The invention provides an isolated or purified T cell receptor (TCR) having antigen specificity for melanoma antigen family A (MAGE A)-3. 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.
ANTI-MAGE-A3 T CELL RECEPTORS AND RELATED MATERIALS AND METHODS OF USE

CROSS-REFERENCE TO A RELATED APPLICATION

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 61/405,668, filed October 22, 2010, which is incorporated by reference in its entirety herein.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 83,081 Byte ASCII (Text) file named "708842_ST25.TXT," dated August 23, 2011.

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 has been successful in causing the regression of tumors in some cancers, e.g., melanoma. One obstacle to the widespread application of adoptive cell therapy is the difficulty in generating human T cells with anti-tumor potential. Another obstacle to the successful application of adoptive cell therapy is that the transferred T cells can also be toxic to normal, i.e., non-cancerous tissues. 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 melanoma antigen family A (MAGE A)-3 (SEQ ID NO: 1). The TCR can comprise specified amino acid sequences as described herein. For instance, the inventive TCR can comprise the amino acid sequence of any one or more of SEQ ID NOs: 9-14; SEQ ID NOs: 15-16, SEQ ID NOs: 17-18; SEQ ID NOs: 9, 10, 20, and 12-14; SEQ ID NOs: 21 and 16; SEQ ID NOs: 22 and 18; SEQ ID NOs: 23-28; SEQ ID NOs: 29-30; or SEQ ID NOs: 31-32.
The invention provides an isolated or purified T cell receptor (TCR) having antigenic specificity for MAGE-A3 and MAGE-12, wherein the TCR also recognizes at least one of MAGE-A2 and MAGE-A6.

The invention also provides an isolated or purified TCR having antigenic specificity for MAGE-A3 and MAGE-A12.

The invention further provides an isolated or purified anti-MAGE-A3 271-279 TCR comprising the amino acid sequences of SEQ ID NOs: 23-28.

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. 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.

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)

Figure 1A is a graph showing IFN-γ (pg/ml) secretion by MAGE-A3 TCR 112-120 (unsubstituted)-transduced peripheral blood cells (PBL) upon co-culture with MAGE 112-120 peptide (ng/ml) pulsed T2 cells.

Figure 1B is a graph showing IFN-γ (pg/ml) secretion by MAGE-A3 TCR 271-279-transduced peripheral blood cells (PBL) upon co-culture with MAGE 271-279 peptide (ng/ml) pulsed T2 cells.
Figure 2A is a graph showing the specific killing (\% lysis) of tumor cell line 1300 Mel (HLA-A*0201 +/MAGE-A3 +) by untransduced PBL (squares) or MAGE-A3 112-120 TCR-transduced (circles) or MAGE-A3 271-279 TCR-transduced (triangles) PBL from a first donor at various effector:target (E:T) ratios.

Figure 2B is a graph showing the specific killing (\% lysis) of tumor cell line 526 Mel (HLA-A*0201 +/MAGE-A3 +) by untransduced PBL (squares) or MAGE-A3 112-120 TCR-transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) PBL from a first donor at various E:T ratios.

Figure 2C is a graph showing the specific killing (\% lysis) of tumor cell line 938 Mel (HLA-A*02017MAGE-A3 +) by untransduced PBL (squares) or MAGE-A3 112-120 TCR-transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) PBL from a first donor at various E:T ratios.

Figure 2D is a graph showing the specific killing (\% lysis) of tumor cell line 1300 Mel (HLA-A*02017MAGE-A3 +) by untransduced PBL (squares) or MAGE-A3 112-120 TCR-transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) PBL from a second donor at various E:T ratios.

Figure 2E is a graph showing the specific killing (\% lysis) of tumor cell line 526 Mel (HLA-A*02017MAGE-A3 +) by untransduced PBL (squares) or MAGE-A3 112-120 TCR transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) PBL from a second donor at various E:T ratios.

Figure 2F is a graph showing the specific killing (\% lysis) of tumor cell line 938 Mel (HLA-A*02017MAGE-A3 +) by untransduced PBL (squares) or MAGE-A3 112-120 TCR transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) PBL from a second donor at various E:T ratios.

Figure 3A is a graph showing the specific killing (\% lysis) of non-small cell lung cancer (NSCLC) cell line H1299-A*0201 (MAGE+/HLA-A2 +) by untransduced PBL (squares) or MAGE-A3 112-120 TCR transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) PBL from a first donor at various E:T ratios.

Figure 3B is a graph showing the specific killing (\% lysis) of NSCLC cell line H1299 (MAGE7HLA-A2-) by untransduced PBL (squares) or MAGE-A3 112-120 TCR transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) PBL from a first donor at various E:T ratios.
[0021] Figure 3C is a graph showing the specific killing (% lysis) of NSCLC cell line H1299-A*0201 (MAGE+/HLA-A2+) by untransduced PBL (squares) or MAGE-A3 112-120 TCR transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) PBL from a second donor at various E:T ratios.

[0022] Figure 3D is a graph showing the specific killing (% lysis) of NSCLC cell line H1299 (MAGE7HLA-A2+) by untransduced PBL (squares) or MAGE-A3 112-120 TCR transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) PBL from a second donor at various E:T ratios.

[0023] Figure 4A is a graph showing the specific killing (% lysis) of tumor cell line 1300 Mel (HLA-A*0201+/MAGE-A3) by untransduced CD8+ T cells (squares) or MAGE-A3 112-120 TCR transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) CD8+ T cells at various E:T ratios.

[0024] Figure 4B is a graph showing the specific killing (% lysis) of tumor cell line 1300 Mel (HLA-A*02017MAGE-A3+) by untransduced CD4+ T cells (squares) or MAGE-A3 112-120 TCR transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) CD4+ T cells at various E:T ratios.

[0025] Figure 4C is a graph showing the specific killing (% lysis) of tumor cell line 526 Mel (HLA-A*02017MAGE-A3+) by untransduced CD8+ T cells (squares) or MAGE-A3 112-120 TCR transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) CD8+ T cells at various E:T ratios.

[0026] Figure 4D is a graph showing the specific killing (% lysis) of tumor cell line 526 Mel (HLA-A*02017MAGE-A3+) by untransduced CD4+ T cells (squares) or MAGE-A3 112-120 TCR transduced (circles) or MAGE-A3 271-279 TCR transduced (triangles) CD4+ T cells at various E:T ratios.

[0027] Figure 5A is a graph showing IFN-γ secretion (pg/ml) by a first donor’s PBL transduced with wild-type (WT) (unsubstituted) MAGE-A3 112-120 TCR (closed bars) or 118AT substituted TCR (open bars) upon co-culture with T2 cells pulsed with various concentrations (pg/ml) of MAGE-A3 112-120 peptide.

[0028] Figure 5B is a graph showing IFN-γ secretion (pg/ml) by a second donor’s PBL transduced with wild-type (WT) (unsubstituted) MAGE-A3 112-120 TCR (closed bars) or 118AT substituted TCR (open bars) upon co-culture with T2 cells pulsed with various concentrations (pg/ml) of MAGE-A3 112-120 peptide.
FIGURE 6

[A] is a graph showing granulocyte-macrophage colony stimulating factor (GM-CSF) secretion (pg/ml) by a first donor's PBL that were untransduced (white bars) or transduced with wild-type (WT) (unsubstituted) MAGE-A3 112-120 TCR (black bars) or 118AT substituted TCR (grey bars) upon co-culture with H1299, H1299-A2, or 624.38 tumor cell targets.

[B] is a graph showing GM-CSF secretion (pg/ml) by a second donor's PBL that were untransduced (white bars) or transduced with wild-type (WT) (unsubstituted) MAGE-A3 112-120 TCR (black bars) or 118AT substituted TCR (grey bars) upon co-culture with H1299, H1299-A2, or 624.38 tumor cell targets.

FIGURE 7

Is a graph showing IFN-γ secretion (pg/ml) by PBL transduced with wild-type (WT) (unsubstituted) MAGE-A3 112-120 TCR upon co-culture with T2 cells pulsed with various concentrations (ng/ml) of MAGE-A1 peptide (SEQ ID NO: 64) (diamonds), MAGE-A2 peptide (SEQ ID NO: 6) (squares), MAGE-A3 peptide (SEQ ID NO: 2) (triangles), MAGE-A4 peptide (SEQ ID NO: 65) (X), MAGE-A6 peptide (SEQ ID NO: 8) (*), MAGE-A8 peptide (SEQ ID NO: 66) (circles), MAGE-A12 peptide (SEQ ID NO: 4) (+), or MAGE-C2 peptide (SEQ ID NO: 67) (—).

FIGURES 8A-8E

Are computed tomography (CT) scans of the lung of Patient 1 before (A) treatment with PBL transduced with the 118AT substituted TCR, one month after treatment (B), two months after treatment (C), and three months after treatment (E). Figure 8D is an enlargement of Figure 8A. The arrow points to a tumor.

FIGURES 9A-9F

Are CT scans of the abdominal wall of Patient 3 before (A-C) treatment with PBL transduced with the 118AT substituted TCR and one month after treatment (D-F). The arrow points to a tumor.

DETAILED DESCRIPTION OF THE INVENTION

[0034] The invention provides a T cell receptor (TCR) having antigenic specificity for melanoma antigen family A (MAGE A)-3 (also known as MAGE-3). MAGE-A3 is a member 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, MAGE-A11, and MAGE-A12. 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), and urothelial cancer. The MAGE-A3 protein may comprise, consist, or consist essentially of, SEQ ID NO: 1. The inventive TCRs described herein include both substituted and unsubstituted TCRs.

[0035] An embodiment of the invention includes a TCR having antigenic specificity for MAGE-A3 and/or MAGE-A12.

[0036] The phrase "antigenic specificity" as used herein means that the TCR can specifically bind to and immunologically recognize MAGE-A3 and/or MAGE-A12 with high avidity. For example, a TCR may be considered to have "antigenic specificity" for MAGE-A3 and/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 target cells pulsed with a low concentration of HLA-A2 restricted MAGE-A3 and/or MAGE-A12 (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 and/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 target cells pulsed with a low concentration of HLA-A2 restricted MAGE-A3 and/or MAGE-A12. The inventive TCRs may also secrete IFN-γ upon co-culture with target cells pulsed with higher concentrations of MAGE-A3 and/or MAGE-A12.

[0037] The TCR may have antigenic specificity for any MAGE-A3 protein, polypeptide or peptide. In an embodiment of the invention, the TCR has antigenic specificity for a MAGE-A3 protein comprising, consisting of, or consisting essentially of, SEQ ID NO: 1. In an embodiment of the invention, the TCR has antigenic specificity for a MAGE-A3 271-279 peptide comprising, consisting of, or consisting essentially of, FLWGPRALV (SEQ ID NO: 63). In a preferred embodiment of the invention, the TCR has antigenic specificity for a MAGE-A3 112-120 peptide comprising, consisting of, or consisting essentially of, KVAELVHFL (SEQ ID NO: 2).

[0038] In addition to having antigenic specificity for MAGE-A3, the inventive TCR may also have antigenic specificity for any MAGE-A12 protein, polypeptide or peptide. In an
embodiment of the invention, the TCR has 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 peptide comprising, consisting of, or consisting essentially of, KMAELVHFL (SEQ ID NO: 4).

[0039] An embodiment of the invention includes a TCR having antigenic reactivity toward MAGE-A3 and any one or more of MAGE-A12, MAGE-A6, and MAGE-A2.

[0040] While the TCRs of the invention have antigenic specificity for MAGE-A3 and/or MAGE-A12, the TCRs of the invention can also recognize any one or more of MAGE-A2 and MAGE-A6. That is, the TCRs of the invention can bind to and immunologically recognize any one or more of MAGE-A2 and MAGE-A6, but with a lower avidity than that which is observed for binding to MAGE-A3 and/or MAGE-A12, such that the binding of the TCR to one of these proteins elicits an immune response at a higher concentration of any one of these proteins than that which is necessary to elicit an immune response with MAGE-A3 and/or MAGE-A12. For example, the TCRs of the invention may be considered to recognize any one or more of MAGE-A2 and MAGE-A6 with low avidity if T cells expressing the TCR do not secrete at least about 200 pg/ml (e.g., secretes less than 200 pg/ml, less than 100 pg/ml) of IFN-γ upon co-culture with target cells pulsed with a low concentration of any one or more of MAGE-A2 and MAGE-A6 (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) but do 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 target cells pulsed with a higher concentration of any one or more of MAGE-A2 and MAGE-A6 (e.g., about 10 ng/ml to about 1000 ng/ml, 10 ng/ml, 50 ng/ml, 100 ng/ml, 500 ng/ml, or 1000 ng/ml). Alternatively or additionally, a TCR may be considered to recognize any one or more of MAGE-A2 and MAGE-A6 with low avidity 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 target cells pulsed with a higher concentration of any one or more of MAGE-A2 and MAGE-A6.

[0041] The TCR may recognize a MAGE-A2 and MAGE-A6 protein, polypeptide or peptide. In an embodiment of the invention, the TCR recognizes a protein comprising, consisting of, or consisting essentially of, SEQ ID NO: 5 (MAGE-A2) and/or SEQ ID NO: 7 (MAGE-A6). In a preferred embodiment of the invention, the TCR recognizes a peptide
comprising, consisting of, or consisting essentially of, MAGE-A2 peptide KMVELVHFL
(SEQ ID NO: 6) and/or MAGE-A6 peptide KVAKLVHFL (SEQ ID NO: 8).

[0042] The inventive TCRs are able to recognize MAGE-A3, MAGE-A12, MAGE-A6,
and/or MAGE-A2 (hereinafter, "MAGE-A cancer antigens") in an HLA-A2-dependent
manner. By "HLA-A2-dependent manner" as used herein means that the TCR elicits an
immune response upon binding to a MAGE-A cancer antigen within the context of an HLA-
A2 molecule.

[0043] The TCRs of the invention provide many advantages, including when used for
adoptive cell transfer. For example, without being bound by a particular theory, it is believed
that because MAGE-A3, MAGE-A12, MAGE-A2, and/or MAGE-A6 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, minimizing or eliminating,
toxicity. In addition, while the inventive TCRs have antigenic specificity for MAGE-A3
and/or MAGE-A12, the inventive TCRs advantageously also recognize any one or more of
MAGE-A2 and MAGE-A6. Without being bound to a particular theory, it is believed that
the ability to recognize multiple cancer antigens advantageously increases the number of
cancer cells that can be destroyed by the inventive TCRs. Additionally, should a MAGE-A
antigen become mutated, the inventive TCRs can still be viable in that they recognize more
than just one MAGE-A antigen,

[0044] 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 MAGE-A3 and/or MAGE-
A12, and/or recognizes any one or more of MAGE-A2 and MAGE-A6.

[0045] 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 271-279 and comprises a first polypeptide chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 23 (CDR1 of a chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 24 (CDR2 of a chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 25 (CDR3 of a chain), and a second polypeptide chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 26 (CDR1 of β chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 27 (CDR2 of β chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 28 (CDR3 of β chain). In a preferred embodiment of the invention, the TCR has antigenic specificity for MAGE-A3 112-120, and comprises a first polypeptide chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 9 (CDR1 of a chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 10 (CDR2 of a chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 11 (CDR3 of a chain), and a second polypeptide chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 12 (CDR1 of β chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 13 (CDR2 of β chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 14 (CDR3 of β chain). In this regard, the inventive TCR can comprise the amino acid sequences selected from the group consisting of any one or more of SEQ ID NOs: 9-11, 12-14, 9-14, 23-25, 26-28, and 23-28. Preferably the TCR comprises the amino acid sequences of SEQ ID NOs: 9-14 or 23-28.

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 271-279 can comprise the amino acid sequence of SEQ ID NO: 29 (the variable region of an a chain) or 30 (the variable region of a β chain), or both SEQ ID NOs: 29 and 30. In a preferred embodiment of the invention, the TCR has antigenic specificity for MAGE-A3 112-120 and comprises the amino acid sequence of SEQ ID NO: 15 (the variable region of an a chain) or 16 (the variable region of a β chain), or both SEQ ID NOs: 15 and 16. Preferably, the inventive TCR comprises the amino acid sequences of both SEQ ID NOs: 29 and 30 or both SEQ ID NOs: 15 and 16. Other examples of variable regions include SEQ ID NOs: 71, 73, 75, and 77.

Alternatively or additionally, the TCR can comprise an a chain of a TCR and a β chain of a TCR. Each of the a chain and β chain of the inventive TCR can independently comprise any amino acid sequence. Preferably, the a chain comprises the variable region of an a chain as set forth above. In this regard, the inventive TCR with antigenic specificity for
MAGE-A3 271-279 can comprise the amino acid sequence of SEQ ID NO: 31 and the inventive TCR with antigenic specificity for MAGE-A3 112-120 can comprise the amino acid sequence of SEQ ID NO: 17. 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 271-279 can comprise the amino acid sequence of SEQ ID NO: 32 and the inventive TCR with antigenic specificity for MAGE-A3 112-120 can comprise the amino acid sequence of SEQ ID NO: 18. The inventive TCR, therefore, can comprise the amino acid sequence of SEQ ID NO: 17, 18, 31, or 32, both SEQ ID NOs: 17 and 18 or both SEQ ID NOs: 31 and 32. Preferably, the inventive TCR comprises the amino acid sequences of both SEQ ID NOs: 17 and 18 or both SEQ ID NOs: 31 and 32.

[0048] The invention also provides substituted amino acid sequences that encode TCRs (or functional portions thereof). In some embodiments, the substituted TCRs (or functional portions thereof) provide an increased specificity for any one or more of MAGE-A3, MAGE-A12, MAGE-A2, and MAGE-A6 as compared to an unsubstituted amino acid sequence. In general, the substituted amino acid sequences SEQ ID NOs: 20-22 correspond with all or portions of the native, unsubstituted SEQ ID NO: 17 (TCR α chain), with SEQ ID NOs: 20-22 having at least one substitution when compared to SEQ ID NO: 17. Preferably, the native Ala at position 118 (Ala118) of SEQ ID NO: 17 is substituted.

[0049] In particular, the invention provides a TCR comprising an amino acid sequence comprising SEQ ID NO: 20 (substituted CDR3 of a chain), wherein Xaa7 is selected from the group consisting of Thr and Val. SEQ ID NO: 20 generally corresponds to positions 112-123 of the native, unsubstituted SEQ ID NO: 17 with the exception that in SEQ ID NO: 20, Ala7 is substituted.

[0050] The invention also provides a TCR comprising an amino acid sequence comprising SEQ ID NO: 21 (substituted variable region of a chain), wherein Xaa 18 is selected from the group consisting of Thr and Val. SEQ ID NO: 21 generally corresponds to positions 1-122 of the native, unsubstituted SEQ ID NO: 17 with the exception that in SEQ ID NO: 21, Ala 18 is substituted. Another example of a substituted variable region is SEQ ID NO: 79.

[0051] Also provided by the invention is a TCR comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 22 (substituted a chain), wherein Xaa 18 is selected from the group consisting of Thr and Val. SEQ ID NO: 22 generally corresponds
to positions 1-271 of the native, unsubstituted SEQ ID NO: 17 with the exception that in SEQ ID NO: 22, Alal 18 is substituted.

[0052] Like the first embodiment of the TCRs of the invention, the substituted 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. Preferably, the first polypeptide chain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 9 (CDR1 of a chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 10 (CDR2 of a chain), and a substituted CDR3 comprising the amino acid sequence of SEQ ID NO: 20 (substituted CDR3 of a chain), and the second polypeptide chain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 12 (CDR1 of β chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 13 (CDR2 of β chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 14 (CDR3 of β chain). In this regard, the inventive substituted TCR can comprise any one or more of the amino acid sequences selected from the group consisting of SEQ ID NOs: 9-10 and 20; SEQ ID NOs: 12-14; and SEQ ID NOs: 9-10, 20, and 12-14. Preferably the inventive substituted TCR comprises the amino acid sequences of SEQ ID NOs: 9-10, 20, and 12-14.

[0053] Alternatively or additionally, the substituted TCR can comprise a substituted amino acid sequence of a variable region of a TCR comprising the CDRs set forth above. In this regard, the TCR can comprise the substituted amino acid sequence of SEQ ID NO: 21 (the substituted variable region of an α chain) or 16 (the variable region of a β chain), or both SEQ ID NOs: 21 and 16. Preferably, the inventive substituted TCR comprises the amino acid sequences of both SEQ ID NOs: 21 and 16.

[0054] Alternatively or additionally, the substituted TCR can comprise a substituted a 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 substituted α chain comprises a substituted variable region of an α chain as set forth above. In this regard, the inventive substituted a chain of the TCR can comprise the amino acid sequence of SEQ ID NO: 22. An inventive substituted a chain 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 can comprise the amino acid sequence of SEQ ID NO: 18. The inventive TCR, therefore, can comprise the amino acid sequence of SEQ ID NO: 22, 18, or both SEQ ID NOs: 22 and 18. Preferably, the inventive TCR comprises the amino acid sequences of both SEQ ID NOs: 22 and 18.
[0055] 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.

[0056] 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 and/or MAGE-A12 and/or recognizes any one or more of MAGE-A2 and MAGE-A6. 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 and/or MAGE-A12 and/or recognize any one or more of MAGE-A2 and MAGE-A6 (e.g., in an HLA-A2-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.

[0057] 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 and/or MAGE-A12; recognizing any one or more of MAGE-A2 and MAGE-A6; 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.

[0058] 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: 9 or 23 (CDR1 of a chain), 10 or 24 (CDR2 of a chain), 11 or 25 (CDR3 of a chain), 12 or 26 (CDR1 of β chain), 13 or 27 (CDR2 of β chain), 14 or 28 (CDR3 of β chain), 20 (substituted CDR3 of a chain), or a combination thereof. Preferably, the inventive polypeptide comprises a functional portion comprising SEQ ID NOs: 9-11; 9-10 and 20; 12-14; 23-25; 26-28; all of SEQ ID NOs: 9-14; all of SEQ ID NOs:
9-10, 20, and 12-14; or all of SEQ ID NOs: 23-28. More preferably, the polypeptide comprises a functional portion comprising the amino acid sequences of SEQ ID NOs: 9-14; SEQ ID NOs: 9-10, 20, and 12-14; or SEQ ID NOs: 23-28.

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: 15 or 29 (the variable region of an a chain); SEQ ID NO: 16 or 30 (the variable region of a β chain); SEQ ID NO: 21 (substituted variable region of an a chain); both SEQ ID NOs: 15 and 16; both SEQ ID NOs: 21 and 16; or both SEQ ID NOs: 29 and 30. Preferably, the polypeptide comprises the amino acid sequences of both SEQ ID NOs: 15 and 16; both SEQ ID NOs: 21 and 16; or both SEQ ID NOs: 29 and 30.

Alternatively or additionally, the inventive polypeptide can comprise the entire length of an a 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: 17, 18, 22, 31, or 32. Alternatively, the polypeptide of the invention can comprise a and β chains of the TCRs described herein. For example, the inventive polypeptide can comprise the amino acid sequences of both SEQ ID NOs: 17 and 18; both SEQ ID NOs: 22 and 18; or both SEQ ID NOs: 31 and 32.

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.

In an embodiment, the protein of the invention can comprise a first polypeptide chain comprising the amino acid sequences of SEQ ID NOs: 9-11; SEQ ID NOs: 9-10 and 20; or SEQ ID NOs: 23-25 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NOs: 12-14 or SEQ ID NOs: 26-28. Alternatively or additionally, the protein of the invention can comprise a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 15, 21, or 29 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 16 or 30. The protein of the invention can, for example, comprise a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 17, 22, or 31 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 18 or 32. 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: 17, 22, or 31 and SEQ ID NO: 18 or 32, 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., CDla, CDlb, CDlc, CDld, etc.

[0063] 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).

[0064] 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 a chain and the β chain. In this regard, the TCRs, polypeptides, and proteins of the invention comprising SEQ ID NO: 17, 22, or 31 and SEQ ID NO: 18 or 32 may further comprise a linker peptide comprising SEQ ID NO: 19. For example, the inventive TCR, polypeptide, and/or protein may comprise any of SEQ ID NO: 60 (unsubstituted and recognizing MAGE-A3 112-120), SEQ ID NO: 61 (substituted and recognizing MAGE-A3 112-120), and SEQ ID NO: 62 (recognizing MAGE-A3 271-279). SEQ ID NO: 61 generally corresponds to positions 1-600 of unsubstituted SEQ ID NO: 60 with the exception that in SEQ ID NO: 61, A[a]18 is substituted. 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.

[0065] 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,
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.

[0066] 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 and/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. Alternatively or additionally, functional variants can also encompass, for example, those variants of the TCR, polypeptide, or protein described herein (the parent TCR, polypeptide, or protein) that retain the ability to recognize any one or more of MAGE-A2 and MAGE-A6, which the parent polypeptide or protein recognizes, 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.

[0067] 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, He, 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, Gin, Ser, Thr, Tyr, etc.), etc.

[0068] 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.

[0069] 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: 17, 18, 22, 31, 32, both SEQ ID NOs: 17 and 18, both SEQ ID NOs: 22 and 18, or both SEQ ID NOs: 31 and 32. Also, for instance, the inventive TCRs, polypeptides, or proteins can consist essentially of the amino acid sequence(s) of SEQ ID NO: 15, 16, 21, 29, 30, both SEQ ID NOs: 15 and 16, both SEQ ID NOs: 21 and 16, or both SEQ ID NOs: 29 and 30. Furthermore, the inventive TCRs, polypeptides, or proteins can consist essentially of the amino acid sequence of SEQ ID NO: 9 or 23 (CDR1 of a chain), SEQ ID NO: 10 or 24 (CDR2 of a chain), SEQ ID NO: 11 or 25 (CDR3 of a chain), SEQ ID NO: 12 or 26 (CDR1 of β chain), SEQ ID NO: 13 or 27 (CDR2 of β chain), SEQ ID NO: 14 or 28 (CDR3 of β chain), SEQ ID NO: 20 (substituted CDR3 of a chain) or any combination thereof, e.g., SEQ ID NOs: 9-11; 9-10 and 20; 12-14; 9-14; 9-10, 20 and 12-14; 23-25; 26-28; or 23-28.

[0070] 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 and/or MAGE-A12; recognize any one or more of MAGE-A2 and MAGE-A6; 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.

[0071] 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, a-amino n-decanoic acid, homoserine, S-acetylaminoethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenyl glycine, a-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indole-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, N′-benzyl-N′-methyl-lysine, N′,N′-dibenzyl-lysine, 6-hydroxyllysine, ornithine, a-aminocyclopentane carboxylic acid, cc-aminocyclohexane carboxylic acid, a-aminocycloheptane carboxylic acid, a-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

[0072] 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.

[0073] 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, ^-toluenesulphonic acid.

[0074] 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.

[0075] 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)).

[0076] 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.

[0077] 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.

[0078] 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-dimethyl guanine, 2-methyliadenine, 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).

[0079] 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: set forth in Table 1. Other examples of nucleotide sequences encoding variable regions include SEQ ID NOs: 72, 74, 76, and 78.
<table>
<thead>
<tr>
<th>SEQ ID NO:</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.</td>
<td>MAGE-A3 112-120 CDR1 α</td>
</tr>
<tr>
<td>34.</td>
<td>MAGE-A3 112-120 CDR2 α</td>
</tr>
<tr>
<td>35.</td>
<td>MAGE-A3 112-120 CDR3 α</td>
</tr>
<tr>
<td>36.</td>
<td>MAGE-A3 112-120 CDR1 β</td>
</tr>
<tr>
<td>37.</td>
<td>MAGE-A3 112-120 CDR2 β</td>
</tr>
<tr>
<td>38.</td>
<td>MAGE-A3 112-120 CDR3 β</td>
</tr>
<tr>
<td>39.</td>
<td>MAGE-A3 112-120 variable region α chain</td>
</tr>
<tr>
<td>40.</td>
<td>MAGE-A3 112-120 variable region β chain</td>
</tr>
<tr>
<td>41.</td>
<td>MAGE-A3 112-120 α chain</td>
</tr>
<tr>
<td>42.</td>
<td>MAGE-A3 112-120 β chain</td>
</tr>
<tr>
<td>43.</td>
<td>MAGE-A3 112-120 linker peptide</td>
</tr>
<tr>
<td>44.</td>
<td>MAGE-A3 112-120 substituted CDR3 α, wherein NNN at positions 19-21 is a codon that encodes Thr or Val</td>
</tr>
<tr>
<td>45.</td>
<td>MAGE-A3 112-120 substituted variable region α chain, wherein NNN at positions 352-354 is a codon that encodes Thr or Val</td>
</tr>
<tr>
<td>46.</td>
<td>MAGE-A3 112-120 substituted α chain, wherein NNN at positions 352-354 is a codon that encodes Thr or Val</td>
</tr>
<tr>
<td>47.</td>
<td>MAGE-A3 271-279 CDR1 α</td>
</tr>
<tr>
<td>48.</td>
<td>MAGE-A3 271-279 CDR2 α</td>
</tr>
<tr>
<td>49.</td>
<td>MAGE-A3 271-279 CDR3 α</td>
</tr>
<tr>
<td>50.</td>
<td>MAGE-A3 271-279 CDR1 β</td>
</tr>
<tr>
<td>51.</td>
<td>MAGE-A3 271-279 CDR2 β</td>
</tr>
<tr>
<td>52.</td>
<td>MAGE-A3 271-279 CDR3 β</td>
</tr>
<tr>
<td>53.</td>
<td>MAGE-A3 271-279 variable region α chain</td>
</tr>
<tr>
<td>54.</td>
<td>MAGE-A3 271-279 variable region β chain</td>
</tr>
<tr>
<td>55.</td>
<td>MAGE-A3 271-279 α chain</td>
</tr>
</tbody>
</table>
**SEQ ID NO:** Description

<table>
<thead>
<tr>
<th>SEQ ID NO</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.</td>
<td>MAGE-A3 271-279 β chain</td>
</tr>
<tr>
<td>57.</td>
<td>MAGE-A3 112-120 (a and β chains and linker peptide)</td>
</tr>
<tr>
<td>58.</td>
<td>MAGE-A3 112-120 substituted (a and β chains and linker peptide), wherein NNN at positions 352-354 is a codon that encodes Thr or Val</td>
</tr>
<tr>
<td>59.</td>
<td>MAGE-A3 271-279 (a and β chains and linker peptide)</td>
</tr>
</tbody>
</table>

[0080] The invention also provides substituted nucleic acid sequences which encode any of the substituted TCRs, substituted polypeptides, substituted proteins, or substituted functional portions or functional variants thereof described herein. In this regard, the inventive nucleic acid may comprise a nucleotide sequence comprising, consisting of, or consisting essentially of, any one or more of SEQ ID NOs: 44-46 and 58 as set forth in Table 1. SEQ ID NO: 44 generally corresponds to positions 334-369 of unsubstituted SEQ ID NO: 41 with the exception that in SEQ ID NO: 44, the nucleotides at positions 19-21 are substituted. SEQ ID NO: 45 generally corresponds to positions 1-369 of unsubstituted SEQ ID NO: 41 with the exception that in SEQ ID NO: 45, the nucleotides at positions 352-354 are substituted. SEQ ID NO: 46 generally corresponds to positions 1-813 of unsubstituted SEQ ID NO: 41 with the exception that in SEQ ID NO: 46, the nucleotides at positions 352-354 are substituted. SEQ ID NO: 58 generally corresponds to positions 1-1803 of unsubstituted SEQ ID NO: 57 with the exception that in SEQ ID NO: 58, the nucleotides at positions 352-354 are substituted.

[0081] The codons of the substituted nucleic acids of the invention that encode any of Thr and Val may be any suitable codon that encodes Thr and Val, respectively. For example, the codons may be any of those set forth in Table 2 below.

**TABLE 2**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Codon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thr</td>
<td>ACA, ACC, ACG, ACT</td>
</tr>
<tr>
<td>Val</td>
<td>GTA, GTC, GTG, GTT</td>
</tr>
</tbody>
</table>

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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, LaJoUa, 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 λ GT1, λ ZAP II (Stratagene), λ EMBL4, and λ NMI 149, also can be used. Examples of plant expression vectors include pBIOL, pBI101.2, pBHOI.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.

[0087] 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.

[0088] 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.

[0089] 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.

[0090] The recombinant expression vector can comprise a native or normative 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.

[0091] 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.

[0092] 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.

[0093] 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 leukocyte (PBL) or a
peripheral blood mononuclear cell (PBMC). More preferably, the host cell is a T cell.

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., Th1 and Th2 cells, CD8+ T
cells (e.g., cytotoxic T cells), tumor infiltrating cells (TILs), memory T cells, naive T cells,
and the like. Preferably, the T cell is a CD8+ T cell or a CD4+ T cell.

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.

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: 9 or 23 (CDR1 of a chain), 10 or 24 (CDR2 of a chain), 11 or 25 (CDR3 of a chain), 20 (substituted CDR3 of a chain), 12 or 26 (CDR1 of β chain), 13 or 27 (CDR2 of β chain), 14 or 28 (CDR3 of β chain), SEQ ID NO: 15 or 29 (variable region of a chain), SEQ ID NO: 21 (substituted variable region of a chain), SEQ ID NO: 16 or 30 (variable region of β chain), or a combination thereof, e.g., 9-11; 9-10 and 20; 12-14; 9-14; 9-10, 20, and 12-14; 15-16; 23-25; 26-28; 23-28; 29-30; 21 and 16. More preferably, the functional portion comprises the amino acid sequences of SEQ ID NOs: 9-14; 9-10, 20, and 12-14; or 23-28. 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.

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).

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 Al.

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).

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.

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).

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.

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.
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).

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% or 80%, 90% or can be 100%.

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.

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.

[0108] 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.

[0109] 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.

[0110] 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.
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.

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, caromers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

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. 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-p-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.
The parenteral formulations will typically contain from about 0.5% to about 25% 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.

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.

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.

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.

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.

[0120] 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, from about 0.01 to about 10 mg/kg body weight/day, about 0.01 mg to about 1 mg/kg body weight/day.

[0121] 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 and/or MAGE-A12; recognize any one or more of MAGE-A2 and MAGE-A6; or to detect, treat, or prevent cancer.

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.

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 and/or MAGE-A12 and may also recognize any one or more of MAGE-A2 and MAGE-A6, 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 and/or MAGE-A12 and may also mediate an immune response against any one or more of target cells expressing MAGE-A2 and MAGE-A6. In this regard, the invention provides a method of treating or preventing cancer in a host, comprising 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 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.

In an embodiment of the invention, the method of treating or preventing cancer in a host further comprises administering deoxyazacytidine (DAC) and/or 3-deazaazacytidine (DZNep) to the host. The method may comprise administering DAC and/or DZNep prior to, concurrently with, or after administering any of the inventive pharmaceutical compositions,
TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, or populations of cells to the host. Without being bound to a particular theory, it is believed that DAC and/or DZNep enhances the recognition of cancer cells by any of the inventive TCR materials by upregulating expression of MAGE by cancer cells. For example, prior exposure of lung cancer cells to DAC and/or DZNep increased IFN-γ release and cell lysis upon coculture with cells transduced to express the inventive TCRs in vitro, as described in Rao et al., Cancer Res., 71(12): 4192-204 (2011).

[0125] 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.

[0126] 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 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.

[0127] 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.

[0128] For purposes of the inventive detecting method, the contacting step can take place in vitro or in vivo with respect to the host. Preferably, the contacting is in vitro.

[0129] 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).

[0130] 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.

[0131] 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 (e.g., pancreatic adenocarcinoma), 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).

[0132] 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 Logomoipha, 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.

[0133] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.
EXAMPLES

[0134] Cell lines and human PBL: HLA-A*0201+/MAGE-A3+ melanoma cell lines 526, 624.38, 1300, 2984; and non HLA-A*0201 cell lines 397, 888, 938, 1359, and 1088 were established from surgically resected metastatic melanoma tumors and maintained at the Surgery Branch, National Cancer Institute (NCI). The 2361R is HLA-A*0201+/MAGE-A3+ cell line isolated from a surgically resected metastatic renal cell carcinoma. Non-small cell lung carcinoma line H1299 (HLA-A*0201+/MAGE-A3+); small cell lung carcinoma lines H2721 (HLA-A*0201+/MAGE-A3+), H2122 (HLA-A*02017MAGE-A3+), H1250 (HLA-A*0201+/MAGE-A3+); and esophageal cancer cell line BE-3 (HLA-A*02017MAGE-A3+) were kindly provided by Dr. David Schrump (Surgery Branch, NCI, National Institutes of Health, Bethesda, MD). Breast cancer cell lines MDA-MB-453S (HLA-A*02017MAGE-A3+) were from American Type Culture Collection (Manassas, VA). Glioma cell line U251 (HLA-A*0201+/MAGE-A3+) was obtained from the Division of Cancer Treatment and Diagnosis Tumor Repositoiy, National Cancer Institute (NCI)-Frederick (Frederick, MD). The COS7-A*0201, 293-A*0201, 397-A*0201, 1359-A*0201, MDA-MB-453S-A*0201 and H1299-A*0201 cells were retrovirally engineered to express HLA-A*0201 as previously described (Robbins et al., J Immunol 180:61 16-6 131 (2008); Parkhurst et al., Clin Cancer Res 15:169-180 (2009)). COS7-A*0201-MAGE-A3, COS7-A*0201-MAGE-A12, 293-A*0201-MAGE-A3, and 293-A*0201-MAGE-A12 cells were transduced with a retroviral vector expressing the respective MAGE genes. T2 is a lymphoblastoid cell line lacking TAP function, whose HLA class I proteins can be loaded with exogenous peptides.

[0135] All of the PBL used in this study were obtained from melanoma patients treated in the Surgery Branch, NCI on IRB approved protocols. Human lymphocytes were cultured in AIM-V medium (Invitrogen, Carlsbad CA) supplemented with 5% human AB serum (Valley Biomedical, Winchester, VA) 50 units/mL penicillin, 50 μg/mL streptomycin (Invitrogen) and 300 IU/mL interleukin-2 (IL-2) and maintained at 37°C with 5% C02. Murine lymphocytes were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, MEM nonessential amino acids, 55 μmol/L 2-mercaptoethanol, 50 units/mL penicillin, 50 μg/mL streptomycin and 30 IU/mL rhIL-2 (R10) (Invitrogen, Carlsbad, CA).

[0136] Quantitative RT-PCR for MAGE-A3/6 and MAGE-A12 expression: Total RNA from tumor cell lines was isolated using RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. 1 μg of RNA was then used for cDNA synthesis using oligo-dT
with the Superscript™ First-Strand Synthesis kit (Invitrogen, Carlsbad, CA). The cDNA was used as the template for subsequent RT-PCR with primers designed specifically for MAGE-A3/6 (Riker et al., Int. J. Cancer 86:818-826 (2000)), MAGE-A12 and β-actin genes (TaqMan® Gene Expression Assays, Applied Biosystems, Foster City, CA). Each experiment was performed in duplicates using the TaqMan® 7900 (Applied Biosystems) real-time PCR machine according to the manufacturer’s instructions. Absolute numbers of copies were estimated using standard curves with plasmid DNA expressing respective genes.

[0137] Synthetic peptides: The following peptides were used in this study: MAGE-A1: 105-113 (KVADLVGFL) (SEQ ID NO: 64), MAGE-A2: 112-120 (KMVELVHFL) (SEQ ID NO: 6), MAGE-A3: 112-120 (KVAELVHFL) (SEQ ID NO: 2), MAGE-A3: 271-279 (FLWGPRALV) (SEQ ID NO: 63), MAGE-A4: 113-121 (KVDELAHFL) (SEQ ID NO: 65), MAGE-A6: 112-120 (KVAKLHFL) (SEQ ID NO: 8), MAGE-A8: 115-123 (KVAELVRF) (SEQ ID NO: 66), MAGE-A12: 112-120 (KMAELVHFL) (SEQ ID NO: 4) and MAGE-C2: 144-152 (KVAELVEFL) (SEQ ID NO: 67). The peptides were synthesized using a solid-phase method based on 9-fluorenylmethoxycarbonyl (Fmoc) chemistry using one of two multiple peptide synthesizers (model AMS 422; Gilson, or Pioneer, Applied Biosystems), and the purity of the peptides were verified by mass spectrometry. Peptides were dissolved in DMSO and diluted in RPMI medium.

[0138] Immunization of HLA-A*0201 transgenic mice and isolation of TCR: Transgenic mice expressing full-length human HLA-A*0201 gene were obtained from the Jackson Laboratory. Mouse studies were conducted according to the protocols approved by the National Cancer Institute Animal Care and Use Committee as described previously (Parkhurst et al., Clin Cancer Res 15:169-180 (2009)). Briefly, eight to twelve weeks old mice were immunized subcutaneously at the base of the tail with 100 µg of MAGE-A3: 112-120 (KVAELVHFL) (SEQ ID NO: 2) or MAGE-A3: 271-279 (FLWGPRALV) (SEQ ID NO: 63) plus 120µg HBVc: 128-140 helper peptide (TPPAYRPNNAPIL) (SEQ ID NO: 68) emulsified in 100µl of incomplete Freund's adjuvant (Montanide ISA 51). A booster immunization was given one week later. One week after the booster immunization mice were sacrificed and splenocytes were harvested and stimulated in vitro with irradiated T2 cells (18,000 rad) loaded with 0.01, 0.1 or 1µg/mL of the immunizing peptide in R10 medium containing 30 IU/ml rhIL-2. Cultures were set up in 24 well plates with 1-3 million splenocytes and 0.2-0.4 million peptide-loaded T2 cells. One week following stimulation bulk murine T-cell cultures were tested in co-culture assays for peptide specific reactivity.
using T2 cells and tumor cell recognition using 1300 mel and H1299-A*0201 cells. Antigen specific IFN-γ secretion was measured by ELISA (Thermo Scientific, Rockford, IL). Peptide-reactive bulk cultures were then cloned at 10 cells per well in U-bottom 96-well plates with 5 x 10^4 peptide-pulsed irradiated T2 cells and 5 x 10^4 irradiated (3,000 rad) C57BL/6 feeder splenocytes in medium containing 30 IU/mL recombinant human IL-2. Two to three weeks later, growth-positive wells were identified and the T lymphocytes from those wells were transferred into 48-well plates and stimulated with 2 x 10^5 peptide-pulsed irradiated T2 cells and 1 x 10^6 irradiated C57BL/6 splenocytes in medium containing 30 IU/mL rhIL-2. One to 2 weeks later, these T-cell cultures were evaluated for specific recognition of peptide and tumor cells by means of specific IFN-γ secretion. T cells from each of the tumor-reactive clones were expanded by restimulation as described before and the total RNA extracted for TCR isolation.

[0139] **Cloning of MAGE-A3 specific, HLA-A*0201 restricted TCR**: Total RNA was extracted from tumor reactive T cell clones using RNeasy® mini kit (Qiagen). TCR a and β-chains from each tumor reactive T cell clones were cloned using SMART™ RACE cDNA amplification kit (Clontec, Mountain View, CA). For the amplification of TCRs gene specific primers were made from the constant region of mouse TCR a and β-chains, TCRAC-5'-ACTGGTACACAGCAGGTCTGG-3' (SEQ ID NO: 69), TCRBC-5'-AAGGAGACCTTTGGGTAGT C-3' (SEQ ID NO: 70). The PCR products of the 5'-RACE were cloned into PCR2.1 TOPO vector (Invitrogen Life Technologies) and the insert DNA fragments were sequenced. The DNA sequence data were analyzed using The International Immunogenetics Information System® (http://imgt.cines.fr/IMGT_vquest/vquest?livret=0&Option=mouseTcR) for the identification of mouse TCR a and β-chains. Following the identification of the variable regions of a and β-chains and the identification of constant region of the β-chain (CB1 or CB2), specific primers were used to amplify the full length TCR α and β-chains from the cDNA.

[0140] **Electroporation of TCR into PBL**: The full-length a and β chains were individually cloned into the RNA expression vector pGEM-4Z/64A. In vitro transcribed mRNA encoding a and β-chains were generated using mMMESSAGE-mMACHINE (Ambion, Austin, TX) and electroporated into OKT3-stimulated human lymphocytes with an ElectroSquarePorator ECM-830 (BTX, San Diego, CA) as previously described (Zhao et al, *Mol Ther* 13:151-159 (2006)). Electroporated PBL were tested for antigen specific reactivity
using peptide loaded T2 cells and tumor cell lines 2361 RCC, 938 mel, H1299-A*0201 and
1300 mel. Antigen specific response of TCR-electroporated T cells was evaluated by
coculture with respective MAGE-A3 peptide loaded T2 cells and tumor cell lines, and the
culture supernatants were tested for the IFN-γ levels by ELISA.

Construction of retroviral vectors expressing MAGE-A3 specific HLA-A*0201 restricted TCRs: Two MSGV1 based retroviral vectors were constructed using overlapping PCR method with the transgene construct in the following order of configuration: TCR α chain, linker peptide furinSGSGP2A (SEQ ID NO: 19) (Wargo et al., Cancer Immunol Immunother 58:383-394 (2009)) and TCR β chain. The cloned TCR inserts were verified by restriction enzyme digestion and DNA sequencing.

Transduction of PBL: Retroviral supernatants were generated by transfecting respective MSGV1-MAGE-TCR vector DNA from each of the constructs with a plasmid encoding RD1 14 envelope into 293-GP cells using the Lipofectamine 2000 reagent (Invitrogen) in Optimem medium (Invitrogen) (Wargo et al, Cancer Immunol Immunother 58:383-394 (2009)). Retroviral vector expressing TCR against NY ESO-1 was used as a positive control in all of the experiments (Zhao et al., J Immunol 174:4415-4423 (2005)). MSGV1 vector expressing GFP was also generated. Viral supernatants were then loaded on to retronectin coated non-tissue culture treated 6 well plates. PBL were stimulated with OKT3 (50 ng/ml) and rhIL2 (300 IU/ml) 48h prior to transduction and the transduction was carried out as described previously (Wargo et al., Cancer Immunol Immunother 58:383-394 (2009); Hughes Hum Gene Ther 16:457-472 (2005)).

Tetramer staining: HLA-A*0201 restricted MAGE-A3-derived peptides MAGE-A3: i 12-120 (KVAELVHFL) (SEQ ID NO: 2) and MAGE-A3: 271-279 (FLWGPRALV) (SEQ ID NO: 63) were used by the NIH Tetramer Core Facility at Emory University, to produce tetramers linking the phycoerythrin (PE) as fluorophore. MAGE-A3 TCR transduced T cells were stained with a FITC-labeled anti-CD8 (BD Pharmingen, San Jose, CA) and with PE-labeled HLA-A*0201 tetramers. A FITC-conjugated monoclonal antibody against the constant region of the murine β chain (eBioscience, San Diego, CA) and PE-conjugated anti-CD8 antibodies were also used to detect the expression of MAGE-A3 TCRs in the human PBL. Cells were analyzed using a FACScan flow cytometer with CellQuest software (BD Biosciences) or FlowJo software (Tree Star Inc, Ashland, OR).

Intracellular cytokine staining: Intracellular cytokine staining was performed using a BD cytofix/cytoperm™ kit (BD Biosciences) according to the manufacturer's
instructions. Briefly, cells were first stained with cell surface markers CD3 and CD8 and then stained with FITC-conjugated anti-IFN-γ and APC conjugated anti-IL-2 Abs for intracellular detection of the cytokines. All antibodies as well as isotype controls were purchased from BD Biosciences. Cells were analyzed using a CANTO II flow cytometer with CellQuest software (BD Biosciences) or FlowJo software (Tree Star, Inc, Ashland, OR).

**CD107a mobilization assay:** The cell surface mobilization of the CD107a molecule was determined as a measure of degranulation and functional reactivity following antigen recognition by the TCR. In these assays 1×10^5 H1299-A2 or H1299 cells were cocultured with an equal number of PBL at 37°C for 2h. The cells were then stained with mouse anti-human antibodies against CD107a and CD8 (BD Biosciences) and analyzed by FACS.

**Cytokine release assay:** TCR engineered PBLs were tested for antigen specific reactivity in cytokine release assays using peptide loaded T2 cells and tumor cells. In these assays, effector cells (1 x 10^5) were cocultured with equal number of target cells in AIM-V medium in a final volume of 0.2 mL in duplicate wells of a 96-well U-bottom microplate. Culture supernatants were harvested 18-24 hours after the initiation of co-culture and assayed for IFN-γ and GM CSF by ELISA (Thermo Scientific, Rockford, IL).

**^{51}Cr release assay:** The ability of the transduced PBL to lyse HLA-A*0201+/MAGE-A3+ tumor cells was measured using a ^{51}Cr release assay as described in detail in prior publications (Zhao et al., *J Immunol* 174:4415-4423 (2005); Morgan et al. *J Immunol* 171:3287-3295 (2003)). In these assays, TCR engineered PBL were coinubated with decreasing ratios of ^{51}Cr-labeled target cells (E:T) in AIM-V medium in 96-well Li-bottom plates at 37°C for 4h. Lysis was measured by ^{51}Cr release in the medium: percent lysis = (sample release - minimum release)/(maximum release - minimum release) x 100%, average of duplicate samples.

**Lymphocyte proliferation assay:** TCR transduced PBL were tested for antigen specific proliferation using [^3H] thymidine incorporation assay. Briefly, effector cells (1 x 10^5) were cocultured with equal number of irradiated (18,000 rad, cesium source) H1299 or H1299-A2 target cells in AIM-V medium in a final volume of 0.2 mL in triplicate wells of a 96-well U-bottom microplate. The cells were co-cultured for 3 days and pulsed with 1 μCi [^3H] thymidine (DuPont, New England Nuclear, Shelton, CT) per well and cultured for an additional 18h. The cells were then harvested onto a glass fiber filter (Wallac Oy, Turku,
Finland) and radionucleotide incorporation was measured using Perkin Elmer Microbeta Trilux counter (Shelton, CT). Results expressed as counts per minute.

[0149] **CD4/CD8 separation:** MAGE TCR engineered CD4+ and CD8+ populations were separated using magnetic beads-based BD IMag™ human CD4 or CD8 T lymphocyte enrichment set-DM kit for negative selection of those subsets (BD Biosciences).

[0150] **Generation of single amino acid substitutions of CDR3 alpha chain MAGE-A3: 112-120 TCR:** 85 single amino acid substituted TCRs were generated in four stages. 1. Site directed mutagenesis using QuickChange® Lightning Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA) was used to substitute all other 19 amino acids at position 115D introducing appropriate nucleotide changes in the PCR primer. 2. Alanine substitutions at 8 of the residues 114F, 115D, 116T, 117N, 119Y, 120K, 121V and 1221 were introduced except at position 118A that already had alanine residue. 3. Conservative amino acid substitutions were introduced at positions 117N to Q/K/R, 118A to V/L/I, and 119Y R/K/Q/N by site directed mutagenesis. 4. Single amino acid substitutions at these 3 residues were synthesized (GENEART AG, Regensburg, Germany) to produce a 16 amino acid substitution library at each position. Retroviral vector supernatants expressing all of the above mentioned single amino acid substituted TCRs were generated by transient transfection into 293-GP cells, PBL from donors were transduced and tested for tetramer binding and IFN-γ production in co-culture assays with tumor cell lines.

**EXAMPLE 1**

[0151] This example demonstrates the generation of MAGE-A3 reactive T cell clones from HLA-A*0201 transgenic mice.

[0152] Transgenic mice expressing the full-length HLA-A*0201 molecule were immunized with one of the two previously identified naturally processed and presented HLA-A*0201 restricted peptides from MAGE-A3 [MAGE-A3: 112-120 (KVAELVHFL) (SEQ ID NO: 2) (Kawashima et al., *Hum. Immunol.*, 59:1-14 (1998)) or MAGE-A3: 271-279 (FLWGPRALV) (SEQ ID NO: 63) (van der Bruggen, *Eur. J. Immunol.*, 24:3038-3043 (1994))] along with a helper peptide, HBVc: 128-140. Following two immunizations, murine T cells were harvested from spleen and lymph nodes and stimulated in vitro with the respective peptide and IL-2. Bulk T cell cultures from mice immunized with the MAGE-A3 peptides demonstrated specific reactivity against T2 cells pulsed with the relevant peptide and
the HLA-A*0201 + MAGE-A3 + tumor cell lines H1299-A*0201, 1300 mel and 624 mel after two in vitro stimulations. Reactive T cells from positive wells were cloned by limiting dilution and tested for antigen specific reactivity. Five clones derived from the mice immunized with the MAGE-A3: 112-120 peptide and six clones from the mice immunized with the MAGE-A3: 271-279 peptide that secreted high levels of IFN-γ in response to tumor cells and peptide loaded T2 cells were expanded and further characterized.

EXAMPLE 2

[0153] This example demonstrates the cloning of MAGE reactive TCRs.
[0154] TCR α and β-chains from each tumor reactive T cell clone were cloned using SMART™ RACE cDNA amplification kit with gene specific primers in the constant region of mouse TCR α and β-chains. After the identification of the variable regions of α and β-chains and the specific constant region of the β-chain, specific primers were used to amplify the full length TCR α and β-chains from the cDNA. The TCR α and β chains were then cloned into RNA expression vector pGEM. In vitro transcribed RNA of TCR α and β chains were electroporated into human PBL and tested for antigen specific reactivity as described previously (Zhao et al., Mol. Ther., 13:151-159 (2006)) using peptide loaded T2 cells, H1299-A*0201 and 1300 mel tumor cell lines. Based on the specific reactivity, a TCR against MAGE-A3: 112-120 peptide (TCRoc-TRAV12D-3, TCRp-TRBV29*01, CB1) (SEQ ID NOs: 17 and 18) and a TCR against MAGE-A3: 271-279 peptide (TRAV17*02, TRBV 15*01, CB2) (SEQ ID NOs: 31 and 32) were selected for further evaluation.

EXAMPLE 3

[0155] This example demonstrates the construction of a MAGE-A3 TCR expressing retroviral vector and transduction of PBL.
[0156] Two MSGV1 based retroviral vectors with expression cassettes including TCRα-TRAV12D-3 and TCRp-TRBV29*01-CB1 (SEQ ID NO: 57), and TRAV17*02 and TRBV15*01-CB2 (SEQ ID NO: 59) were constructed. The TCR expression in these vectors is driven by the viral LTR, α and β chains are expressed as a single open reading frame using the 2A linker peptide (SEQ ID NO: 19) (Wargo et al., Cancer Immunol. Immunother., 58:383-394 (2009); Yang et al., Gene Ther., 15:141 1-1423 (2008)). Human PBL were stimulated for 2 days and then transduced. FACS analysis of transduced PBL using the anti-
mouse TCR-β chain revealed that both CD8+ and CD4+ cells had been transduced with these TCR vectors; however, specific tetramer binding was observed only in transduced CD8+ and not CD4+ T cells.

EXAMPLE 4

[0157] This example demonstrates the function of the MAGE-TCR engineered PBL.

[0158] To evaluate the recognition of the respective MAGE-A3 TCRs, PBL were transduced as described in Example 3 and subjected to a co-culture assay with peptide pulsed T2 cells. TCR transduced PBL specifically secreted IFN-γ upon encounter with the antigenic peptide in a dose dependent manner (Figures 1A, IB). PBL transduced with either anti-MAGE-A3: 112-120 or anti-MAGE-A3: 271-279 TCRs recognized T2 cells pulsed with as little as 0.1 ng/ml MAGE-A3 peptides, indicating that both of the TCRs were relatively high avidity receptors. Co-culture of PBL expressing TCR against MAGE-A3: 112-120 or MAGE-A3: 271-279 with control T2 cells that were not pulsed with any peptides produced background levels of IFN-γ.

[0159] To assess the specific recognition of tumor cells, TCR engineered PBL were co-cultured with a panel of HLA-A*0201+ and HLA-A*0201− melanoma and lung tumor derived cell lines. Specific release of IFN-γ was observed when the TCR engineered PBL were co-cultured with HLA-A*0201+/MAGE-A3+ cell lines but not HLA-A*02017MAGE-A3+ or HLA-A*0201+/MAGE-A3+ cell lines (Table 3). A comparison of the two TCRs revealed that T cells transduced with the anti-MAGE-A3: 112-120 TCR released approximately 10-fold higher levels of IFN-γ in response to HLA-A*0201+/MAGE-A3+ tumor cell targets (Table 3). These responses were specific because low levels of IFN-γ were released in response to MAGE+/HLA-A*0201− cell lines and MAGE7HLA-A*0201+ cell lines. Although anti-MAGE-A3: 271-279 TCR transduced PBL efficiently recognized the peptide loaded on T2 cells, the recognition of MAGE-A3+/HLA-A*0201+ tumor cells as measured by the release of IFN-γ production was relatively weak (Table 3).
Table 3. Interferon-γ production by the TCR transduced PBL following co-culture with tumor cell lines.

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Antigen-specific IFN-γ secretion measured by ELISA. RCC-renal cell carcinoma, SCLC-small cell lung carcinoma, Mel-melanoma, NSCLC-Non-small cell lung carcinoma. MAGE-A3/6 and MAGE-A12 mRNA expression levels were quantified using a quantitative real time PCR assay. Co-culture assay was done with PBL from three donors and the result of a representative donor is presented. Values are mean of duplicate samples. Results are presented here as representative of three experiments of PBL from separate donors.
The specific lysis of melanoma cell lines by the TCR engineered PBL was next measured. Anti-MAGE-A3: 112-120 TCR transduced PBL demonstrated superior lytic function against MAGE-A3+/HLA-A*0201+ tumor cell lines 1300 mel and 526 mel cells compared to anti-MAGE-A3: 271-279 TCR transduced PBL (Figures 2A-2F). There was little or no lysis of HLA-A*0201 tumor cells line 938 mel, and the untransduced PBL showed little reactivity against any of the target cells (Figures 2A-2F). Anti-MAGE-A3: 112-120 TCR transduced PBL also showed superior lytic function against NSCLC cell line H1299-A*0201+ and did not recognize the parental non-HLA-A*0201 cell line H1299 (Figures 3A-3D). Because tetramer binding was observed only in anti-MAGE-A3 TCR transduced CD8+ cells and not in CD4+ cells, IFN-γ production and cytolytic activity were investigated using purified lymphocytes. As shown in Figures 4A-4D, antigen specific lysis of melanoma cell lines 1300 mel and 526 mel was evident only in CD8+ cells and not seen in CD4+ cells. Similarly, IFN-γ production following co-culture with tumor cells was observed only in CD8+ cells and was not seen in CD4+ cells. Based on these data, the anti-MAGE-A3: 112-120 TCR was chosen for further analysis.

This example demonstrated that PBLs transduced with a retroviral vector expressing either the anti-MAGE-A3 271-279 TCR or the unsubstituted anti-MAGE-A3 112-120 TCR have high avidity and high reactivity for MAGE-A3+/HLA-A*0201+ target cells and specifically lyse tumor cells. This example also demonstrates that the unsubstituted anti-MAGE-A3 112-120 TCR shows superior reactivity and specific cell lysis in comparison to the anti-MAGE-A3 271-279 TCR.

EXAMPLE 5

This example demonstrates that amino acid substitutions within the CDR3 region of the TCR α chain enhance the function of TCR engineered PBL.

Retroviral vectors expressing 85 single amino acid substitutions in the CDR3 region of the TCR-α chain were generated and their function was tested in PBL. Preliminary screening experiments were carried out by single amino acid residue alanine substitution in the CDR3 region of the α chain. Alanine substitution at 114F, 115D, 116T, 117N, 119Y, 120K and 121V (with respect to SEQ ID NO: 17) completely abolished the activity of the TCR. This was seen by the complete loss of tetramer binding as well as lack of production of IFN-γ following co-culture with peptide pulsed T2 cell or MAGE+/HLA-A*0201+ tumor cells. 19 amino acids substitutions were created at position 115 (with respect to SEQ ID NO:
17). Complete loss of TCR activity as seen by the complete lack of tetramer binding and loss of IFN-γ production was observed when aspartic acid at position 115 was substituted with any of the other amino acids. A retroviral vector library of single amino acid substitutions at positions 117, 118 and 119 of the a-chain (SEQ ID NO: 17) was created. This screening determined that a substitution of valine or threonine for the alanine residue present at position 118 in the wild type a-chain retained TCR function.

[0164] Table 4 shows the IFN-γ secretion results of a co-culture assay with tumor cells of different histologies using the substituted TCRs. Antigen specific HLA-A*0201 restricted recognition of tumor cells from diverse histologies was observed following co-culture of TCR engineered PBL with breast cancer line MDA-454S-A2, glioma line U251, melanoma lines 624 and 526, esophageal line BE-3 and non-small cell lung cancer line H1299-A*0201.
Table 4. Interferon-γ production by the modified anti-MAGE-A3-TCR-118AT transduced PBL or substituted anti-MAGE-A3-TCR-118AV transduced PBL following co-culture with tumor cell lines of different histologies.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Histology</th>
<th>mRNA Copies/10⁶ 6-actin</th>
<th>Interferon-γ (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAGE-A3/6</td>
<td>MAGE-A12</td>
<td>Untransduced</td>
</tr>
<tr>
<td>1299</td>
<td>NSCLC</td>
<td>32346</td>
<td>7862</td>
</tr>
<tr>
<td>2361</td>
<td>RCC</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>888</td>
<td>Melanoma</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>MDA-453S-A'0201</td>
<td>Breast</td>
<td>4437</td>
<td>629</td>
</tr>
<tr>
<td>U251</td>
<td>CNS-Glioma</td>
<td>3643</td>
<td>1087</td>
</tr>
<tr>
<td>624.38</td>
<td>Melanoma</td>
<td>41541</td>
<td>1040</td>
</tr>
<tr>
<td>BE-3</td>
<td>Esophageal</td>
<td>2554</td>
<td>1228</td>
</tr>
<tr>
<td>526</td>
<td>Melanoma</td>
<td>3002</td>
<td>6966</td>
</tr>
<tr>
<td>1299-A'0201</td>
<td>NSCLC</td>
<td>57045</td>
<td>18875</td>
</tr>
</tbody>
</table>

Donor PBL transduced with a retroviral vector expressing wild type or single amino acid substitutions of anti-MAGE-A3: 112-120 TCR were co-cultured with tumor cell lines. Untransduced PBL were used as controls. Antigen specific IFN-γ secretion was measured by ELISA. MAGE-A3/6 and MAGE-A12 mRNA expression levels were estimated using a quantitative real time PCR assay. Values are mean of duplicate samples. Results are presented here as a representative of two experiments using PBL from separate donors.

¹ Comprises SEQ ID NO: 22, wherein Xaa at position 118 is Val (substituted α chain) and SEQ ID NO: 18 (β chain).
² Comprises SEQ ID NO: 22, wherein Xaa at position 118 is Thr (substituted α chain) and SEQ ID NO: 18 (β chain).
The results demonstrated that T cells transduced with the 118AV or 118AT substituted TCR secreted higher levels of IFN-γ than cells transduced with the wild type (unsubstituted) TCR. The results of several experiments indicated that T cells transduced with a TCR including SEQ ID NO: 18 (β chain) and SEQ ID NO: 22, wherein Xaa at position 118 is Thr (substituted α chain) (hereinafter, "118AT substituted TCR") secreted higher levels of IFN-γ than the TCR including SEQ ID NO: 18 (β chain) and SEQ ID NO: 22, wherein Xaa at position 118 is Val (substituted α chain) (hereinafter, "118AV substituted TCR") when tested against multiple MAGE+/HLA-A*0201+ cells. The HLA-A*020T cell lines H1299 and 888 cells as well as MAGEVHLA-A*0201+ cell line 2361-RCC were not recognized by the PBL engineered to express either the wild type or the 118AT substituted TCR, indicating that this amino acid alteration did not alter the specificity of this TCR. This example demonstrated that PBLs transduced with a retroviral vector expressing the 118AT substituted MAGE-A3 112-120 TCR or the 118AV substituted MAGE-A3 112-120 TCR demonstrate enhanced specific lytic activity against tumor cells as compared to the wild-type (unsubstituted) TCR.

EXAMPLE 6

This example demonstrates the enhanced function of 118AT substituted TCR transduced PBL as compared to PBL transduced with wild-type TCR. To further test the function of the substituted MAGE-TCRs, the CD4+ cells were purified and tested in a co-culture assay with 1300 mel, H1299-A*0201 NSCLC, U251 Glioma cells and peptide loaded T2 cells. CD4+ cells engineered with the anti-MAGE-A3: 112-120 118AT substituted TCR specifically secreted IFN-γ in response to MAGE+/HLA-A*0201+ tumor cells whereas no response was observed in CD4+ T cells transduced with the wild type (unsubstituted) TCR (Table 5).
In addition, the 118AT substituted TCR led to enhanced recognition of peptide pulsed target cells. Tetramer analysis of anti-MAGE-A3: 112-120 118AT substituted TCR transduced PBL showed MAGE-A3: 112-120/HLA-A*0201 specific tetramer binding in CD4+ cells though with lesser intensity than in CD8+ cells.

In an effort to compare the function of anti-MAGE-A3: 112-120 wild type and 118AT substituted TCR expressing PBLs, co-culture assays were performed with MAGE-A3: 112-120 peptide pulsed T2 cells. Anti-MAGE-A3: 112-120 118AT substituted TCR expressing PBL produced higher levels of IFN-γ than the wild type (unsubstituted) TCR transduced PBL (Figures 5A and 5B).

Secretion of GM-CSF by the anti-MAGE-A3 TCR transduced PBL was also measured following co-culture with H1299, H1299-A2 and 624.38 cells. Anti-MAGE-A3: 112-120 118AT substituted TCR expressing PBL produced higher levels of GM-CSF than the wild type (unsubstituted) TCR transduced PBL (Figures 6A and 6B).

A cell proliferation assay was then performed to measure the antigen specific proliferation of MAGE TCR engineered PBL. When co-cultured with H1299-A2 tumor cell lines, both anti-MAGE-A3: 112-120 wild type as well as 118AT substituted TCR transduced PBL proliferated extensively as measured by the radiolabeled thymidine incorporation, but not with H1299 cells. Untransduced PBL exhibited background level of proliferation in response to both H1299 and H1299-A2 cells.

The percentage of anti-MAGE-A3: 112-120 wild-type (WT) or 118AT substituted TCR transduced PBL that produced IFN-γ and IL-2 after overnight co-culture with H1299-

### TABLE 5

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>IFN-γ fpg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-MAGE</td>
<td></td>
</tr>
<tr>
<td>WT TCR</td>
<td>anti-MAGE</td>
</tr>
<tr>
<td></td>
<td>118AV</td>
</tr>
<tr>
<td></td>
<td>substituted</td>
</tr>
<tr>
<td>1300 melanoma</td>
<td>&lt;30</td>
</tr>
<tr>
<td>1299-A*0201 NSCLC</td>
<td>&lt;30</td>
</tr>
<tr>
<td>U251 Glioma</td>
<td>&lt;30</td>
</tr>
<tr>
<td>T2 cells pulsed-MAGE peptide</td>
<td>395</td>
</tr>
</tbody>
</table>

[0169] In addition, the 118AT substituted TCR led to enhanced recognition of peptide pulsed target cells. Tetramer analysis of anti-MAGE-A3: 112-120 118AT substituted TCR transduced PBL showed MAGE-A3: 112-120/HLA-A*0201 specific tetramer binding in CD4+ cells though with lesser intensity than in CD8+ cells.

[0170] In an effort to compare the function of anti-MAGE-A3: 112-120 wild type and 118AT substituted TCR expressing PBLs, co-culture assays were performed with MAGE-A3: 112-120 peptide pulsed T2 cells. Anti-MAGE-A3: 112-120 118AT substituted TCR expressing PBL produced higher levels of IFN-γ than the wild type (unsubstituted) TCR transduced PBL (Figures 5A and 5B).

[0171] Secretion of GM-CSF by the anti-MAGE-A3 TCR transduced PBL was also measured following co-culture with H1299, H1299-A2 and 624.38 cells. Anti-MAGE-A3: 112-120 118AT substituted TCR expressing PBL produced higher levels of GM-CSF than the wild type (unsubstituted) TCR transduced PBL (Figures 6A and 6B).

[0172] A cell proliferation assay was then performed to measure the antigen specific proliferation of MAGE TCR engineered PBL. When co-cultured with H1299-A2 tumor cell lines, both anti-MAGE-A3: 112-120 wild type as well as 118AT substituted TCR transduced PBL proliferated extensively as measured by the radiolabeled thymidine incorporation, but not with H1299 cells. Untransduced PBL exhibited background level of proliferation in response to both H1299 and H1299-A2 cells.

[0173] The percentage of anti-MAGE-A3: 112-120 wild-type (WT) or 118AT substituted TCR transduced PBL that produced IFN-γ and IL-2 after overnight co-culture with H1299-
A2 cells was determined by intracellular cytokine staining. The percentage of cells that produced IFN-γ in response to antigen exposure was almost twice in number in the case of 118AT substituted TCR transduced PBL as compared to WT TCR expressing cells, whereas a marginal increase in the number of IL-2 producing cells was observed.

The functional ability of anti-MAGE-A3 wild type and 118AT substituted TCR engineered PBLs was also compared in a CD107a mobilization assay. Anti-MAGE TCR transduced PBL were co-cultured with H1299 or H1299-A2 cells for 2 hrs and analyzed for CD107a antigen expression. A significantly higher number of anti-MAGE-A3 118AT substituted TCR transduced cells stained positive for CD107a expression compared to WT TCR transduced PBL following co-culture with H1299-A2 cells. Untransduced cells showed background levels of staining.

This example demonstrated that 118AT substituted TCR transduced PBL demonstrate enhanced recognition of peptide pulsed target cells and secrete higher levels of IFN-γ and GM-CSF upon co-culture with tumor cells as compared to PBL transduced with wild-type TCR.

EXAMPLE 7

This example demonstrates that the unsubstituted anti-MAGE-A3 112-120 TCR has antigenic specificity for MAGE-A3 and MAGE-A12 and also recognizes MAGE-A2 and MAGE-A6.

MAGE-A3 TCR transduced PBL were tested for the recognition of peptides derived from other members of the MAGE-A family by loading T2 cells with synthetic peptides and performing co-culture assays to test the peptide recognition and secretion of IFN-γ. As shown in Figure 7, in addition to the MAGE-A3: 112-120 (KVAELVHFL) peptide (SEQ ID NO: 2), MAGE-A12: 112-120 (KMAELVHFL) (SEQ ID NO: 4) was also recognized efficiently by the unsubstituted MAGE-A3 112-120 TCR engineered PBL. Peptides derived from MAGE-A2 (SEQ ID NO: 6) and MAGE-A6 (SEQ ID NO: 8) were also recognized by the TCR engineered PBL, albeit with lower avidity (Figure 7).

It was then investigated whether TCR engineered PBL were able to recognize the endogenously processed peptides from MAGE-A3 and A12 expressing cells. The recognition of COS7-A*0201 and 293-A*0201 cells that were retrovirally transduced with full length MAGE-A3 or A12 genes was tested in co-culture assay using PBL transduced with the unsubstituted and substituted TCRs. As seen in Table 6, superior recognition of
MAGE-A12 was evident in both COS7-A*0201 and 293-A*0201 cells, and again the 118AT substituted TCR demonstrated the most activity.
Table 6. Interferon-γ production by the TCR transduced PBL following co-culture with COS-A2 and 293-A2 cell lines expressing full-length MAGE-A3 or A12 genes.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>MAGE-A3/6 (copies/μg)</th>
<th>MAGE-A12 (copies/μg)</th>
<th>Untransduced (copies/μg)</th>
<th>Interferon (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anti-MAGE-118AV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anti-MAGE-118AT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(substituted) TCR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TCR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TCR</td>
</tr>
<tr>
<td>H1299-A2</td>
<td>57045</td>
<td>18875</td>
<td>48</td>
<td>27191</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45158</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>83294</td>
</tr>
<tr>
<td>COS7-A2</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;30</td>
<td>&lt;30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45158</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>293-A2</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;30</td>
<td>&lt;30</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>45158</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>COS7-A2-MAGE A3</td>
<td>602975</td>
<td>&lt;100</td>
<td>93</td>
<td>3644</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6063</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25920</td>
</tr>
<tr>
<td>293-A2-MAGE A3</td>
<td>547966</td>
<td>673</td>
<td>108</td>
<td>27375</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>33884</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72582</td>
</tr>
<tr>
<td>293-A2-MAGE A12</td>
<td>334</td>
<td>285454</td>
<td>88</td>
<td>4456</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>829</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72582</td>
</tr>
</tbody>
</table>

PBLs transduced with retroviral vector expressing TCR were co-cultured with COS7-A2 or 293-A2 cells expressing MAGE-A3 or A12 genes. Antigen specific IFN-γ secretion was measured by ELISA. MAGE-A3/6 and MAGE-A12 mRNA expression levels were estimated using a quantitative real time PCR assay. Values are mean of duplicate samples. Results are presented here as a representative of two independent experiments using PBL from separate donors.
[0179] This example demonstrated that the unsubstituted anti-MAGE-A3 12-120 TCR has antigenic specificity for MAGE-A3 and MAGE-A12 and also recognizes MAGE-A2 and MAGE-A6.

EXAMPLE 8

[0180] This example demonstrates the persistence of anti-MAGE-TCR engineered PBL and that the anti-MAGE-TCR engineered PBL mediate tumor regression.

[0181] Prior to administering PBL transduced with the 118AT substituted TCR to Patient 1, a pre-infusion co-culture assay was performed. Untransduced (UT) cells or cells transduced with the 118AT substituted TCR were cultured alone (none) or were co-cultured with various melanoma cell lines (Table 7A) or T2 cells pulsed with 1 µM MAGE-A3 peptide (or MART peptide as a control) Table 7B. IFN-γ secretion (pg/ml) was measured and the results are set forth in Tables 7A and 7B.

<table>
<thead>
<tr>
<th>TABLE 7A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma Cell Line</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Melanoma Cell Line</td>
</tr>
<tr>
<td>UT</td>
</tr>
<tr>
<td>118AT substituted TCR Td(bag)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 7B</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 cells pulsed with i µM peptide</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>
HLA-A*0201 patients with metastatic cancer having tumors expressing MAGE-A3 and/or MAGE-A12 were treated with a non-myeloablative but lymphoid depleting preparative regimen, followed by IL-2 and PBL transduced with the 118AT substituted TCR.

One month after the 118AT substituted TCR engineered PBL were infused into Patients 1 and 2, persistence of the 118AT substituted TCR engineered PBL was measured using flow cytometry. 16.2% of the 118AT substituted TCR engineered PBL persisted in Patient 1 one month after infusion, while 50.6% of the 11SAT substituted TCR engineered PBL persisted in Patient 2 one month after infusion.

Clinical tumor regression was evaluated in Patients 1-3. Evaluation of Patients 4-6 is ongoing. The results are set forth in Table 8.
TABLE 8

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Cancer</th>
<th>MAGE-A Expression</th>
<th>Cells (10 x 10^6)</th>
<th>%CD4/%CD8</th>
<th>% cells expressing transduced TCR+ (prior to administration)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59/M</td>
<td>Melanoma</td>
<td>&gt;80%</td>
<td>27.9</td>
<td>36/64</td>
<td>92</td>
<td>Complete Response (at 5 months)</td>
</tr>
<tr>
<td>2</td>
<td>38/F</td>
<td>Melanoma</td>
<td>&gt;70%</td>
<td>30</td>
<td>87/13</td>
<td>90</td>
<td>No Response</td>
</tr>
<tr>
<td>3</td>
<td>57/F</td>
<td>Melanoma</td>
<td>&gt;90%</td>
<td>30</td>
<td>63/35</td>
<td>92</td>
<td>Partial Response (at 3 months)</td>
</tr>
<tr>
<td>4</td>
<td>54/M</td>
<td>Melanoma</td>
<td>&gt;70%</td>
<td>78.8</td>
<td>39/56</td>
<td>81</td>
<td>Not yet determined</td>
</tr>
<tr>
<td>5</td>
<td>44/M</td>
<td>Melanoma</td>
<td>&gt;95%</td>
<td>53</td>
<td>55/43</td>
<td>77</td>
<td>Not yet determined</td>
</tr>
<tr>
<td>6</td>
<td>21/F</td>
<td>Synovial cell</td>
<td>&gt;90%</td>
<td>41</td>
<td>42/58</td>
<td>61</td>
<td>Not yet determined</td>
</tr>
</tbody>
</table>

[0185] Computed tomography (CT) scans of the lung of Patient 1 (Figures 8A-E) before and after treatment with the 118AT substituted TCR engineered PBL demonstrate the presence of a tumor before treatment (Figure 8A and 8D) and significant shrinkage of the tumor one month (Figure 8B) and two months (Figure 8C) after treatment. The tumor was not visible in the CAT scan three months after treatment (Figure 8E).

[0186] CT scans of the abdominal wall of Patient 3 (Figures 9A-F) before and after treatment with the 118AT substituted TCR engineered PBL demonstrate the presence of a tumor before treatment (Figures 9A-9C) and significant shrinkage of the tumor one month after treatment (Figures 9D-9F).
EXAMPLE 9

[0187] This example demonstrates the expression of MAGE-A3/6 and MAGE-A12 in melanoma patients.

[0188] MAGE-A3/6 mRNA and MAGE-A12 mRNA expression was measured in fresh melanoma tumor digests from 44 metastatic melanoma patients using quantitative reverse transcriptase PCT (qRT-PCR). 66% of the patients expressed more than 1,000 copies of MAGE-A3/6 mRNA. 50% of the patients expressed more than 1,000 copies of MAGE-A12 mRNA.

[0189] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0190] The use of the terms "a" and "an" and "the" 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 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.

[0191] 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.
CLAIM(S):

1. An isolated or purified T cell receptor (TCR) having antigenic specificity for melanoma antigen family A (MAGE A)-3 and MAGE-A12, wherein the TCR also recognizes at least one of MAGE-A2 and MAGE-A6.

2. An isolated or purified TCR having antigenic specificity for MAGE-A3 and MAGE-A12.

3. The TCR of claim 1 or 2, comprising the amino acid sequences of SEQ ID NOs: 9-14.

4. The TCR of claim 3, comprising the amino acid sequences of SEQ ID NOs: 15 and 16.

5. The TCR of claim 4, comprising the amino acid sequences of SEQ ID NOs: 17 and 18.


7. The TCR of claim 6, comprising the amino acid sequences of SEQ ID NOs: 29-30.

8. The TCR of claim 7, comprising the amino acid sequences of SEQ ID NOs: 31-32.

9. The TCR of claim 5 or 8, further comprising a linker peptide comprising SEQ ID NO: 19.

10. The TCR of claim 9, comprising the amino acid sequence of SEQ ID NO: 60 or 62.

11. A TCR comprising the amino acid sequence of SEQ ID NO: 20, wherein Xaa7 is Thr or Val.
12. The TCR of claim 11 comprising the amino acid sequence of SEQ ID NO: 21, wherein XaaL 18 is Thr or Val.

13. The TCR of claim 12 comprising the amino acid sequence of SEQ ID NO: 22, wherein XaaL 18 is Thr or Val.

14. The TCR of claim 13 comprising SEQ ID NO: 61.

15. A polypeptide comprising a functional portion of the TCR of any of claims 1 to 14, wherein the functional portion comprises:
   a) the amino acid sequences of SEQ ID NOs: 9-14;
   b) the amino acid sequences of SEQ ID NOs: 23-28; or
   c) the amino acid sequence of SEQ ID NO: 20, wherein Xaa7 is Thr or Val; wherein the polypeptides of a) and b) are isolated or purified.

16. The polypeptide of claim 15, wherein the portion comprises:
   d) the amino acid sequences of SEQ ID NOs: 15-16;
   e) the amino acid sequences of SEQ ID NOs: 29-30; or
   f) the amino acid sequence of SEQ ID NO: 21, wherein XaaL 18 is Thr or Val; wherein the polypeptides of d) and e) are isolated or purified.

17. The polypeptide of claim 16, wherein the portion comprises:
   g) the amino acid sequences of SEQ ID NOs: 17-18;
   h) the amino acid sequences of SEQ ID NOs: 31-32; or
   i) the amino acid sequence of SEQ ID NO: 22, wherein XaaL 18 is Thr or Val; wherein the polypeptides of g) and h) are isolated or purified.

18. A protein comprising at least one of the polypeptides of any of claims 15-17, wherein the proteins comprising any of a), b), d), e), g), and h) are isolated or purified.

19. The protein of claim 18, comprising:
   a first polypeptide chain comprising the amino acid sequence of
(k) SEQ ID NOs: 9-11;
   (l) SEQ ID NOs: 23-25; or

(m) the amino acid sequence of SEQ ID NO: 20, wherein Xaa7 is Thr or Val;

and

a second polypeptide chain comprising the amino acid sequence of

(n) SEQ ID NOs: 12-14; or

(o) SEQ ID NOs: 26-28, wherein the polypeptides comprising any of (k), (l), (n),

and (o) are isolated or purified.

20. The protein of claim 19, comprising:

a first polypeptide chain comprising the amino acid sequence of

(p) SEQ ID NO: 15;

(q) SEQ ID NOs: 29; or

(r) the amino acid sequence of SEQ ID NO: 21, wherein Xaa18 is Thr or Val;

and

a second polypeptide chain comprising the amino acid sequence of

(s) SEQ ID NO: 16; or

(t) SEQ ID NOs: 30, wherein the polypeptides comprising any of (p), (q), (s), and

(t) are isolated or purified.

21. The protein of claim 20, comprising:

a first polypeptide chain comprising the amino acid sequence of

(u) SEQ ID NO: 17;

(v) SEQ ID NOs: 31; or

(w) the amino acid sequence of SEQ ID NO: 22, wherein Xaa18 is Thr or Val;

and

a second polypeptide chain comprising the amino acid sequence of

(x) SEQ ID NO: 18; or

(y) SEQ ID NOs: 32, wherein the polypeptides comprising any of (u), (v), (x),

and (y) are isolated or purified.

22. The protein of claim 18, comprising an amino acid sequence comprising any one

of SEQ ID NOs: 60; SEQ ID NO: 61, wherein Xaa18 is Thr or Val; and SEQ ID NO: 62.
23. The protein of any of claims 18-22, wherein the protein is a fusion protein.

24. The protein of any of claims 18-23, wherein the protein is a recombinant antibody.

25. A nucleic acid comprising a nucleotide sequence encoding the TCR of any one of claims 1-14, the polypeptide of any one of claims 15-17, or the protein of any one of claims 18-24, wherein the nucleic acid encoding any of the TCR of any one of claims 1-8, the polypeptides of any one of a), b), d), e) g), and h), and the protein of any one of k), l), m), o), p), q) s), t), u), v), x), and y) are isolated or purified.

26. The nucleic acid of claim 25, comprising a nucleotide sequence comprising:
   z) SEQ ID NOs: 33-38;
   aa) SEQ ID NOs: 47-52; or
   bb) SEQ ID NOs: 44, wherein NNN at positions 19-21 is a codon that encodes Thr or Val, and wherein the nucleic acids of z) and aa) are isolated or purified.

27. The nucleic acid of claim 26, comprising a nucleotide sequence comprising:
   cc) SEQ ID NOs: 39-40;
   dd) SEQ ID NOs: 53-54; or
   ee) SEQ ID NO: 45, wherein NNN at positions 352-354 is a codon that encodes Thr or Val, and wherein the nucleic acids of cc) and dd) are isolated or purified.

28. The nucleic acid of claim 27, comprising a nucleotide sequence comprising:
   ff) SEQ ID NOs: 41-42;
   gg) SEQ ID NOs: 55-56; or
   hh) SEQ ID NO: 46, wherein NNN at positions 352-354 is a codon that encodes Thr or Val, and wherein the nucleic acids of ff) and gg) are isolated or purified.

29. The nucleic acid of claim 28, comprising a nucleotide sequence comprising:
   ii) SEQ ID NO: 57;
jj) SEQ ID NO: 58, wherein NNN at positions 352-354 is a codon that encodes Thr or Val; or
    kk) SEQ ID NO: 59, wherein the nucleic acids of ii) and kk) are isolated or purified.

30. An isolated or purified nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of the nucleic acid of any one of claims 25-29.

31. An isolated or purified nucleic acid comprising a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of the nucleic acid of any one of claims 25-29.

32. A recombinant expression vector comprising the nucleic acid of any one of claims 25-31.

33. An isolated host cell comprising the recombinant expression vector of claim 32.

34. The isolated host cell of claim 33, wherein the cell is a peripheral blood lymphocyte (PBL).

35. The isolated host cell of claim 34, wherein the PBL is a T cell.

36. A population of cells comprising at least one host cell of any one of claims 33-35.

37. An antibody, or antigen binding portion thereof, which specifically binds to a functional portion of the TCR of any of claims 1 to 14, wherein the functional portion comprises the amino acid sequences of:
    11) SEQ ID NOs: 9-14;
    mm) SEQ ID NOs: 23-28; or
    nn) SEQ ID NO: 20, wherein Xaa7 is Thr or Val.

38. A pharmaceutical composition comprising the TCR of any of claims 1 to 14, the polypeptide of any of claims 15 to 17, the protein of any of claims 18 to 24, the nucleic acid of any of claims 25 to 31, the recombinant expression vector of claim 32, the host cell of any
of claims 33 to 35, the population of cells of claim 36, the antibody, or antigen binding portion thereof, of claim 37, and a pharmaceutically acceptable carrier.

39. A 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 of claims 1 to 14, the polypeptide of any of claims 15 to 17, the protein of any of claims 18 to 24, the nucleic acid of any of claims 25 to 31, the recombinant expression vector of claim 32, the host cell of any of claims 33 to 35, the population of cells of claim 36, the antibody, or antigen binding portion thereof, of claim 37, thereby forming a complex, and
   (ii) detecting the complex,
wherein detection of the complex is indicative of the presence of cancer in the host.

40. A method of treating or preventing cancer in a host, comprising administering to the host the TCR of any of claims 1 to 14, the polypeptide of any of claims 15 to 17, the protein of any of claims 18 to 24, the nucleic acid of any of claims 25 to 31, the recombinant expression vector of claim 32, the host cell of any of claims 33 to 35, the population of cells of claim 36, the antibody, or antigen binding portion thereof, of claim 37, or the pharmaceutical composition of claim 38, in an amount effective to treat or prevent cancer in the host.

41. The method of claims 39 or 40, 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, or urothelial cancer.

42. The method of any of claims 39-41, wherein the host cell is a cell that is autologous to the host.

43. The method of any of claims 39-42, wherein the cells of the population are cells that are autologous to the host.

44. Use of the TCR of any of claims 1 to 14, the polypeptide of any of claims 15 to 17, the protein of any of claims 18 to 24, the nucleic acid of any of claims 25 to 31, the
recombinant expression vector of claim 32, the host cell of any of claims 33 to 35, the population of cells of claim 36, the antibody, or antigen binding portion thereof, of claim 37, or the pharmaceutical composition of claim 38, for the treatment or prevention of cancer in a host.

45. The use of claim 44, 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, or urothelial cancer.

46. The use of claim 44 or 45, wherein the host cell is a cell that is autologous to the host.

47. The use of any of claims 44-46, wherein the cells of the population are cells that are autologous to the host.
FIG. 1

(A) MAGE-A3 TCR:112-120

(B) MAGE-A3 TCR:271-279

IFN-γ (pg/ml)

ng/ml of MAGE peptide
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K14/705

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>US 2006/263283 AI (KOSMATOPULOS KOSTAS [FR] ET AL) 23 November 2006 (2006-11-23) e.g. table 1, II; paragraph 53, 61; example 3; the whole document</td>
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Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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Date of the actual completion of the international search: 20 February 2012

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