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(54) Titre : RECEPTEURS DES LYMPHOCYTES T RECONNAISSANT UN GENE MAGE RESTREINT PAR HLA-A1 OU HLA-CW7
(54) Title: T CELL RECEPTORS RECOGNIZING HLA-A1- OR HLA-CW7-RESTRICTED MAGE

(57) Abrégé/Abstract:
The invention provides an isolated or purified T cell receptor (TCR) having antigenic specificity for a) melanoma antigen family A (MAGE A)-3 in the context of HLA-A1 or b) MAGE-A12 in the context of HLA-Cw7. The invention further provides related polypeptides and proteins, as well as related nucleic acids, recombinant expression vectors, host cells, and populations of cells. Further provided by the invention are antibodies, or an antigen binding portion thereof, and pharmaceutical compositions relating to the TCRs of the invention. Methods of detecting the presence of cancer in a host and methods of treating or preventing cancer in a host are further provided by the invention.

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(54) Title: T CELL RECEPTORS RECOGNIZING HLA-A1- OR HLA-CW7-RESTRICTED MAGE

(57) **Abstract**: The invention provides an isolated or purified T cell receptor (TCR) having antigenic specificity for a) melanoma antigen family A (MAGE A)-3 in the context of HLA-A1 or b) MAGE-A12 in the context of HLA-Cw7. The invention further provides related polypeptides and proteins, as well as related nucleic acids, recombinant expression vectors, host cells, and populations of cells. Further provided by the invention are antibodies, or an antigen binding portion thereof, and pharmaceutical compositions relating to the TCRs of the invention. Methods of detecting the presence of cancer in a host and methods of treating or preventing cancer in a host are further provided by the invention.

T CELL RECEPTORS RECOGNIZING HLA-A1- OR HLA-CW7-RESTRICTED MAGE

[0001] This application claims priority to United States Application No. 61/535,086, filed on September 15, 2011.

[0001A] This invention was made with U.S. Government support under project number ZIABC10984 by the National Institutes of Health, National Cancer Institute. The U.S. Government has certain rights in this invention.

MATERIAL SUBMITTED ELECTRONICALLY

[0002] A computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 52,162 Byte ASCII (Text) file named "710922ST25.TXT," dated August 22, 2012.

BACKGROUND OF THE INVENTION

[0003] Adoptive cell therapy (ACT) involves the transfer of reactive T cells into patients, including the transfer of tumor-reactive T cells into cancer patients. Adoptive cell therapy using T-cells that target human leukocyte antigen (HLA)-A2 restricted T-cell epitopes has been successful in causing the regression of tumors in some patients. However, patients that lack HLA-A2 expression cannot be treated with T-cells that target HLA-A2 restricted T-cell epitopes. Such a limitation creates an obstacle to the widespread application of adoptive cell therapy. Accordingly, there exists a need for improved immunological compositions and methods for treating cancer.

BRIEF SUMMARY OF THE INVENTION

[0004] The invention provides an isolated or purified T cell receptor (TCR) having antigenic specificity for a) melanoma antigen family A (MAGE A)-3 in the context of HLA-A1 or b) MAGE-A12 in the context of HLA-Cw7. The invention further provides related polypeptides and proteins, as well as related nucleic acids, recombinant expression vectors, host cells, and populations of cells. Further provided by the invention are antibodies, or antigen binding portions thereof, and pharmaceutical compositions relating to the TCRs of the invention.

[0005] Methods of detecting the presence of cancer in a host and methods of treating or preventing cancer in a host are further provided by the invention. The inventive method of detecting the presence of cancer in a host comprises (i) contacting a sample comprising cells of the cancer with any of the inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of host cells, or antibodies, or antigen binding portions thereof, described herein, thereby forming a complex, and (ii) detecting the complex, wherein detection of the complex is indicative of the presence of cancer in the host.

[0006] The inventive method of treating or preventing cancer in a host comprises administering to the host any of the TCRs, polypeptides, or proteins described herein, any nucleic acid or recombinant expression vector comprising a nucleotide sequence encoding any of the TCRs, polypeptides, proteins described herein, or any host cell or population of host cells comprising a recombinant vector which encodes any of the TCRs, polypeptides, or proteins described herein, in an amount effective to treat or prevent cancer in the host.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0007] Figure 1A is a bar graph showing interferon (IFN)-γ secretion (pg/ml) of untransduced (UT) cells (black bars) or cells transduced with anti-MAGE-A3 TCR A10 (SEQ ID NO: 46) (unshaded bars) or anti-MAGE-A3 TCR 13-18 (SEQ ID NO: 48) (grey bars) in response to co-culture with various tumor cell lines.

[0008] Figure 1B is a bar graph showing IFN-γ secretion (pg/ml) of UT cells or cells transduced with anti-MAGE-A3 TCR A10 (SEQ ID NO: 46) or anti-MART-1 TCR DMF5 in response to co-culture with HLA-A1+/MAGE-A3+ fresh tumors FrTu 2767 (black bars), FrTu 3178 (grey bars), FrTu 2823 (unshaded bars) or FrTu 3068 (diagonal lined bars) or HLA-A*0201+/MART-1+ fresh tumors FrTu 2851 (horizontal lined bars) or FrTu 3242 (vertical lined bar). Checkered bars indicate cells co-cultured with no tumor cells.

[0009] Figures 2A and 2B are bar graphs showing IFN-γ secretion (pg/ml) of cells from first (Figure 2A) and second (Figure 2B) donors transduced with a control construct encoding the truncated human low affinity nerve growth factor receptor (NGFR) (black bars), anti-MAGE-A12 TCR 502 (SEQ ID NO: 47) (unshaded bars), or anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49) (grey bars) in response to co-culture with various tumor cell lines.

[0010] Figure 3A is a bar graph illustrating the cumulative percentage of the normal Caucasian population that expresses HLA-A1 (unshaded portion of bar), HLA-A2 (grey portion of bar), and/or HLA-Cw7 (black portion of bar).

[0011] Figures 3B and 3C are bar graphs illustrating the cumulative percentage of the human melanoma (Figure 3B) and synovial cell sarcoma (Figure 3C) patient populations that would be expected to express HLA-A2 and NY-ESO-1 (diagonal lined portion of bar); HLA-A1 and MAGE-A3 (unshaded portion of bar); HLA-A2, MAGE-A3, and MAGE-A12 (grey portion of bar); and/or HLA-Cw7 and MAGE-A12 (black portion of bar).

[0012] Figure 4 is a bar graph showing IFN-γ secretion (pg/ml) of cells transduced with NGFR (black bars), anti-MAGE-A12 TCR 502 (SEQ ID NO: 47) (unshaded bars), or anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49) (grey bars) in response to co-culture with HLA-Cw*0701 and HLA-Cw*0702 target cells pulsed with peptides from MAGE-A12 (VRIGHLYIL; SEQ ID NO: 4), MAGE-A2 (VPISHLYIL; SEQ ID NO: 50), MAGE-A3 (DPIGHLYIF; SEQ ID NO: 51), MAGE-A6 (DPIGHVYIF; SEQ ID NO: 52), or control peptide (EDGCPAAEK; SEQ ID NO: 53).

[0013] Figures 5A-5D are line graphs showing percent lysis of 397 mel (A), 624 mel (B), 2984 mel (C), and 2661 RCC (D) cells by PBMC that were untransduced (closed circles) or transduced with anti-MAGE-A12 TCR 502 (SEQ ID NO: 47) (▼), anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49) (diamonds), anti-MAGE-A3 TCR A10 (SEQ ID NO: 46) (squares), anti-MAGE-A3 TCR 13-18 (SEQ ID NO: 48) (▲), or anti-MAGE-A3 TCR 112-120 (open circles) at the indicated effector to target (E:T) ratios. Representative results from one of two independent experiments are presented.

[0014] Figure 6A is a bar graph showing IFN-γ secretion (pg/ml) of untransduced (control) cells (striped bars) or cells transduced with anti-MAGE-A3 TCR A10 (SEQ ID NO: 46) (shaded bars) or anti-MAGE-A3 TCR 13-18 (SEQ ID NO: 48) (checkered bars) in response to co-culture with various tumor cell lines. Representative results from two of three independent experiments assessing responses of T cells transduced with these TCRs are presented.

[0015] Figure 6B is a bar graph showing estimated relative copies of vector DNA measured for cells transduced with anti-MAGE-A3 TCR A10 (SEQ ID NO: 46), anti-MAGE-A3 TCR 13-18 (SEQ ID NO: 48), anti-MAGE-A12 TCR 502 (SEQ ID NO: 47), or anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49).

[0016] Figure 6C is a line graph showing the amount of IFN-gamma secreted by cells transduced with anti-MAGE-A3 TCR A10 (SEQ ID NO: 46) (circles) or anti-MAGE-A3 TCR 13-18 (SEQ ID NO: 48) (squares) in response to co-culture with target cells incubated with various concentrations of MAGE-A3 168-176 peptide.

[0017] Figure 6D is a bar graph showing IFN-γ secretion (pg/ml) of cells transduced with anti-MAGE-A12 TCR 502 (SEQ ID NO: 47) (shaded bars) or anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49) (checkered bars) in response to co-culture with various tumor cell lines. Representative results from two of three independent experiments assessing responses of T cells transduced with these TCRs are presented.

[0018] Figure 6E is a line graph showing the amount of IFN-gamma secreted by cells transduced with anti-MAGE-A12 TCR 502 (SEQ ID NO: 47) (circles) or anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49) (squares) in response to co-culture with target cells incubated with various concentrations of MAGE-A12:170-178 peptide.

[0019] Figure 6F is a bar graph showing IFN-γ secretion (pg/ml) of cells untransduced (control) (striped bars) or transduced with anti-MAGE-A12 TCR 502 (SEQ ID NO: 47) (shaded bars) or anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49) (checkered bars) in response to co-culture with various tumor cell lines. Representative results from two of three independent experiments assessing responses of T cells transduced with these TCRs are presented.

[0020] Figure 7A is a bar graph showing IFN-γ secretion (pg/ml) of untransduced cells (control) (striped bars) or cells transduced with anti-MAGE-A3 TCR A10 (SEQ ID NO: 46) (shaded bars) or anti-MAGE-A3 TCR 13-18 (checkered bars) in response to co-culture with various fresh uncultured tumors. Representative results from one of three independent experiments assessing responses of T cells transduced with these TCRs are presented.

[0021] Figure 7B is a bar graph showing IFN-γ secretion (pg/ml) of untransduced cells (control) (striped bars) or cells transduced with anti-MAGE-A12 TCR 502 (SEQ ID NO: 47) (shaded bars) or anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49) (checkered bars) in response to co-culture with various fresh uncultured tumors. Representative results from one of three independent experiments assessing responses of T cells transduced with these TCRs are presented.

[0022] Figure 8A is a bar graph showing IFN-γ secretion (pg/ml) of untransduced cells (control) (striped bars) or cells transduced with anti-MAGE-A3 TCR A10 (SEQ ID NO: 46) (shaded bars) or anti-MAGE-A3 TCR 13-18 (checkered bars) co-cultured with target cells transfected with HLA-A*01 plus either MAGE-A3, A1, A2, A4, A6, A9, A10 or A12 overnight.

[0023] Figure 8B is a bar graph showing IFN-γ secretion (pg/ml) of untransduced cells (control) (striped bars) or cells transduced with anti-MAGE-A12 TCR 502 (SEQ ID NO: 47) (shaded bars) or anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49) (checkered bars) co-cultured with target cells transfected with HLA-C*07:02 plus either MAGE-A3, A1, A2, A4, A6, A9, A10 or A12 overnight.

[0024] Figure 8C is a bar graph showing IFN-γ secretion (pg/ml) of untransduced cells (control) (striped bars) or cells transduced with anti-MAGE-A12 TCR 502 (SEQ ID NO: 47) (shaded bars) or anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49) (checkered bars) co-cultured with target cells transfected with HLA-C*07:01 plus either MAGE-A3, A1, A2, A4, A6, A9, A10 or A12 overnight.

[0025] Figures 9A and 9B are bar graphs showing IFN-gamma secretion of CD8+ (Fig. 9A) or CD4+ cells (Fig. 9B) that were untransduced (control) (striped bars) or cells transduced with anti-MAGE-A3 TCR A10 (SEQ ID NO: 46) (shaded bars) or anti-MAGE-A3 TCR 13-18 (checkered bars) co-cultured with various tumor targets. Representative results from one of two independent experiments are presented.

[0026] Figure 9C is a bar graph showing IFN-gamma secretion of CD8+ cells that were untransduced (control) (striped bars) or cells transduced with anti-MAGE-A12 TCR 502 (SEQ ID NO: 47) (shaded bars) or anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49) (checkered bars) co-cultured with various tumor targets. Representative results from one of two independent experiments are presented.

DETAILED DESCRIPTION OF THE INVENTION

[0027] An embodiment of the invention provides a T cell receptor (TCR) having antigenic specificity for a) melanoma antigen family A (MAGE A)-3 (also known as MAGE-3) in the context of HLA-A1 or b) MAGE-A12 (also known as MAGE-12) in the context of HLA-Cw7.

[0028] MAGE-A3 and MAGE-A12 are members of the MAGE-A family of twelve homologous proteins also including MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, and MAGE-A11. The MAGE-A proteins are cancer testis antigens (CTA), which are expressed only in tumor cells and non-MHC expressing germ cells of the testis and placenta. MAGE-A proteins are expressed in a variety of human cancers including, but not limited to, melanoma, breast cancer, leukemia, thyroid cancer, gastric cancer, pancreatic cancer, liver cancer (e.g., hepatocellular carcinoma), lung cancer (e.g., non-small cell lung carcinoma), ovarian cancer, multiple myeloma, esophageal cancer, kidney cancer, head cancers (e.g., squamous cell carcinoma), neck cancers (e.g., squamous cell carcinoma), prostate cancer, and urothelial cancer.

[0029] The TCRs of the invention provide many advantages, including when used for adoptive cell transfer. For example, by targeting a) MAGE-A3 that is presented in the context of HLA-A1 or b) MAGE-A12 that is presented in the context of HLA-Cw7, the inventive TCRs make it possible to treat patients who are unable to be treated using TCRs that target MAGE antigens that are presented in the context of other HLA molecules, e.g., HLA-A2. Because HLA-A1 and HLA-Cw7 are highly prevalent alleles, the inventive TCRs advantageously greatly expand the patient population that can be treated. Additionally, without being bound by a particular theory, it is believed that because MAGE-A3 and/or MAGE-A12 are expressed by cells of multiple cancer types, the inventive TCRs advantageously provide the ability to destroy cells of multiple types of cancer and, accordingly, treat or prevent multiple types of cancer. Additionally, without being bound to a particular theory, it is believed that because the MAGE-A proteins are cancer testis antigens that are expressed only in tumor cells and non-MHC expressing germ cells of the testis and placenta, the inventive TCRs advantageously target the destruction of cancer cells while minimizing or eliminating the destruction of normal, non-cancerous cells, thereby reducing, for example, by minimizing or eliminating, toxicity.

[0030] The phrase "antigenic specificity" as used herein means that the TCR can specifically bind to and immunologically recognize MAGE-A3 or MAGE-A12 with high avidity. For example, a TCR may be considered to have "antigenic specificity" for MAGE-A3 or MAGE-A12 if T cells expressing the TCR secrete at least about 200 pg/ml or more (e.g., 200 pg/ml or more, 300 pg/ml or more, 400 pg/ml or more, 500 pg/ml or more, 600 pg/ml or more, 700 pg/ml or more, 1000 pg/ml or more, 5,000 pg/ml or more, 7,000 pg/ml or more, 10,000 pg/ml or more) of IFN-γ upon co-culture with antigen-negative HLA-A1+ target cells or HLA-Cw7+ target cells, respectively, pulsed with a low concentration of MAGE-A3 peptide or MAGE-A12 peptide, respectively (e.g., about 0.05 ng/ml to about 5 ng/ml, 0.05 ng/ml, 0.1 ng/ml, 0.5 ng/ml, 1 ng/ml, or 5 ng/ml). Alternatively or additionally, a TCR may be considered to have "antigenic specificity" for MAGE-A3 or MAGE-A12 if T cells expressing the TCR secrete at least twice as much IFN-γ as the untransduced PBL background level of IFN-γ upon co-culture with antigen-negative HLA-A1+ target cells or HLA-Cw7+ target cells, respectively, pulsed with a low concentration of MAGE-A3 peptide or MAGE-A12 peptide, respectively. The inventive TCRs may also secrete IFN-γ upon co-culture with antigen-negative HLA-A1+ target cells or HLA-Cw7+ target cells pulsed with higher concentrations of MAGE-A3 peptide or MAGE-A12 peptide, respectively.

[0031] An embodiment of the invention provides a TCR with antigenic specificity for any MAGE-A3 protein, polypeptide or peptide. The inventive TCR may have antigenic specificity for a MAGE-A3 protein comprising, consisting of, or consisting essentially of, SEQ ID NO: 1. In a preferred embodiment of the invention, the TCR has antigenic specificity for a MAGE-A3 168-176 peptide comprising, consisting of, or consisting essentially of, EVDPIGHLY (SEQ ID NO: 2).

[0032] The inventive TCRs are able to recognize MAGE-A3 in a human leukocyte antigen (HLA)-A1-dependent manner. By "HLA-A1-dependent manner" as used herein means that the TCR elicits an immune response upon binding to a MAGE-A3 cancer antigen within the context of an HLA-A1 molecule. The inventive TCRs are able to recognize MAGE-A3 that is presented by an HLA-A1 molecule and may bind to the HLA-A1 molecule in addition to MAGE-A3. Exemplary HLA-A1 molecules, in the context of which the inventive TCRs recognize MAGE-A3, include those encoded by the HLA-A*0101, HLA-A*0102, and/or HLA-A*0103 alleles.

[0033] An embodiment of the invention provides a TCR with antigenic specificity for any MAGE-A12 protein, polypeptide or peptide. The inventive TCR may have antigenic specificity for a MAGE-A12 protein comprising, consisting of, or consisting essentially of, SEQ ID NO: 3. In a preferred embodiment of the invention, the TCR has antigenic specificity for a MAGE-A12 170-178 peptide comprising, consisting of, or consisting essentially of, VRIGHLYIL (SEQ ID NO: 4).

[0034] The inventive TCRs are able to recognize MAGE-A12 in an HLA-Cw7-dependent manner. By "HLA-Cw7-dependent manner" as used herein means that the TCR elicits an immune response upon binding to a MAGE-A12 cancer antigen within the context of an HLA-Cw7 molecule. The inventive TCRs are able to recognize MAGE-A12 that is presented by an HLA-Cw7 molecule and may bind to the HLA-Cw7 molecule in addition to MAGE-A12. Exemplary HLA-Cw7 molecules, in the context of which the inventive TCRs recognize MAGE-A12, include those encoded by the HLA-Cw*0701 and/or HLA-Cw*0702 alleles.

[0035] The invention provides a TCR comprising two polypeptides (i.e., polypeptide chains), such as an alpha (α) chain of a TCR, a beta (β) chain of a TCR, a gamma (γ) chain of a TCR, a delta (δ) chain of a TCR, or a combination thereof. Such polypeptide chains of

TCRs are known in the art. The polypeptides of the inventive TCR can comprise any amino acid sequence, provided that the TCR has antigenic specificity for a) MAGE-A3 in the context of HLA-A1 or b) MAGE-A12 in the context of HLA-Cw7.

[0036] In an embodiment of the invention, the TCR comprises two polypeptide chains, each of which comprises a variable region comprising a complementarity determining region (CDR) 1, a CDR2, and a CDR3 of a TCR. In an embodiment of the invention, the TCR has antigenic specificity for MAGE-A3 168-176 and comprises a first polypeptide chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 5 or 16 (CDR1 of α chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 6 or 17 (CDR2 of α chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 7 or 18 (CDR3 of α chain), and a second polypeptide chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 8 or 19 (CDR1 of β chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 9 or 20 (CDR2 of β chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 10 or 21 (CDR3 of β chain). In another embodiment of the invention, the TCR has antigenic specificity for MAGE-A12 170-178, and comprises a first polypeptide chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 26 or 36 (CDR1 of α chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 27 or 37 (CDR2 of α chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 28 or 38 (CDR3 of α chain), and a second polypeptide chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 29 or 39 (CDR1 of β chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 30 or 40 (CDR2 of β chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 31 or 41 (CDR3 of β chain). In this regard, the inventive TCR can comprise any one or more of the amino acid sequences selected from the group consisting of any one or more of SEQ ID NOs: 5-7, 8-10, 16-18, 19-21, 26-28, 29-31, 36-38, and 39-41. Preferably the TCR comprises the amino acid sequences of SEQ ID NOs: 5-10, 16-21, 26-31, or 36-41. More preferably the TCR comprises the amino acid sequences of SEQ ID NOs: 5-10 or 26-31.

[0037] Alternatively or additionally, the TCR can comprise an amino acid sequence of a variable region of a TCR comprising the CDRs set forth above. In this regard, the TCR with antigenic specificity for MAGE-A3 168-176 can comprise the amino acid sequence of SEQ ID NO: 11 or 22 (the variable region of an α chain) or 12 or 23 (the variable region of a β chain), both SEQ ID NOs: 11 and 12 or both SEQ ID NOs: 22 and 23. In another embodiment of the invention, the TCR has antigenic specificity for MAGE-A12 170-178 and comprises the amino acid sequence of SEQ ID NO: 32 or 42 (the variable region of an α chain) or 33 or 43 (the variable region of a β chain), both SEQ ID NOs: 32 and 33, or both SEQ ID NOs: 42 and 43. Preferably, the inventive TCR comprises the amino acid sequences of both SEQ ID NOs: 11 and 12 or both SEQ ID NOs: 32 and 33.

[0038] Alternatively or additionally, the TCR can comprise an α chain of a TCR and a β chain of a TCR. Each of the α chain and β chain of the inventive TCR can independently comprise any amino acid sequence. Preferably, the α chain comprises the variable region of an α chain as set forth above. In this regard, the inventive TCR with antigenic specificity for MAGE-A3 168-176 can comprise the amino acid sequence of SEQ ID NO: 13 or 24 and the inventive TCR with antigenic specificity for MAGE-A12 170-178 can comprise the amino acid sequence of SEQ ID NO: 34 or 44. An inventive TCR of this type can be paired with any β chain of a TCR. Preferably, the β chain of the inventive TCR comprises the variable region of a β chain as set forth above. In this regard, the inventive TCR with antigenic specificity for MAGE-A3 168-176 can comprise the amino acid sequence of SEQ ID NO: 14 or 25 and the inventive TCR with antigenic specificity for MAGE-A12 170-178 can comprise the amino acid sequence of SEQ ID NO: 35 or 45. The inventive TCR, therefore, can comprise the amino acid sequence of SEQ ID NO: 13, 14, 24, 25, 34, 35, 44, or 45, both SEQ ID NOs: 13 and 14, both SEQ ID NOs: 24 and 25, both SEQ ID NOs: 34 and 35, or both SEQ ID NOs: 44 and 45. Preferably, the inventive TCR comprises the amino acid sequences of both SEQ ID NOs: 13 and 14 or both SEQ ID NOs: 34 and 35.

[0039] Also provided by the invention is a polypeptide comprising a functional portion of any of the TCRs described herein. The term "polypeptide" as used herein includes oligopeptides and refers to a single chain of amino acids connected by one or more peptide bonds.

[0040] With respect to the inventive polypeptides, the functional portion can be any portion comprising contiguous amino acids of the TCR of which it is a part, provided that the functional portion specifically binds to MAGE-A3 or MAGE-A12. The term "functional portion" when used in reference to a TCR refers to any part or fragment of the TCR of the invention, which part or fragment retains the biological activity of the TCR of which it is a part (the parent TCR). Functional portions encompass, for example, those parts of a TCR that retain the ability to specifically bind to MAGE-A3 (e.g., in an HLA-A1-dependent manner) or MAGE-A12 (e.g., in an HLA-Cw7-dependent manner), or detect, treat, or prevent cancer, to a similar extent, the same extent, or to a higher extent, as the parent TCR. In reference to the parent TCR, the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent TCR.

[0041] The functional portion can comprise additional amino acids at the amino or carboxy terminus of the portion, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent TCR. Desirably, the additional amino acids do not interfere with the biological function of the functional portion, e.g., specifically binding to MAGE-A3 or MAGE-A12; and/or having the ability to detect cancer, treat or prevent cancer, etc. More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent TCR.

[0042] The polypeptide can comprise a functional portion of either or both of the α and β chains of the TCRs of the invention, such as a functional portion comprising one of more of CDR1, CDR2, and CDR3 of the variable region(s) of the α chain and/or β chain of a TCR of the invention. In this regard, the polypeptide can comprise a functional portion comprising the amino acid sequence of SEQ ID NO: 5, 16, 26, or 36 (CDR1 of α chain), 6, 17, 27, or 37 (CDR2 of α chain), 7, 18, 28, or 38 (CDR3 of α chain), 8, 19, 29, or 39 (CDR1 of β chain), 9, 20, 30, or 40 (CDR2 of β chain), 10, 21, 31, or 41 (CDR3 of β chain), or a combination thereof. Preferably, the inventive polypeptide comprises a functional portion comprising SEQ ID NOs: 5-7; 8-10; 16-18; 19-21; 26-28; 29-31; 36-38; 39-41; all of SEQ ID NOs: 5-10; all of SEQ ID NOs: 16-21; all of SEQ ID NOs: 26-31; or all of SEQ ID NOs: 36-41. More preferably, the polypeptide comprises a functional portion comprising the amino acid sequences of all of SEQ ID NOs: 5-10 or all of SEQ ID NOs: 26-31.

[0043] Alternatively or additionally, the inventive polypeptide can comprise, for instance, the variable region of the inventive TCR comprising a combination of the CDR regions set forth above. In this regard, the polypeptide can comprise the amino acid sequence of SEQ ID NO: 11, 22, 32, or 42 (the variable region of an α chain), SEQ ID NO: 12, 23, 33, or 43 (the variable region of a β chain), both SEQ ID NOs: 11 and 12, both SEQ ID NOs: 22 and 23, both SEQ ID NOs: 32 and 33, or both SEQ ID NOs: 42 and 43. Preferably, the polypeptide comprises the amino acid sequences of both SEQ ID NOs: 11 and 12 or both SEQ ID NOs: 32 and 33.

[0044] Alternatively or additionally, the inventive polypeptide can comprise the entire length of an α or β chain of one of the TCRs described herein. In this regard, the inventive polypeptide can comprise an amino acid sequence of SEQ ID NOs: 13, 14, 24, 25, 34, 35, 44, or 45. Alternatively, the polypeptide of the invention can comprise α and β chains of the

TCRs described herein. For example, the inventive polypeptide can comprise the amino acid sequences of both SEQ ID NOs: 13 and 14; both SEQ ID NOs: 24 and 25; both SEQ ID NOs: 34 and 35; or both SEQ ID NOs: 44 and 45. Preferably, the polypeptide comprises the amino acid sequences of both SEQ ID NOs: 13 and 14 or both SEQ ID NOs: 34 and 35.

[0045] The invention further provides a protein comprising at least one of the polypeptides described herein. By "protein" is meant a molecule comprising one or more polypeptide chains.

[0046] In an embodiment, the protein of the invention can comprise a first polypeptide chain comprising the amino acid sequences of SEQ ID NOs: 5-7; SEQ ID NOs: 16-18; SEQ ID NOs: 26-28; or SEQ ID NOs: 36-38 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NOs: 8-10; SEQ ID NOs: 19-21; SEQ ID NOs: 29-31; or SEQ ID NOs: 39-41. Alternatively or additionally, the protein of the invention can comprise a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 11, 22, 32, or 42 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 12, 23, 33, or 43. The protein of the invention can, for example, comprise a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 13, 24, 34, or 44 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 14, 25, 35, or 45. In this instance, the protein of the invention can be a TCR. Alternatively, if, for example, the protein comprises a single polypeptide chain comprising SEQ ID NO: 13, 24, 34, or 44 and SEQ ID NO: 14, 25, 35, or 45, or if the first and/or second polypeptide chain(s) of the protein further comprise(s) other amino acid sequences, e.g., an amino acid sequence encoding an immunoglobulin or a portion thereof, then the inventive protein can be a fusion protein. In this regard, the invention also provides a fusion protein comprising at least one of the inventive polypeptides described herein along with at least one other polypeptide. The other polypeptide can exist as a separate polypeptide of the fusion protein, or can exist as a polypeptide, which is expressed in frame (in tandem) with one of the inventive polypeptides described herein. The other polypeptide can encode any peptidic or proteinaceous molecule, or a portion thereof, including, but not limited to an immunoglobulin, CD3, CD4, CD8, an MHC molecule, a CD1 molecule, e.g., CD1a, CD1b, CD1c, CD1d, etc.

[0047] The fusion protein can comprise one or more copies of the inventive polypeptide and/or one or more copies of the other polypeptide. For instance, the fusion protein can comprise 1, 2, 3, 4, 5, or more, copies of the inventive polypeptide and/or of the other polypeptide. Suitable methods of making fusion proteins are known in the art, and include, for example, recombinant methods. See, for instance, Choi et al., *Mol. Biotechnol.* 31: 193-202 (2005).

[0048] In some embodiments of the invention, the TCRs, polypeptides, and proteins of the invention may be expressed as a single protein comprising a linker peptide linking the α chain and the β chain. In this regard, the TCRs, polypeptides, and proteins of the invention comprising SEQ ID NO: 13, 24, 34, or 44 and SEQ ID NO: 14, 25, 35, or 45 may further comprise a linker peptide comprising SEQ ID NO: 15 or 54. The linker peptide may advantageously facilitate the expression of a recombinant TCR, polypeptide, and/or protein in a host cell. Upon expression of the construct including the linker peptide by a host cell, the linker peptide may be cleaved, resulting in separated α and β chains.

[0049] The protein of the invention can be a recombinant antibody comprising at least one of the inventive polypeptides described herein. As used herein, "recombinant antibody" refers to a recombinant (e.g., genetically engineered) protein comprising at least one of the polypeptides of the invention and a polypeptide chain of an antibody, or a portion thereof. The polypeptide of an antibody, or portion thereof, can be a heavy chain, a light chain, a variable or constant region of a heavy or light chain, a single chain variable fragment (scFv), or an Fc, Fab, or $F(ab)_2'$ fragment of an antibody, etc. The polypeptide chain of an antibody, or portion thereof, can exist as a separate polypeptide of the recombinant antibody. Alternatively, the polypeptide chain of an antibody, or portion thereof, can exist as a polypeptide, which is expressed in frame (in tandem) with the polypeptide of the invention. The polypeptide of an antibody, or portion thereof, can be a polypeptide of any antibody or any antibody fragment, including any of the antibodies and antibody fragments described herein.

[0050] Included in the scope of the invention are functional variants of the inventive TCRs, polypeptides, and proteins described herein. The term "functional variant" as used herein refers to a TCR, polypeptide, or protein having substantial or significant sequence identity or similarity to a parent TCR, polypeptide, or protein, which functional variant retains the biological activity of the TCR, polypeptide, or protein of which it is a variant. Functional variants encompass, for example, those variants of the TCR, polypeptide, or protein described herein (the parent TCR, polypeptide, or protein) that retain the ability to specifically bind to MAGE-A3 or MAGE-A12 for which the parent TCR has antigenic specificity or to which the parent polypeptide or protein specifically binds, to a similar extent, the same extent, or to a higher extent, as the parent TCR, polypeptide, or protein. In reference to the parent TCR, polypeptide, or protein, the functional variant can, for instance, be at least about 30%, 50%, 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more identical in amino acid sequence to the parent TCR, polypeptide, or protein.

[0051] The functional variant can, for example, comprise the amino acid sequence of the parent TCR, polypeptide, or protein with at least one conservative amino acid substitution. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic amino acid substituted for another acidic amino acid (e.g., Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (e.g., Ala, Gly, Val, Ile, Leu, Met, Phe, Pro, Trp, Val, etc.), a basic amino acid substituted for another basic amino acid (Lys, Arg, etc.), an amino acid with a polar side chain substituted for another amino acid with a polar side chain (Asn, Cys, Gln, Ser, Thr, Tyr, etc.), etc.

[0052] Alternatively or additionally, the functional variants can comprise the amino acid sequence of the parent TCR, polypeptide, or protein with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the functional variant. Preferably, the non-conservative amino acid substitution enhances the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent TCR, polypeptide, or protein.

[0053] The TCR, polypeptide, or protein can consist essentially of the specified amino acid sequence or sequences described herein, such that other components of the functional variant, e.g., other amino acids, do not materially change the biological activity of the functional variant. In this regard, the inventive TCR, polypeptide, or protein can, for example, consist essentially of the amino acid sequence of SEQ ID NO: 13, 14, 24, 25, 34, 35, 44, or 45, both SEQ ID NOs: 13 and 14, both SEQ ID NOs: 24 and 25, both SEQ ID NOs: 34 and 35, or both SEQ ID NOs: 44 and 45. Also, for instance, the inventive TCRs, polypeptides, or proteins can consist essentially of the amino acid sequence(s) of SEQ ID NO: 11, 12, 22, 23, 32, 33, 42, or 43, both SEQ ID NOs: 11 and 12, both SEQ ID NOs: 22 and 23, both SEQ ID NOs: 32 and 33, or both SEQ ID NOs: 42 and 43. Furthermore, the inventive TCRs, polypeptides, or proteins can consist essentially of the amino acid sequence of SEQ ID NO: 5, 16, 26, or 36 (CDR1 of α chain), SEQ ID NO: 6, 17, 27, or 37 (CDR2 of α chain), SEQ ID NO: 7, 18, 28, or 38 (CDR3 of α chain), SEQ ID NO: 8, 19, 29, or 39 (CDR1 of β chain), SEQ ID NO: 9, 20, 30, or 40 (CDR2 of β chain), SEQ ID NO: 10, 21, 31, or 41 (CDR3 of β chain), or any combination thereof, e.g., SEQ ID NOs: 5-7; 8-10; 5-10; 16-18; 19-21; 16-21; 26-28; 29-31; 26-31; 36-38; 39-41; or 36-41.

[0054] The TCRs, polypeptides, and proteins of the invention (including functional portions and functional variants) can be of any length, i.e., can comprise any number of amino acids, provided that the TCRs, polypeptides, or proteins (or functional portions or functional variants thereof) retain their biological activity, e.g., the ability to specifically bind to MAGE-A3 or MAGE-A12; detect cancer in a host; or treat or prevent cancer in a host, etc. For example, the polypeptide can be in the range of from about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids in length. In this regard, the polypeptides of the invention also include oligopeptides.

[0055] The TCRs, polypeptides, and proteins of the invention (including functional portions and functional variants) of the invention can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4- nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

[0056] The TCRs, polypeptides, and proteins of the invention (including functional portions and functional variants) can be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

[0057] When the TCRs, polypeptides, and proteins of the invention (including functional portions and functional variants) are in the form of a salt, preferably, the polypeptides are in the form of a pharmaceutically acceptable salt. Suitable pharmaceutically acceptable acid addition salts include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids, for example, *p*-toluenesulphonic acid.

[0058] The TCR, polypeptide, and/or protein of the invention (including functional portions and functional variants thereof) can be obtained by methods known in the art. Suitable methods of *de novo* synthesizing polypeptides and proteins are described in references, such as Chan et al., Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2005; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2000; and U.S. Patent No. 5,449,752. Also, polypeptides and proteins can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. See, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 1994. Further, some of the TCRs, polypeptides, and proteins of the invention (including functional portions and functional variants thereof) can be isolated and/or purified from a source, such as a plant, a bacterium, an insect, a mammal, e.g., a rat, a human, etc. Methods of isolation and purification are well-known in the art. Alternatively, the TCRs, polypeptides, and/or proteins described herein (including functional portions and functional variants thereof) can be commercially synthesized by companies, such as Synpep (Dublin, CA), Peptide Technologies Corp. (Gaithersburg, MD), and Multiple Peptide Systems (San Diego, CA). In this respect, the inventive TCRs, polypeptides, and proteins can be synthetic, recombinant, isolated, and/or purified.

[0059] Included in the scope of the invention are conjugates, e.g., bioconjugates, comprising any of the inventive TCRs, polypeptides, or proteins (including any of the functional portions or variants thereof), nucleic acids, recombinant expression vectors, host cells, populations of host cells, or antibodies, or antigen binding portions thereof. Conjugates, as well as methods of synthesizing conjugates in general, are known in the art (See, for instance, Hudecz, F., *Methods Mol. Biol.* 298: 209-223 (2005) and Kirin et al., *Inorg Chem.* 44(15): 5405-5415 (2005)).

[0060] By "nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. It is generally preferred that the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

[0061] Preferably, the nucleic acids of the invention are recombinant. As used herein, the term "recombinant" refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be *in vitro* replication or *in vivo* replication.

[0062] The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Sambrook et al., *supra*, and Ausubel et al., *supra*. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N^6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N^6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N^6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Macromolecular Resources (Fort Collins, CO) and Synthegen (Houston, TX).

[0063] The nucleic acid can comprise any nucleotide sequence which encodes any of the TCRs, polypeptides, or proteins, or functional portions or functional variants thereof described herein. For example, the nucleic acid can comprise, consist, or consist essentially of any one or more of the nucleotide sequence SEQ ID NOs: 46-49.

[0064] The invention also provides a nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

[0065] The nucleotide sequence which hybridizes under stringent conditions preferably hybridizes under high stringency conditions. By "high stringency conditions" is meant that the nucleotide sequence specifically hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions which would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (e.g., 3-10 bases) that matched the nucleotide sequence. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70 °C. Such high stringency conditions tolerate little, if any, mismatch between the nucleotide sequence and the template or target strand, and are particularly suitable for detecting expression of any of the inventive TCRs. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

[0066] The invention also provides a nucleic acid comprising a nucleotide sequence that is at least about 70% or more, e.g., about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to any of the nucleic acids described herein.

[0067] The nucleic acids of the invention can be incorporated into a recombinant expression vector. In this regard, the invention provides recombinant expression vectors comprising any of the nucleic acids of the invention. For purposes herein, the term "recombinant expression vector" means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors of the invention are not naturally-occurring as a whole. However, parts of the vectors can be naturally-occurring. The inventive recombinant expression vectors can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring, non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages does not hinder the transcription or replication of the vector.

[0068] The recombinant expression vector of the invention can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA). Bacteriophage vectors, such as λGT10, λGT11, λZapII (Stratagene), λEMBL4, and λNM1149, also can be used. Examples of plant expression vectors include pBI01, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech). Preferably, the recombinant expression vector is a viral vector, e.g., a retroviral vector.

[0069] The recombinant expression vectors of the invention can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., *supra*, and Ausubel et al., *supra*. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColEl, 2 μ plasmid, λ, SV40, bovine papilloma virus, and the like.

[0070] Desirably, the recombinant expression vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based.

[0071] The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected hosts. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[0072] The recombinant expression vector can comprise a native or nonnative promoter operably linked to the nucleotide sequence encoding the TCR, polypeptide, or protein (including functional portions and functional variants thereof), or to the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the TCR, polypeptide, or protein. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter, e.g., a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, and a promoter found in the long-terminal repeat of the murine stem cell virus.

[0073] The inventive recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression. Further, the recombinant expression vectors can be made to include a suicide gene.

[0074] As used herein, the term "suicide gene" refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, e.g., a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art (see, for example, Suicide Gene Therapy: Methods and Reviews, Springer, Caroline J. (Cancer Research UK Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, UK), Humana Press, 2004) and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine daminase, purine nucleoside phosphorylase, and nitroreductase.

[0075] Another embodiment of the invention further provides a host cell comprising any of the recombinant expression vectors described herein. As used herein, the term "host cell" refers to any type of cell that can contain the inventive recombinant expression vector. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5α *E. coli* cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell is preferably a prokaryotic cell, e.g., a DH5α cell. For purposes of producing a recombinant TCR, polypeptide, or protein, the host cell is preferably a mammalian cell. Most preferably, the host cell is a human cell. While the host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage, the host cell preferably is a peripheral blood lymphocyte (PBL) or a peripheral blood mononuclear cell (PBMC). More preferably, the host cell is a T cell.

[0076] For purposes herein, the T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupT1, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. Preferably, the T cell is a human T cell. More preferably, the T cell is a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, $CD4^+/CD8^+$ double positive T cells, $CD4^+$ helper T cells, e.g., Th_1 and Th_2 cells, $CD8^+$ T cells (e.g., cytotoxic T cells), tumor infiltrating lymphocytes (TILs), memory T cells (e.g., central memory T cells and effector memory T cells), naïve T cells, and the like. Preferably, the T cell is a $CD8^+$ T cell or a $CD4^+$ T cell.

[0077] Also provided by the invention is a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, e.g., a host cell (e.g., a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, e.g., a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cells, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly of host cells (e.g., consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

[0078] The invention further provides an antibody, or antigen binding portion thereof, which specifically binds to a functional portion of any of the TCRs described herein. Preferably, the functional portion specifically binds to the cancer antigen, e.g., the functional portion comprising the amino acid sequence SEQ ID NO: 5, 16, 26, or 36 (CDR1 of α chain), 6, 17, 27, or 37 (CDR2 of α chain), 7, 18, 28, or 38 (CDR3 of α chain), 8, 19, 29, or 39 (CDR1 of β chain), 9, 20, 30, or 40 (CDR2 of β chain), 10, 21, 31, or 41 (CDR3 of β chain), SEQ ID NO: 11, 22, 32, or 42 (variable region of α chain), SEQ ID NO: 12, 23, 33, or 43 (variable region of β chain), or a combination thereof, e.g., 5-7; 8-10; 5-10; 16-18, 19-21; 16-21; 26-28; 29-31; 26-31; 36-38; 39-41; or 36-41. More preferably, the functional portion comprises the amino acid sequences of SEQ ID NOs: 5-10 or SEQ ID NOs: 26-31. In a preferred embodiment, the antibody, or antigen binding portion thereof, binds to an epitope which is formed by all 6 CDRs (CDR1-3 of the alpha chain and CDR1-3 of the beta chain). The antibody can be any type of immunoglobulin that is known in the art. For instance, the antibody can be of any isotype, e.g., IgA, IgD, IgE, IgG, IgM, etc. The antibody can be monoclonal or polyclonal. The antibody can be a naturally-occurring antibody, e.g., an antibody isolated and/or purified from a mammal, e.g., mouse, rabbit, goat, horse, chicken, hamster, human, etc. Alternatively, the antibody can be a genetically-engineered antibody, e.g., a humanized antibody or a chimeric antibody. The antibody can be in monomeric or polymeric form. Also, the antibody can have any level of affinity or avidity for the functional portion of the inventive TCR. Desirably, the antibody is specific for the functional portion of the inventive TCR, such that there is minimal cross-reaction with other peptides or proteins.

[0079] Methods of testing antibodies for the ability to bind to any functional portion of the inventive TCR are known in the art and include any antibody-antigen binding assay, such as, for example, radioimmunoassay (RIA), ELISA, Western blot, immunoprecipitation, and competitive inhibition assays (see, e.g., Janeway et al., *infra*, and U.S. Patent Application Publication No. 2002/0197266 A1).

[0080] Suitable methods of making antibodies are known in the art. For instance, standard hybridoma methods are described in, e.g., Köhler and Milstein, *Eur. J.* Immunol., *5*, 511-519 (1976), Harlow and Lane (eds.), *Antibodies: A Laboratory Manual*, CSH Press (1988), and C.A. Janeway et al. (eds.), *Immunobiology*, 5th Ed., Garland Publishing, New York, NY (2001)). Alternatively, other methods, such as EBV-hybridoma methods (Haskard and Archer, *J. Immunol. Methods*, *74*(2), 361-67 (1984), and Roder et al., *Methods Enzymol.*, *121*, 140-67 (1986)), and bacteriophage vector expression systems (see, e.g., Huse et al., *Science*, *246*, 1275-81 (1989)) are known in the art. Further, methods of producing antibodies in non-human animals are described in, e.g., U.S. Patents 5,545,806, 5,569,825, and 5,714,352, and U.S. Patent Application Publication No. 2002/0197266 A1.

[0081] Phage display furthermore can be used to generate the antibody of the invention. In this regard, phage libraries encoding antigen-binding variable (V) domains of antibodies can be generated using standard molecular biology and recombinant DNA techniques (see, e.g., Sambrook et al. (eds.), *Molecular Cloning, A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, New York (2001)). Phage encoding a variable region with the desired specificity are selected for specific binding to the desired antigen, and a complete or partial antibody is reconstituted comprising the selected variable domain. Nucleic acid sequences encoding the reconstituted antibody are introduced into a suitable cell line, such as a myeloma cell used for hybridoma production, such that antibodies having the characteristics of monoclonal antibodies are secreted by the cell (see, e.g., Janeway et al., *supra*, Huse et al., *supra*, and U.S. Patent 6,265,150).

[0082] Antibodies can be produced by transgenic mice that are transgenic for specific heavy and light chain immunoglobulin genes. Such methods are known in the art and described in, for example U.S. Patents 5,545,806 and 5,569,825, and Janeway et al., *supra*.

[0083] Methods for generating humanized antibodies are well known in the art and are described in detail in, for example, Janeway et al., *supra*, U.S. Patents 5,225,539, 5,585,089 and 5,693,761, European Patent No. 0239400 B1, and United Kingdom Patent No. 2188638. Humanized antibodies can also be generated using the antibody resurfacing technology described in, for example, U.S. Patent 5,639,641 and Pedersen et al., *J. Mol. Biol.*, *235*, 959-973 (1994).

[0084] The invention also provides antigen binding portions of any of the antibodies described herein. The antigen binding portion can be any portion that has at least one antigen binding site, such as Fab, $F(ab')_2$, dsFv, sFv, diabodies, and triabodies.

[0085] A single-chain variable region fragment (sFv) antibody fragment, which consists of a truncated Fab fragment comprising the variable (V) domain of an antibody heavy chain linked to a V domain of a light antibody chain via a synthetic peptide, can be generated using routine recombinant DNA technology techniques (see, e.g., Janeway et al., *supra*). Similarly, disulfide-stabilized variable region fragments (dsFv) can be prepared by recombinant DNA technology (see, e.g., Reiter et al., *Protein Engineering, 7*, 697-704 (1994)). Antibody fragments of the invention, however, are not limited to these exemplary types of antibody fragments.

[0086] Also, the antibody, or antigen binding portion thereof, can be modified to comprise a detectable label, such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), and element particles (e.g., gold particles).

[0087] The inventive TCRs, polypeptides, proteins, (including functional portions and functional variants thereof), nucleic acids, recombinant expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof), can be isolated and/or purified. The term "isolated" as used herein means having been removed from its natural environment. The term "purified" as used herein means having been increased in purity, wherein "purity" is a relative term, and not to be necessarily construed as absolute purity. For example, the purity can be at least about 50%, can be greater than 60%, 70%, 80%, 90%, 95%, or can be 100%.

[0088] The inventive TCRs, polypeptides, proteins (including functional portions and variants thereof), nucleic acids, recombinant expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof), all of which are collectively referred to as "inventive TCR materials" hereinafter, can be formulated into a composition, such as a pharmaceutical composition. In this regard, the invention provides a pharmaceutical composition comprising any of the TCRs, polypeptides, proteins, functional portions, functional variants, nucleic acids, expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof), and a pharmaceutically acceptable carrier. The inventive pharmaceutical compositions containing any of the inventive TCR materials can comprise more than one inventive TCR material, e.g., a polypeptide and a nucleic acid, or two or more different TCRs. Alternatively, the pharmaceutical composition can comprise an inventive TCR material in combination with another pharmaceutically active agents or drugs, such as a chemotherapeutic agents, e.g., asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc.

[0089] Preferably, the carrier is a pharmaceutically acceptable carrier. With respect to pharmaceutical compositions, the carrier can be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the active compound(s), and by the route of administration. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active agent(s) and one which has no detrimental side effects or toxicity under the conditions of use.

[0090] The choice of carrier will be determined in part by the particular inventive TCR material, as well as by the particular method used to administer the inventive TCR material. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention. The following formulations for oral, aerosol, parenteral, subcutaneous, intravenous, intramuscular, intraarterial, intrathecal, and interperitoneal administration are exemplary and are in no way limiting. More than one route can be used to administer the inventive TCR materials, and in certain instances, a particular route can provide a more immediate and more effective response than another route.

[0091] Topical formulations are well-known to those of skill in the art. Such formulations are particularly suitable in the context of the invention for application to the skin.

[0092] Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the inventive TCR material dissolved in diluents, such as water, saline, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and other pharmacologically compatible excipients. Lozenge forms can comprise the inventive TCR material in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the inventive TCR material in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to, such excipients as are known in the art.

[0093] The inventive TCR material, alone or in combination with other suitable components, can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer. Such spray formulations also may be used to spray mucosa.

[0094] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The inventive TCR material can be administered in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol or hexadecyl alcohol, a glycol, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol, ketals such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, poly(ethyleneglycol) 400, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

[0095] Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[0096] Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-β-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[0097] The parenteral formulations will typically contain from about 0.5% to about 25%, or more, by weight of the inventive TCR material in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0098] Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J.B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)). Preferably, when administering cells, e.g., T cells, the cells are administered via injection.

[0099] It will be appreciated by one of skill in the art that, in addition to the above-described pharmaceutical compositions, the inventive TCR materials of the invention can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes.

[0100] For purposes of the invention, the amount or dose of the inventive TCR material administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject or animal over a reasonable time frame. For example, the dose of the inventive TCR material should be sufficient to bind to a cancer antigen, or detect, treat or prevent cancer in a period of from about 2 hours or longer, e.g., 12 to 24 or more hours, from the time of administration. In certain embodiments, the time period could be even longer. The dose will be determined by the efficacy of the particular inventive TCR material and the condition of the animal (e.g., human), as well as the body weight of the animal (e.g., human) to be treated.

[0101] Many assays for determining an administered dose are known in the art. For purposes of the invention, an assay, which comprises comparing the extent to which target cells are lysed or IFN-γ is secreted by T cells expressing the inventive TCR, polypeptide, or protein upon administration of a given dose of such T cells to a mammal among a set of mammals of which is each given a different dose of the T cells, could be used to determine a starting dose to be administered to a mammal. The extent to which target cells are lysed or IFN-γ is secreted upon administration of a certain dose can be assayed by methods known in the art.

[0102] The dose of the inventive TCR material also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular inventive TCR material. Typically, the attending physician will decide the dosage of the inventive TCR material with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, inventive TCR material to be administered, route of administration, and the severity of the condition being treated. By way of example and not intending to limit the invention, the dose of the inventive TCR material can be about 0.001 to about 1000 mg/kg body weight of the subject being treated/day or more, from about 0.01 to about 10 mg/kg body weight/day or more, or about 0.01 mg to about 1 mg/kg body weight/day or more. In an embodiment in which the inventive TCR material is a population of cells, the number of cells administered may vary, e.g., from about 1×10^6 to about 1×10^{11} cells or more.

[0103] One of ordinary skill in the art will readily appreciate that the inventive TCR materials of the invention can be modified in any number of ways, such that the therapeutic or prophylactic efficacy of the inventive TCR materials is increased through the modification. For instance, the inventive TCR materials can be conjugated either directly or indirectly through a bridge to a targeting moiety. The practice of conjugating compounds, e.g., inventive TCR materials, to targeting moieties is known in the art. See, for instance, Wadwa et al., *J. Drug Targeting* 3: 111 (1995) and U.S. Patent 5,087,616. The term "targeting moiety" as used herein, refers to any molecule or agent that specifically recognizes and binds to a cell-surface receptor, such that the targeting moiety directs the delivery of the inventive TCR materials to a population of cells on which surface the receptor is expressed. Targeting moieties include, but are not limited to, antibodies, or fragments thereof, peptides, hormones, growth factors, cytokines, and any other natural or non-natural ligands, which bind to cell surface receptors (e.g., Epithelial Growth Factor Receptor (EGFR), T-cell receptor (TCR), B-cell receptor (BCR), CD28, Platelet-derived Growth Factor Receptor (PDGF), nicotinic acetylcholine receptor (nAChR), etc.). The term "bridge" as used herein, refers to any agent or molecule that links the inventive TCR materials to the targeting moiety. One of ordinary skill in the art recognizes that sites on the inventive TCR materials, which are not necessary for the function of the inventive TCR materials, are ideal sites for attaching a bridge and/or a targeting moiety, provided that the bridge and/or targeting moiety, once attached to the inventive TCR materials, do(es) not interfere with the function of the inventive TCR materials, i.e., the ability to bind to MAGE-A3 or MAGE-A12; or to detect, treat, or prevent cancer.

[0104] Alternatively, the inventive TCR materials can be modified into a depot form, such that the manner in which the inventive TCR materials is released into the body to which it is administered is controlled with respect to time and location within the body (see, for example, U.S. Patent 4,450,150). Depot forms of inventive TCR materials can be, for example, an implantable composition comprising the inventive TCR materials and a porous or non-porous material, such as a polymer, wherein the inventive TCR materials is encapsulated by or diffused throughout the material and/or degradation of the non-porous material. The depot is then implanted into the desired location within the body and the inventive TCR materials are released from the implant at a predetermined rate.

[0105] It is contemplated that the inventive pharmaceutical compositions, TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, or populations of cells can be used in methods of treating or preventing cancer. Without being bound to a particular theory, the inventive TCRs are believed to bind specifically to MAGE-A3 MAGE-A12, such that the TCR (or related inventive polypeptide or protein) when expressed by a cell is able to mediate an immune response against a target cell expressing MAGE-A3 or MAGE-A12. In this regard, the invention provides a method of treating or preventing cancer in a host, comprising administering to the host any of the pharmaceutical compostions, TCRs, polypeptides, or proteins described herein, any nucleic acid or recombinant expression vector comprising a nucleotide sequence encoding any of the TCRs, polypeptides, proteins described herein, or any host cell or population of cells comprising a recombinant vector which encodes any of the TCRs, polypeptides, or proteins described herein, in an amount effective to treat or prevent cancer in the host.

[0106] The terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of treatment or prevention of cancer in a host. Furthermore, the treatment or prevention provided by the inventive method can include treatment or prevention of one or more conditions or symptoms of the disease, e.g., cancer, being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

[0107] Also provided is a method of detecting the presence of cancer in a host. The method comprises (i) contacting a sample comprising cells of the cancer with any of the inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or antibodies, or antigen binding portions thereof, described herein, thereby forming a complex, and detecting the complex, wherein detection of the complex is indicative of the presence of cancer in the host.

[0108] With respect to the inventive method of detecting cancer in a host, the sample of cells of the cancer can be a sample comprising whole cells, lysates thereof, or a fraction of the whole cell lysates, e.g., a nuclear or cytoplasmic fraction, a whole protein fraction, or a nucleic acid fraction.

[0109] For purposes of the inventive detecting method, the contacting can take place *in vitro* or *in vivo* with respect to the host. Preferably, the contacting is *in vitro*.

[0110] Also, detection of the complex can occur through any number of ways known in the art. For instance, the inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or antibodies, or antigen binding portions thereof, described herein, can be labeled with a detectable label such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), and element particles (e.g., gold particles).

[0111] For purposes of the inventive methods, wherein host cells or populations of cells are administered, the cells can be cells that are allogeneic or autologous to the host. Preferably, the cells are autologous to the host.

[0112] With respect to the inventive methods, the cancer can be any cancer, including any of sarcomas (e.g., synovial sarcoma, osteogenic sarcoma, leiomyosarcoma uteri, and alveolar rhabdomyosarcoma), lymphomas (e.g., Hodgkin lymphoma and non-Hodgkin lymphoma), hepatocellular carcinoma, glioma, head cancers (e.g., squamous cell carcinoma), neck cancers (e.g., squamous cell carcinoma), acute lymphocytic cancer, leukemias (e.g., acute myeloid leukemia and chronic lymphocytic leukemia), bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic myeloid cancer, colon cancers (e.g., colon carcinoma), esophageal cancer, cervical cancer, gastric cancer, gastrointestinal carcinoid tumor, hypopharynx cancer, larynx cancer, liver cancers (e.g., hepatocellular carcinoma), lung cancers (e.g., non-small cell lung carcinoma), malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, kidney cancers (e.g., renal cell carcinoma), small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, and urothelial cancers (e.g., ureter cancer and urinary bladder cancer).

[0113] The host referred to in the inventive methods can be any host. Preferably, the host is a mammal. As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). An especially preferred mammal is the human.

[0114] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0115] This example demonstrates the cloning of TCR genes from T cell clones and the generation of TCR constructs.

[0116] Four T cell clones were initially identified that recognized epitopes of the MAGE-A gene family in the context of the dominant class I alleles HLA-A*01 and C*07. Approximately 30% of the melanoma patient population expresses HLA-A*01, and more than 95% of HLA-A*01$^+$ individuals express the HLA-A*0101 sub-type, while more than 50% of melanoma patients express one of the two dominant HLA-C*07 sub-types, C*07:01 and C* 07:02.

[0117] The expressed TCR α and β chains were isolated from two clones, A10 and 13-18, that recognized residues 168-176 of protein MAGE-A3 (MAGE-A3:168-176) in the context of HLA-A*01. In addition, HLA-C*07 restricted TCRs recognizing a peptide corresponding to residues 170-178 of the MAGE-A12 protein (MAGE-A12:170-178) were isolated from clones 502 and FM8.

[0118] The α and β chains encoding functional TCRs were isolated from two MAGE-A12 reactive, HLA-C*07 reactive T cell clones, PHIN LB831-501D/19, referred to "502" (Heidecker et al., *J. Immunol.*, 164: 6041-6045 (2000)) and "FM8" (Panelli et al., *J. Immunol.*, 164: 4382-4392 (2000)), as well as two MAGE-A3 reactive, HLA-A*01 restricted T cell clones, LAU147 CTL1 or 810/A10, referred to "A10" (Parmentier et al., *Nat. Immunol.*, 11: 449-454 (2010)) and NW1000 AVP-1 13-18, referred to "13-18." Briefly, oligo-dT was used to reverse transcribe total RNA isolated from the T cell clones into cDNA using the SMART RACE cDNA amplification kit (Clontech, Mountain View, CA). The TCR α and β chains expressed by the T cell clones were identified by carrying out 5'-RACE reactions using a primer 5'-CACTGTTGCTCTTGAA GTCC-3' (SEQ ID NO: 55) that is complementary to the TCR α chain constant region and 5'-CAGGCAGTAT CTGGAGTCATTGAG-3' (SEQ ID NO: 56) that is complementary to the TCR β chain constant region in combination with adaptor primers from the SMART RNA synthesis kit. After sequencing of the 5'-RACE products, full length gene products were amplified using specific primers designed to amplify the appropriate full length TCR α and β chains. The A10 TCR expresses AV12-1/BV24-1, 13-18 expresses AV12-3/BV15, 502 TCR expresses AV13-1/BV25-1, and FM8 expresses AV38-2/BV4-3.

[0119] Transcripts encoding the paired α and β chains for each of the four T cell clones were inserted into the MSGV1 retroviral expression vectors.

EXAMPLE 2

[0120] This example demonstrates the reactivity of cells expressing anti-MAGE-A3 TCR-A10 (SEQ ID NOs: 13 and 14) and anti-MAGE-A3 TCR 13-18 (SEQ ID NOs: 24 and 25) in response to HLA-A1+/MAGE-A3+ cells.

[0121] Anti-CD3 stimulated T cells transduced with TCR-A10 (SEQ ID NO: 46) and TCR 13-18 (SEQ ID NO: 48) were evaluated for their ability to recognize a panel of HLA-A*01+ melanoma cell lines that express MAGE-A3. Untransduced (UT) and transduced cells were co-cultured overnight with various tumor cell lines (Tables 1A, 1B and Figure 6A), and interferon-gamma (IFN-γ) (pg/ml) was measured.

TABLE 1A

Tumor	HLA-A*01	Copies MAGE-A3
1860 mel	+	12,100
397 mel	+	32,700
SK23 mel	+	18,400
2984 mel	+	14,900
2951 mel	+	12,300
A375 mel	+	3,670
537 mel	+	4,270
1300-A1 mel	+	7,280
1300 mel	-	13,600
2661 RCC	+	<1,000

TABLE 1B

Tumor	HLA-A*01	MAGE-A3
2984 mel	+	+
397 mel	+	+
2630 mel	+	+
2556 mel	+	+
526 mel	-	+
624 mel	-	+
2359 mel	-	+
2661 RCC	+	-

[0122] The results indicate that six of the eight HLA-A*01+/MAGE-A3+ melanoma cell lines that were evaluated stimulated higher levels of IFN-γ release from TCR A10 than from TCR 13-18-transduced T cells (Figure 1A). Lower levels of IFN-γ were released following the co-culture of TCR-transduced T cells with two HLA-A1+ melanoma cell lines that expressed relatively low levels of MAGE-A3, A375 mel and 537 mel, but TCR A10-transduced T cells released higher levels of IFN-γ than TCR 13-18 transduced T cells in response to these target cells. These responses were restricted by HLA-A1 because 1300 mel, which lacked expression of HLA-A1, failed to stimulate IFN-γ release from TCR A10 and TCR 13-18-transduced cells, whereas a cell line generated by transfection of the parental 1300 mel cell line with HLA-A*01, designated 1300-A1, stimulated IFN-γ release from TCR A10 and TCR 13-18 transduced T cells. An HLA-A*01+ renal cancer cell line that lacked expression of MAGE-A3, 2661 RCC, failed to stimulate significant IFN-γ release from TCR A10 and TCR 13-18 transduced T cells. These results demonstrate that cells expressing TCR A10 release higher levels of IFN-γ than cells expressing TCR-13-18 when co-cultured with MAGE-A3$^+$/HLA-A1$^+$ target cells. These results also demonstrate that TCR A10 and TCR-13-18 are stimulated in the presence of MAGE-A3+/HLA-A1+ target cells.

[0123] The results of co-culture assays carried out with transduced PBMC demonstrated that TCR A10-transduced T cells generated high levels of IFN-gamma in response to the HLA-A*01+/MAGE-A3+ tumor cell lines 397 mel, 2984 mel, and 2556 mel. The cytokine levels were between five and ten times those generated from TCR 13-18 transduced T cells (Fig. 6A). The MAGE-A3+ but HLA-A*01 negative cell lines 562, 624 and 2359 mel, as well as the MAGE-A3 negative but HLA-A*01+ renal cancer cell line 2661 RCC failed to stimulate significant levels of cytokine from either TCR A10 or 13-18 transduced T cells (Fig. 6A).

[0124] The levels of transduction of the TCRs were evaluated using a quantitative PCR assay carried out using genomic DNA with forward (SEQ ID NO: 58) and reverse (SEQ ID NO: 59) primers and a probe (SEQ ID NO: 60) designed to specifically detect the MSGV1 retroviral LTR but not human endogenous retroviral sequences. Levels of the amplified products were normalized to a positive control sample of PBMC that had been transduced with a TCR directed against the NY-ESO-1:157-165 epitope that was estimated to contain approximately 80% transduced T cells by staining with an NY-ESO-1 tetramer.

[0125] The differences in activity of T cells transduced with the A10 or 13-18 TCR did not appear to be due to differences in the frequency of transduction with the two TCRs, as they appeared to be equivalent (Fig. 6B). In addition, T cells transduced with the A10 TCR recognized target cells incubated with a minimum concentration of 0.5 nM MAGE-A3 168-176 peptide, which was a 10-fold lower concentration than required for recognition by cells transduced with the 13-18 TCR (Fig. 6C), indicating that the A10 TCR possessed a higher functional avidity than the 13-18 TCR.

[0126] The ability of fresh, un-cultured tumor cells to stimulate T cells transduced with either TCR A10 (SEQ ID NO: 46) or DMF5 (a TCR directed against the HLA-A*0201/MART-1:27-35 T cell epitope), was evaluated. Untransduced (UT) and transduced cells were co-cultured with various fresh tumors, and IFN-γ (pg/ml) was measured.

[0127] The results indicated that TCR A10 transduced T cells recognized four of the four HLA-A*01+/MAGE-A3+ fresh tumors that were tested (FrTu 2767, FrTu 3178, FrTu 2823 and FrTu 3068), and DMF5-transduced T cells recognized both of the HLA-A*0201+/MART-1+ fresh tumor cells that were tested (FrTu 2851 and FrTu 3242) (Figure 1B). The TCR A10 transduced T cells failed to recognize HLA-A*0201+ fresh tumors, while DMF5 transduced T cells failed to recognize HLA-A*01+ fresh tumors, indicating that the IFN-γ secretion by TCR A10 was a HLA-A1+/MAGE-A3+-specific response.

[0128] T cells that were transduced with TCR A10 and 13-18 recognized five of six MAGE-A3+ and HLA-A*01^{+} fresh tumors (FrTu), FrTu 3178, 2767, 2823, 2830 and 3068, but did not recognize either FrTu 2685, an HLA-A*01^{+} fresh tumor that lacked expression of MAGE-A3 or the three MAGE-A3^{+} fresh tumors, FrTu 2181, 3242 and 2803, that lacked expression of HLA-A*01 (Fig. 7A; Table 1C).

TABLE 1C

Fresh Tumor	HLA-A*01	MAGE-A3
3178	+	+
2767	+	+
2823	+	+
2830	+	+
3068	+	+
2268	+	+
2685	+	-
2181	-	+
3242	-	+
2803	-	+

EXAMPLE 3

[0129] This example demonstrates the reactivity of cells expressing anti-MAGE-A12 TCR 502 (SEQ ID NOs: 34 and 35) or anti-MAGE-A12 TCR FM8 (SEQ ID NOs: 44 and 45) in response to co-culture with HLA-Cw*07+/MAGE-A12+ cells.

[0130] Anti-CD3 stimulated CD8+ T cells isolated from two patient PBMC samples were transduced with a control construct encoding the truncated human low affinity nerve growth factor receptor (NGFR), TCR 502 (SEQ ID NO: 47), or TCR FM8 (SEQ ID NO: 49) were evaluated for their ability to recognize a panel of Cw*07+ melanoma cell lines that express MAGE-A12.

[0131] Expression of the MAGE-A12 gene product was evaluated by Q-PCR using two primers (SEQ ID NOs: 61 and 62) designed to specifically amplify the MAGE-A12 gene product but not other members of the MAGE-A gene family as well as a MAGE-A12 specific probe (SEQ ID NO: 63). Antigen expression was determined using plasmid controls as standards for estimating copy numbers and using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for normalization. Tumor cell lines and fresh tumors expressing greater than 1,000 copies of MAGE-A12 per 106 copies of GAPDH were denoted as positive for MAGE-A12 expression.

[0132] Transduced cells were co-cultured overnight with various tumor cell lines (Table 2A; Figures 6D and 6F), and IFN-γ (pg/ml) was measured.

TABLE 2A

Stimulator	HLA-C allele	MAGE-A12
1910 mel	0701, 0303	+
586 mel	0701	+
2359 mel	0701, 16	+
F002 mel	0701, 1203	+
1300 mel	0702	+
624 mel	0702, 0802	+
SK23 mel	0701, 0702	+
1909 mel	0701, 0702	+
1011 mel	0702	-
397 mel	0701	+
526 mel	-	+
2556 mel	-	+
2984 mel	-	+
2630 mel	0701	-

[0133] The results demonstrated that T-cells transduced with TCR 502 recognized eight of the eight MAGE-A12+ melanoma cell lines tested that express either HLA-Cw*0701 or 0702, whereas T cells transduced with TCR FM8 only recognized melanoma cell lines that express HLA-Cw*0702 (Figures 2A and 2B; see also Fig. 6F). In addition, TCR 502 transduced T cells released higher levels of IFN-γ in response to the HLA-Cw*0702+ targets SK23 mel, 1300 mel and 624 mel as compared to TCR FM8 transduced T cells (Figs. 6D and 6F). The 1011 mel cells, which expressed HLA-Cw*0702 but lacked expression of MAGE-A12, did not stimulate significant cytokine release from cells transduced with TCR 502 or TCR FM8. T cells transduced with a control construct encoding NGFR failed to respond significantly to any of the targets tested. These results demonstrate that cells expressing TCR 502 release higher levels of IFN-γ than cells expressing TCR FM8 when co-cultured with MAGE-A12+/HLA-Cw7 target cells and that TCR 502 recognizes MAGE-A12 in the context of either HLA-Cw0701 or HLA-Cw0702. These results also demonstrate that TCR A502 and TCR FM8 are stimulated in the presence of MAGE-A12+ cells.

[0134] The TCR 502 transduced T cells recognized the HLA-C*0702+, MAGE-A12+ tumor 624 mel as well as two HLA-C*07:01+, MAGE-A12+ tumors, 397 and 2359 mel, whereas FM8 transduced T cells recognized the HLA-C*07:02+ tumor cell line 624 mel but failed to recognize 397 and 2359 mel (Fig. 6D). Neither population of transduced T cells recognized 526, 2556, or 2984 mel, MAGE-A3+ melanoma cell lines that lacked expression of HLA-C*07, or 2630 mel, an HLA-C*07:01+ tumor cell line that lacked expression of MAGE-A12 (Fig. 6D). These differences did not appear to be due to differences in transduction frequencies of the two TCRs (measured as described in Example 2), which appeared to be similar in cells transduced with either TCR (Fig. 6B). In addition, cells transduced with the 502 TCR recognized target cells incubated with a minimum concentration of 2.5 nM MAGE-A12:170-178, a 100-fold lower concentration than that required for recognition by cells transduced with the FM8 TCR (Fig. 6E), indicating that the 502 TCR possessed a higher functional avidity than the FM8 TCR.

[0135] The T cells that were transduced with MAGE-A12 reactive TCRs were then evaluated for their responses to enzymatic digests of fresh, un-cultured tumor cells. The T cells transduced with TCR 502 recognized one of the four MAGE-A12$^+$ fresh tumors that expressed HLA-C*0701, FrTu 3068, and TCR 502 as well as FM8 transduced T cells recognized one of the two MAGE-A12$^+$ tumors that expressed HLA-C*07:02, FrTu 2181 (Fig. 7B; Table 2B). Neither population of TCR transduced T cells recognized the HLA-C*07:01$^-$ and 07:02$^-$ fresh tumors 2767 or 2823, or the MAGE-A12$^-$ tumors 2685, 3242 and 2803 that lacked expression of MAGE-A12.

TABLE 2B

Fresh Tumor	HLA-C*07	MAGE-A12
3068	01	+
2181	02	+
3178	01	+
2830	01	+
2268	01, 02	+
2767	-	+
2823	-	+
2685	01	-
3242	01	-
2803	02	-

EXAMPLE 4

[0136] This example demonstrates the population that may be treated using the inventive TCRs.

[0137] Approximately 28% of the patient population expresses HLA-A*01, and approximately 54% of the patient population expresses HLA-Cw*07. Two dominant subtypes of HLA-Cw*07, Cw*0701 and Cw*0702, are expressed by approximately 27% and approximately 31% of the patient population, respectively. Figure 3A illustrates the cumulative percentage of the population that would be expected to be treatable by the use of TCRs restricted by HLA-A1, HLA-A2, and/or HLA-Cw7 (based upon the percentages of these alleles in the normal Caucasian population).

[0138] Because approximately 30% of patients express high levels of MAGE-A3 and MAGE-A12, the use of the inventive TCRs will allow a significantly higher percentage of patients to be eligible for TCR-based adoptive immunotherapies. Figures 3B and 3C illustrate the cumulative percentage of the human melanoma (Figure 3B) and synovial cell sarcoma (Figure 3C) patient populations that would be expected to be treatable using TCRs that recognize NY-ESO-1 in the context of HLA-A2; MAGE-A3 in the context of HLA-A1; MAGE-A3 and MAGE-A12 in the context of HLA-A2; and/or MAGE-A12 in the context of HLA-Cw7.

EXAMPLE 5

[0139] This example demonstrates the reactivity of cells expressing TCR 502 or TCR FM8 in response to co-culture with HLA-Cw*0701 or HLA-Cw*0702-expressing target cells pulsed with peptides of various proteins from the MAGE family.

[0140] Cells transduced with NGFR, TCR 502 (SEQ ID NO: 47), or TCR FM8 (SEQ ID NO: 49) were co-cultured with cells expressing HLA-Cw*0701 or HLA-Cw*0702. IFN-γ (pg/ml) secretion was measured.

[0141] The results demonstrated that cells transduced with TCR 502 recognize cells expressing HLA-Cw*0701 or HLA-Cw*0702 when pulsed with MAGE-A12 (VRIGHLYIL; SEQ ID NO: 4), and that cells transduced with TCR FM8 recognized cells expressing HLA-Cw*0702 when pulsed with MAGE-A12 (VRIGHLYIL; SEQ ID NO: 4) (Figure 4). Cells transduced with NGFR showed no significant reactivity.

EXAMPLE 6

[0142] This example demonstrates the specificity of the anti-MAGE-A3 and anti-MAGE-A12 TCRs.

[0143] Following stimulation with anti-CD3 antibody, PMBC from a single donor were transduced with PBMC that were untransduced or transduced with anti-MAGE-A12 TCR 502 (SEQ ID NO: 47), anti-MAGE-A12 TCR FM8 (SEQ ID NO: 49), anti-MAGE-A3 TCR A10 (SEQ ID NO: 46), anti-MAGE-A3 TCR 13-18 (SEQ ID NO: 48), or anti-MAGE-A3 TCR 112-120. Thirteen days after stimulation, transduced T cells were incubated with the tumor targets set forth in Table 3 in a standard 4 hour ^{51}Cr release assay.

TABLE 3

	MAGE-A3	MAGE-A12	HLA-A	HLA-C
397 mel	+	+	01/02	0401/0701
624 mel	+	+	02/03	0702/0802
2984 mel	+	+	01/02	06
2661 RCC	-	-	01/02	07

[0144] As shown in Figures 5(A)-5(D), anti-MAGE-A12 TCR 502 specifically lysed tumor cells that expressed MAGE-A12 and HLA-Cw7 and did not lyse tumor cells that lacked expression of MAGE-A12 or HLA-Cw7. Anti-MAGE-A3 TCR A10 specifically lysed tumor cells that expressed MAGE-A3 and HLA-A1 and did not lyse tumor cells that lacked expression of MAGE-A3 or HLA-A1.

EXAMPLE 7

[0145] This example demonstrates the specificity of the anti-MAGE-A3 and anti-MAGE-A12 TCRs.

[0146] The monkey kidney cell line COS-7 was transiently transfected with either HLA-A*01, C*07:01 or C*07:02 plus either MAGE-A3, A1, A2, A4, A6, A9, A10 or A12 overnight. The following day T cells transduced with either TCR A10, 13-18 or un-transduced control cells or TCR 502, FM8 or un-transduced control cells were added and the release of soluble IFN-gamma was evaluated following an overnight co-culture by ELISA.

[0147] T cells transduced with the MAGE-A3-reactive TCR A10 recognize HLA-A1^{+} target cells transfected with MAGE-A3 but failed to recognize targets transfected with

MAGE-A1, A2, A4, A6, A9, A10 or A12 constructs (Fig. 8A) that encoded peptides that differed at between one and three positions from the MAGE-A3:170-178 epitope. The T cells that were transduced with either TCR 502 or FM8 recognized HLA-C*07:02^{+} targets transfected with MAGE-A12 but not MAGE-A3, A1, A2, A4,A6, A9, A10 (Fig. 8B), while T cells transduced with TCR 502 but not FM8 recognized HLA-C*07:01^{+} targets transfected with MAGE-A12 but not the additional MAGE-A family members tested (Fig. 8C).

EXAMPLE 8

[0148] This example demonstrates the reactivity of transduced T cells.

[0149] Purified CD8+ and CD4+ T cells were isolated by negative selection using CD8 and CD4 T lymphocyte enrichment kits (Becton/Dickinson, Franklin Lakes, N.J.), followed by positive selection using CD8 and CD4 magnetic beads (Becton/Dickinson). The isolated CD8+ and CD4+ cells were estimated by fluorescence activated cell sorting (FACS) analysis to contain less than 1% contaminating CD4+ and CD8+ T cells, respectively.

[0150] The responses of separated populations of CD8+ and CD4+ T cells transduced with TCRs to tumor cell targets was then evaluated. Highly purified CD4+ T cells transduced with TCR A10 containing fewer than 1% contaminating CD8+ T cells released low but significant levels of IFN-gamma in response to MAGE-A3+ tumor cell line 397 mel as well as the MAGE-A3+ tumor cell line 1300A1 mel that was stably transfected with HLA-A*01 (Table 4A; Fig. 9B). CD8+ T cells transduced with TCR A10 released interferon-gamma in response to 397 mel or 1300-A1 mel (Table 4A; Fig. 9A). CD8+ T cells transduced with TCR 502 or TCR FM8 released interferon-gamma in response to 624 mel, and TCR 502 released interferon-gamma in response to 397 mel (Figure 9C and Table 4B).

TABLE 4A

Cell Line	HLA-A*01	MAGE-A3
397 mel	+	+
1300 mel	+	+
1300-A1 mel	+	+
624 mel	-	+
2359 mel	-	+
2661 RCC	+	-

TABLE 4B

Cell Line	HLA-C*07	MAGE-A12
397 mel	01	+
624 mel	02	+
2359 mel	01	+
526 mel	-	+
2661 RCC	-	-

[0151] [BLANK]

[0152] The use of the terms "a" and "an" and "the" and "at least one" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term "at least one" followed by a list of one or more items (for example, "at least one of A and B") is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

WE CLAIM:

1. An isolated or purified T cell receptor (TCR) having antigenic specificity for melanoma antigen family A (MAGE A)-3 in the context of HLA-A1 and comprising (a) an alpha chain comprising the amino acid sequences of all of SEQ ID NOs: 5-7 and a beta chain comprising the amino acid sequences of all of SEQ ID NOs: 8-10; or (b) an alpha chain comprising the amino acid sequences of all of SEQ ID NOs: 16-18 and a beta chain comprising the amino acid sequences of all of SEQ ID NOs: 19-21.

2. The isolated or purified TCR of claim 1 having antigenic specificity for a MAGE-A3 epitope comprising EVDPIGHLY (SEQ ID NO: 2).

3. The isolated or purified TCR of claim 2, comprising (a) the amino acid sequences of both of SEQ ID NOs: 11-12 or (b) the amino acid sequences of both of SEQ ID NOs: 22-23.

4. The isolated or purified TCR of claim 3, comprising (a) the amino acid sequences of both of SEQ ID NOs: 13-14 or (b) the amino acid sequences of both of SEQ ID NOs: 24-25.

5. An isolated or purified polypeptide comprising a functional portion of the TCR of any one of claims 1 to 4, wherein the functional portion comprises (a) the amino acid sequences of all of SEQ ID NOs: 5-10 or (b) the amino acid sequences of all of SEQ ID NOs: 16-21.

6. The isolated or purified polypeptide of claim 5, wherein the portion comprises (a) the amino acid sequences of both of SEQ ID NOs: 11-12 or (b) the amino acid sequences of both of SEQ ID NOs: 22-23.

7. The isolated or purified polypeptide of claim 6, wherein the portion comprises (a) the amino acid sequences of both of SEQ ID NOs: 13-14 or (b) both of SEQ ID NOs: 24-25.

8. An isolated or purified protein comprising at least one of the polypeptides of any one of claims 5-7.

Date Reçue/Date Received 2020-10-08

9. An isolated or purified protein, comprising:

(a) a first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 5-7 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 8-10; or

(b) a first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 16-18 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 19-21.

10. The isolated or purified protein of claim 9, comprising:

(a) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 11 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 12; or

(b) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 22 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 23.

11. The isolated or purified protein of claim 10, comprising:

(a) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 13 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 14; or

(b) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 24 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 25.

12. The protein of any one of claims 8-11, wherein the protein is a fusion protein.

13. The protein of any one of claims 8-12, wherein the protein is a recombinant antibody.

14. An isolated or purified nucleic acid comprising a nucleotide sequence encoding the TCR of any one of claims 1-4, the polypeptide of any one of claims 5-7, or the protein of any one of claims 8-13.

Date Reçue/Date Received 2020-10-08

15. The nucleic acid of claim 14, comprising a nucleotide sequence comprising any one of SEQ ID NO: 46 or 48.

16. An isolated or purified nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of the nucleic acid of claim 15.

17. An isolated or purified nucleic acid comprising a nucleotide sequence which hybridizes to the nucleotide sequence of the nucleic acid of any one of claims 14-16 in about 0.02-0.1 M NaCl and at a temperature of about 50 to about 70 °C, wherein the nucleotide sequence encodes a TCR having antigenic specificity for MAGE-A3 in the context of HLA-A1.

18. A recombinant expression vector comprising the nucleic acid of any one of claims 14-17.

19. An isolated host cell comprising the recombinant expression vector of claim 18.

20. The isolated host cell of claim 19, wherein the cell is a peripheral blood lymphocyte (PBL).

21. The isolated host cell of claim 20, wherein the PBL is a T cell.

22. The isolated host cell of claim 19, wherein the cell is a tumor infiltrating lymphocyte (TIL).

23. An isolated or purified population of peripheral blood lymphocytes or TIL comprising at least one host cell of any one of claims 19-22.

24. An antibody, or antigen binding portion thereof, which specifically binds to a functional portion of the TCR of any one of claims 1 to 4, wherein the functional portion

Date Reçue/Date Received 2020-10-08

comprises (a) the amino acid sequences of all of SEQ ID NOs: 5-10 or (b) the amino acid sequences of all of SEQ ID NOs: 16-21.

25. A pharmaceutical composition comprising the TCR of any one of claims 1 to 4, the polypeptide of any one of claims 5 to 7, the protein of any one of claims 8 to 13, the nucleic acid of any one of claims 14 to 17, the recombinant expression vector of claim 18, the host cell of any one of claims 19 to 22, the isolated or purified population of cells of claim 23, or the antibody, or antigen binding portion thereof, of claim 24, and a pharmaceutically acceptable carrier.

26. An *in vitro* method of detecting the presence of cancer in a host, comprising:

(i) contacting a sample comprising cells of the cancer with the TCR of any one of claims 1 to 4, the polypeptide of any one of claims 5 to 7, the protein of any one of claims 8 to 13, the nucleic acid of any one of claims 14 to 17, the recombinant expression vector of claim 18, the host cell of any one of claims 19 to 22, the isolated or purified population of cells of claim 23, or the antibody, or antigen binding portion thereof, of claim 24, thereby forming a complex, and

(ii) detecting the complex,

wherein detection of the complex is indicative of the presence of cancer in the host.

27. Use of the TCR of any one of claims 1 to 4, the polypeptide of any one of claims 5 to 7 the protein of any one of claims 8 to 13, the nucleic acid of any one of claims 14 to 17, the recombinant expression vector of claim 18, the host cell of any one of claims 19 to 22, the isolated or purified population of cells of claim 23, the antibody, or antigen binding portion thereof, of claim 24, or the pharmaceutical composition of claim 25, in the manufacture of a medicament for the treatment or prevention of cancer in the host.

28. The method of claim 26 or the use of claim 27, wherein the cancer is melanoma, breast cancer, leukemia, thyroid cancer, gastric cancer, pancreatic cancer, liver cancer, lung cancer, ovarian cancer, multiple myeloma, esophageal cancer, kidney cancer, head cancers, neck cancers, prostate cancer, or urothelial cancer.

29. The method of claim 26 or 28 or the use of claim 27 or 28, wherein the host cell is a cell that is autologous to the host.

30. The method of any one of claims 26 and 28-29 or the use of any one of claims 27-29, wherein the cells of the population are cells that are autologous to the host.

31. The TCR of any one of claims 1 to 4, the polypeptide of any one of claims 5 to 7, the protein of any one of claims 8 to 13, the nucleic acid of any one of claims 14 to 17, the recombinant expression vector of claim 18, the host cell of any one of claims 19 to 22, the isolated or purified population of cells of claim 23, the antibody, or antigen binding portion thereof, of claim 24, or the pharmaceutical composition of claim 25, for use in the treatment or prevention of cancer in a host.

32. The TCR, polypeptide, protein, nucleic acid, recombinant expression vector, host cell, the isolated or purified population of cells, antibody, or antigen binding portion thereof, or the pharmaceutical composition for the use of claim 31, wherein the cancer is melanoma, breast cancer, leukemia, thyroid cancer, gastric cancer, pancreatic cancer, liver cancer, lung cancer, ovarian cancer, multiple myeloma, esophageal cancer, kidney cancer, head cancers, neck cancers, prostate cancer, or urothelial cancer.

33. The host cell for the use of claim 31 or 32, wherein the host cell is a cell that is autologous to the host.

34. The isolated or purified population of cells for the use of claim 31 or 32, wherein the cells of the isolated or purified population are cells that are autologous to the host.

Date Reçue/Date Received 2020-10-08

FIG. 1A

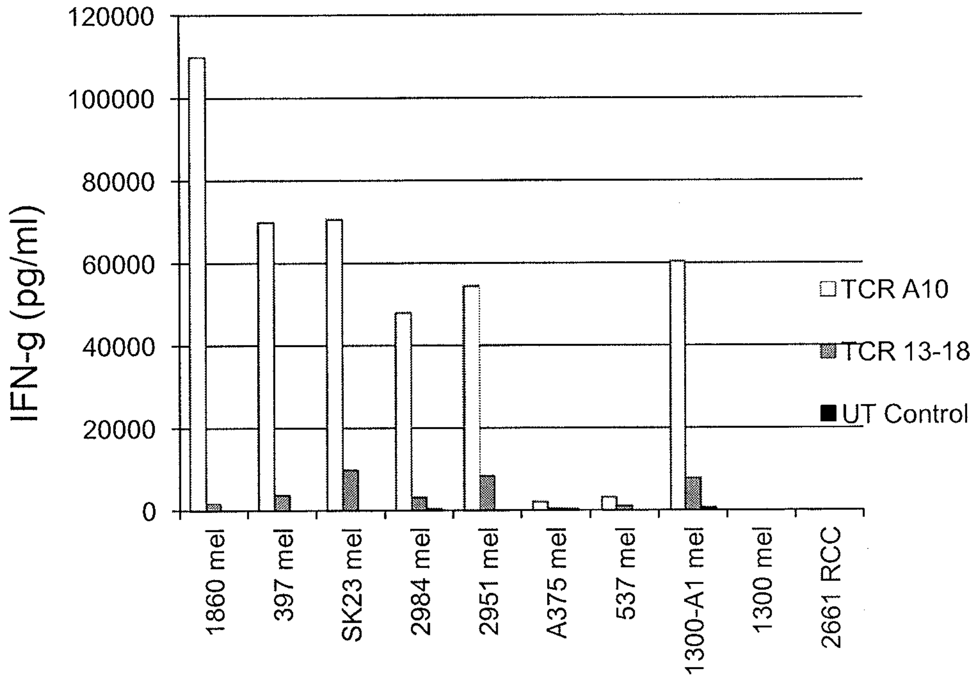

120000
100000
80000
60000
40000
20000
0
IFN-g (pg/ml)
TCR A10
TCR 13-18
UT Control
1860 mel
397 mel
SK23 mel
2984 mel
2951 mel
A375 mel
537 mel
1300-A1 mel
1300 mel
2661 RCC

FIG. 1B

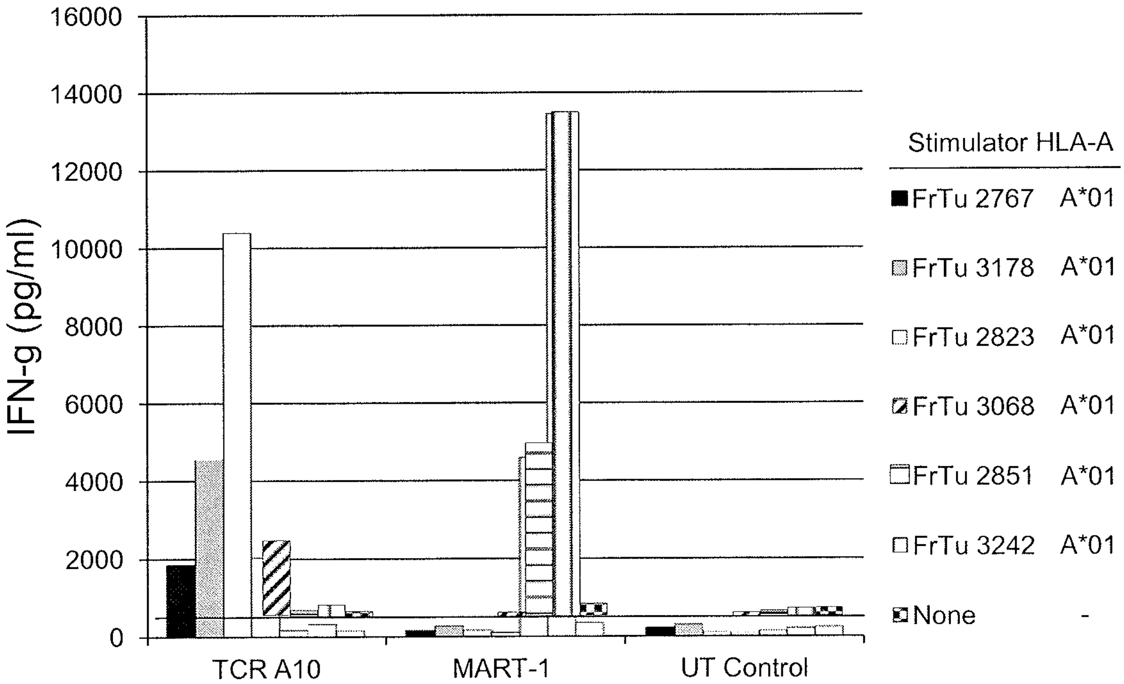

16000
14000
12000
10000
8000
6000
4000
2000
0
IFN-g (pg/ml)
Stimulator HLA-A
FrTu 2767 A*01
FrTu 3178 A*01
FrTu 2823 A*01
FrTu 3068 A*01
FrTu 2851 A*01
FrTu 3242 A*01
None -
TCR A10
MART-1
UT Control

FIG. 2A

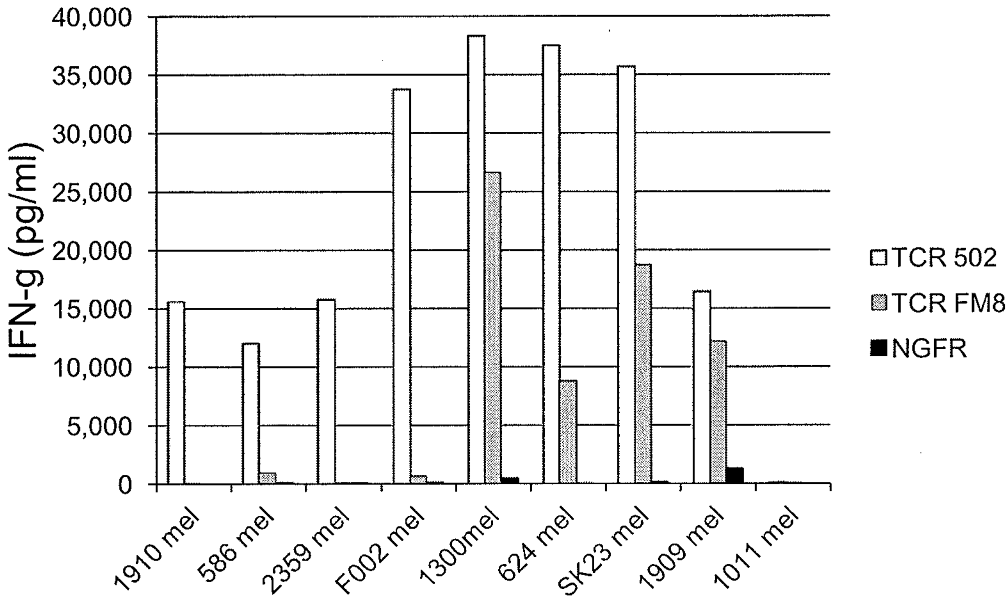

40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
IFN-g (pg/ml)
TCR 502
TCR FM8
NGFR
1910 mel
586 mel
2359 mel
F002 mel
1300mel
624 mel
SK23 mel
1909 mel
1011 mel

FIG. 2B

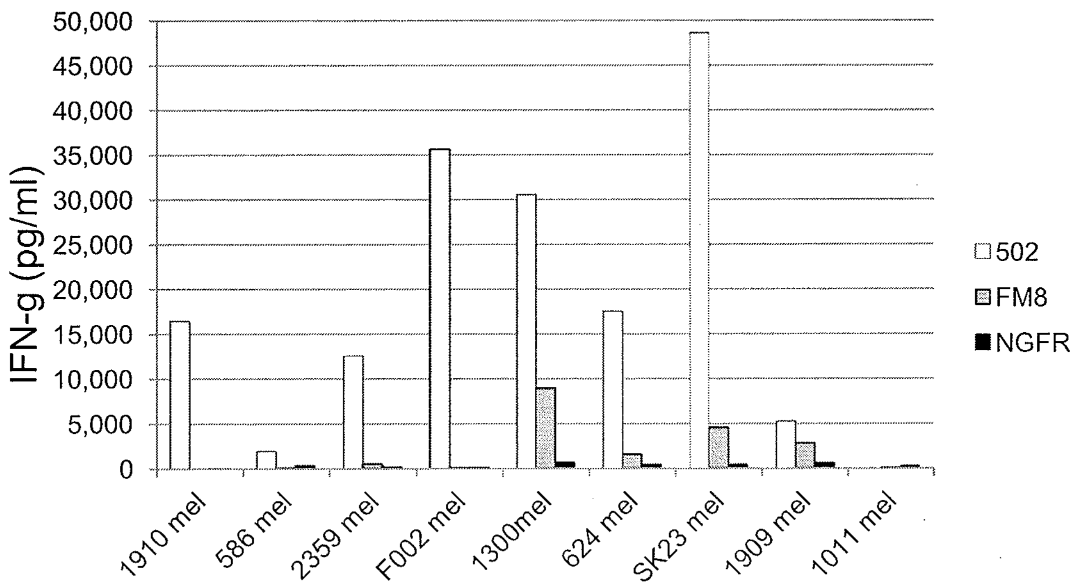

50,000
45,000
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
IFN-g (pg/ml)
502
FM8
NGFR
1910 mel
586 mel
2359 mel
F002 mel
1300mel
624 mel
SK23 mel
1909 mel
1011 mel

FIG. 3

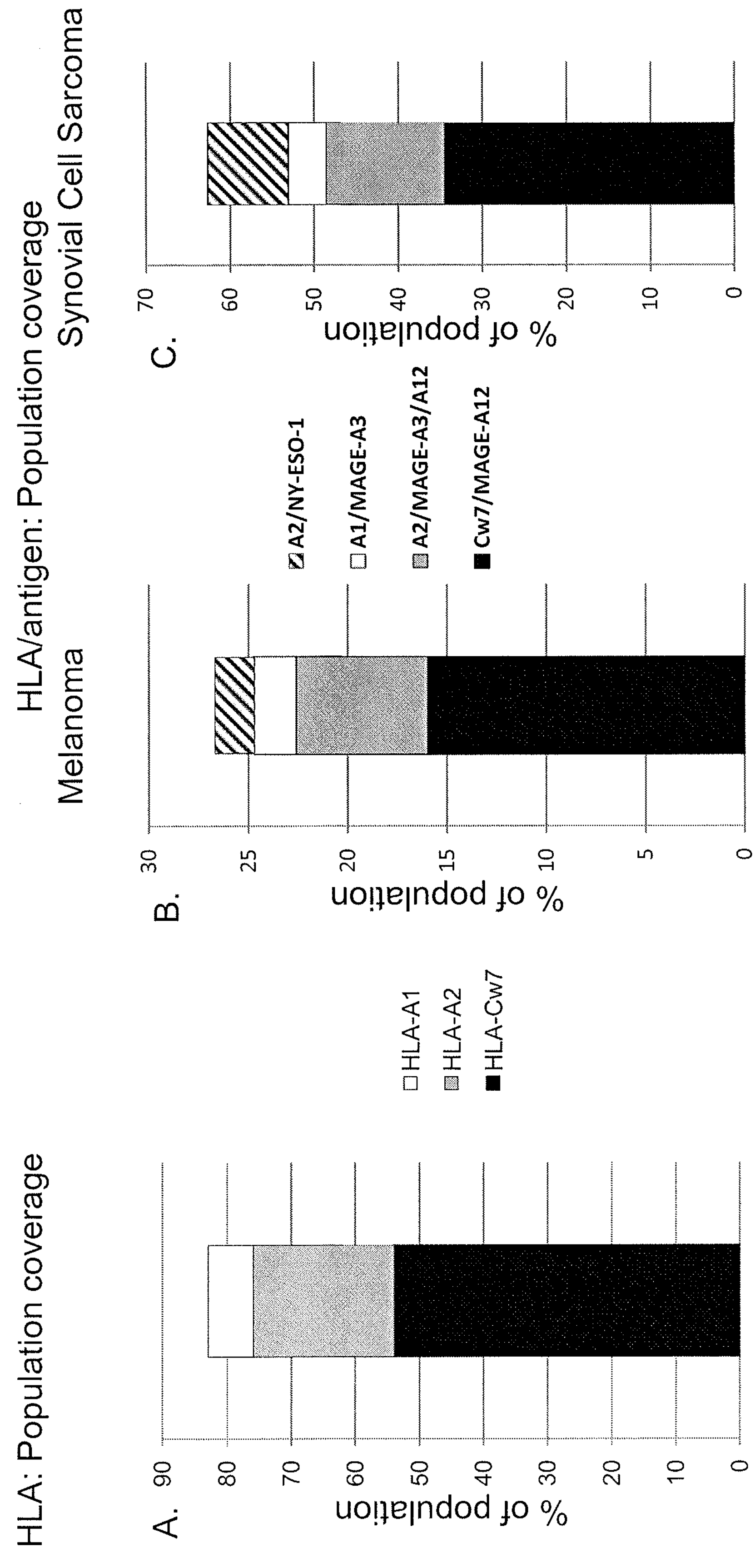

HLA: Population coverage
A.
% of population
HLA-A1
HLA-A2
HLA-Cw7
HLA/antigen: Population coverage
Melanoma
B.
% of population
A2/NY-ESO-1
A1/MAGE-A3
A2/MAGE-A3/A12
Cw7/MAGE-A12
Synovial Cell Sarcoma
C.
% of population

FIG. 4

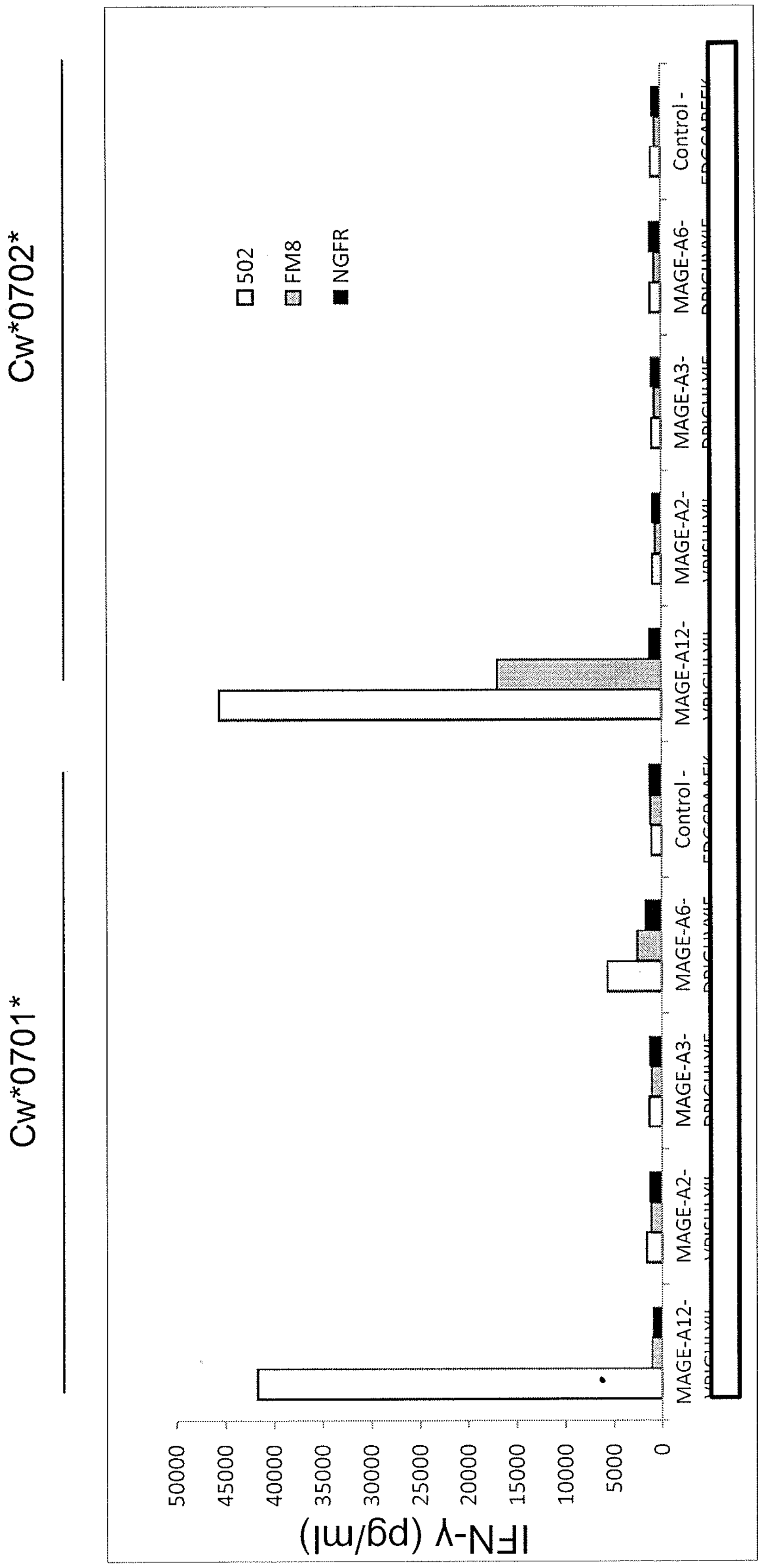
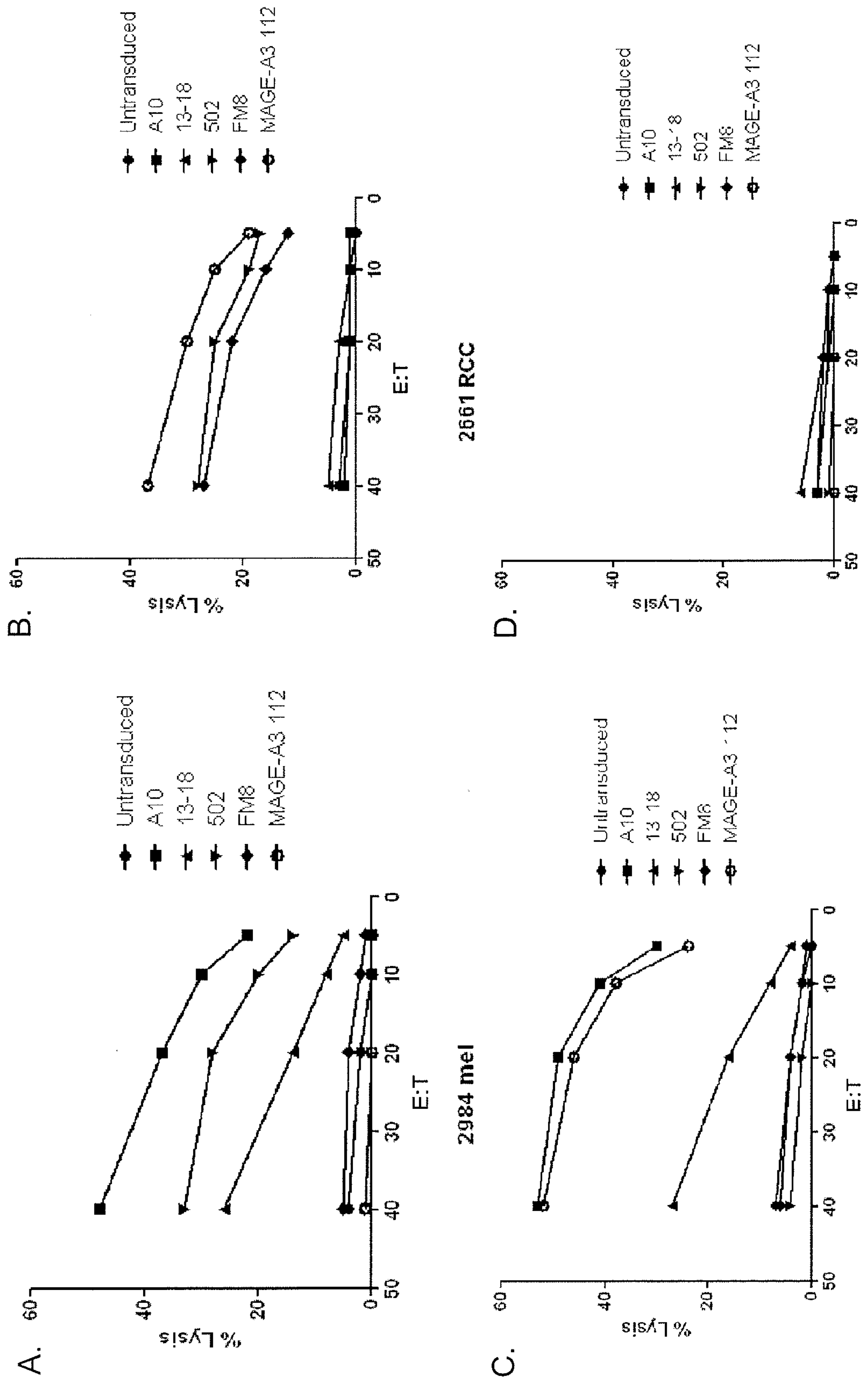

EBV B
Cw*0701*
Cw*0702*
IFN-γ (pg/ml)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
MAGE-A12-
MAGE-A2-
MAGE-A3-
MAGE-A6-
Control -
502
FM8
NGFR

FIG. 5


A.
397 mel
% Lysis
E:T
B.
624 mel
C.
2984 mel
D.
2661 RCC
Untransduced
A10
13-18
502
FM8
MAGE-A3 112

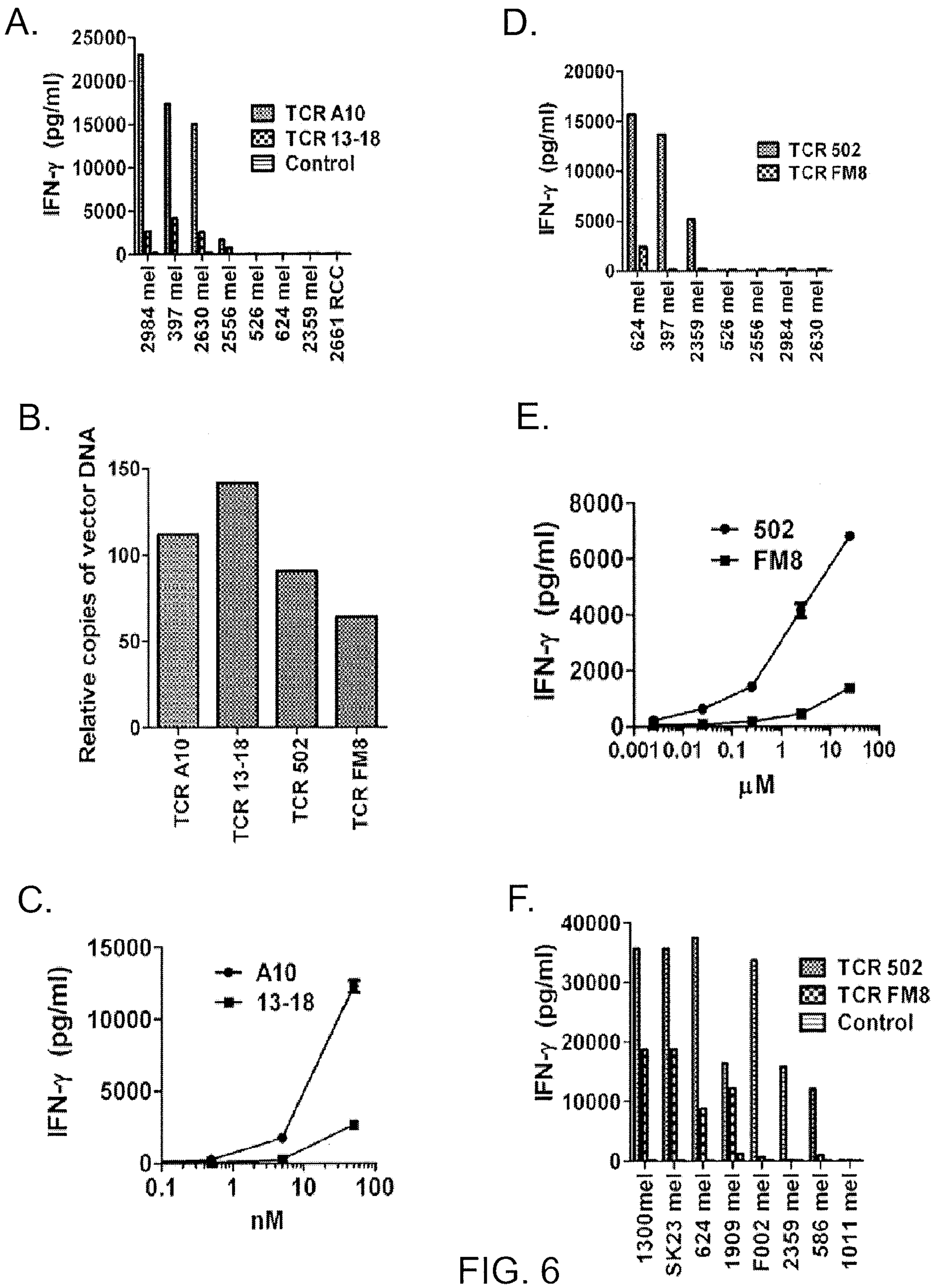

A.
IFN-γ (pg/ml)
25000
20000
15000
10000
5000
0
TCR A10
TCR 13-18
Control
2984 mel
397 mel
2630 mel
2556 mel
526 mel
624 mel
2359 mel
2661 RCC
D.
IFN-γ (pg/ml)
20000
15000
10000
5000
0
TCR 502
TCR FM8
624 mel
397 mel
2359 mel
526 mel
2556 mel
2984 mel
2630 mel
B.
Relative copies of vector DNA
150
100
50
0
TCR A10
TCR 13-18
TCR 502
TCR FM8
E.
IFN-γ (pg/ml)
8000
6000
4000
2000
0
502
FM8
0.001 0.01 0.1 1 10 100
μM
C.
IFN-γ (pg/ml)
15000
10000
5000
0
A10
13-18
0.1 1 10 100
nM
F.
IFN-γ (pg/ml)
40000
30000
20000
10000
0
TCR 502
TCR FM8
Control
1300mel
SK23 mel
624 mel
1909 mel
F002 mel
2359 mel
586 mel
1011 mel

FIG. 6

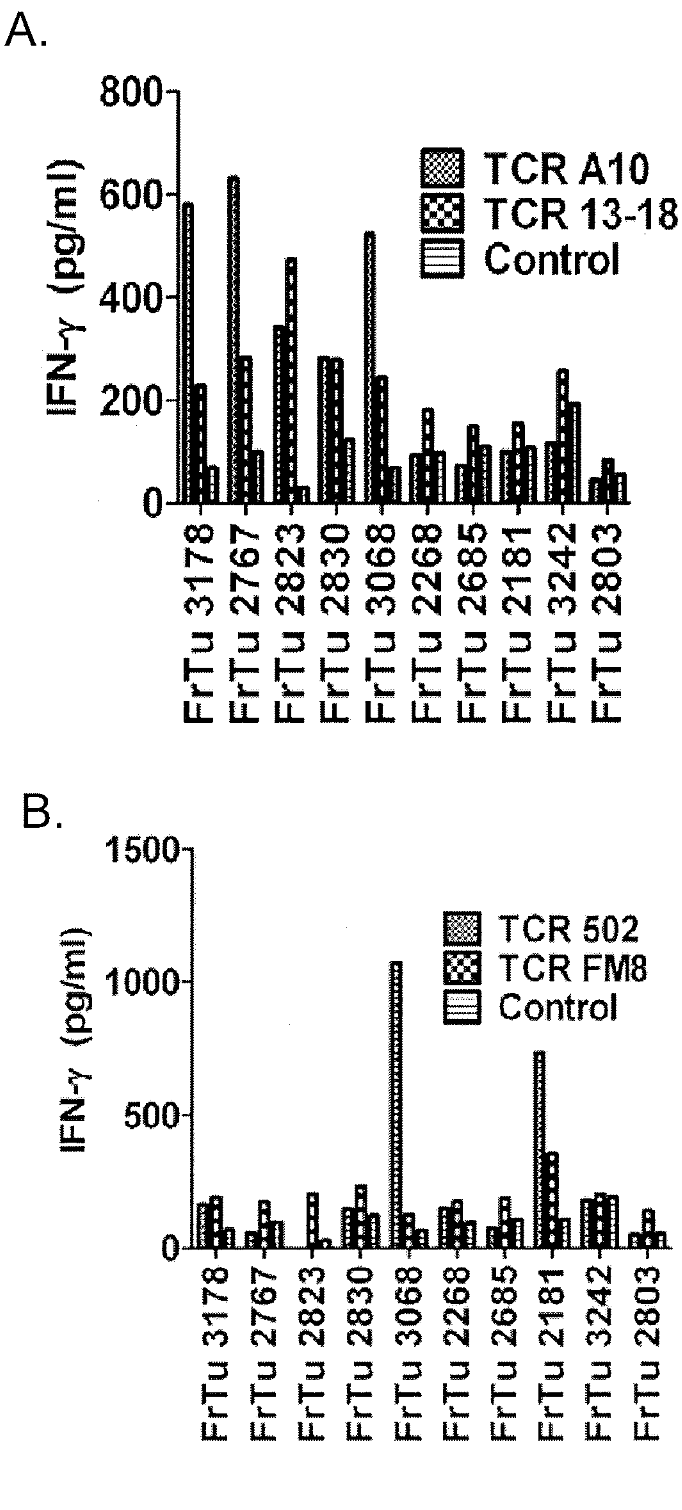

A.
800
600
400
200
0
IFN-γ (pg/ml)
TCR A10
TCR 13-18
Control
FrTu 3178
FrTu 2767
FrTu 2823
FrTu 2830
FrTu 3068
FrTu 2268
FrTu 2685
FrTu 2181
FrTu 3242
FrTu 2803
B.
1500
1000
500
0
IFN-γ (pg/ml)
TCR 502
TCR FM8
Control
FrTu 3178
FrTu 2767
FrTu 2823
FrTu 2830
FrTu 3068
FrTu 2268
FrTu 2685
FrTu 2181
FrTu 3242
FrTu 2803

FIG. 7

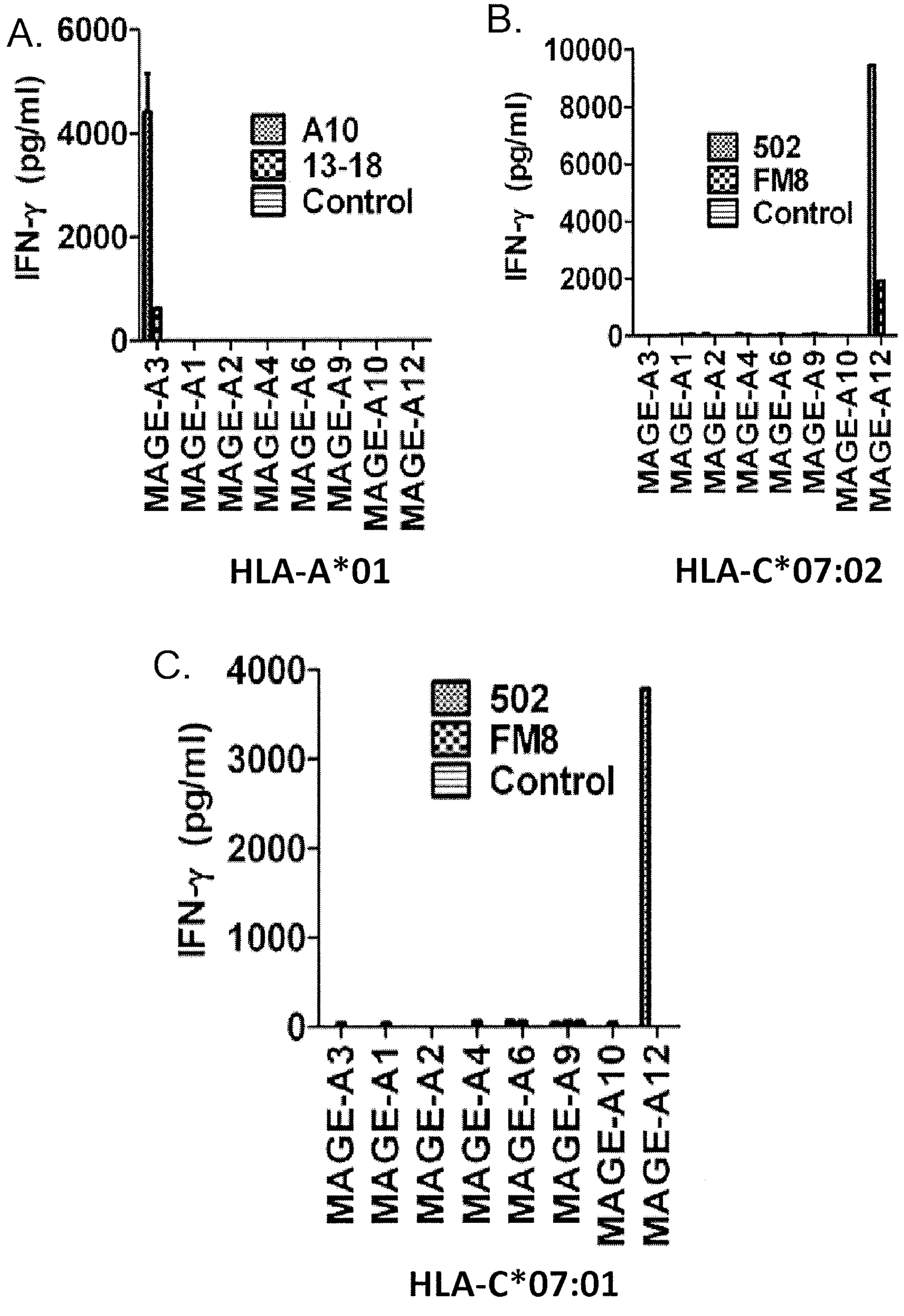

A.
6000
4000
2000
0
IFN-γ (pg/ml)
A10
13-18
Control
MAGE-A3
MAGE-A1
MAGE-A2
MAGE-A4
MAGE-A6
MAGE-A9
MAGE-A10
MAGE-A12
HLA-A*01
B.
10000
8000
6000
4000
2000
0
IFN-γ (pg/ml)
502
FM8
Control
MAGE-A3
MAGE-A1
MAGE-A2
MAGE-A4
MAGE-A6
MAGE-A9
MAGE-A10
MAGE-A12
HLA-C*07:02
C.
4000
3000
2000
1000
0
IFN-γ (pg/ml)
502
FM8
Control
MAGE-A3
MAGE-A1
MAGE-A2
MAGE-A4
MAGE-A6
MAGE-A9
MAGE-A10
MAGE-A12
HLA-C*07:01

FIG. 8

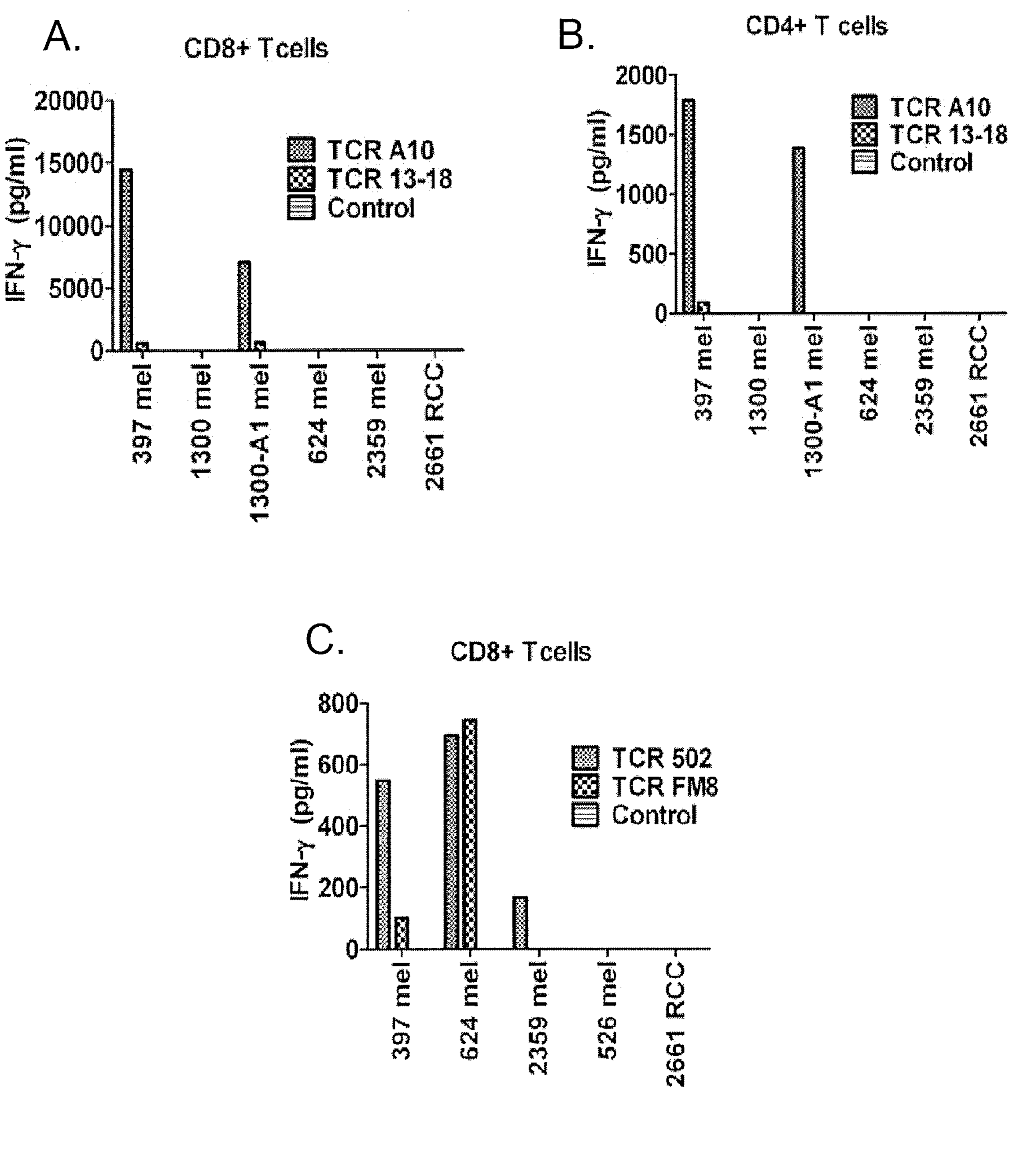

A.
CD8+ Tcells
IFN-γ (pg/ml)
20000
15000
10000
5000
0
TCR A10
TCR 13-18
Control
397 mel
1300 mel
1300-A1 mel
624 mel
2359 mel
2661 RCC
B.
CD4+ T cells
IFN-γ (pg/ml)
2000
1500
1000
500
0
TCR A10
TCR 13-18
Control
397 mel
1300 mel
1300-A1 mel
624 mel
2359 mel
2661 RCC
C.
CD8+ Tcells
IFN-γ (pg/ml)
800
600
400
200
0
TCR 502
TCR FM8
Control
397 mel
624 mel
2359 mel
526 mel
2661 RCC

FIG. 9