFGTGTSLTVIP</u> (SEQ ID NO: 519) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCGACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 520)

PN41829 V β	DVKVTQSSRYLVKRTGEKVFLCVDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP</u>EGYSVSREKKERFSLILESASTNQTSMYLC<u>ASSL</u>FALN <u>TEAFFGQ</u>GTRLTVV (SEQ ID NO: 527) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT TCGCATTGAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAG TTGTA (SEQ ID NO: 528)
PN41839 V α	DAKTTQPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIHG LKNNETNEMASLIITEDRKSSTLILPHATLRDТАVYYCIVYGGSQGNLIFG KGTKLSVKP (SEQ ID NO: 535) GATGCTAAGACCACCCAGCCCCCTCCATGGATTGCGCTGAAGGAAG AGCTGCAAACCTGCCTTGTAACTACTCTACCATCAGTGGAATGAGTA TGTGTATTGGTATCGACAGATTCACTCCCAGGGGCCACAGTATATCAT TCATGGTCTAAAAACAATGAAACCAATGAAATGGCCTCTCTGATCAT CACAGAAGACAGAAAGTCCAGCACCTTGATCCTGCCCCACGCTACGCT GAGAGACACTGCTGTGTACTATTGCATCGTTTATGGAGGAAGCCAAGG AAATCTCATCTTTGGAAAAGGCACTAACTCTCTGTТАAACCA (SEQ ID NO: 536)
PN41839 V β	DAGVTQSPHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQY YEEEEQRGNFPDRFSGHQFPNYSSSELNVNALLLGDSALYLCASSFSRVQ PQHFGDGTRLSIL (SEQ ID NO: 543) GACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGG ACAGCAAGTGACTCTGAGATGCTCTCCTAAGTCTGGGCATGACACTGT GTCCTGGTACCAACAGGCCCTGGGTCAGGGGGCCCCAGTTTATCTTTCA GTATTATGAGGAGGAAGAGAGACAGAGAGGCAACTTCCCTGATCGAT TCTCAGGTCACCAGTTCCCTAACTATAGCTCTGAGCTGAATGTGAACG CCTTGTTGCTGGGGGACTCGGCCCTCTATCTCTGTGCCAGCAGCTTCAG CAGGGTCCAGCCCCAGCATTTTGGTGATGGGACTCGACTCTCCATCCT A (SEQ ID NO: 544)
PN41852 V α	AQTVTQSQPEMSVQEAETVTLSCITYDTSESDYYLFWYKQPPSRQMILVIR QEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYRSALG NDMRFGAGTRLTVKP (SEQ ID NO: 551) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACCTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCGTTGGGCAATGACATGCGCTTTGGAGCAGGGACCAGACTGACAG TAAACCA (SEQ ID NO: 552)

PN41852 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP</u>EGYSVSREKKERFSLILESASTNQTSMYLC<u>ASSLWAL</u> <u>NTEAFFGQ</u>GTRLTVV (SEQ ID NO: 559) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGCACTGAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAG TTGTA (SEQ ID NO: 560)
PN41867 V α	AQTVTQSQPEMSVQEAETVTLSTYDTSENYYLFWYKQPPSRQMILVIR <u>QEAYKQQN</u>ATENRFSVNFQKAAKSFSCLKISDSQLGDTAMYFCAFMKTQG <u>GSEKLVFG</u>GTKLTVNP (SEQ ID NO: 567) GCCCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGC AGAGACTGTGACCCTGAGTTGCACATATGACACCAAGTGAGAATAATTA TTATTTGTTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACGGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGACACTGCGATGTATTTCTGTGCTTTCATGAA AACTCAGGGCGGATCTGAAAAGCTGGTCTTTGGAAAGGGAACGAAAC TGACAGTAAACCCA (SEQ ID NO: 568)
PN41867 V β	AAGVIQSPRHLIKEKRETATLKCYPHPRHDTVYWYQQGPGQDPQFLISEYE <u>KMQSDKGS</u>IPDRFSAQQFSDYHSELNMSLELGDSALYFCASSLVGYGYT FGSGTRLTVV (SEQ ID NO: 575) GCTGCTGGAGTCATCCAGTCCCCAAGACATCTGATCAAAGAAAAGAG GGAAACAGCCACTCTGAAATGCTATCCTATCCCTAGACACGACACTGT CTACTGGTACCAGCAGGGTCCAGGTCAGGACCCCCAGTTCCCTCATTTT GTTTTATGAAAAGATGCAGAGCGATAAAGGAAGCATCCCTGATCGATT CTCAGCTCAACAGTTCACTGACTATCATTCTGAACTGAACATGAGCTC CTTGGAGCTGGGGGACTCAGCCCTGTACTTCTGTGCCAGCAGCTTAGT TGGGTATGGCTACACCTTCGGTTCGGGGACCAGGTTAACCGTTGTA (SEQ ID NO: 576)
PN41868 V α	AQTVTQSQPEMSVQEAETVTLSTYDTSESDDYYLFWYKQPPSRQMILVIR <u>QEAYKQQN</u>ATENRFSVNFQKAAKSFSCLKISDSQLGDAAMYFCAYRSATG <u>NQFYFG</u>TGTSLTVIP (SEQ ID NO: 583) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CGCAACCGGTAACCAAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 584)
PN41868 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIP</u>EGYSVSREKKERFSLILESASTNQTSMYLC<u>ASSLWGT</u> <u>HEKLFFGSGT</u>QLSVL (SEQ ID NO: 591)

	GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGGGACACATGAAAAACTGTTTTTTGGCAGTGGAACCCAGCTCTCTG TCTTG (SEQ ID NO: 592)
PN41869 V α	AQTVTQSQPEMSVQEAETVTLSCYDTSSESDYYLFWYKQPPSRQMILVIR QEAYKQONATENRFSVNFQKAAKSFSCLKISDSQLGDAAMYFCAYRSSTG NQFYFGTGTSLTVIP (SEQ ID NO: 599) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTT ATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTT CTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTC AGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAG CTCCACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGT CATTCCA (SEQ ID NO: 600)
PN41869 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF SYDVKMKEKGDIPGYSVSREKKERFSLILESASTNQTSMYLCASSLWGT NEKLFFGSGTQLSVL (SEQ ID NO: 607) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAT GGGGA ACTAATGAAAAACTGTTTTTTGGCAGTGGAACCCAGCTCTCTG TCTTG (SEQ ID NO: 608)

Example 2. Identification of MAGE-A4 specific T cell receptors

[00187] Mice humanized for cellular immune system components, Veloci-T® mice (see, *e.g.*, PCT Publication No. WO 2016/164492, the entire contents of which are incorporated herein by reference), were immunized with MAGE-A4 (230-239) peptide (GVYDGREHTV, SEQ ID NO: 612) presented specifically by human HLA-A2, diluted in PBS and mixed with adjuvant, *e.g.* in equal volume with Complete Freund's Adjuvant (CFA; Chondrex, Inc.). Spleen suspensions from immunized mice were obtained and dissociated. Red blood cells were lysed in ACK lysis buffer (Life Technologies), and splenocytes were suspended in RPMI complete media. Isolated splenocytes are sorted and single T cells that bind MAGE-A4 (230-239) peptide in the context of MHC are isolated by fluorescent-

activated cell sorting (FACS). Isolated T cells are single well plated and mixed with TCR alpha and beta variable region-specific PCR primers. cDNAs for each single T cell were synthesized *via* a reverse transcriptase (RT) reaction. Each resulting RT product was then split and transferred into two corresponding wells for subsequent TCR beta and alpha PCRs. One set of the resulting RT products was first amplified by PCR using a 5' degenerate primer specific for TCR beta variable region leader sequence or a 5' degenerate primer specific for TCR alpha chain variable region leader sequence and a 3' primer specific for TCR constant region, to form an amplicon. The amplicons were then amplified again by PCR using a 5' degenerate primer specific for TCR beta variable region framework 1 or a 5' degenerate primer specific for TCR alpha chain variable region framework 1 and a 3' primer specific for TCR constant region, to generate amplicons for cloning. The TCR beta and alpha derived PCR products were cloned into expression vectors containing beta constant region and alpha constant region, respectively. Expression vectors expressing full-length beta and alpha chain pairs were transfected into CHO cells and tested for binding to a commercial MAGE-A4/HLA tetramer reagent (HLA-A02:01 MAGE-A4 tetramer; MBL International Corporation) or to a commercial MAGE-A8/HLA tetramer reagent (HLA-A02:01 MAGE-A8 tetramer; MBL International Corporation). CHO cells were incubated with an antibody specific for mouse TCR constant region (clone H57-597) (Biolegend, San Diego, CA), and with either a soluble HLA-A2 MAGE-A4 (GVYDGREHTV) (SEQ ID NO: 612) tetramer or a soluble HLA-A2 MAGE-A8 (GLYDGREHSV) (SEQ ID NO: 613). Samples were then analyzed on an LSRFortessa X-20 (BD Biosciences, San Jose, CA). To calculate percentage of tetramer positive cells for MAGE-A4 (230-239), antigen positive (Ag+) gates were set based on a negative control TCR that does not bind to the HLA-A2 (GVYDGREHTV) (SEQ ID NO: 612) tetramer. To calculate percentage of tetramer positive cells for MAGE-A8, antigen positive (Ag+) gates were set based on a negative control TCR that does not bind to the HLA-A2 (GLYDGREHSV) (SEQ ID NO: 613), using FlowJo (LLC, Ashland, OR). All Ag+ TCRs had a FlowJo criteria of $\geq 1\%$ of cells in Ag+ gate with the mean fluorescence intensity (MFI) > 250. However, a more stringent cutoff, such as 3%, could be used to separate out Ag+ TCRs that behave better in the binding assay. Ag+ TCRs were determined by Next Generation Sequencing. Table 5 shows the total number of TCRs that express identical TCR alpha and beta nucleotide sequences, the % tetramer in the Ag+ gate (MAGE-A4 (230-239)), the % tetramer in the Ag+ gate (MAGE-A8 (232-241)), and the ratio of the latter two numbers (% tetramer in the Ag+ gate (MAGE-A4 (230-239)) divided by the %

tetramer in the Ag+ gate (MAGE-A8 (232-241))). This ratio is an indicator of the specificity of MAGE-A4 (230-239) binding.

Table 5. TCR-antigen binding, on-target and off-target

TCR ID	Total Ag+ TCRs (CHot)	% tetramer in Ag+ gate (MAGE-A4)	% tetramer in Ag+ gate (MAGE-A8)	MAGE-A4/MAGE-A8
PN45515	1	11.50	0.00	1150.00
PN45593	34	56.50	0.06	991.23
PN45539	37	59.80	0.12	498.33
PN45545	1	11.50	0.08	149.35
PN45584	1	69.10	1.30	53.15
PN45581	1	3.18	0.17	18.71
PN45428	26	27.40	1.92	14.27
PN45418	1	12.30	1.24	9.92
PN45446	5	52.20	16.90	3.09
PN45489	1	16.00	6.39	2.50
PN45460	1	34.70	16.70	2.08
PN45494	1	3.70	1.88	1.97
PN45590	15	77.90	46.80	1.66
PN45557	10	94.70	66.30	1.43
PN45546	5	74.80	60.60	1.23
PN45481	41	87.90	72.90	1.21

[00188] A detailed list of the beta chain variable CDR1, CDR2, and CDR3 amino acid sequences, and the alpha chain variable domain CDR1, CDR2, and CDR3 amino acid sequences variable of the TCRs that were determined as described above are provided in Table 6, and the corresponding nucleic acid sequences are provided in Table 7. Table 8 provides the amino acid and nucleotide sequences of the beta chain variable and alpha chain variable regions of the TCRs.

[00189] Table 9 provides the TCR gene families for the alpha and beta variable and joining regions of the isolated TCRs, and Table 10 provides the amino acid and polynucleic acid sequence identifiers for alpha and beta variable chains and CDRs.

Table 6. Amino acid sequences for Veloci-T® TCRs specific for MAGE-A4 (230-239) / HLA-A2

TCR ID	TCR No.	Vα CDR1	SEQ ID NO	Vα CDR2	SEQ ID NO	Vα CDR3	SEQ ID NO	Vβ CDR1	SEQ ID NO	Vβ CDR2	SEQ ID NO	Vβ CDR3	SEQ ID NO
39	PN45515	TISGNEY	614	GLKNN	615	IVRPYNAGNNRKL	616	MDHEN	622	SYDVVKM	623	ASSFGGGYYGYT	624
40	PN45593	TSDPSYG	630	QGSYDQQ	631	AMREGPGNNARL	632	MNHEY	638	SVGEGT	639	ASSYWDRGSPLH	640
41	PN45539	TISGNEY	646	GLKN	647	IVRPYNAGNNRKL	648	MDHEN	654	SYDVVKM	655	ASSFEGGYYGYT	656
42	PN45545	TSDPSYG	662	QGSYDQQ	663	AMRGGSGGSYIPT	664	MDHEN	670	SYDVVKM	671	ASSFTGPYNSPLH	672
43	PN45584	TSDPSYG	678	QGSYDEQ	679	AMREGPGSGNTGKL	680	MNHNY	686	SVGAGI	687	ASSYSEWQNYGYT	688
44	PN45581	TSDPSYG	694	QGSYDQQ	695	AMREGPGNNARL	696	MNHEY	702	SMNVEV	703	ASSLWTGGGYT	704
45	PN45428	VSGLRG	710	LYSAGEE	711	AVQPLNAGNNRKL	712	DFQATT	718	SNEGSKA	719	SAREWGGTEAF	720
46	PN45418	VSGLRG	726	LYSAGE	727	AVQPSYSGAGSYQL	728	MNHEY	734	SMNVEV	735	ASSPGTGGFSPLH	736
47	PN45446	VSGLRG	742	LYSAGEE	743	AVQPSYSGAGSYQL	744	DFQATT	750	SNEGSKA	751	SAREWRGTEAF	752
48	PN45489	SVFSS	758	VVTGGE	759	AEDGGSQGNLI	760	MDHEN	766	SYDVVKM	767	ASSLQGRYYGYT	768
49	PN45460	ATGYPS	774	ATKADD	775	ALSDTRDDKII	776	MNHEY	782	SMNVEV	783	ASSPGTGGFSPLH	784
50	PN45494	ATGYPS	790	ATKADD	791	ALSDTRDDKTI	792	MDHEN	798	SYDVVKM	799	ASSLQGRYYGYT	800
51	PN45590	YGATPY	806	YFSGDTL	807	AVGAGSARQLT	808	SGHDT	814	YEEEE	815	ASSFDTEAF	816
52	PN45557	TSDPSYG	822	QGSYDQQ	823	AMREGPGSGNTGKL	824	MNHNY	830	SVGAGI	831	ASSYSEWQNYGYT	832
53	PN45546	TSEDYY	838	QEAYKQQ	839	AYRSGAGGTSYGKL	840	SGHKS	846	YEEKEE	847	ASSIRDYGYT	848
54	PN45481	ATGYPS	854	ATKADD	855	ALSDTRDDKII	856	MDHEN	862	SYDVVKM	863	ASSLQGRYYGYT	864

Table 7. Nucleic acid CDR sequences for Veloci-T® TCRs specific for MAGE-A4 (230-239) / HLA-A2

TCR No.	TCR ID	V α CDR1 SEQ ID NO	V α CDR2 SEQ ID NO	V α CDR3 SEQ ID NO	V β CDR1 SEQ ID NO	V β CDR2 SEQ ID NO	V β CDR3 SEQ ID NO
39	PN45515	ACCATCA ⁶¹⁷ GTGGAA ATGAGTA T	GGTCTAAAA ⁶¹⁸ AACAAAT	ATCGTCAGACCTTA TAATGCTGGCAACA ⁶¹⁹ ACCGTAAGCTG	ATGGAC CATGAA AAT	TCATATG ATGTTAA AATG	GCCAGCAGTTTGG GGGGGATACTATG GCTACACC
40	PN45593	ACCAGT ⁶³³ GATCCA AGTTATG GT	CAGGGGTCT ⁶³⁴ TATGACCCAG CAA	GCAATGAGAGAGG GCCCTGGTAACAAT ⁶³⁵ GCCAGACTC	ATGAAC CATGAA TAC	TCAGTTG GTGAGG GTACA	GCCAGCAGTTACTG GGACAGGGGCTCAC CCCTCCAC
41	PN45539	ACCATCA ⁶⁴⁹ GTGGAA ATGAGTA T	GGTCTAAAA ⁶⁵⁰ AAC	ATCGTCAGACCTTA TAATGCTGGCAACA ⁶⁵¹ ACCGTAAGCTG	ATGGAC CATGAA AAT	TCATATG ATGTTAA AATG	GCCAGCAGTTTGA GGGGGATACTATG GCTACACC
42	PN45545	ACCAGT ⁶⁶⁵ GATCCA AGTTATG GT	CAGGGGTCT ⁶⁶⁶ TATGACCCAG CAA	GCAATGAGAGGGG GGGATCAGAGAGG ⁶⁶⁷ AAGCTACATACCTA CA	ATGGAC CATGAA AAT	TCATATG ATGTTAA AATG	GCCAGCAGTTTCAC AGGGCCCTATAATT CACCCCTCCAC
43	PN45584	ACCAGT ⁶⁸¹ GATCCA AGTTATG GT	CAGGGGTCT ⁶⁸² TATGACCCAG CAA	GCAATGAGAGAGG GCCCCGGCTCTGGC ⁶⁸³ AACACAGGCAAAAC TAATC	ATGAAC CATAAC TAC	TCAGTTG GTGCTGG TATC	GCCAGCAGTTACTC GGAGTGGCAGAACT ATGGCTACACC
44	PN45581	ACCAGT ⁶⁹⁷ GATCCA AGTTATG GT	CAGGGGTCT ⁶⁹⁸ TATGACCCAG CAA	GCAATGAGAGAGG GCCCTGGTAACAAT ⁶⁹⁹ GCCAGACTC	ATGAAC CATGAG TAT	TCAATGA ATGTTGA GGTG	GCCAGCAGTTTATG GACAGGGGAGGCT ACACC
45	PN45428	GTCAGC ⁷¹³ GGTTAA GAGGG	CTGTATTCA ⁷¹⁴ GCTGGGGAA	GCTGTGAGCCCT TAATGCTGGCAACA ⁷¹⁵ ACCGTAAGCTG	GACTTT CAGGCC ACAACT	TCCAATG AGGGCT	AGTGCTAGAGAATG GGGTGGCACTGAAG CTTTC

46	PN45418	<u>GTCAGC</u> <u>GGTTTAA</u> <u>GAGGG</u>	729	<u>CTGTATTCA</u> <u>GCTGGGGAA</u>	730	<u>GCTGTGCAGCCCTC</u> <u>ATACTCTGGGGCTG</u> <u>GGAGTTACCAACTC</u> <u>ACT</u>	<u>ATGAAC</u> <u>CATGAG</u> <u>TAT</u>	737	<u>CCAAGG</u> <u>CC</u>	738	<u>GCCAGCAGTCCCGG</u> <u>GACAGGGGGATTTT</u> <u>CACCCCTCCAC</u>	739
47	PN45446	<u>GTCAGC</u> <u>GGTTTAA</u> <u>GAGGG</u>	745	<u>CTGTATTCA</u> <u>GCTGGGGAA</u>	746	<u>GCTGTGCAGCCCTC</u> <u>ATACTCTGGGGCTG</u> <u>GGAGTTACCAACTC</u> <u>ACT</u>	<u>GACTTT</u> <u>CAGGCC</u> <u>ACAAC</u>	753	<u>TCCAATG</u> <u>AGGGCT</u> <u>CCAAGG</u> <u>CC</u>	754	<u>AGTGTAGAGAGTG</u> <u>GAGGGGCACTGAAG</u> <u>CTTTC</u>	755
48	PN45489	<u>AGTGTTT</u> <u>TTTCCAG</u> <u>C</u>	761	<u>GTAGTTACG</u> <u>GGTGGAGA</u> <u>A</u>	762	<u>GCAGAAGATGGAG</u> <u>GAAGCCAAGGAAA</u> <u>TCTCATC</u>	<u>ATGGAC</u> <u>CATGAA</u> <u>AAT</u>	769	<u>TCATATG</u> <u>ATGTTAA</u> <u>AATG</u>	770	<u>GCCAGCAGTTTACA</u> <u>GGGGAGGTACTATG</u> <u>GCTACACC</u>	771
49	PN45460	<u>GCCACA</u> <u>GGATAC</u> <u>CCTTCC</u>	777	<u>GCCACGAA</u> <u>GGCTGATGA</u> <u>C</u>	778	<u>GCTCTGAGTGATAC</u> <u>CAGAGATGACAAAG</u> <u>ATCATC</u>	<u>ATGAAC</u> <u>CATGAG</u> <u>TAT</u>	785	<u>TCAATGA</u> <u>ATGTTGA</u> <u>GGTG</u>	786	<u>GCCAGCAGTCCCGG</u> <u>GACAGGGGGATTTT</u> <u>CACCCCTCCAC</u>	787
50	PN45494	<u>GCCACA</u> <u>GGATAC</u> <u>CCTTCC</u>	793	<u>GCCACGAA</u> <u>GGCTGATGA</u> <u>C</u>	794	<u>GCTCTGAGTGATAC</u> <u>CAGAGATGACAAAG</u> <u>ACCATC</u>	<u>ATGGAC</u> <u>CATGAA</u> <u>AAT</u>	801	<u>TCATATG</u> <u>ATGTTAA</u> <u>AATG</u>	802	<u>GCCAGCAGTTTACA</u> <u>GGGGAGGTACTATG</u> <u>GCTACACC</u>	803
51	PN45590	<u>TATGGGG</u> <u>CAACAC</u> <u>CTTAT</u>	809	<u>TACTTTTCA</u> <u>GGAGACACT</u> <u>CTG</u>	810	<u>GCTGTGGGTGCTGG</u> <u>TTCTGCAAGGCAAC</u> <u>TGACC</u>	<u>TCTGGG</u> <u>CATGAC</u> <u>ACT</u>	817	<u>TATTATG</u> <u>AGGAGG</u> <u>AAGAG</u>	818	<u>GCCAGCAGCTTTGA</u> <u>CACTGAAGCTTTC</u>	819
52	PN45557	<u>ACCAGT</u> <u>GATCCA</u> <u>AGTTATG</u> <u>GT</u>	825	<u>CAGGGGTCT</u> <u>TATGACCCAG</u> <u>CAA</u>	826	<u>GCAATGAGAGAGG</u> <u>GCCCCGGGCTCTGGC</u> <u>AACACAGGCAAAAC</u> <u>TAATC</u>	<u>ATGAAC</u> <u>CATAAC</u> <u>TAC</u>	833	<u>TCAGTTG</u> <u>GTGCTGG</u> <u>TATC</u>	834	<u>GCCAGCAGTTACTC</u> <u>GGAGTGGCAGAACT</u> <u>ATGGCTACACC</u>	835
53	PN45546	<u>ACCAGT</u> <u>GAGAGT</u> <u>GATTATT</u> <u>AT</u>	841	<u>CAAGAAGCT</u> <u>TATAAGCAA</u> <u>CAG</u>	842	<u>GCTTATAGGAGCCGG</u> <u>TGCTGGTGGTACTA</u> <u>GCTATGGAAGCTG</u> <u>ACA</u>	<u>TCTGGG</u> <u>CACAAG</u> <u>AGT</u>	849	<u>TATTATG</u> <u>AGAAAG</u> <u>AAGAG</u>	850	<u>GCCAGCAGCATCCG</u> <u>GGACACCTATGGCT</u> <u>ACACC</u>	851

54	PN45481	GCCACA	857	GCCACGAA	858	GCTCTGAGTGATAC	859	ATGGAC	865	TCATATG	866	GCCAGCAGTTTACA	867
		GGATAC		GGCTGATGA		CAGAGATGACAAG		CATGAA		ATGTTAA		GGGGAGGTACTATG	
		CCTTCC		C		ATCATC		AAT		AATG		GCTACACC	

Table 8: Amino acid and nucleic acid sequences for Veloci-T® TCRs specific for MAGE-A4 (230-239) / HLA-A2

<i>TCR name Domain name</i>	<i>Domain Sequences</i> Amino Acid Sequence (SEQ ID NO); CDR1, CDR2, and CDR3 sequences are <u>underlined</u> Nucleic Acid Sequence (SEQ ID NO) ; CDR1, CDR2, and CDR3 sequences are <u>underlined</u>
PN45515 V α	DAKTTQPPSMDCAEGRAANLPCNHST <u>ISGNEYVYWYRQIHSQGPQYIIHG</u> <u>LKNNETNEMASLIITEDRKSSTLILPHATLRDTAVYYCIVRPYNAGNNRKL</u> IWGLGTS LAVNP (SEQ ID NO: 620) GATGCTAAGACCACCCAGCCCCCTCCATGGATTGCGCTGAAGGAAG AGCTGCAAACCTGCCTTGTAATCACTCT <u>ACCATCAGTGGAAATGAGTA</u> <u>TGTGTATTGGTATCGACAGATTCCTCCAGGGGCCACAGTATATCAT</u> <u>TCATGGTCTAAAAACAATGAAACCAATGAAATGGCCTCTCTGATCAT</u> CACAGAAGACAGAAAGTCCAGCACCTTGATCCTGCCCCACGCTACGCT GAGAGACACTGCTGTGTACTATTGC <u>ATCGTCAGACCTTATAATGCTGG</u> <u>CAACAACCGTAAGCTGATTTGGGGATTGGGAACAAGCCTGGCAGTAA</u> ATCCG (SEQ ID NO: 621)
PN45515 V β	DVKVTQSSRYLVKRTGEKVFLQVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIPGYSVSREKKERFSLILESASTNQTSMYLCASSFGGG</u> <u>YYGYTFGSGTRLTVV</u> (SEQ ID NO: 628) GATGTGAAAGTAAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT <u>CTCATATGATGTTAAAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT</u> ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATCCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTTG GGGGGGGATACTATGGCTACACCTTCGGTTCGGGGACCAGGCTAACC GTTGTA (SEQ ID NO: 629)
PN45593 V α	AQKITQTQPGMFVQEKEAVTLDCYDTSYGLFWYKQPSSGEMIFLIY <u>QGSYDQQNATEGRYSLNFQKARKSANLVISASQLGDSAMYFCAMREGP</u> <u>GNNARLMFGDGTQLVVKP</u> (SEQ ID NO: 636) GCCCAGAAGATAACTCAAACCCAACCAGGAATGTTTCGTGCAGGAAAA GGAGGCTGTGACTCTGGACTGCACATATGAC <u>ACCAGTGATCCAAGTTA</u> <u>TGGTCTATTCTGGTACAAGCAGCCCAGCAGTGGGGAAATGATTTTTCT</u> <u>TATTTATCAGGGGTCTTATGACCAGCAAAATGCAACAGAAGGTCGCTA</u> CTCATTGAATTTCCAGAAGGCAAGAAAATCCGCCAACCTTGTCATCTC CGCTTCACAACCTGGGGGACTCAGCAATGTACTTCTGT <u>GCAATGAGAGA</u> <u>GGGCCCTGGTAACAATGCCAGACTCATGTTTGGAGATGGAACCTCAGCT</u> GGTGGTGAAGCCC (SEQ ID NO: 637)
PN45593 V β	NAGVTQTPKFRVLKTGQSMTLLCAQDMNHEYMYWYRQDPGMGLRLIH <u>YSVGEGTAKGEVPDGYNVSRLLKQNFLLGLESAAPSQTSVYFCASSYW</u> <u>DRGSPLHFGNGTRLTVT</u> (SEQ ID NO: 644)

	AATGCTGGTGTCACTCAGACCCCAAAATTCCGGGTCCTGAAGACAGGA CAGAGCATGACACTGCTGTGTGCCCAGGATATGAACCATGAATACATG TACTGGTATCGACAAGACCCAGGCATGGGGCTGAGGCTGATTCATTAC <u>TCAGTTGGTGAGGGTACA</u> ACTGCCAAAGGAGAGGTCCCTGATGGCTA CAATGTCTCCAGATTAAAAAAACAGAATTCCTGCTGGGGTTGGAGTC GGCTGCTCCCTCCCAAACATCTGTGTACTTCTGTGCCAGCAGTTACTGG <u>GACAGGGGCTCACCCCTCCACTTT</u> GGGAACGGGACCAGGCTCACTGTG ACA (SEQ ID NO: 645)
PN45539 V α	DAKTTQPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIHG <u>LKNNETNEMASLIITEDRKSSTLILPHATLRD</u> TA VYYCIVRPYNAGNNRKL IWGLGTS LAVNP (SEQ ID NO: 652) GATGCTAAGACCACCCAGCCCCCTCCATGGATTGCGCTGAAGGAAG AGCTGCAAACCTGCCTTGTAATCACTCTACCATCAGTGGAATGAGTA <u>TGTGTATTGGTATCGACAGATTC</u> ACTCCCAGGGGCCACAGTATATCAT TCATGGTCTAAAAAAACAATGAAACCAATGAAATGGCCTCTCTGATCAT CACAGAAGACAGAAAGTCCAGCACCTTGATCCTGCCCCACGCTACGCT GAGAGACACTGCTGTGTACTATTGCATCGTCAGACCTTATAATGCTGG <u>CAACAACCGTAAGCTGATTT</u> GGGGATTGGGAACAAGCCTGGCAGTAA ATCCG (SEQ ID NO: 653)
PN45539 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGD</u> IEGYSVSREKKERFSLILESASTNQTSMYLCASSFEGG <u>YYGYTFGSGTRLTVV</u> (SEQ ID NO: 660) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT <u>CTCATATGATGT</u> TAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTTG <u>AGGGGGGATACTATGGCTACACCTTC</u> GGTTCGGGGACCAGGTAAACCG TTGTA (SEQ ID NO: 661)
PN45545 V α	AQKITQTQPGMFVQEKEAVTLDCTYDTS ~~SD~~ PSYGLFWYKQPSSGEMIFLIY <u>QGSYDQQN</u> ATEGRYSLNFQKARKSANLVISASQLGDSAMYFCAMRGGG <u>SGGSYIPTF</u> FRGRTSLIVHP (SEQ ID NO: 668) GCCCAGAAGATAACTCAAACCCAACCAGGAATGTTTCGTGCAGGAAAA GGAGGCTGTGACTCTGGACTGCACATATGACACCAAGTGATCCAAGTTA <u>TGGTCTATTCTGGTACAAGCAGCCCAGCAGTGGGGAAATGATTTTTCT</u> TATTTATCAGGGGTCTTATGACCAGCAAATGCAACAGAAGGTCGCTA CTCATTGAATTTCCAGAAGGCAAGAAAATCCGCCAACCTTGTCATCTC CGCTTCACAACCTGGGGGACTCAGCAATGTACTTCTGTGCAATGAGAGG <u>GGGGGGATCAGGAGGAAGCTACATACCTACATTT</u> GGAAGAGGAACCA GCCTTATTGTTTCATCCG (SEQ ID NO: 669)
PN45545 V β	DVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGD</u> IEGYSVSREKKERFSLILESASTNQTSMYLCASSFTGPY <u>NSPLHFGNGTRLTVT</u> (SEQ ID NO: 676) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT

	GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTTCTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGTACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGTCCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTCA <u>CAGGGCCCTATAATTCACCCCTCCACTTTGGGAACGGGACCAGGCTCACTGTGACA</u> (SEQ ID NO: 677)
PN45584 V α	AQKITQTQPGMFVQEKEAVTLDCTYDTS ~~DP~~ SYGLFWYKQPSSGEMIFLIYQGSYDEQNATEGRYSLNFQKARKSANLVISASQLGDSAMYFC <u>AMREGPGSGNTGKLIFGQGTTLQVKP</u> (SEQ ID NO: 684) GCCCAGAAGATAACTCAAACCCAACCAGGAATGTTTCGTGCAGGAAAAAGAGGCTGTGACTCTGGACTGCACATATGACACCAGTGATCCAAGTTA <u>TGGTCTATTCTGGTACAAGCAGCCCAGCAGTGGGGAAATGATTTTTCTTATTTATCAGGGGTCTTATGACGAGCAAAATGCAACAGAAGGTCGCTACTCATTGAATTTCCAGAAGGCAAGAAAATCCGCCAACCTTGTCATCTCGCTTCACAACCTGGGGGACTCAGCAATGTACTTCTGTGCAATGAGAGAGGGCCCGGGCTCTGGCAACACAGGCAAACTAATCTTTGGGCAAGGGA</u> CAACTTTACAAGTAAACCA (SEQ ID NO: 685)
PN45584 V β	NAGVTQTPKFRILKIGQSM ~~TL~~ QCAQDMNHN ~~Y~~ MYWYRQDPGMGLKLIYYSVGAGITDKGEVPNGYNVSRSTTEYFPLRLELAAPSQTSVYFC <u>ASSYSEWQNYGYTFGSGTRLTVV</u> (SEQ ID NO: 692) AATGCTGGTGTCACTCAGACCCCAAAATTCCGCATCCTGAAGATAGGACAGAGCATGACACTGCAGTGTGCCCAGGATATGAACCATAACTACATGTACTGGTATCGACAAGACCCAGGCATGGGGCTGAAGCTGATTTATTATTCAGTTGGTGCTGGTATCACTGATAAAGGAGAAGTCCCGAATGGCTACAACGTCTCCAGATCAACCACAGAGTATTTCCCGCTCAGGCTGGAGTTGGCTGCTCCCTCCCAGACATCTGTGTACTTCTGTGCCAGCAGTTACTCGGAGTGGCAGAACTATGGCTACACCTTCGGTTCGGGGACCAGGTTAACCGTTGTA (SEQ ID NO: 693)
PN45581 V α	AQKITQTQPGMFVQEKEAVTLDCTYDTS ~~DP~~ SYGLFWYKQPSSGEMIFLIYQGSYDQ ~~Q~~ NATEGRYSLNFQKARKSANLVISASQLGDSAMYFC <u>AMREGPGNNARLMFGDGTQLVVKP</u> (SEQ ID NO: 700) GCCCAGAAGATAACTCAAACCCAACCAGGAATGTTTCGTGCAGGAAAAAGAGGCTGTGACTCTGGACTGCACATATGACACCAGTGATCCAAGTTA <u>TGGTCTATTCTGGTACAAGCAGCCCAGCAGTGGGGAAATGATTTTTCTTATTTATCAGGGGTCTTATGACCAGCAAAATGCAACAGAAGGTCGCTACTCATTGAATTTCCAGAAGGCAAGAAAATCCGCCAACCTTGTCATCTCGCTTCACAACCTGGGGGACTCAGCAATGTACTTCTGTGCAATGAGAGAGGGCCCTGGTAACAATGCCAGACTCATGTTTGGAGATGGAACCTCAGCTGGTGGTGAAGCCC</u> (SEQ ID NO: 701)
PN45581 V β	EAQVTQTPRYLITVTGKKLTVTCSQN ~~MN~~ HEYMSWYRQDPGLGLRQIYY ~~S~~ <u>MNVEVTDKGDVPEGYKVS</u> RKERNFPLILESPSPNQTSLYFC <u>ASSLWTGGGYTFGSGTRLTVV</u> (SEQ ID NO: 708) GAAGCCCAAGTGACCCAGACCCCAAGATACCTCATCACAGTGACTGGAAGAAGTTAACAGTGACTTGTTCTCAGAATATGAACCATGAGTATATGTCCTGGTATCGACAAGACCCAGGGCTGGGCTTAAGGCAGATCTACTATTCAATGAATGTTGAGGTGACTGATAAGGGAGATGTTCTGAAGGGTA

	CAAAGTCTCTCGAAAAGAGAAGAGGAATTTCCCCCTGATCCTGGAGTC GCCCAGCCCCAACCAGACCTCTCTGTACTTCTGTGCCAGCAGTTTATG <u>GACAGGGGGAGGCTACACCTTCGGTTCGGGGACCAGGTAAACCGTTGT</u> A (SEQ ID NO: 709)
PN45428 V α	EDQVTQSPEALRLQEGESSLNCSYTVSGLRGLFWYRQDPGKGPEFLFTL <u>YSAGEEKEKERLKATLTKKESFLHITAPKPEDSATYLC</u> <u>AVQPLNAGNNRK</u> <u>LIWGLGTS LAVNP</u> (SEQ ID NO: 716) GAAGACCAGGTGACGCAGAGTCCCGAGGCCCTGAGACTCCAGGAGGG AGAGAGTAGCAGTCTCAACTGCAGTTACACAGTCAGCGGTTTAAGAG <u>GGCTGTTCTGGTATAGGCAAGATCCTGGGAAAGGCCCTGAATTCCTCT</u> <u>TCACCCTGTATTCAGCTGGGGAAGAAAAGGAGAAAGAAAGGCTAAAA</u> <u>GCCACATTAACAAAGAAGGAAAGCTTTCTGCACATCACAGCCCCTAA</u> <u>ACCTGAAGACTCAGCCACTTATCTCTGTGCTGTGCAGCCCCTTAATGCT</u> <u>GGCAACAACCGTAAGCTGATTTGGGGATTGGGAACAAGCCTGGCAGT</u> <u>AAATCCG</u> (SEQ ID NO: 717)
PN45428 V β	GAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFWYRQFPKQSLMLMAT <u>SNEGSKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYICSAREWG</u> <u>GTEAFFGQGTRLTVV</u> (SEQ ID NO: 724) GGTGTGTCTGCTCTCAACATCCGAGCTGGGTTATCTGTAAGAGTGGA ACCTCTGTGAAGATCGAGTGCCGTTCCCTGGACTTTCAGGCCACAAC ATGTTTTGGTATCGTCAGTTCCTGAAACAGAGTCTCATGCTGATGGCA ACTTCCAATGAGGGCTCCAAGGCCACATACGAGCAAGGCGTCGAGAA GGACAAGTTTCTCATCAACCATGCAAGCCTGACCTTGCTCCACTCTGAC AGTGACCAGTGCCCATCCTGAAGACAGCAGCTTCTACATCTGCAGTGC <u>TAGAGAATGGGGTGGCACTGAAGCTTTCTTTGGACAAGGCACCAGACT</u> <u>CACAGTTGTA</u> (SEQ ID NO: 725)
PN45418 V α	EDQVTQSPEALRLQEGESSLNCSYTVSGLRGLFWYRQDPGKGPEFLFTL <u>YSAGEEKEKERLKATLTKKESFLHITAPKPEDSATYLC</u> <u>AVQPSYSGAGSY</u> <u>QLTFGKGTKLSVIP</u> (SEQ ID NO: 732) GAAGACCAGGTGACGCAGAGTCCCGAGGCCCTGAGACTCCAGGAGGG AGAGAGTAGCAGTCTCAACTGCAGTTACACAGTCAGCGGTTTAAGAG <u>GGCTGTTCTGGTATAGGCAAGATCCTGGGAAAGGCCCTGAATTCCTCT</u> <u>TCACCCTGTATTCAGCTGGGGAAGAAAAGGAGAAAGAAAGGCTAAAA</u> <u>GCCACATTAACAAAGAAGGAAAGCTTTCTGCACATCACAGCCCCTAA</u> <u>ACCTGAAGACTCAGCCACTTATCTCTGTGCTGTGCAGCCCCTCATACTCT</u> <u>GGGGCTGGGAGTTACCAACTCACTTTCGGGAAGGGGACCAAACCTCTC</u> <u>GGTCATACCA</u> (SEQ ID NO: 733)
PN45418 V β	EAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYY <u>MNVEVTDKGDVPEGYKVS RKEKRNFLILESPSPNQTSLYFC</u> <u>ASSPGTGG</u> <u>FSPLHFGNGTRLTVT</u> (SEQ ID NO: 740) GAAGCCCAAGTGACCCAGAACCCAAGATACCTCATCACAGTGACTGG AAAGAAGTTAACAGTGACTTGTTCTCAGAATATGAACCATGAGTATAT GTCCTGGTATCGACAAGACCCAGGGCTGGGCTTAAGGCAGATCTACTA TTCAATGAATGTTGAGGTGACTGATAAGGGAGATGTTCTGAAGGGTA CAAAGTCTCTCGAAAAGAGAAGAGGAATTTCCCCCTGATCCTGGAGTC GCCCAGCCCCAACCAGACCTCTCTGTACTTCTGTGCCAGCAGTCCCGG

	<u>GACAGGGGGATTTCACCCCTCCACTTTGGGAACGGGACCAGGCTCAC</u> TGTGACA (SEQ ID NO: 741)
PN45446 V α	<u>EDQVTQSPEALRLQEGESSLNCSYTVSGLRGLFWYRQDPGKGPEFLFTL</u> <u>YSAGEEKEKERLKATLTKKESFLHITAPKPEDSATYLC</u> <u>AVQPSYSGAGSY</u> <u>QLTFGKGTKLSVIP</u> (SEQ ID NO: 748) GAAGACCAGGTGACGCAGAGTCCCGAGGCCCTGAGACTCCAGGAGGG AGAGAGTAGCAGTCTCAACTGCAGTTACACAGTCAGCGGTTTAAGAG GGCTGTTCTGGTATAGGCAAGATCCTGGGAAAGGCCCTGAATTCCTCT TCACCCTGTATTCAGCTGGGGAAAGAAAAGGAGAAAGAAAGGCTAAAA GCCACATTAACAAAGAAGGAAAGCTTTCTGCACATCACAGCCCCTAA ACCTGAAGACTCAGCCACTTATCTCTGTGCTGTGCAGCCCTCATACTCT GGGGCTGGGAGTTACCAACTCACTTTTCGGGAAGGGGACCAAACCTCTC GGTCATACCA (SEQ ID NO: 749)
PN45446 V β	<u>GAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFWYRQFPKQSLMLMAT</u> <u>SNEGSKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYICSAREWR</u> <u>GTEAFFGQGTRLTVV</u> (SEQ ID NO: 756) GGTGCTGTCGTCTCTCAACATCCGAGCTGGGTTATCTGTAAGAGTGGA ACCTCTGTGAAGATCGAGTGCCGTTCCCTGGACTTTCAGGCCACAACCT ATGTTTTGGTATCGTCAGTTCCTGAAACAGAGTCTCATGCTGATGGCA ACTTCCAATGAGGGCTCCAAGGCCACATACGAGCAAGGCGTCGAGAA GGACAAGTTTCTCATCAACCATGCAAGCCTGACCTTGTCCACTCTGAC AGTGACCAGTGCCCATCCTGAAGACAGCAGCTTCTACATCTGCAGTGC TAGAGAGTGGAGGGGCACTGAAGCTTTCTTTGGACAAGGCACCAGAC TCACAGTTGTA (SEQ ID NO: 757)
PN45489 V α	<u>TQLLEQSPQFLSIQEGENLTVYCNSSSVFSSLQWYRQEPGEGPVLLVTVVT</u> <u>GGEVKKLRLTFQFGDARKDSSLHITAAQPGDTGLYLCA</u> <u>AEDGGSQGNLIF</u> <u>GKGTKLSVKP</u> (SEQ ID NO: 764) ACCCAGCTGCTGGAGCAGAGTCCTCAGTTTCTAAGCATCCAAGAGGGA GAAAATCTCACTGTGTACTGCAACTCCTCAAGTGTTTTTCCAGCTTAC AATGGTACAGACAGGAGCCTGGGGAAGGTCCTGTCTCCTGGTGACA GTAGTTACGGGTGGAGAAAGTGAAGAAGCTGAAGAGACTAACCTTTCA GTTTGGTGATGCAAGAAAGGACAGTTCTCTCCACATCACTGCGGCCCA GCCTGGTGATACAGGCCTCTACCTCTGTGCAGAAGATGGAGGAAGCC AAGGAAATCTCATCTTTGGAAAAGGCACTAAACTCTCTGTAAACCA (SEQ ID NO: 765)
PN45489 V β	<u>DVKVTQTSRYLVKRTGEKVFLCEVQDMDHENMFWYRQDPGLGLRLIYF</u> <u>SYDVKMKEKGDIPGYSVSREKKERFSLILESASTNQTSMYLC</u> <u>ASSLOQR</u> <u>YYGYTFGSGTRLTVV</u> (SEQ ID NO: 772) GATGTGAAAGTAACCCAGACCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAC AGGGGAGGTACTATGGCTACACCTTCGGTTCGGGGACCAGGTAAACCG TTGTA (SEQ ID NO: 773)

PN45460 V α	GNSVTQMEGPVTLSEEAFLTINCTYTATGYPSLFWYVQYPGEGLQLLLKA TKADDKGSNKGFEATYRKETTSFHLEKGSVQVSDSAVYFCALSDTRDDK IFGKGTRLHILP (SEQ ID NO: 780) GGAAATTCAGTGACCCAGATGGAAGGGCCAGTGACTCTCTCAGAAGA GGCCTTCCTGACTATAAACTGCACGTACACAGCCACAGGATACCCCTC CCTTTTCTGGTATGTCCAATATCCTGGAGAAGGTCTACAGCTCCTCCTG AAAGCCACGAAGGCTGATGACAAGGGAAGCAACAAAGGTTTTGAAGC CACATACCGTAAAGAAACCACTTCTTTCCACTTGGAGAAAGGCTCAGT TCAAGTGTCAGACTCAGCGGTGTACTTCTGTGCTCTGAGTGATACCAG AGATGACAAGATCATCTTTGGAAAAGGGACACGACTTCATATTCTCCC C (SEQ ID NO: 788)
PN45460 V β	EAQVTQSPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYS MNVEVTDKGDVPEGYKVS RKEKRNFLILESPSPNQTSLYFCASSPGTGG ESPLHFGNGTRLTVT (SEQ ID NO: 781) GAAGCCCAAGTGACCCAGAGCCCAAGATACCTCATCACAGTGACTGG AAAGAAGTTAACAGTGACTTGTCTCAGAATATGAACCATGAGTATAT GTCCTGGTATCGACAAGACCCAGGGCTGGGCTTAAGGCAGATCTACTA TTCAATGAATGTTGAGGTGACTGATAAGGGAGATGTTCTGAAGGGTA CAAAGTCTCTCGAAAAGAGAAGAGGAATTTCCCCCTGATCCTGGAGTC GCCAGCCCCAACCAGACCTCTCTGTACTTCTGTGCCAGCAGTCCCGG GACAGGGGGATTTTCACCCCTCCACTTTGGGAACGGGACCAGGCTCAC TGTGACA (SEQ ID NO: 789)
PN45494 V α	GNSVTQIEGPVTLSEEAFLTINCTYTATGYPSLFWYVQYPGEGLQLLLKAT KADDKGSNKGFEATYRKETTSFHLEKGSVQVSDSAVYFCALSDTRDDKT IFGKGTRLHILP (SEQ ID NO: 796) GGAAATTCAGTGACCCAGATTGAAGGGCCAGTGACTCTCTCAGAAGA GGCCTTCCTGACTATAAACTGCACGTACACAGCCACAGGATACCCCTC CCTTTTCTGGTATGTCCAATATCCTGGAGAAGGTCTACAGCTCCTCCTG AAAGCCACGAAGGCTGATGACAAGGGAAGCAACAAAGGTTTTGAAGC CACATACCGTAAAGAAACCACTTCTTTCCACTTGGAGAAAGGCTCAGT TCAAGTGTCAGACTCAGCGGTGTACTTCTGTGCTCTGAGTGATACCAG AGATGACAAGACCATCTTTGGAAAAGGGACACGACTTCATATTCTCCC C (SEQ ID NO: 804)
PN45494 V β	DVKVTQSSRYLVKRTGEKVFLQVQDMDHENMFWYRQDPGLGLRLIYF SYDVKMKEKGDIPGYVSREKKERFSLILESASTNQTSMYLCASSLQGR YYGYTFGSGTRLTVV (SEQ ID NO: 797) GATGTGAAAGTAACCCAGAGTTTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAC AGGGGAGGTACTATGGCTACACCTTCGGTTCGGGGACCAGGTAAACCG TTGTA (SEQ ID NO: 805)

PN45590 V α	AQSVTPDIHITVSEGASLELRNYSYGATPYLFWYVQSPGQGLQLLLKY <u>FSGDTLVQGIKGFEAEFKRSQSSFNLRKPSVHWSDAAEYFC</u><u>AVGAGSARQ</u> <u>LTFGSGTQLTVLP</u> (SEQ ID NO: 812) GCCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGA GCCTCACTGGAGTTGAGATGTAACCTATTCCTATGGGGCAACACCTTAT CTCTTCTGGTATGTCCAGTCCCCCGGCCAAGGCCTCCAGCTGCTCCTG AAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAG GCTGAATTTAAGAGGAGTCAATCTTCCTTCAATCTGAGGAAACCCTCT GTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTGGGTGCTGGT <u>TCTGCAAGGCAACTGACCTTTGGATCTGGGACACAATTGACTGTTT</u> <u>CCT</u> (SEQ ID NO: 820)
PN45590 V β	DAGVTQSPTHLIKTRGQQVTLRCSKSGHDTVSWYQQALGQGPQFIFQY YEEEEQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLC<u>ASSFDTEA</u> <u>EFGQGTRLTVV</u> (SEQ ID NO: 813) GACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGG ACAGCAAGTGACTCTGAGATGCTCTCCTAAGTCTGGGCATGACACTGT GTCCTGGTACCAACAGGCCCTGGGTCAGGGGCCCCAGTTTATCTTTCA GTATTATGAGGAGGAAGAGAGACAGAGAGGCAACTTCCCTGATCGAT TCTCAGGTCACCAGTTCCCTAAGTATAGCTCTGAGCTGAATGTGAACG CCTTGTTGCTGGGGGACTCGGCCCTCTATCTCTGTGCCAGCAGCTTTGA <u>CACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTA</u> (SEQ ID NO: 821)
PN45557 V α	AQKITQTQPGMFVQEKEAVTLDCTYDTS<u>SDPSYGLFWYKQPSSGEMIFLIY</u> <u>QGSYDQQNATEGRYSLNFQKARKSANLVISASQLGDSAMYFC</u><u>AMREGP</u> <u>GSGNTGKLIFGQGTTLQVKP</u> (SEQ ID NO: 828) GCCCAGAAGATAACTCAAACCCAACCAGGAATGTTTCGTGCAGGAAAA GGAGGCTGTGACTCTGGACTGCACATATGAC<u>ACCAGTGATCCAAGTTA</u> <u>TGGTCTATTCTGGTACAAGCAGCCCAGCAGTGGGGAAATGATTTTCT</u> TATTTAT<u>CAGGGGTCTTATGACCAGCAA</u>AATGCAACAGAAGGTCGCTA CTCATTGAATTTCCAGAAGGCAAGAAAATCCGCCAACCTTGTCATCTC CGCTTCACAACCTGGGGGACTCAGCAATGTACTTCTGT<u>GCAATGAGAGA</u> <u>GGGCCCCGGGCTCTGGCAACACAGGCAAACTAATCTTTGGGCAAGGGA</u> CAACTTTACAAGTAAACCA (SEQ ID NO: 836)
PN45557 V β	NAGVTQTPKFRILKIGQSMTLQCAQDMNHNYMYWYRQDPGMGLKLIYY <u>SVGAGITDKGEVPNGYNVSRSTTEYFPLRLELAAPSQTSVYFC</u><u>ASSYSEW</u> <u>QNYGYTFGSGTRLTVV</u> (SEQ ID NO: 829) AATGCTGGTGTCACTCAGACCCCAAAATTCCGCATCCTGAAGATAGGA CAGAGCATGACACTGCAGTGTGCCAGGATATGAACCATAACTACAT GTACTGGTATCGACAAGACCCAGGCATGGGGCTGAAGCTGATTTATTA TTCAGTTGGTGCTGGTATCACTGATAAAGGAGAAGTCCCGAATGGCTA CAACGTCTCCAGATCAACCACAGAGTATTTCCCGCTCAGGCTGGAGTT GGCTGCTCCCTCCCAGACATCTGTGTACTTCTGTGCCAGCAGTTACTCG <u>GAGTGGCAGAACTATGGCTACACCTTCGGTTCGGGGACCAGGTTAACC</u> GTTGTA (SEQ ID NO: 837)

PN45546 $V\alpha$	AQTVTQSQPEMSVQEAETVTLSCITYDT<u>SESDYYLFWYKQPPSRQMILVIRQEAYKQQNATENRFSVNFQKAAKSFSLSKISDSQLGDAAMYFCAYRSGAGGTSYGKLTFGQGTLTVHP</u> (SEQ ID NO: 844) GCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCA GAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATTAT <u>TATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTTATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTTCTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTCAGACTCACAGCTGGGGGATGCCGCGATGTATTTCTGTGCTTATAGGAGCGGTGCTGGTGGTACTAGCTATGGAAAGCTGACATTTGGACAAGGGA</u> CCATCTTGACTGTCCATCCA (SEQ ID NO: 852)
PN45546 $V\beta$	DAGVTQSPTHLIKTRGQQVTLRCSPISGHKS VSWYQQVVGQGPQFIFQYY <u>EKEERGRGNFPDRFSARQFPNYSSELNVNALLLGDSALYLCASSIRDITYG</u> YTFGSGTRLTVV (SEQ ID NO: 845) GACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGG ACAGCAAGTGACTCTGAGATGCTCTCCTATCTCTGGGCACAAGAGTGT GTCCTGGTACCAACAGGTCTCTGGGTCAGGGGCCCCAGTTTATCTTTCA <u>GTATTATGAGAAAGAAGAGAGAGGAAGAGGAACTTCCCTGATCGAT</u> TCTCAGCTCGCCAGTTCCCTAACTATAGCTCTGAGCTGAATGTGAACG CCTTGTTGCTGGGGGACTCGGCCCTGTATCTCTGTGCCAGCAGCATCC <u>GGGACACCTATGGCTACACCTTCGGTTCGGGGACCAGGTAAACCGTTG</u> TA (SEQ ID NO: 853)
PN45481 $V\alpha$	GNSVTQMEGPVTLSEEAFLTINCTYTATGYPSLFWYVQYPGEGLQLLLKA <u>TKADDKGSNKGFEATYRKETTSFHLEKGSVQVSDSAVYFCALSDTRDDK</u> IIFGKGTRLHILP (SEQ ID NO: 860) GGAAATTCAGTGACCCAGATGGAAGGGCCAGTGACTCTCTCAGAAGA GGCCTTCCTGACTATAAACTGCACGTACACAGCCACAGGATACCCCTC <u>CCTTTTCTGGTATGTCCAATATCCTGGAGAAGGTCTACAGCTCCTCCTG</u> AAAGCCACGAAGGCTGATGACAAGGGAAGCAACAAAGGTTTTGAAGC CACATACCGTAAAGAAACCACTTCTTTCCACTTGGAGAAAGGCTCAGT TCAAGTGTCAGACTCAGCGGTGTACTTCTGTGCTCTGAGTGATACCAG AGATGACAAGATCATCTTTGGAAGGACACGACTTCATATTCTCCC C (SEQ ID NO: 868)
PN45481 $V\beta$	DVKVTQSSRYLVKRTGEKVFLFCVQDMDHENMFWYRQDPGLGLRLIYF <u>SYDVKMKEKGDIPGYSVSREKKERFSLILESASTNQTSMYLCASSLOGR</u> YGYTTFGSGTRLTVV (SEQ ID NO: 861) GATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGG AGAGAAAGTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATAT GTTCTGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTT <u>CTCATATGATGTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGT</u> ACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGT CCGCCAGCACCAACCAGACATCTATGTACCTCTGTGCCAGCAGTTTAC <u>AGGGGAGGTACTATGGCTACACCTTCGGTTCGGGGACCAGGTAAACCG</u> TTGTA (SEQ ID NO: 869)

Table 9: Variable (V) and joining (J) region gene families for the α and β chains of Veloci-T[®] TCRs specific for MAGE-A4 (230-239) / HLA-A2

TCR ID	V β	J β	V α	J α
PN45515	28	1-2	26-1	38
PN45593	6-2	1-6	14/DV4	31
PN45539	28	1-2	26-1	38
PN45545	28	1-6	14/DV4	6
PN45584	6-6	1-2	14/DV4	37
PN45581	27	1-2	14/DV4	31
PN45428	20-1	1-1	20	38
PN45418	27	1-6	20	28
PN45446	20-1	1-1	20	28
PN45489	28	1-2	27	42
PN45460	27	1-6	9-2	30
PN45494	28	1-2	9-2	30
PN45590	5-6	1-1	8-3	22
PN45557	6-6	1-2	14/DV4	37
PN45546	5-5	1-2	38- 2/DV8	52
PN45481	28	1-2	9-2	30

Table 10: Amino acid and polynucleic acid sequence identifiers for TCR alpha and beta variable chains and CDRs

	Amino Acid Sequences						Polynucleic Acid Sequences											
	α CDRs			β CDRs			α CDRs			β CDRs								
	Vα	CDR1	CDR2	CDR3	Vβ	CDR1	CDR2	CDR3	Vα	CDR1	CDR2	CDR3	Vβ	CDR1	CDR2	CDR3		
TCR ID	SEQ ID NO:						SEQ ID NO:											
PN45515	620	614	615	616	628	622	623	624	621	617	618	619	629	625	626	627		
PN45593	636	630	631	632	644	638	639	640	637	633	634	635	645	641	642	643		
PN45539	652	646	647	648	660	654	655	656	653	649	650	651	661	657	658	659		
PN45545	668	662	663	664	676	670	671	672	669	665	666	667	677	673	674	675		
PN45584	684	678	679	680	692	686	687	688	685	681	682	683	693	689	690	691		
PN45581	700	694	695	696	708	702	703	704	701	697	698	699	709	705	706	707		
PN45428	716	710	711	712	724	718	719	720	717	713	714	715	725	721	722	723		
PN45418	732	726	727	728	740	734	735	736	733	729	730	731	741	737	738	739		
PN45446	748	742	743	744	756	750	751	752	749	745	746	747	757	753	754	755		
PN45489	764	758	759	760	772	766	767	768	765	761	762	763	773	769	770	771		
PN45460	780	774	775	776	788	782	783	784	781	777	778	779	789	785	786	787		
PN45494	796	790	791	792	804	798	799	800	797	793	794	795	805	801	802	803		
PN45590	812	806	807	808	820	814	815	816	813	809	810	811	821	817	818	819		
PN45557	828	822	823	824	836	830	831	832	829	825	826	827	837	833	834	835		
PN45546	844	838	839	840	852	846	847	848	845	841	842	843	853	849	850	851		
PN45481	860	854	855	856	868	862	863	864	861	857	858	859	869	865	866	867		

Example 3. Cytotoxicity of MAGE-A4 TCRs Against A375 Melanoma Cells

[00190] This example describes the ability of certain Mage-A4 TCRs of the invention to selectively kill A375 melanoma cells.

Methods*T cell manipulation*

[00191] Pan T cells were purified by negative selection from human peripheral blood monocyte cells (PBMCs) using the EasySep Human T cell isolation kit (StemCell Technologies 17951), then cryopreserved. On experiment day 0, T cells were thawed and plated at 1×10^6 cells/ml in media (CTS OpTmizer media [Life Technologies A1048501] supplemented with 10 mg/ml gentamicin, 4mM L-glutamine) containing 200 U/ml human IL-2 and anti-CD3/anti-CD28 activation beads at a 1:1 bead:cell ratio (Life Technologies 111.32D). On day 3, activation beads were removed, and 5×10^6 cells were transfected with 20 mg Cas9 protein (Life Technologies A36499) complexed with sgRNAs targeting the TRAC and TRBC loci using the Nucleofector IIb device and Human T cell Nucleofection Kit (Lonza VVPA-1002). After nucleofection, cells were transduced with adeno-associated virus (AAV) vectors engineered to target TCRa and TCRb expression cassettes to the human TRAC locus. Every 2-3 days, cells were re-plated in fresh supplemented media to about 1×10^6 cells/ml. At various points after TCR construct transduction, cells were stained with antibodies against surface markers and peptide-MHC tetramer reagents, then analyzed by flow cytometry to quantitate transduction efficiency.

Cytotoxicity assay

[00192] Antigen-specific, cytotoxic T cell function was assessed by calcein release assay. Target tumor cells were labeled in the presence of 8 mM Calcein AM dye (Life Technologies C1430) for 30 minutes at 37°C, then washed with media. 1×10^4 target cells were plated per assay well in a 96-well plate, along with varying dilutions of transduced T cells or untransduced (UTD) controls. After two hours, released fluorescence dye was measured in culture supernatants. Spontaneous release (SR) was measured in wells containing labeled target cells but no T cells, and maximum release (MR) was measured in wells with labeled target cells in the presence of 0.5% Triton X-100. Percent specific cytotoxicity was determined as $100 \times (\text{test release} - \text{SR})/(\text{MR} - \text{SR})$.

Results

[00193] Primary human T cells were engineered to express MAGE-A4 specific TCRs against two HLA-A2 restricted peptides (MAGE-A4 286-294 (KVLEHVVRV; SEQ ID NO: 609) and MAGE-A4 230-239 (GVYDGREHTV; SEQ ID NO: 294)), or an irrelevant HLA-A2 restricted peptide derived from a viral protein (HPV). As depicted in FIG. 1, flow cytometry analysis using peptide MHC tetramer reagents confirmed expression and expected antigen specificity of transduced TCRs.

[00194] To validate cytotoxic activity of the TCRs, engineered T cells were tested for their ability to direct cytolytic function against MAGE-A4 expressing tumor cells in a Calcein AM dye release assay, which was performed in duplicate (FIG. 2A, FIG. 2B, FIG. 3A, FIG. 3B). These assays confirmed that the MAGE-A4 specific TCRs (against both the MAGE-A4 286-294 and the MAGE-A4 230-239 peptides), but not the TCR against the irrelevant HLA-A2 restricted peptide, mediated killing of A375 melanoma cells in a dose dependent manner.

Example 4. MAGE-A4 (286-294) TCRs in Jurkat Cell TCR Signaling Bioassays

[00195] This example describes the ability of selected MAGE-A4 TCRs of the invention to activate TCR signaling in Jurkat cells

Method

Jurkat cell line generation

[00196] A Jurkat cell line lacking endogenous TCR α and TCR β expression was generated by knockout of those genes, and then engineered to allow single-copy Cre recombinase-mediated insertion of transgenic TCR constructs. An AP1 response element-driven luciferase reporter was then incorporated into this parental bioassay line. TCR bioassay lines were generated by Cre mediated insertion of customized TCR α/β expression constructs.

TCR activation bioassay

[00197] Jurkat bioassay lines expressing TCR constructs were FACS sorted to homogeneity, then tested in peptide-MHC stimulation assays. 293T cells (HLA-A2*01) were plated in assay wells with varying dilutions of antigenic (MAGE-A4 286-294 (KVLE)) or irrelevant (MAGE-A4 230-239 (GVY)) peptide. After 2 hours of incubation, engineered

Jurkat cells were added to wells at a 3:1 Jurkat:293T cell ratio and incubated a further 5 hours. Luciferase reporter activity was determined by measuring luminescence output in assay cells.

Results

[00198] Jurkat bioassay lines with an AP1 response element-driven luciferase transgene and engineered to express MAGE-A4 specific TCRs, or a TCR against an irrelevant antigen (NY-ESO-1), were tested in peptide stimulation assays. As depicted in FIG. 4A and FIG. 4B, TCRs specific for HLA-A2*01-restricted MAGE-A4 (286-294 (KVLE)) were reactive only to the cognate (KVLE) peptide, but not to an irrelevant MAGE-A4 (230-239 (GVY)) peptide. Parental TCR-negative (TCR-) cells and cells expressing irrelevant TCR (to NY ESO) showed no reactivity. In addition, FIG. 4A and FIG. 4B demonstrate that the AP1 reporter activation induced by the MAGE-A4 specific TCRs was dose-dependent.

Example 5. Specific Activity of MAGE-A4 (230-239) TCRs in T cells

[00199] This example describes the ability of selected Mage-A4 TCRs of the invention to activate TCR signaling in T cells.

[00200] Human peripheral blood mononuclear cells (PBMCs) (n=3 donors) were activated, transduced with a lentiviral vector encoding TCRs binding to HLA-A2 restricted MAGE-A4 (230-239 (GVY)), and expanded *in vitro* for 10 days before freezing. After thawing, T cells containing the aforementioned TCRs were cultured at a 1:1 ratio with T2 cells pulsed with the MAGE-A4 230-239 peptide. Reactivity was measured as interferon gamma release 24 hours after co-culture. T cells alone were used as control for antigen-independent interferon gamma release. T cells cultured with unpulsed T2 cells or T2 cells pulsed with an irrelevant peptide were used as a control for specificity. FIG. 5 depicts the average interferon gamma signal +/- standard error of the mean (SEM) of n=3 donors, and shows that MAGE-A4 (230-239)-specific TCRs of the present disclosure exhibit specific activity against T2 cells pulsed with the MAGE-A4 (230-239) peptide.

[00201] To test activity against tumor cells expressing MAGE-A4, human PBMCs (n=3 donors) were activated, transduced with a lentiviral vector encoding MAGE-A4 (230-239) TCRs, and expanded *in vitro* for 10 days before freezing. After thawing, MAGE-A4 (230-239) TCR T cells were cultured at a 5:1 ratio with tumor cells expressing engineered

levels of HLA-A2 and MAGE-A4 (A549.A2.MAGEA4 cells) or endogenous levels of HLA-A2 and MAGE-A4 (A375 cells). Reactivity was measured as interferon gamma release 24 hours after co-culture. T cells alone were used as control for antigen-independent interferon gamma release. T cells cultured with unmodified A549 cells (which are MAGE-A4 negative and HLA-A2 negative) or A549 cells modified to express HLA-A2 (A549.A2) were used as a control for specificity. FIG. 6 depicts the average interferon gamma signal +/- standard error of the mean (SEM) of n=3 donors, and shows that MAGE-A4 (230-239)-specific TCRs of the present disclosure exhibit specific activity against tumor cells expressing HLA-A2 and MAGE-A4. In a separate assay, the T cells were co-cultured with A549 cells, A549.A2 cells, NCI-H520 (HLA-A2 negative/MAGE-A4 positive) cells, and MCF-7 (HLA-A2 positive/MAGE-A4 negative) cells to provide further controls for specificity to the HLA-A2/MAGE-A4 complex. FIG. 7 depicts the average interferon gamma signal +/- standard error of the mean (SEM) of n=3 donors, and shows that MAGE-A4 (230-239)-specific TCRs of the present disclosure exhibit specific activity against tumor cells expressing HLA-A2 and MAGE-A4. In a further assay, the MAGE-A4 (230-239) TCR-expressing T cells were cultured at 5:1 ratio with tumor cells expressing low or medium endogenous levels of MAGE-A4 (U2-OS and NCI-H1703, respectively). Reactivity was measured as IFN γ release 24 hours after co-culture. FIG. 8 depicts the average interferon gamma signal +/- standard error of the mean (SEM) of n=3 donors, and shows that MAGE-A4 (230-239)-specific TCRs of the present disclosure exhibit specific activity against HLA-A2-expressing tumor cells expressing even just low or medium endogenous levels of MAGE-A4.

Equivalents

[00202] Those skilled in the art will recognize or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. such equivalents are intended to be encompassed by the following claims. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.