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(54) Title: ANTI-MAGE-A3/A6 ANTIBODIES

(57) Abstract: Disclosed are isolated or purified antibodies, or antigen-binding fragments thereof, which specifically bind to one or both of MAGE-A3 and MAGE-A6 and does not bind to any one or more of MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11 and MAGE-A12. Related polypeptides, proteins, anti-MAGE-A3, and anti-MAGE-A6 binding moieties are also disclosed. Also disclosed are methods of assaying a biological sample for the presence of one or both of MAGE-A3 and MAGE-A6. Relating methods of diagnosing, treating, and prognosing cancer in a mammal are also disclosed.

ANTI-MAGE-A3/A6 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 62/425,231, filed November 22, 2016, which is incorporated by reference in its entirety herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government support under project number BC010985 by the National Institutes of Health, National Cancer Institute. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 15,630 Byte ASCII (Text) file named "730839_ST25.txt," dated October 23, 2017.

BACKGROUND OF THE INVENTION

[0004] Immunotherapy may result in positive clinical results in some cancer patients by targeting an antigen expressed by the patient's cancer cells. To determine whether a cancer patient is eligible for a particular immunotherapy, the patient's tumor specimen may be tested for expression of the cancer antigen which is targeted by the immunotherapy. Despite advancements in the field of antigen detection, obstacles to the accurate detection of the expression of cancer antigens exist. Accordingly, there exists a need for improved methods and compositions for detecting cancer antigens.

BRIEF SUMMARY OF THE INVENTION

[0005] An embodiment of the invention provides an isolated or purified antibody, or an antigen-binding fragment thereof, which specifically binds to one or both of MAGE-A3 and

MAGE-A6 and does not bind to any one or more of MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11 and MAGE-A12.

[0006] Another embodiment of the invention provides a polypeptide comprising the amino acid sequences of (a) all of SEQ ID NOs: 1-6; (b) both of SEQ ID NO: 20 and SEQ ID NO: 21; or (c) both of SEQ ID NO: 7 and SEQ ID NO: 8.

[0007] Another embodiment of the invention provides a protein comprising: (a) a first polypeptide comprising the amino acid sequences of all of SEQ ID NOs: 1-3 and a second polypeptide comprising the amino acid sequences of all of SEQ ID NOs: 4-6; (b) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 20 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 21; or (c) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 7 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 8.

[0008] Further embodiments of the invention provide related anti-MAGE-A3, anti-MAGE-A6 binding moieties, nucleic acids, recombinant expression vectors, host cells, and populations of host cells.

[0009] Another embodiment of the invention provides a method of assaying a biological sample for the presence of one or both of MAGE-A3 and MAGE-A6, the method comprising: (a) contacting the biological sample with a composition, wherein the composition is the inventive antibody, or antigen binding fragment thereof, polypeptide, protein, or anti-MAGE-A3, anti-MAGE-A6 binding moiety; (b) forming a complex between the composition and the biological sample; and (c) detecting the complex, wherein detection of the complex is indicative of the presence of one or both of MAGE-A3 and MAGE-A6 in the biological sample.

[0010] Another embodiment of the invention provides a method of diagnosing cancer in a mammal, the method comprising: obtaining a biological sample from the mammal; assaying the biological sample for the presence of one or both of MAGE-A3 and MAGE-A6 by the inventive method; and diagnosing the mammal with cancer when the complex is detected.

[0011] Still another embodiment of the invention provides a method of diagnosing and treating cancer in a mammal, the method comprising: diagnosing the mammal with cancer by the inventive method; and treating the mammal by administering immunotherapy targeting one or both of MAGE-A3 and MAGE-A6 in an amount effective to treat cancer in the mammal.

[0012] Another embodiment of the invention provides a method of determining the prognosis of cancer in a mammal, the method comprising: obtaining a biological sample from the mammal; and assaying the biological sample for the presence of one or both of MAGE-A3 and MAGE-A6 by the inventive method, wherein the presence of the complex is indicative of a poor prognosis and the absence of the complex is indicative of a good prognosis.

DETAILED DESCRIPTION OF THE INVENTION

[0013] An embodiment of the invention provides an isolated or purified antibody, or an antigen-binding fragment thereof, which specifically binds to one or both of MAGE-A3 and MAGE-A6 and does not bind to any one or more of MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11 and MAGE-A12 (hereinafter referred to as "anti-MAGE-A3, anti-MAGE-A6 antibody, or antigen binding fragment thereof").

[0014] MAGE-A3 and MAGE-A6 are members of the MAGE-A family of twelve homologous proteins, also including MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, 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-A3 and MAGE-A6 proteins are expressed in a variety of human cancers including, but not limited to, melanoma, breast cancer, leukemia, thyroid cancer, gastric cancer, pancreatic cancer, liver cancer (e.g., hepatocellular carcinoma), lung cancer (e.g., non-small cell lung carcinoma), ovarian cancer, multiple myeloma, esophageal cancer, kidney cancer, head cancers (e.g., squamous cell carcinoma), neck cancers (e.g., squamous cell carcinoma), prostate cancer, synovial cell sarcoma, and urothelial cancer. Preferably, the MAGE proteins referred to herein are human MAGE proteins. In an embodiment of the invention, the antibody, and antigen binding fragment thereof, specifically bind to human MAGE-A3 and human MAGE-A6, and the antibody is a rabbit antibody.

[0015] In an embodiment of the invention, the antibody, or antigen binding fragment thereof, specifically binds to human MAGE-$A3_{54-64}$. MAGE-$A3_{54-64}$ has the amino acid sequence of PAAESPDPPQS (SEQ ID NO: 9), which is also shared with human MAGE-A6. The antibody, or antigen binding fragment thereof, does not bind to any one or more of the MAGE-A1 amino acid sequence of PTAGSTDPPQS (SEQ ID NO: 11), the MAGE-A2

amino acid sequence of PAADSPSPPHS (SEQ ID NO: 12), the MAGE-A4 amino acid sequence of PAAESAGPPQS (SEQ ID NO: 13), the MAGE-A5 amino acid sequence of PAAGSPGPLKS (SEQ ID NO: 14), the MAGE-A8 amino acid sequence of TDSGSPSPPQS (SEQ ID NO: 15), the MAGE-A9 amino acid sequence of SAAGSSSPPQS (SEQ ID NO: 16), the MAGE-A11 amino acid sequence of PAAESPSPPQS (SEQ ID NO: 17), the MAGE-A12 amino acid sequence of PAAESPSPPHS (SEQ ID NO: 18), and the MAGE-A10 amino acid sequence of SADDETPNPPQS (SEQ ID NO: 19). Preferably, the antibody, or antigen binding fragment thereof, does not bind to any of the amino acid sequences of SEQ ID NOs: 11-19.

[0016] In an embodiment of the invention, the antibody, or antigen binding fragment thereof, comprises two polypeptides (i.e., polypeptide chains), such as a heavy chain and a light chain (or antigen-binding fragments thereof). The polypeptides of the inventive antibody, or antigen binding fragment thereof, can comprise any amino acid sequence, provided that the antibody, and antigen binding fragment thereof, specifically binds to one or both of MAGE-A3 and MAGE-A6 and does not bind to any one or more of (preferably all of) MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11 and MAGE-A12.

[0017] In an embodiment of the invention, the antibody, or antigen binding fragment thereof, comprises two polypeptide chains, each of which comprises a variable region comprising a complementarity determining region (CDR) 1, a CDR2, and a CDR3 of an antibody. Preferably, the first polypeptide chain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 1 (CDR1 of heavy chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 2 (CDR2 of heavy chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 3 (CDR3 of heavy chain), and the second polypeptide chain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 4 (CDR1 of light chain), a CDR2 comprising the amino acid sequence of SEQ ID NO: 5 (CDR2 of light chain), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 6 (CDR3 of light chain). In this regard, the inventive antibody, or antigen binding fragment thereof, can comprise, consist, or consist essentially of the amino acid sequences of (a) SEQ ID NOs: 1-3, (b) SEQ ID NOs: 4-6, or (c) SEQ ID NOs: 1-6. Preferably, the antibody, or antigen binding fragment thereof, comprises the amino acid sequences of SEQ ID NOs: 1-6.

[0018] In an embodiment of the invention, the antibody, or antigen binding fragment thereof, can comprise an amino acid sequence of a variable region of an antibody comprising

the CDRs set forth above. In this regard, the antibody, or antigen binding fragment thereof, can comprise, consist of, or consist essentially of, the amino acid sequence of SEQ ID NO: 20 (the variable region of a heavy chain) or SEQ ID NO: 21 (the variable region of a light chain), or both SEQ ID NOs: 20 and 21. Preferably, the inventive antibody, or antigen binding fragment thereof, comprises the amino acid sequences of SEQ ID NOs: 20 and 21.

[0019] In an embodiment of the invention, the antibody, or antigen binding fragment thereof, can further comprise an amino acid sequence of a constant region of an antibody. In this regard, the antibody, or antigen binding fragment thereof, can further comprise the amino acid sequence of SEQ ID NO: 22 (the constant region of a heavy chain) or SEQ ID NO: 23 (the constant region of a light chain), or both SEQ ID NOs: 22 and 23. Preferably, the inventive antibody, or antigen binding fragment thereof, comprises the amino acid sequences of SEQ ID NOs: 22 and 23.

[0020] In an embodiment of the invention, the antibody, or antigen binding fragment thereof, can comprise a heavy chain of an antibody and a light chain of an antibody. Each of the heavy chain and light chain can independently comprise any amino acid sequence. Preferably, the heavy chain comprises the variable region of the heavy chain as set forth above. In this regard, the heavy chain of the inventive antibody, or antigen binding fragment thereof, can comprise, consist, or consist essentially of the amino acid sequence of SEQ ID NO: 7. An inventive heavy chain of this type can be paired with any light chain of an antibody. Preferably, the light chain of the inventive antibody, or antigen binding fragment thereof, comprises the variable region of a light chain as set forth above. In this regard, the light chain of the inventive antibody, or antigen binding fragment thereof, can comprise, consist, or consist essentially of the amino acid sequence of SEQ ID NO: 8. The inventive antibody, or antigen binding fragment thereof, therefore, can comprise the amino acid sequence of SEQ ID NO: 7 or 8, or both SEQ ID NOs: 7 and 8. Preferably, the inventive antibody, or antigen binding fragment thereof, comprises the amino acid sequences of SEQ ID NOs: 7 and 8.

[0021] The antibody of the invention can be any type of immunoglobulin that is known in the art. For instance, antibody may be a recombinant antibody. The antibody may be of any isotype, e.g., IgA, IgD, IgE, IgG (e.g., IgG1, IgG2, IgG3, or IgG4), 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, or antigen-binding fragment thereof, can have any level of affinity or avidity for one or both of MAGE-A3 and MAGE-A6.

[0022] The antigen-binding fragment of the antibody may be any fragment of the antibody which specifically binds to one or both of MAGE-A3 and MAGE-A6. Examples of antigen-binding fragments include, but are not limited to, Fab fragments, Fab' fragments, (Fab')2 fragments, and Fv fragments.

[0023] Suitable methods of making antibodies are known in the art and include, for example, standard hybridoma methods, Epstein-Barr virus (EBV)-hybridoma methods, and bacteriophage vector expression systems. Antibodies may be produced in non-human animals such as, for example, rabbits.

[0024] Another embodiment of the invention provides a polypeptide which specifically binds to one or both of MAGE-A3 and MAGE-A6 and does not bind to any one or more of MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11 and MAGE-A12. Preferably, the polypeptide does not bind to all of MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11 and MAGE-A12.

[0025] The term "polypeptide," as used herein, includes oligopeptides and refers to a single chain of amino acids connected by one or more peptide bonds. The polypeptide may comprise the antigen binding domain of the anti-MAGE-A3, anti-MAGE-A6 antibody described herein with respect to other aspects of the invention. The polypeptide may comprise one or more variable regions (e.g., two variable regions) of an antigen binding domain of the inventive anti-MAGE-A3, anti-MAGE-A6 antibody described herein with respect to other aspects of the invention, each variable region comprising a complementarity determining region (CDR) 1, a CDR2, and a CDR3.

[0026] In this regard, the polypeptide may comprise a first variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 1 (CDR1 of first variable region), a CDR2 comprising the amino acid sequence of SEQ ID NO: 2 (CDR2 of first variable region), a CDR3 comprising the amino acid sequence of SEQ ID NO: 3 (CDR3 of first variable region), and a second variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 4 (CDR1 of second variable region), a CDR2 comprising the amino acid sequence of SEQ ID NO: 5 (CDR2 of second variable region), and a CDR3 comprising the amino acid sequence of SEQ ID NO: 6 (CDR3 of second variable region). In this regard,

the inventive polypeptide can comprise, consist of, or consist essentially of the amino acid sequences of SEQ ID NOs: (a) 1-3, (b) SEQ ID NOs: 4-6, or (c) SEQ ID NOs: 1-6. Preferably, an embodiment of the invention provides a polypeptide comprising the amino acid sequences of all of SEQ ID NOs: 1-6.

[0027] In an embodiment, the polypeptide comprises one or more variable regions (e.g., first and second variable regions) of an antigen binding domain of the inventive anti-MAGE-A3, anti-MAGE-A6 antibody, each comprising the CDRs as described above. For example, the polypeptide may comprise the heavy chain variable region and the light chain variable region of the inventive anti-MAGE-A3, anti-MAGE-A6 antibody. The first variable region may comprise the amino acid sequence of SEQ ID NO: 20. The second variable region may comprise the amino acid sequence of SEQ ID NO: 21. Accordingly, in an embodiment of the invention, the polypeptide comprises, consists of, or consists essentially of the amino acid sequence(s) of (a) SEQ ID NO: 20, (b) SEQ ID NO: 21, or (c) SEQ ID NOs: 20 and 21. Preferably, the polypeptide comprises the amino acid sequences of both of SEQ ID NOs: 20 and 21.

[0028] In an embodiment of the invention, the polypeptide can further comprise an amino acid sequence of a constant region of an antibody. In this regard, the polypeptide can further comprise the amino acid sequence of SEQ ID NO: 22 (the constant region of a heavy chain) or SEQ ID NO: 23 (the constant region of a light chain), or both SEQ ID NOs: 22 and 23.

[0029] In an embodiment, the polypeptide comprises one or both of the heavy chain and the light chain of the inventive anti-MAGE-A3, anti-MAGE-A6 antibody, each comprising a variable region described above. The heavy chain may comprise the amino acid sequence of SEQ ID NO: 7. The light chain may comprise the amino acid sequence of SEQ ID NO: 8. Accordingly, in an embodiment of the invention, the polypeptide comprises, consists of, or consists essentially of the amino acid sequence(s) of (a) SEQ ID NO: 7, (b) SEQ ID NO: 8, or (c) SEQ ID NOs: 7 and 8. Preferably, the polypeptide comprises the amino acid sequences of both of SEQ ID NOs: 7 and 8.

[0030] In an embodiment of the invention, the amino acid sequences of the polypeptide are rabbit amino acid sequences and the polypeptide specifically binds to one or both of human MAGE-A3 and human MAGE-A6.

[0031] In an embodiment of the invention, the variable regions of the polypeptide may be joined by a linker. The linker may comprise any suitable amino acid sequence. In an embodiment of the invention, the linker is a Gly/Ser linker from about 1 to about 100, from

about 3 to about 20, from about 5 to about 30, from about 5 to about 18, or from about 3 to about 8 amino acids in length and consists of glycine and/or serine residues in sequence. Accordingly, the Gly/Ser linker may consist of glycine and/or serine residues. In some embodiments, the Gly/Ser linker is a peptide of the formula: $(Xaa1)_n$ wherein each amino acid residue Xaa1 is selected independently from glycine and serine and n is an integer from 3 to 15.

[0032] 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. An embodiment of the invention provides a protein which specifically binds to one or both of MAGE-A3 and MAGE-A6 and does not bind to any one or more of (preferably all of) MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11 and MAGE-A12. In an embodiment of the invention, the amino acid sequences of the protein are rabbit amino acid sequences and the protein specifically binds to one or both of human MAGE-A3 and human MAGE-A6.

[0033] The protein of the invention can comprise a first polypeptide chain comprising the amino acid sequences of SEQ ID NOs: 1-3 and a second polypeptide chain comprising the amino acid sequences of SEQ ID NOs: 4-6. The protein of the invention can, for example, comprise a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 20 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 21. In an embodiment of the invention, the first polypeptide chain of the protein may further comprise the amino acid sequence of SEQ ID NO: 22 and the second polypeptide chain may further comprise the amino acid sequence of SEQ ID NO: 23. In an embodiment of the invention, the protein may comprise a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 7 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 8.

[0034] The protein may further comprise a linker as described herein with respect to other aspects of the invention.

[0035] The protein of the invention can be, for example, a fusion protein. If, for example, 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 Fc region, an immunoglobulin constant region, etc.

[0036] 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.

[0037] It is contemplated that the polypeptides and proteins of the invention may be useful as anti-MAGE-A3, anti-MAGE-A6 binding moieties which specifically bind to one or both of MAGE-A3 and MAGE-A6 and does not bind to any one or more of (preferably all of) MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11 and MAGE-A12. In this regard, an embodiment of the invention provides an anti-MAGE-A3, anti-MAGE-A6 binding moiety comprising any of the antibodies (or antigen-binding fragments thereof), polypeptides or proteins described herein with respect to other aspects of the invention. The antigen binding portion can be any portion that has at least one antigen binding site. In an embodiment, the anti-MAGE-A3, anti-MAGE-A6 binding moiety is an antibody, Fab fragment (Fab), F(ab')2 fragment, Fab' fragment, Fv fragment, diabody, triabody, tetrabody, single-chain variable region fragment (scFv), disulfide-stabilized variable region fragment (dsFv), IgGΔCH2, scFv2CH3, scFv4, scFv3, scFv2, scFv-Fc, (scFv)2, or a bivalent antibody. The anti-MAGE-A3, anti-MAGE-A6 binding moieties of the invention, however, are not limited to these exemplary types of anti-MAGE-A3, anti-MAGE-A6 binding moieties.

[0038] In a preferred embodiment, the anti-MAGE-A3, anti-MAGE-A6 binding moiety is a single-chain variable region fragment (scFv). A single-chain variable region fragment (scFv), which is a fusion protein including 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. Similarly, disulfide-stabilized variable region fragments (dsFv) can be prepared by recombinant DNA technology.

[0039] The antibody may be, for example, 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 or proteins of the invention and one or more polypeptide chains of an antibody, or a portion thereof. The polypeptide of an antibody, or portion thereof, can be, for example, a constant region of a heavy or light chain, or an Fc 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 or protein of the invention. The polypeptide of an antibody, or portion thereof, can be a polypeptide of any antibody or any antibody fragment.

[0040] Methods of testing antibodies, antigen-binding fragments thereof, polypeptides, proteins, and anti-MAGE-A3, anti-MAGE-A6 binding moieties for the ability to bind to one or both of MAGE-A3 and MAGE-A6 are known in the art and include, for example, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), Western blot, immunoprecipitation, and competitive inhibition assays.

[0041] The inventive antibodies, antigen-binding fragments thereof, polypeptides, proteins, and anti-MAGE-A3, anti-MAGE-A6 binding moieties (hereinafter, "anti-MAGE-A3, anti-MAGE-A6 binding agents") may provide any of a variety of advantages. For example, the inventive anti-MAGE-A3, anti-MAGE-A6 binding agents may, advantageously, overcome the difficulty of distinguishing MAGE-A3 and MAGE-A6 from other MAGE family members which have a high sequence homology to MAGE-A3 and MAGE-A6. Accordingly, the inventive anti-MAGE-A3, anti-MAGE-A6 binding agents make it possible to accurately test whether a patient's cancer expresses one or both of MAGE-A3 and MAGE-A6 to determine whether the patient would be eligible for treatment with immunotherapy targeting one or both of anti-MAGE-A3 and anti-MAGE-A6. In addition, methods of detection using the inventive anti-MAGE-A3, anti-MAGE-A6 binding agents may be less expensive and/or less labor-intensive as compared to methods which detect the expression of one or both of MAGE-A3 mRNA and MAGE-A6 mRNA (e.g., reverse transcription polymerase chain reaction (RT-PCR), RNA sequencing (RNA-Seq), and digital detection of mRNA molecules (e.g., NANOSTRING NCOUNTER Gene Expression Assay, NanoString, Seattle, WA). The inventive anti-MAGE-A3, anti-MAGE-A6 binding agents may also reduce or avoid the disadvantages presented by mRNA-based detection methods, which may fail due to, e.g., a failure to isolate mRNA from patients' tumor specimens due to, e.g., the improper preparation of the formalin-fixed, paraffin-embedded (FFPE) tissue blocks.

[0042] Also, the anti-MAGE-A3, anti-MAGE-A6 binding agents 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).

[0043] Included in the scope of the invention are functional variants of the anti-MAGE-A3, anti-MAGE-A6 binding agents described herein. The term "functional variant," as used herein, refers to an anti-MAGE-A3, anti-MAGE-A6 binding agent having substantial or significant sequence identity or similarity to a parent anti-MAGE-A3, anti-MAGE-A6 binding agent, which functional variant retains the biological activity of the anti-MAGE-A3, anti-MAGE-A6 binding agent of which it is a variant. Functional variants encompass, for example, those variants of the anti-MAGE-A3, anti-MAGE-A6 binding agents described herein that retain the ability to specifically bind to one or both of MAGE-A3 and MAGE-A6 to a similar extent, the same extent, or to a higher extent, as the parent anti-MAGE-A3, anti-MAGE-A6 binding agent. In reference to the parent anti-MAGE-A3, anti-MAGE-A6 binding agent, the functional variant can, for instance, be at least about 30%, about 50%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identical in amino acid sequence to the parent anti-MAGE-A3, anti-MAGE-A6 binding agent.

[0044] A functional variant can, for example, comprise the amino acid sequence of the parent anti-MAGE-A3, anti-MAGE-A6 binding agent with at least one conservative amino acid substitution. Alternatively or additionally, the functional variants can comprise the amino acid sequence of the parent anti-MAGE-A3, anti-MAGE-A6 binding agent 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. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent anti-MAGE-A3, anti-MAGE-A6 binding agent.

[0045] Amino acid substitutions of the inventive anti-MAGE-A3, anti-MAGE-A6 binding agents are preferably conservative amino acid substitutions. 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 or similar chemical or physical properties. For instance, the

conservative amino acid substitution can be an acidic/negatively charged polar amino acid substituted for another acidic/negatively charged polar amino acid (e.g., Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (e.g., Ala, Gly, Val, Ile, Leu, Met, Phe, Pro, Trp, Cys, Val, etc.), a basic/positively charged polar amino acid substituted for another basic/positively charged polar amino acid (e.g. Lys, His, Arg, etc.), an uncharged amino acid with a polar side chain substituted for another uncharged amino acid with a polar side chain (e.g., Asn, Gln, Ser, Thr, Tyr, etc.), an amino acid with a beta-branched side-chain substituted for another amino acid with a beta-branched side-chain (e.g., Ile, Thr, and Val), an amino acid with an aromatic side-chain substituted for another amino acid with an aromatic side chain (e.g., His, Phe, Trp, and Tyr), etc.

[0046] The anti-MAGE-A3, anti-MAGE-A6 binding agents of the invention can consist essentially of the specified amino acid sequence or sequences described herein, such that other components, e.g., other amino acids, do not materially change the biological activity of the antibody (or antigen-binding fragment thereof), polypeptide, protein, or anti-MAGE-A3, anti-MAGE-A6 binding moiety.

[0047] The anti-MAGE-A3, anti-MAGE-A6 binding agents of embodiments of the invention (including functional variants) can be of any length, i.e., can comprise any number of amino acids, provided that the anti-MAGE-A3, anti-MAGE-A6 binding agents retain their biological activity, e.g., the ability to specifically bind to one or both of MAGE-A3 and MAGE-A6, or detect cancer cells in a mammal, etc. For example, the anti-MAGE-A3, anti-MAGE-A6 binding agent can be 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.

[0048] The anti-MAGE-A3, anti-MAGE-A6 binding agents of embodiments of the invention (including functional variants of the invention) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4- nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid

monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

[0049] The anti-MAGE-A3, anti-MAGE-A6 binding agents of embodiments of the invention (including 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.

[0050] The inventive anti-MAGE-A3, anti-MAGE-A6 binding agents (including functional variants thereof) can be obtained by methods known in the art. The polypeptides and proteins may be made by any suitable method of making polypeptides or proteins. Suitable methods of *de novo* synthesizing polypeptides and proteins are known in the art. Also, polypeptides and proteins can be recombinantly produced using nucleic acids and standard recombinant methods. Further, some of the anti-MAGE-A3, anti-MAGE-A6 binding agents of the invention (including 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 anti-MAGE-A3, anti-MAGE-A6 binding agents described herein (including 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 anti-MAGE-A3, anti-MAGE-A6 binding agents can be synthetic, recombinant, isolated, and/or purified.

[0051] Further provided by an embodiment of the invention is a nucleic acid comprising a nucleotide sequence encoding any of the anti-MAGE-A3, anti-MAGE-A6 binding agents (including functional variants thereof) described herein. In this regard, the nucleic acid comprises a nucleotide sequence encoding the amino acid sequence(s) of heavy chain SEQ ID NO: 7, light chain SEQ ID NO: 8, or both SEQ ID NOs: 7 and 8. Another embodiment of the invention provides a nucleic acid comprising a nucleotide sequence encoding any of the variable regions described herein. In this regard, the nucleic acid comprises a nucleotide sequence encoding the amino acid sequence(s) of the variable region of heavy chain SEQ ID NO: 20, variable region of light chain SEQ ID NO: 21, or both SEQ ID NOs: 20 and 21. Another embodiment of the invention provides a nucleic acid comprising a nucleotide

sequence encoding any of the CDR regions described herein. In this regard, the nucleic acid comprises a nucleotide sequence encoding the amino acid sequences of SEQ ID NOs: (a) 1-3, (b) SEQ ID NOs: 4-6, or (c) SEQ ID NOs: 1-6.

[0052] "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. In some embodiments, 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. In some embodiments, the nucleic acid may encode additional amino acid sequences that do not affect the function of the anti-MAGE-A3, anti-MAGE-A6 binding agents (including functional variants thereof) and which may or may not be translated upon expression of the nucleic acid by a host cell. In an embodiment of the invention, the nucleic acid is complementary DNA (cDNA). In an embodiment of the invention, the nucleic acid comprises a codon-optimized nucleotide sequence.

[0053] The nucleic acids of an embodiment of the invention may be 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.

[0054] The nucleic acids can consist essentially of the specified nucleotide sequence or sequences described herein, such that other components, e.g., other nucleotides, do not materially change the biological activity of the encoded anti-MAGE-A3, anti-MAGE-A6 binding agents (including functional variants thereof).

[0055] A recombinant nucleic acid may be one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of

nucleic acids, e.g., by genetic engineering techniques, such as those described in Green and Sambrook, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 4th Ed. (2012). The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Green et al., *supra*. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N^6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N^6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N^6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Macromolecular Resources (Fort Collins, CO) and Synthegen (Houston, TX).

[0056] An embodiment of the invention also provides an isolated or purified nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of any of the nucleic acids described herein.

[0057] In an embodiment, the nucleic acids of the invention can be incorporated into a recombinant expression vector. In this regard, an embodiment of 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 or non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

[0058] In an embodiment, 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 cell. 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, Glen Burnie, MD), the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA). Bacteriophage vectors, such as λGT10, λGT11, λZapII (Stratagene), λEMBL4, and λNM1149, also can be used. Examples of plant expression vectors include pBI01, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM, and pMAMneo (Clontech). The recombinant expression vector may be a viral vector, e.g., a retroviral vector.

[0059] A number of transfection techniques are generally known in the art. Transfection methods include calcium phosphate co-precipitation, direct micro injection into cultured cells, electroporation, liposome mediated gene transfer, lipid mediated transduction, and nucleic acid delivery using high velocity microprojectiles.

[0060] In an embodiment, the recombinant expression vectors of the invention can be prepared using standard recombinant DNA techniques described in, for example, Green, *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.

[0061] The recombinant expression vector may comprise regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type

of host cell (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. The recombinant expression vector may comprise restriction sites to facilitate cloning.

[0062] The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected host cells. 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.

[0063] The recombinant expression vector can comprise a native or nonnative promoter operably linked to the nucleotide sequence encoding the inventive anti-MAGE-A3, anti-MAGE-A6 binding agents (including functional variants thereof), or to the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the inventive anti-MAGE-A3, anti-MAGE-A6 binding agents (including functional variants thereof). 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 ordinary 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, or a promoter found in the long-terminal repeat of the murine stem cell virus.

[0064] 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.

[0065] An 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 may be a prokaryotic cell, e.g., a DH5α cell. For purposes of producing a recombinant anti-MAGE-A3, anti-MAGE-A6 binding agents (including functional variants thereof), the host cell may be a mammalian cell. The host cell may be 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 may be a peripheral blood lymphocyte (PBL) or a peripheral blood mononuclear cell (PBMC). The host cell may be a B cell or a T cell.

[0066] Also provided by an embodiment of 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 B 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 cell, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly 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.

[0067] The antibodies (and antigen-binding fragments thereof), polypeptides, proteins, anti-MAGE-A3, anti-MAGE-A6 binding moieties, (including functional variants thereof), nucleic acids, recombinant expression vectors, host cells (including populations thereof), all of which are collectively referred to as "inventive anti-MAGE-A3, anti-MAGE-A6 materials" hereinafter, can be isolated and/or purified. The term "isolated" as used herein means having been removed from its natural environment. The term "purified" or "isolated" does not require absolute purity or isolation; rather, it is intended as a relative term. Thus, for example, a purified (or isolated) antibody preparation is one in which the antibody is more pure than antibodies in their natural environment within the body. Such antibodies may be produced, for example, by standard purification techniques. In some embodiments, a preparation of an antibody is purified such that the antibody represents at least about 50%, for

example, at least about 70%, of the total antibody content of the preparation. For example, the purity can be at least about 50%, can be greater than about 60%, about 70% or about 80%, about 90%, about 95%, or can be about 100%.

[0068] It is contemplated that the inventive anti-MAGE-A3, anti-MAGE-A6 binding agents (including functional variants thereof) may be useful in detecting one or both of MAGE-A3 and MAGE-A6 in a biological sample. Accordingly, an embodiment of the invention provides a method of assaying a biological sample for the presence of one or both of MAGE-A3 and MAGE-A6, the method comprising: (a) contacting the biological sample with a composition, wherein the composition is any of the inventive antibodies, or antigen binding fragments thereof, polypeptides, proteins, or anti-MAGE-A3, anti-MAGE-A6 binding moieties described herein; (b) forming a complex between the composition and the biological sample; and (c) detecting the complex, wherein detection of the complex is indicative of the presence of one or both of MAGE-A3 and MAGE-A6 in the biological sample.

[0069] The biological sample may be obtained by any suitable method, e.g., biopsy or necropsy. A biopsy is the removal of tissue and/or cells from a mammal. Such removal may be to collect tissue and/or cells from the mammal in order to perform experimentation on the removed tissue and/or cells, for example, to test for the expression of one or both of MAGE-A3 and MAGE-A6.

[0070] The biological sample may be, for example, a tumor sample. In an embodiment of the invention, the biological sample comprises cancer cells (e.g., tumor cells). The biological sample may comprise 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. If the sample comprises whole cells, the cells can be any cells of the mammal, e.g., the cells of any organ or tissue, including blood cells or endothelial cells.

[0071] MAGE-A3 and MAGE-A6 are intracellular proteins. Accordingly, when the biological sample comprises whole cells, the method may comprise permeabilizing the cells to facilitate the contacting of the inventive anti-MAGE-A3, anti-MAGE-A6 binding agents described herein with the one or both of the MAGE-A3 and MAGE-A6 expressed inside the cell. In another embodiment of the invention, the whole cells may be lysed prior to contacting the inventive anti-MAGE-A3, anti-MAGE-A6 binding agents described herein with the biological sample.

[0072] The contacting can take place *in vitro* or *in vivo* with respect to the mammal. Preferably, the contacting is *in vitro*. Contacting may comprise physically contacting the biological sample with the inventive anti-MAGE-A3, anti-MAGE-A6 binding agent under conditions that facilitate the formation of one or more complexes including the inventive anti-MAGE-A3, anti-MAGE-A6 binding agent bound to one or both of MAGE-A3 and MAGE-A6 in the biological sample. The method may further comprise washing any unbound inventive anti-MAGE-A3, anti-MAGE-A6 binding agent and any unbound antigen from the one or more complexes.

[0073] Detection of the complex can occur through any number of ways known in the art. For instance, the inventive anti-MAGE-A3, anti-MAGE-A6 binding agents 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). Detection of the complex can be carried out using immunochemical methods. Such immunochemical methods are known to persons of ordinary skill in the art and include methods such as immunoprecipitation, immunonephelometry, radioimmunoassay (RIA), enzyme immunoassay (EIA), fluorescent immunoassay (FIA), luciferase immunoprecipitation system (LIPS), and lateral flow immunochromatographic assay (also referred to as "lateral flow test") and the like. Preferably, detection of the complex is carried out using an enzyme-linked immunosorbent assay (ELISA) or enzyme immunoassay (EIA) (e.g., sandwich ELISA).

[0074] In an embodiment, the method may further comprise comparing the level of expression of one or both of MAGE-A3 and MAGE-A6 in the biological sample to that of a control sample. Generally, the method may include contacting the biological sample with an inventive anti-MAGE-A3, anti-MAGE-A6 binding agent and determining the amount of anti-MAGE-A3, anti-MAGE-A6 binding agent that specifically binds to one or both of MAGE-A3 and MAGE-A6 in the biological sample to determine the level of one or both of MAGE-A3 and MAGE-A6 in the biological sample. The method may include contacting a control sample with the same inventive anti-MAGE-A3, anti-MAGE-A6 binding agent used to contact the biological sample and determining the amount of anti-MAGE-A3, anti-MAGE-A6 binding agent that specifically binds to one or both of MAGE-A3 and MAGE-A6 in the control sample to determine the level of one or both of MAGE-A3 and MAGE-A6 in the control sample. The control sample may be a positive control sample or a negative control

sample. A negative control sample is preferably a biological sample which is known to be negative for expression of both of MAGE-A3 and MAGE-A6 such as, for example, a normal, non-cancerous tissue which does not comprise non-MHC expressing germ cells of the testis and placenta. A positive control sample is preferably a biological sample which is known to be negative for expression of one or both of MAGE-A3 and MAGE-A6 such as, for example, a tumor sample. A significantly higher amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the negative control sample, or a similar or significantly higher amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the positive control sample, is indicative of the presence of one or both of MAGE-A3 and MAGE-A6 in the biological sample. A significantly lower amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the positive control sample, or a similar or significantly lower amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the negative control sample, is indicative of the absence of one or both of MAGE-A3 and MAGE-A6 in the biological sample. Methods of using the anti-MAGE-A3, anti-MAGE-A6 binding agents of the invention to determine one or both of MAGE-A3 and MAGE-A6 expression levels can include any method known in the art such as, for example, immunoassays such as immuno-(Western) blotting, enzyme-linked immunosorbent assay (ELISA), and flow cytometry, e.g., fluorescence-activated cell sorting (FACS) analysis.

[0075] Another embodiment of the invention provides a method of diagnosing cancer in a mammal, the method comprising: obtaining a biological sample from the mammal; assaying the biological sample for the presence of one or both of MAGE-A3 and MAGE-A6 as described herein with respect to other aspects of the invention; and diagnosing the mammal with cancer when the complex is detected. The biological sample may be as described herein with respect to other aspects of the invention.

[0076] The inventive method of diagnosing cancer in a mammal may further comprise comparing the level of expression of one or both of MAGE-A3 and MAGE-A6 in the biological sample to that of a control sample, as described herein with respect to other aspects of the invention. A significantly higher amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the negative control sample, or a similar or significantly higher amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the positive control

sample, is indicative of the presence of cancer in the mammal. A significantly lower amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the positive control sample, or a similar or significantly lower amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the negative control sample, is indicative of the absence of cancer in the biological sample.

[0077] Still another embodiment of the invention provides a method of diagnosing and treating cancer in a mammal, the method comprising: diagnosing the mammal with cancer as described herein with respect to other aspects of the invention; and treating the mammal by administering immunotherapy targeting one or both of MAGE-A3 and MAGE-A6 in an amount effective to treat cancer in the mammal.

[0078] The immunotherapy may be any suitable immunotherapy targeting one or both of MAGE-A3 and MAGE-A6. In an embodiment of the invention, the immunotherapy comprises cells expressing a T cell receptor (TCR), wherein the TCR specifically binds to one or both of MAGE-A3 and MAGE-A6 (also referred to herein as "a MAGE-A3, MAGE-A6-specific TCR").

[0079] The TCR can be an endogenous TCR, i.e., the antigen-specific TCR that is endogenous or native to (naturally-occurring on) a T cell. In such a case, the cell comprising the endogenous TCR can be a T cell that was isolated from a mammal which is known to express one or both of MAGE-A3 and MAGE-A6. In certain embodiments, the T cell is a primary T cell isolated from a mammal afflicted with a cancer. In some embodiments, the T cell is a tumor infiltrating lymphocyte (TIL) or a peripheral blood lymphocyte (PBL) isolated from a human cancer patient.

[0080] In some embodiments, the mammal from which a T cell is isolated is immunized with one or both of MAGE-A3 and MAGE-A6. Desirably, the mammal is immunized prior to obtaining the T cell from the mammal. In this way, the isolated T cells can include T cells induced to have specificity for one or both of MAGE-A3 and MAGE-A6, or can include a higher proportion of cells specific for one or both of MAGE-A3 and MAGE-A6.

[0081] Alternatively, a T cell comprising an endogenous antigen-specific TCR can be a T cell within a mixed population of cells isolated from a mammal, and the mixed population can be exposed to one or both of MAGE-A3 and MAGE-A6 which is recognized by the endogenous TCR while being cultured *in vitro*. In this manner, the T cell which comprises the TCR that recognizes one or both of MAGE-A3 and MAGE-A6 expands or proliferates *in*

vitro, thereby increasing the number of T cells having the endogenous receptor that is specific for one or both of MAGE-A3 and MAGE-A6.

[0082] The TCR can be an exogenous TCR, i.e., a MAGE-A3, MAGE-A6-specific TCR that is not native to (not naturally-occurring on) the T cell. A recombinant TCR is a TCR which has been generated through recombinant expression of one or more exogenous TCR α-, β-, γ-, and/or δ-chain encoding genes. A recombinant TCR can comprise polypeptide chains derived entirely from a single mammalian species, or the MAGE-A3, MAGE-A6-specific-specific TCR can be a chimeric or hybrid TCR comprised of amino acid sequences derived from TCRs from two different mammalian species. For example, the MAGE-A3, MAGE-A6-specific TCR can comprise a variable region derived from a murine TCR, and a constant region of a human TCR such that the TCR is "humanized." Methods of making recombinant TCRs which recognize one or both of MAGE-A3 and MAGE-A6 are known in the art. See, for example, Yao et al., *J. Immunother.*, 39(5): 191-201 (2016) and U.S. Patent Application Publication Nos. 2015/0246959 and 2014/0378389 and WO 2012/054825.

[0083] In an embodiment of the invention, the immunotherapy comprises a vaccine targeting one or both of MAGE-A3 and MAGE-A6 (that is, a vaccine which elicits an immune response against one or both of MAGE-A3 and MAGE-A6). The vaccine may comprise the full-length MAGE-A3 protein or an immunogenic portion thereof, the full-length MAGE-A6 protein or an immunogenic portion thereof, or a nucleic acid encoding the same. In another embodiment of the invention, the vaccine may comprise an antigen presenting cell (APC) (e.g., a dendritic cell) presenting one or both of an immunogenic MAGE-A3 peptide and an immunogenic MAGE-A6 peptide. Vaccines targeting one or both of MAGE-A3 and MAGE-A6 are known in the art and are described in, for example, Rapoport et al., *Clin. Cancer Res.*, 20(5):1355-65 (2014) and Vansteenkiste et al., *J. Clin. Oncol.*, 31(19):2396-403 (2013).

[0084] Another embodiment of the invention provides a method of determining the prognosis of cancer in a mammal. The method may comprise obtaining a biological sample from the mammal and assaying the biological sample for the presence of one or both of MAGE-A3 and MAGE-A6 as described herein with respect to other aspects of the invention. The biological sample may be as described herein with respect to other aspects of the invention. The expression of one or both of MAGE-A3 and MAGE-A6 has been associated with the poor survival of breast cancer patients and non-small cell lung cancer patients. Mecklenberg et al., *Clin. Cancer Res.*, (2016) and Ayyoub et al., *J. Immunother.*, 37(2): 73-6

(2014). Accordingly, the presence of the complex is indicative of a poor prognosis and the absence of the complex is indicative of a good prognosis.

[0085] The inventive method of determining the prognosis of cancer in a mammal may further comprise comparing the level of expression of one or both of MAGE-A3 and MAGE-A6 in the biological sample to that of a control sample, as described herein with respect to other aspects of the invention. A significantly higher amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the negative control sample, or a similar or significantly higher amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the positive control sample, is indicative of a poor prognosis. A significantly lower amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the positive control sample, or a similar or significantly lower amount of binding of the anti-MAGE-A3, anti-MAGE-A6 binding agent in the biological sample relative to that in the negative control sample, is indicative of a good prognosis.

[0086] The term "treat," as well as words stemming therefrom, as used herein, does not necessarily imply 100% or complete treatment. Rather, there are varying degrees of treatment 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 of cancer in a mammal. Furthermore, the treatment provided by the inventive method can include treatment of one or more conditions or symptoms of the cancer being treated.

[0087] With respect to the inventive methods, the cancer can be any cancer, including any of sarcomas (e.g., synovial sarcoma, osteogenic sarcoma, leiomyosarcoma uteri, and alveolar rhabdomyosarcoma), lymphomas (e.g., Hodgkin lymphoma and non-Hodgkin lymphoma), hepatocellular carcinoma, glioma, head cancers (e.g., squamous cell carcinoma), neck cancers (e.g., squamous cell carcinoma), acute lymphocytic cancer, leukemias (e.g., acute myeloid leukemia and chronic lymphocytic leukemia), bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic myeloid cancer, colon cancers (e.g., colon carcinoma), esophageal cancer, cervical cancer, gastric cancer, gastrointestinal carcinoid tumor, hypopharynx cancer, larynx cancer, liver cancers (e.g., hepatocellular carcinoma), lung cancers (e.g., non-small cell lung carcinoma),

malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, kidney cancers (e.g., renal cell carcinoma), small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, and urothelial cancers (e.g., ureter cancer and urinary bladder cancer). Preferably, the cancer is melanoma, bone cancer, breast cancer, leukemia, thyroid cancer, gastric cancer, pancreatic cancer, liver cancer, lung cancer, ovarian cancer, multiple myeloma, esophageal cancer, kidney cancer, head cancers, neck cancers, prostate cancer, synovial cell sarcoma, urothelial cancer, or uterine cervical cancer. Preferably, the cancer expresses one or both of MAGE-A3 and MAGE-A6.

[0088] The mammal referred to herein can be any mammal. As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. The mammals may be from the order Carnivora, including Felines (cats) and Canines (dogs). The mammals may be from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). The mammals may be of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). Preferably, the mammal is a human.

[0089] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0090] This example demonstrates the generation of a rabbit monoclonal antibody which recognizes human MAGE-A3 and human MAGE-A6.

[0091] Both MAGE-A3 and MAGE-A6 contain the amino acid sequence of the immunized peptide PAAESPDPPQS (SEQ ID NO: 9). Other members of the MAGE-A family contain the amino acid sequence of the immunized peptide PAAESPDPPQS (SEQ ID NO: 9) with 1–5 amino acid substitutions. Table 1 shows the amino acid sequences of the immunized peptide and MAGE-A family members. MAGE-A10 also contains an aspartic acid insertion as compared to the immunized peptide (shown in Table 1 in larger font). In Table 1, the amino acid residues which differ from the corresponding residue in the immunized peptide are underlined and in bold. MAGE-A7 is a pseudogene and is not included in Table 1.

[0092] A BLAST search of the NCBI database indicated that other potential human proteins contained the amino acid sequence of the immunized peptide PAAESPDPPQS (SEQ ID NO: 9) with at least 3 amino acid substitutions.

TABLE 1

Name	Amino Acid Sequence	SEQ ID NO:
Immunized Peptide	PAAESPDPPQS	9
MAGE-A1	PTAGSTDPPQS	11
MAGE-A2	PAADSPSPPHS	12
MAGE-A3	PAAESPDPPQS	9
MAGE-A4	PAAESAGPPQS	13
MAGE-A5	PAAGSPGPLKS	14
MAGE-A6	PAAESPDPPQS	9
MAGE-A8	TDSGSPSPPQS	15
MAGE-A9	SAAGSSSPPQS	16
MAGE-A11	PAAESPSPPQS	17
MAGE-A12	PAAESPSPPHS	18
MAGE-A10	SADDETPNPPQS	19

[0093] Two rabbits (*Oryctolagus cuniculus*) were immunized with a KLH (keyhole limpet hemocyanin)-modified peptide PAAESPDPPQS (MAGE-A3$_{54-64}$; SEQ ID NO: 9). Another KLH-modified peptide, LVFGIELMEVDPIGH (MAGE-A3$_{160-174}$; SEQ ID NO: 10), was also injected into these rabbits at the same time, but ultimately failed to generate any specific antibody. One rabbit died unexpectedly.

[0094] Lymphocytes were isolated from the living rabbit, and hybridoma fusion was performed. Multiclone cultures in a total of eighty 96-well plates were screened for recognition to PAAESPDPPQS (SEQ ID NO: 9) or LVFGIELMEVDPIGH (SEQ ID NO: 10) by enzyme-linked immunosorbent assay (ELISA). Some multiclone cultures recognized PAAESPDPPQS (SEQ ID NO: 9), some recognized LVFGIELMEVDPIGH (SEQ ID NO: 10), and some cultures recognized both peptides (because they are mixed clones).These samples were further screened by flow cytometric analysis of intracellularly stained COS-7 cells overexpressing MAGE-A3, as described in more detail in Example 2.

[0095] Six multiclone cultures were selected to isolate antibody heavy and light chain cDNA sequences. Recombinant antibodies were produced in HEK293 cells, and the supernatant was analyzed by ELISA, fluorescence activated cell sorting (FACS) and immunohistochemistry (IHC). Only the supernatant from clone NCI-186-24 showed specific staining to both of MAGE-A3 and MAGE-A6, but not to MAGE-A1, A2, A4, A5, A8, A9, A10, A11 or A12. As a result, the antibody clone NCI-186-24 was selected for large-scale production and protein A purification. The reactivity of the NCI-186-24 antibody to the immunized peptide PAAESPDPPQS (SEQ ID NO: 9) was confirmed by ELISA.

[0096] The amino acid sequences of the heavy and light chains of NCI-186-24 were SEQ ID NO: 7 and 8, respectively.

EXAMPLE 2

[0097] This example demonstrates that the NCI-186-24 antibody binds to either MAGE-A3 or MAGE-A6 expressed by transfected COS-7 cells but not to MAGE-A1, A2, A4, A5, A8, A9, A10, A11 or A12 expressed by transfected COS-7 cells, as measured by flow cytometry.

[0098] A cDNA plasmid encoding each MAGE-A family member (MAGE-A1, A2, A3, A4, A5, A6, A8, A9, A10, A11 and A12) was independently transfected into COS-7 cells. As a negative control, an empty vector was transfected into COS-7 cells (mock). After 3 days, transfected cells were fixed, permeabilized, and intracellularly stained with NCI-186-24 or 6C1 antibody (Santa Cruz Biotechnology) at 40 μg/mL. The 6C1 antibody is described in Rimoldi et al., *Int. J. Cancer*, 86(5): 749-51 (2000). After 30 minutes (min.), the cells were washed and stained with either fluorescein (FITC)-labeled anti-rabbit or anti-mouse secondary antibody (BD Biosciences or Jackson ImmunoResearch, respectively) at 10 μg/mL for an additional 30 min. After washing, the stained cells were analyzed by flow cytometry.

[0099] The flow cytometry results showed that the NCI-186-24 antibody bound to MAGE-A3- and A6-expressing cells, but not to MAGE-A1, A2, A4, A8, A9, A10, A11 or A12-expressing cells. On the other hand, the 6C1 antibody bound to MAGE-A1, A2, A3, A4, A6, A8, A9, A11 and A12. Both NCI-186-25 and 6C1 did not bind to MAGE-A5, which contained 4 amino acid substitutions as compared to the immunized peptide (Table 1). Both NCI-186-25 and 6C1 did not bind to mock-transfected control cells.

EXAMPLE 3

[0100] This example demonstrates that the NCI-186-24 antibody binds to either MAGE-A3 or MAGE-A6 expressed by transfected COS-7 cells but not to MAGE-A1, A2, A4, A8, A9, A10, A11 or A12 expressed by transfected COS-7 cells, as measured by immunocytochemistry.

[0101] For immunocytochemistry analysis, a cDNA plasmid encoding each MAGE-A family member (MAGE-A1, A2, A3, A4, A6, A8, A9, A10, A11 and A12) was transfected into COS-7 cells. As a negative control, an empty vector was transfected into COS-7 cells (mock). After 3 days, 2.5 x 10^4 transfected COS-7 cells were transferred to slides by cytocentrifuge. Cytospin slides were stained with NCI-186-24 or 6C1 antibody at 20 μg/mL, and incubated at 4 °C overnight. The samples were washed twice in TBST buffer (Tris-buffered saline + Tween-20). After washing, VECTASTAIN avidin-biotin complex (ABC) kit (Vector Laboratories) was used to detect the NCI-186-24 or 6C1 antibody, followed by hematoxylin counterstaining.

[0102] The MAGE-A3 and MAGE-A6 transfected cells showed strong NCI-186-24 staining, while the other transfected cells, including mock, showed only slight background staining. On the other hand, the 6C1 antibody bound to MAGE-A1, A2, A3, A4, A6, A8, A9, A11 and A12-transfected COS-7 cells.

EXAMPLE 4

[0103] This example demonstrates that normal testis tissue samples and melanoma tissue samples stain more lightly with the NCI-186-24 antibody as compared to the 6C1 antibody.

[0104] For immunohistochemistry analysis, formalin-fixed, paraffin-embedded (FFPE) normal testis tissue array (TEN241) and melanoma array (ME1004c) (Biomax) slides were processed with heat-induced epitope retrieval before staining. FFPE slides were stained with NCI-186-24 or 6C1 antibody at 20 μg/mL, and incubated at 4 °C overnight. The samples were washed twice in TBST buffer. After washing, VECTASTAIN avidin-biotin complex (ABC) kit (Vector Laboratories) was used to detect the NCI-186-24 or 6C1 antibody, followed by hematoxylin counterstaining.

[0105] The staining for NCI-186-24 was lighter than the staining for 6C1. One possible explanation for this result was that the 6C1 antibody could recognize multiple MAGE-A

family members expressed in the tissue (which would provide a darker stain), but NCI-186-24 could only recognize MAGE-A3 and MAGE-A6 (which would provide a lighter stain).

[0106] 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.

[0107] The use of the terms "a" and "an" and "the" and "at least one" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term "at least one" followed by a list of one or more items (for example, "at least one of A and B") is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0108] 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 antibody, or an antigen-binding fragment thereof, which specifically binds to one or both of MAGE-A3 and MAGE-A6 and does not bind to any one or more of MAGE-A1, MAGE-A2, MAGE-A4, MAGE-A5, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11 and MAGE-A12.

2. The isolated or purified antibody, or antigen-binding fragment thereof, of claim 1, wherein the MAGE-A3 and the MAGE-A6 are human MAGE-A3 and human MAGE-A6, respectively, and the antibody is a rabbit antibody.

3. The isolated or purified antibody, or antigen-binding fragment thereof, of claim 1 or 2, wherein the antibody and antigen-binding fragment thereof specifically bind to the amino acid sequence of PAAESPDPPQS (SEQ ID NO: 9).

4. The isolated or purified antibody, or antigen-binding fragment thereof, of any one of claims 1-3 comprising the amino acid sequences of (a) all of SEQ ID NOs: 1-6; (b) both of SEQ ID NO: 20 and SEQ ID NO: 21; or (c) both of SEQ ID NO: 7 and SEQ ID NO: 8.

5. A polypeptide comprising the amino acid sequences of (a) all of SEQ ID NOs: 1-6; (b) both of SEQ ID NO: 20 and SEQ ID NO: 21; or (c) both of SEQ ID NO: 7 and SEQ ID NO: 8.

6. The polypeptide of claim 5, wherein the amino acid sequences of (a) and (b) are rabbit amino acid sequences and the polypeptide specifically binds to one or both of human MAGE-A3 and human MAGE-A6.

7. A protein comprising:

(a) a first polypeptide comprising the amino acid sequences of all of SEQ ID NOs: 1-3 and a second polypeptide comprising the amino acid sequences of all of SEQ ID NOs: 4-6;

(b) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 20 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 21; or

(c) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 7 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 8.

8. The protein of claim 7, wherein the amino acid sequences of (a) and (b) are rabbit amino acid sequences and the protein specifically binds to one or both of human MAGE-A3 and human MAGE-A6.

9. An anti-MAGE-A3, anti-MAGE-A6 binding moiety comprising the antibody, or antigen-binding fragment thereof, of any one of claims 1-4, the polypeptide of claim 5 or 6, or the protein of claim 7 or 8.

10. The anti-MAGE-A3, anti-MAGE-A6 binding moiety of claim 9, wherein the anti-MAGE-A3, anti-MAGE-A6 binding moiety is an antibody, Fab fragment (Fab), F(ab')2 fragment, Fab' fragment, Fv fragment, diabody, triabody, tetrabody, single-chain variable region fragment (scFv), disulfide-stabilized variable region fragment (dsFv), IgGΔCH2, scFv2CH3, scFv4, scFv3, scFv2, scFv-Fc, (scFv)2, or a bivalent antibody.

11. The antibody, or antigen binding fragment thereof, of any one of claims 1-4, the polypeptide of claim 5 or 6, the protein of claim 7 or 8, or the anti-MAGE-A3, anti-MAGE-A6 binding moiety of claim 9 or 10, further comprising a detectable label.

12. A nucleic acid encoding the antibody, or antigen binding fragment thereof, of any one of claims 1-4, the polypeptide of claim 5 or 6, the protein of claim 7 or 8, or the anti-MAGE-A3, anti-MAGE-A6 binding moiety of claim 9 or 10.

13. A recombinant expression vector comprising the nucleic acid of claim 12.

14. A host cell comprising the recombinant expression vector of claim 13.

15. A population of host cells comprising at least two host cells of claim 14.

16. A method of assaying a biological sample for the presence of one or both of MAGE-A3 and MAGE-A6, the method comprising:

(a) contacting the biological sample with a composition, wherein the composition is the antibody, or antigen binding fragment thereof, of any one of claims 1-4 and 11, the polypeptide of any one of claims 5, 6, and 11, the protein of any one of claims 7, 8, and 11, or the anti-MAGE-A3, anti-MAGE-A6 binding moiety of any one of claims 9-11;

(b) forming a complex between the composition and the biological sample; and

(c) detecting the complex, wherein detection of the complex is indicative of the presence of one or both of MAGE-A3 and MAGE-A6 in the biological sample.

17. The method of claim 16, wherein the biological sample comprises cancer cells.

18. A composition for use in the diagnosis of cancer in a mammal, wherein the composition is the antibody, or antigen binding fragment thereof, of any one of claims 1-4 and 11, the polypeptide of any one of claims 5, 6, and 11, the protein of any one of claims 7, 8, and 11, or the anti-MAGE-A3, anti-MAGE-A6 binding moiety of any one of claims 9-11.

19. A set for use in the diagnosis and treatment of cancer in a mammal, wherein the set comprises (a) the composition for the use of claim 18 and (b) immunotherapy targeting one or both of MAGE-A3 and MAGE-A6.

20. The set of claim 19, wherein the immunotherapy comprises cells expressing a T cell receptor (TCR), wherein the TCR specifically binds to one or both of MAGE-A3 and MAGE-A6.

21. The set of claim 19, wherein the immunotherapy comprises a vaccine targeting one or both of MAGE-A3 and MAGE-A6.

22. A composition for use in determining the prognosis of cancer in a mammal, wherein the composition is the antibody, or antigen binding fragment thereof, of any one of claims 1-4 and 11, the polypeptide of any one of claims 5, 6, and 11, the protein of any one of claims 7, 8, and 11, or the anti-MAGE-A3, anti-MAGE-A6 binding moiety of any one of claims 9-11.

23. The method of claim 17, the composition for the use of claim 18 or 22, or the set for the use of any one of claims 19-21, wherein the cancer is melanoma, bone cancer, breast cancer, leukemia, thyroid cancer, gastric cancer, pancreatic cancer, liver cancer, lung cancer, ovarian cancer, multiple myeloma, esophageal cancer, kidney cancer, head cancers, neck cancers, prostate cancer, synovial cell sarcoma, urothelial cancer, or uterine cervical cancer.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/060097

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 C07K16/30
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 data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/010635 A1 (GLAXOSMITHKLINE BIOLOG SA [BE]; BERGERON ALAIN [CA]; BLAIS NORMAND [CA) 26 January 2012 (2012-01-26) See claims -----	1-23
A	WEON JENNY L ET AL: "The MAGE protein family and cancer", CURRENT OPINION IN CELL BIOLOGY, CURRENT SCIENCE, LONDON, GB, vol. 37, 3 September 2015 (2015-09-03), pages 1-8, XP029343219, ISSN: 0955-0674, DOI: 10.1016/J.CEB.2015.08.002 the whole document -----	1-23

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 2017	15/12/2017

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/060097

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/060097

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012010635 A1	26-01-2012	BR 112013001637 A2	24-05-2016
		CA 2805035 A1	26-01-2012
		CN 103108887 A	15-05-2013
		EP 2596027 A1	29-05-2013
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		JP 2013538557 A	17-10-2013
		US 2013123472 A1	16-05-2013
		WO 2012010635 A1	26-01-2012