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(71) Applicant: **LUDWIG INSTITUTE FOR CANCER RESEARCH** [US/US]; 605 Third Avenue, New York, NY 10158 (US).

(72) Inventors: **KNUTH, Alexander**; Steinbacher Hohl 2-26, 60488 Frankfurt am Main (DE). **JAGER, Elke**; Steinbacher Hohl 2-26, 60488 Frankfurt am Main (DE). **JAGER, Dirk**; Steinbacher Hohl 2-26, 60488 Frankfurt am Main (DE).

(74) Agent: **HANSON, Norman, D.**; Fulbright & Jaworski L.L.P., 666 Fifth Avenue, New York, NY 10103 (US).

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(54) Title: METHODS FOR DETECTION OF DISEASE-ASSOCIATED ANTIBODIES IN URINE

(57) Abstract: The present invention provides methods and diagnostic kits for the detection of disease-specific antibodies in urine containing sample. The present invention is also directed toward methods for determining that a patient suffers from a disorder characterized by aberrant expression of a naturally occurring gene. Methods for monitoring the status of a disorder in a patient characterized by aberrant expression of a naturally occurring gene are also provided.



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METHODS FOR DETECTION OF DISEASE-ASSOCIATED ANTIBODIES IN URINE

FIELD OF THE INVENTION

This invention relates to methods of detecting disease-associated antibodies in patient urine. These methods allow the status of a disorder in a patient characterized by aberrant expression of a naturally occurring protein to be monitored. The present invention also relates to diagnostic kits. The present invention also relates to methods of identifying disease-associated markers.

BACKGROUND AND PRIOR ART

1. Introduction

It is well established that many pathological conditions, including but not limited to infections, cancer, and autoimmune disorders, are characterized by aberrant expression of certain molecules. It is also known that the altered expression of these molecules leads to the induction of a humoral-immune response and the production of circulating antibodies against those molecules. Both the disease-associated molecules and the presence of serum antibodies are useful "markers" for the detection and monitoring of particular pathologies and for the generation of diagnostic and/or therapeutic agents.

2. Detection of disease-associated antibodies in patient samples

Although accurate assays for determining the presence of disease markers in a subject are commercially available, these assays rely on the detection of serum antibodies against particular markers and are both complex and inconvenient. There has long been a need for non-invasive, simple assays for antibodies specific for the presence of disease markers. Assays that rely on the detection of specific antibodies in urine, for example, are non-invasive, require less sample preparation and are generally less complex than serum-based assays. High-titer antibody responses have been detected in both serum and urine of HIV-, *Helicobacter pylori*-, *Schistosoma mansoni*-, *Schistosoma haematobium*-, and *Schistosoma japonicum*-infected individuals. (Wu *et al.* (2001) *Hepatogastroenterology* **48**: 614-7; Zhu *et al.* (2000) *Diagn. Microbiol. Infect. Dis.* **38**: 237-41; Yamamoto *et al.* (2000) *Helicobacter* **5**: 160-4; Bauer *et al.* (1992) *Lancet* **340**: 559). A strong correlation between serum-antibody and urine-antibody levels, combined with the beneficial features of urine-based assays compared to serum-dependent tests, makes urine-based diagnostics particularly attractive. Although the presence of antibody against infectious agents in urine has been reported (Wu *et al.* (2001) *Hepatogastroenterology* **48**: 614-7; Zhu *et al.* (2000) *Diagn.*

Microbiol. Infect. Dis. **38**: 237-41; Yamamoto *et al.* (2000) *Helicobacter* **5**: 160-4; Bauer *et al.* (1992) *Lancet* **340**: 559), the utility of these methods for the detection of antibodies against autologous molecules such as tumor-associated antigens and self-antigens, in diagnostic methods for cancer or autoimmune disease, has never been demonstrated.

Both humoral- and cellular-immune responses against human tumor-associated antigens have been observed in patients with different types of cancer (Knuth *et al.* (1984) *Proc. Natl. Acad. Sci. USA* **81**: 3511-5; Sahin *et al.* (1995) *Proc. Natl. Acad. Sci. USA* **92**: 11810-3). Based on the specific recognition by humoral and/or cellular effectors of the immune system, a large number of tumor-associated antigens—and thus, likely diagnostic reagents—have been identified (Knuth *et al.* (1984) *Proc. Natl. Acad. Sci. USA* **81**: 3511-5; Old (1981) *Cancer Res.* **41**: 361-75; Angelopoulou *et al.* (1994) *Int. J. Cancer* **58**: 480-7; Jager *et al.* (1998) *J. Exp. Med.* **187**: 265-70; Tureci *et al.* (1997) *Mol. Med. Today* **3**: 342-9). These antigens can be categorized into six groups according to their expression patterns, function or origin. Specifically, these groups include: a) Cancer-Testis (CT) antigens, which are expressed in normal germ cells and aberrantly expressed in different tumors (van der Bruggen *et al.* (1991) *Science* **254**: 1643-7; Tureci *et al.* (1998) *Proc. Natl. Sci. USA* **95**: 5211-6; Gure *et al.* (2000) *Int. J. Cancer* **85**: 726-32; Gure *et al.* (1997) *Int. J. Cancer* **72**: 965-71; Chen *et al.* (1997) *Proc. Natl. Acad. Sci. USA* **94**: 1914-8); b) differentiation antigens, which are expressed in tumor cells and the corresponding normal tissue of origin (Brichard *et al.* (1993) *J. Exp. Med.* **178**: 489-95; Kawakami *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91**: 3515-9; Kawakami *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91**: 6458-62; Jager *et al.* (2001) *Cancer Res.* **61**: 2055-61); c) mutated normal genes (Disis *et al.* (1997) *Adv. Cancer Res.* **71**: 343-71; Coulie *et al.* (1995) *Proc. Natl. Acad. Sci. USA* **92**: 7976-80; Wolfel *et al.* (1995) *Science* **269**: 1281-4); d) overexpressed 'self' antigens (Disis *et al.* (1997) *Adv. Cancer Res.* **71**: 343-71; Gnjatic *et al.* (1998) *J. Immunol.* **160**: 328-33); e) viral antigens (Lennette *et al.* (1995) *Eur. J. Cancer* **B31A**: 1875-8; Tindle *et al.* (1996) *Curr. Opin. Immunol.* **8**: 643-50); and f) splice variants of normal genes (Scanlan *et al.* (1998) *Int. J. Cancer* **76**: 652-8; Jager *et al.* (1999) *Cancer Res.* **59**: 6197-204).

The CT antigen NY-ESO-1, initially identified by serological expression cloning of a recombinant cDNA library obtained from a squamous cell carcinoma of the esophagus, elicits both humoral- and cellular immune responses in patients with NY-ESO-1-positive cancers (Chen *et al.* (1997) *Proc. Natl. Acad. Sci. USA* **94**: 1914-8; Jager *et al.* (1998) *J. Exp. Med.* **187**: 265-70; Jager *et al.* (1999) *Int. J. Cancer* **84**: 506-10; Stockert *et al.* (1998) *J. Exp.*

Med. **187**: 1349-54). NY-ESO-1 serum antibody is a reliable indicator of CD4- and CD8-positive T-cell responses against NY-ESO-1-derived peptide epitopes presented by different MHC class I and class II alleles (Chen *et al.* (1997) *Proc. Natl. Acad. Sci. USA* **94**: 1914-8; Jager *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**: 4760-5; Jager *et al.* (2000) *J. Exp. Med.* **191**: 625-30). High-titer NY-ESO-1 serum antibody was found in patients with advanced NY-ESO-1-positive malignancies. Changes in NY-ESO-1 antibody titers over extended periods of time correlate with the clinical development of NY-ESO-1-positive disease (Jager *et al.* (1998) *J. Exp. Med.* **187**: 265-70).

As with NY-ESO-1, other tumor-associated antigens have been shown to elicit both humoral and cellular immune responses that are demonstrated by detection of both autologous antibodies and T cells in cancer patients. Examples of these tumor-associated antigens are SSX2 (Ayyoub *et al.* (2002) *J. Immunol.* **168**: 1717-1722; Sahin *et al.* (1995) *Proc. Nat'l. Acad. Sci. USA* **92**: 11810-11813; both incorporated herein by reference), MAGE-1 (Sahin *et al.* (1995) *Proc. Nat'l. Acad. Sci. USA* **92**: 11810-11813; van der Bruggen *et al.* (1991) *Science* **254**: 1643-1647; both incorporated herein by reference) and tyrosinase (Sahin *et al.* (1995) *Proc. Nat'l. Acad. Sci. USA* **92**: 11810-11813; Brichard *et al.* (1993) *J. Exp. Med.* **173**: 489-495; both incorporated herein by reference). In view of the finding that disease specific antibodies are detected in the urine of patients infected with various organisms (Wu *et al.* (2001) *Hepatogastroenterology* **48**: 614-7; Zhu *et al.* (2000) *Diagn. Microbiol. Infect. Dis.* **38**: 237-41; Yamamoto *et al.* (2000) *Helicobacter* **5**: 160-4; Bauer *et al.* (1992) *Lancet* **340**: 559), and the finding that—in addition to activating cellular immunity—tumor-associated antigens induce production of detectable levels of serum antibody, the inventors investigated whether aberrations in the expression of autologous proteins in non-infectious diseases and disorders, such as tumor-associated antigens, result in the excretion of antibodies urine. The results of these investigations provide the basis for the development of urine-based tests for the detection and monitoring of spontaneous and vaccine-induced immunity against defined disease-associated antigens in patients suffering from disorders characterized by the aberrant expression of the antigen.

The present invention demonstrates that specific antibodies against an aberrantly expressed molecule can be detected in urine samples taken from patients believed to be expressing that molecule, and that these molecules and their derivatives are useful diagnostic and therapeutic reagents. This discovery has important implications for the detection and

monitoring of spontaneous and vaccine-induced immunity against defined disease-associated antigens.

3. Identification of disease-associated antigens: SEREX

The identification and preparation of disease-associated antigens is laborious, unpredictable and expensive. Two main methods have been used for the detection of such antigens. In the gene-based approach, host cells are transformed or transfected with a tumor-derived cDNA library and tested for the expression of the specific antigen. *See, e.g., dePlaen et al. (1988) Proc. Natl. Sci. USA 85: 2275; incorporated herein by reference.* In contrast, the biochemical approach is based on the elution of peptides from MHC-class I molecules of tumor cells, followed by isolation and purification of those peptides by reverse-phase high performance liquid chromatography (RP-HPLC) and testing for the ability of the peptides to bind MHC-class I molecules and activate cytotoxic T-lymphocytes (CTLs), including induction of CTL proliferation, release of tumor necrosis factor (TNF), and the lysis of target cells. The disadvantages of these approaches are highlighted by the relatively few new antigens identified by these methods. *See, e.g., van der Bruggen et al. (1991) Science 254: 1643-1647; Brichard et al. (1993) J. Exp. Med. 178: 489-495; Coulie, et al. (1994) J. Exp. Med. 180: 35-42; Kawakami et al. (1994) Proc. Natl. Acad. Sci. USA 91: 3515-3519.* Furthermore, these methods require established, permanent tumor-cell lines of the cancer type under consideration. Stable tumor-cell lines are very difficult to establish and maintain. *See, e.g., Oettgen et al. (1990) Immunol. Allerg. Clin. North. Am. 10: 607-637.* Also, numerous tumor types, including epithelial cell-tumors, are not responsive to CTLs *in vitro*, thereby precluding routine assay. These problems have led to the development of new methods for identifying disease-associated antigens.

The SEREX method ("Serological identification of antigens by Recombinant Expression Cloning") has been used to both identify new disease-associated antigens and confirm expression of previously identified antigens. *Sahin et al. (1995) Proc. Natl. Acad. Sci. USA 92: 11810-11913; Crew et al. (1995) EMBO J. 144: 2333-2340; U.S. Patent No. 5,698,396; U.S. Patent No. 6,251,603; and U.S. Patent No. 6,252,052; each incorporated herein by reference.* In brief, the SEREX method involves the expression of tumor-derived cDNA libraries in a prokaryotic host followed by screening with absorbed and diluted sera. The binding of serum antibodies to the expressed protein antigens identifies those proteins as antigens likely to elicit a high titer humoral-immune response. SEREX has led to the identification, isolation and cloning of a large number of immunogenic molecules that induce

autologous humoral antibody responses. Several of these molecules have also been shown to induce autologous T-cell responses. NY-ESO-1 was identified by SEREX using serum from a patient with an esophageal tumor. *See, e.g.*, U.S. Patent Nos. 5,698,396; 6,251,603; 6,252,052; and U.S. Patent Application Serial No. 09/262,422; each incorporated herein by reference. SEREX has also been used to monitor the status of a disease or disorder by assaying for antibodies specific to that disease or disorder. *See, e.g.*, U.S. Patent Nos. 5,698,396; 6,251,603; 6,252,052; and U.S. Patent Application Serial No. 09/262,422; each incorporated herein by reference.

The search for disease- and tumor-associated antigens may be improved by screening disease- and tumor-derived cDNA expression libraries with autologous or allogeneic urine samples. The inventors have demonstrated that urine represents a source of highly specific, high-titer antibodies that are useful in diagnostic and therapeutic applications and in methods for identifying new disease-associated antigens.

SUMMARY OF THE INVENTION

The present invention provides methods for determining whether a patient suffers from a disorder characterized by aberrant expression of a naturally occurring gene, comprising: i) contacting a sample containing urine from a patient that is believed to suffer from said disorder with an agent that specifically binds to an antibody present in said urine and characteristic of said condition, under conditions favoring complex formation between the agent and the antibody; and ii) detecting the described complex, wherein detection of the complex indicates that the patient suffers from said disorder. In one embodiment, the agent is a cell that presents a binding partner for said antibody on its surface. In another embodiment, the agent is a binding partner for said antibody and is not cell-associated. In a preferred embodiment, the agent is a tumor-associated antigen. The tumor-associated antigens of the present invention include antigens identified by SEREX. The tumor-associated antigen of the present invention preferably induces the production of a cellular-immune response and/or a humoral-immune response. More preferably, the tumor-associated antigen is a cancer-testis (CT) antigen, a differentiation antigen, a mutated normal gene, an overexpressed self antigen, or a viral antigen. In a preferred embodiment the tumor-associated antigen is NY-ESO-1, NY-BR-1 (GenBank Accession Nos. XM165546 and XM166711 (both version 1 and submitted May 13, 2002); Cross *et al.* (2000) *Mamm. Genome* **11(5)**: 373-383; all incorporated herein by reference), SCP-1 (GenBank Accession No. D67035 (February 2, 1999; version 1); Kondoh *et al.* (1997) *Cytogenet. Cell. Genet.* **78(2)**: 103-104; all

incorporated herein by reference) and UniGene designation Hs.339651 or the clone 18CGI1F2 (GenBank Accession No. AJ236609 (May 2, 2000; version 1) and the UniGene designation Hs.339651; both incorporated herein by reference). Most preferably, the tumor-associated antigen is NY-ESO-1.

The present invention also provides a method for monitoring the status of a disorder in a patient characterized by aberrant expression of a naturally occurring gene, comprising: i) contacting a sample containing urine from a patient that is believed to suffer from said disorder with an agent that specifically binds to an antibody present in said urine and characteristic of said condition, under conditions favoring complex formation between the agent and the antibody; and ii) detecting the described complex; and iii) comparing a value obtained to a prior value obtained following assay of a prior sample taken from said patient, wherein any difference is indicative of a change in status of said disorder. In another embodiment, the agent is a binding partner for said antibody and is not cell-associated. In a preferred embodiment, the agent is a tumor-associated antigen. The tumor-associated antigens of the present invention include antigens identified by SEREX. The tumor-associated antigen of the present invention preferably induces the production of a cellular-immune response and/or a humoral-immune response. More preferably, the tumor-associated antigen is a cancer-testis (CT) antigen, a differentiation antigen, a mutated normal gene, an overexpressed self antigen, or a viral antigen. In a preferred embodiment the tumor-associated antigen is NY-ESO-1, NY-BR-1 (GenBank Accession Nos. XM165546 and XM166711 (both version 1 and submitted May 13, 2002); Cross *et al.* (2000) *Mamm. Genome* **11**(5): 373-383; all incorporated herein by reference), SCP-1 (GenBank Accession No. D67035 (February 2, 1999; version 1); Kondoh *et al.* (1997) *Cytogenet. Cell. Genet.* **78**(2): 103-104; all incorporated herein by reference) or the clone 18CGI1F2 (GenBank Accession No. AJ236609 (May 2, 2000; version 1) and the UniGene designation Hs.339651; both incorporated herein by reference). Most preferably, the tumor-associated antigen is NY-ESO-1.

In one embodiment of the present invention, the complex between the tumor-associated antigen and the antibody is detected by a Western Blot. In a preferred embodiment, the described complex is detected by the specific binding of a second antibody to the antibody of the complex. The second antibody specifically binds any portion of the antibody present in the urine of the patient when it is complexed with an agent. This antibody preferably binds the F_c portion of the antibody of the described complex. For

example, if the antibody of the complex is human, the second antibody may be an antibody that specifically binds human antibodies. In one embodiment, the antibody that specifically binds the antibody present in the urine of a patient contains a detectable label. In a preferred embodiment, the detectable label includes fluorescent molecules, radioactive molecules, proteins, magnetic beads, or metals. In a more preferred embodiment, the complex between the tumor-associated antigen and the antibody is detected by an enzyme-linked immunosorbent assay (ELISA).

In another embodiment of the present invention, the agent or the second antibody is immobilized on a solid matrix. The solid matrix may be plastic, glass, paper, nitrocellulose, nylon, poly(vinyl-chloride), cellulose, polyacrylamide, polystyrene or polypropylene. In a preferred embodiment, the solid matrix is a magnetic bead.

The present invention also provides a kit comprising: i) an agent that binds an antibody present in urine of a patient suffering from a disorder characterized by aberrant expression of a naturally occurring gene; and ii) a second component selected from the group consisting of: a control antibody, a control antigen, a positive control sample containing urine, a negative control sample containing urine, an antibody that specifically binds the antibody present in the urine of said patient, and instructions for use of the agent for the detection of an antibody that is present in urine and specifically binds to the agent. In the kits of the present invention, the preferred agent is a tumor-associated antigen. More preferably, the tumor-associated antigen is NY-ESO-1, SCP-1 (GenBank Accession No. D67035 (February 2, 1999; version 1); Kondoh *et al.* (1997) *Cytogenet. Cell. Genet.* **78(2)**: 103-104; all incorporated herein by reference), NY-BR-1 (GenBank Accession Nos. XM165546 and XM166711 (both version 1 and submitted May 13, 2002); Cross *et al.* (2000) *Mamm. Genome* **11(5)**: 373-383; all incorporated herein by reference) or clone 18CGI1F2 (GenBank Accession No. AJ236609 (May 2, 2000; version 1) and the UniGene designation Hs.339651; both incorporated herein by reference). Most preferably, the tumor-associated antigen is NY-ESO-1.

In another embodiment, the kits of the present invention further comprise an antibody that specifically binds the antibody present in the urine of a patient suffering from a disorder. In a preferred embodiment, the antibody that specifically binds the antibody present in the urine of a patient suffering from a disorder contains a detectable label. In a more preferred embodiment the label is a fluorescent molecule, a radioactive molecule, a protein, a magnetic bead, or a metal.

In another embodiment, the kits of the present invention contain an agent that is immobilized on a solid matrix. In yet another embodiment, the kits of the present invention contain an antibody that specifically binds the antibody present in the urine of a patient suffering from a disorder, wherein that antibody is immobilized on a solid matrix. In a preferred embodiment, the solid matrix is plastic, glass, paper, nitrocellulose, nylon, poly(vinyl-chloride), cellulose, polyacrylamide, polystyrene, or polypropylene. In a more preferred embodiment, the solid matrix is a magnetic bead.

The present invention also provides a method for determining an immunoreactive substance produced by a subject, wherein said subject is capable of eliciting an immune response, comprising: i) producing a cDNA library from a cell taken from said subject; ii) inserting said cDNA library into an expression vector; iii) transfecting said vector into a host cell to produce a transfected host cell; iv) culturing said transfected host cell to express said immunoreactive substance; v) contacting a urine sample which contains an antibody specific to said immunoreactive substance with a sample of non-transfected host cells, to remove an immunologic binding partner from said sample which is specific for said non-transfected host cell, to produce a stripped urine sample; vi) contacting said stripped urine sample with a sample of host cells transfected with the same vector into which said cDNA library has been inserted, wherein said vector does not contain any library cDNA, to remove any immunologic binding partners specific for vector produced antigens thereby producing a twice stripped urine sample; vii) contacting said twice stripped urine sample to the transfected host cell of (iv), thereby any immunologic binding partners specific for said immunoreactive substance bind thereto; and vii) determining binding in (vii) to determine said immunoreactive substance. In a preferred embodiment, the cultured transfected cell of step (iv) is lysed to produce cell lysate and said cell lysate is contacted with urine as in step (v). In one embodiment of the present invention the immunoreactive substance is a tumor-associated antigen. In a preferred embodiment, the immunoreactive substance is an autoimmune-disease-associated antigen.

DETAILED DESCRIPTION OF THE INVENTION

As used herein the term “agent that specifically binds to an antibody” refers to a binding partner of an antibody present in serum or urine. The agents of the present invention specifically bind to the antigen-binding portion of an antibody present in serum or urine. Agents include, but are not limited to, antigens and antibody-binding fragments thereof—either naturally occurring or recombinant or engineered—cells expressing antigens and

antibody-binding fragments thereof. Agents may be molecules which are or contain protein, carbohydrate, nucleic acid, or any compound that mimics the structure of a binding partner of an antibody present in patient serum or urine.

In one embodiment of the present invention, the agent is a cell that presents a molecule on its surface that is a binding partner for an antibody present in the urine of a patient suffering from a disease or disorder characterized by aberrant expression of a naturally occurring gene. In this embodiment, cells are first contacted with urine that may contain an antibody that is specific for a molecule on the surface of the cell. Detection of a complex between the cell and a urine antibody indicates that the patient's urine contains an antibody specific for a molecule on the surface of the cell and that the patient suffers from a disorder characterized by aberrant expression of a naturally occurring gene. The complex between an agent and an antibody present in the urine of a patient may be detected by the specific binding of a second antibody that specifically binds the urine antibody. The second antibody may contain a detectable label. This label may be attached directly or indirectly through a linker or conjugating agent such as glutaraldehyde, periodate, and the like. A wide variety of different conjugation techniques exist, which are well known to the skilled artisan. Cells labeled with a second antibody may be detected by flow cytometry.

The agents and antibodies of the disclosed kits may be formulated with acceptable carriers such that they can be used directly from the container without further preparation, in the kits of the present invention. Further, the agents and antibodies may be lyophilized with lyoprotective agents known in the art, such as trehalose and tricine, to enhance stability and increase shelf-life. Also, the container(s) may be presented in a pack or dispenser device. The pack or dispenser device may be accompanied by instructions for use of the agents and antibodies for determining that a patient suffers from a disorder characterized by aberrant expression of a naturally-occurring gene. The kits may also comprise additional materials necessary to conduct the assay, including but not limited to control antibodies, control antigens, and/or materials necessary for calibration of the detector.

As used herein, the term "antibody", unless indicated otherwise, is used broadly to refer to both antibody molecules and a variety of antibody-derived molecules. Such antibody-derived molecules comprise at least one variable region (either a heavy chain or light chain variable region) and include molecules such as Fab fragments, F(ab)₂ fragments, single chain (sc) antibodies, diabodies, triabodies, tetrabodies, individual antibody light chains, individual antibody heavy chains, chimeric fusions between antibody chains and other molecules, and

the like. As used herein, "antigen-binding fragment" or "antigen-binding domain" or "Fab fragment" refer to the about 45 kDa fragment obtained by papain digestion of an immunoglobulin molecule and consist of one intact light chain linked by disulfide bond to the N-terminal portion of the contiguous heavy chain. As used herein, "F(ab)₂ fragment refers to the about 90 kDa protein produced by pepsin hydrolysis of an immunoglobulin molecule. It consists of the N-terminal pepsin cleavage product and contains both antigen binding fragments of a divalent immunoglobulin, such as IgD, IgE, and IgG. Neither the "antigen-binding fragment" nor "F(ab)₂ fragment" contain the about 50 kDa F_c fragment produced by papain digestion of an immunoglobulin molecule that contains the C-terminal halves of the immunoglobulin heavy chains, which are linked by two disulfide bonds, and contain sites necessary for complement fixation. The term, as used herein, also includes antibodies with engineered F_c regions, such as humanized antibodies.

As used herein the term "antigen" refers to any molecule, substance or complex that stimulates the production of a specific antibody or antibodies when introduced into an immunocompetent animal.

As used herein, the term "autologous gene" or "autologous protein" refers to a gene or protein that is self-produced. A protein that is autologous to a subject is produced by that subject.

As used herein, the term "binding partner" refers to a molecule, compound or complex that will bind to another molecule, compound or complex, under the appropriate conditions in the presence of that molecule, compound or complex. Binding partners useful in the present invention include specific binding pairs such as antigens and antibodies, or fragments of antibodies, both polyclonal and monoclonal, lectins and carbohydrates, hormones and hormone receptors, enzymes and enzyme substrates, biotin and (strept) avidin vitamins and vitamin binding proteins, complementary polynucleotide sequences, drugs and receptors, enzymes and inhibitors, apoproteins and cofactors, growth factors and receptors, and the like. Biotin and (strept) avidin derivatives may also be used, including biotin analogs/avidin, biotin/streptavidin, and biotin analogs/streptavidin. Binding partners may be naturally occurring or "engineered." Such techniques are well known in the art, and include the design and production of chimeric and humanized antibodies and fragments thereof, synthetic peptides, modified enzymes or substrates, synthetic RNA and DNA oligonucleotides, and the like.

As used herein the term “cancer-testis (CT) antigen” refers to antigens that are exclusively expressed in a variety of tumors and normal germ cells. In a preferred embodiment, the CT antigens are selected from the group consisting of: the MAGE-family of proteins; the BAGE-family of proteins; the GAGE-family of proteins; the LAGE-family of proteins; NY-ESO-1 proteins; SSX, SCP-1 (GenBank Accession No. D67035 (February 2, 1999; version 1); Kondoh *et al.* (1997) *Cytogenet. Cell. Genet.* **78(2)**: 103-104; all incorporated herein by reference), CT-10, clone 18CGI1F2 (GenBank Accession No. AJ236609 (May 2, 2000; version 1) and the UniGene designation Hs.339651; both incorporated herein by reference), and the like. In a more preferred embodiment the CT antigens are selected from the group consisting of: NY-ESO-1, and SCP-1 (GenBank Accession No. D67035 (February 2, 1999; version 1); Kondoh *et al.* (1997) *Cytogenet. Cell. Genet.* **78(2)**: 103-104; all incorporated herein by reference). The MAGE-family of proteins include MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-5, MAGE-6, MAGE-7, MAGE-8, MAGE-9, MAGE-10, MAGE-11, MAGE-12, MAGE-13, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, and antibody-binding fragments thereof. The BAGE-family of proteins include BAGE-1, and antibody-binding fragments thereof. The GAGE-family of proteins include GAGE-1, GAGE-2, GAGE-3, GAGE4, GAGE-5, GAGE-6, GAGE-7, GAGE-8 and antibody-binding fragments thereof. The RAGE-family of proteins include RAGE-1, and antibody-binding fragments thereof. The LAGE-family of proteins include LAGE-1, and antibody-binding fragments thereof. The PRAME-family of proteins include PRAME-1, and antibody-binding fragments thereof. NY-ESO-1 includes NY-ESO-1 and antibody-binding fragments thereof. CT7 includes CT7 and antigen-binding fragments thereof. CT-10 includes CT-10 and antigen-binding fragments thereof. The SSX-family of proteins include SSX-1, SSX-2 (HOM-MEL-40), SSX-4, SSX-5 and antibody-binding fragments thereof. The OY-TES-1-family of proteins includes OY-TES-1 and antibody-binding fragments thereof.

As used herein the term “complex” refers to a combination of two or more molecules that have spatial and/or polar features that permit specific binding to each other. Examples of complexes of the present invention include ligand-receptor complexes, enzyme-substrate complexes, protein-protein complexes, and agent-antibody complexes, and the like. Binding may be either covalent or non-covalent.

As used herein, the term “a control antibody” refers to an antibody that binds a particular agent, under conditions favoring the formation of an agent-antibody complex

(positive control) or does not bind a particular agent, under conditions favoring the formation of an agent-antibody complex (negative control).

As used herein, the term “a control antigen” refers to an antigen that binds a particular antibody, under conditions favoring the formation of an antigen-antibody complex (positive control) or does not bind a particular antibody, under conditions favoring the formation of an antigen-antibody complex (negative control).

As used herein, the term “a positive control sample containing urine” refers to a urine sample that contains an antibody that specifically binds a defined agent, under conditions favoring the formation of an antigen-antibody complex. Specifically, in a kit of the present invention, “a positive control sample containing urine” refers to a urine sample that contains an antibody that specifically binds the agent provided in the kit, under conditions favoring the formation of an antigen-antibody complex.

As used herein, the term “a negative control sample containing urine” refers to a urine sample that contains an antibody that does not specifically bind a defined agent, under conditions favoring the formation of an antigen-antibody complex. Specifically, in a kit of the present invention, “a negative control sample containing urine” refers to a urine sample that does not specifically bind the agent provided in the kit.

As used herein the term “detecting the complex” or “detection of the complex” refers to the ability to identify the formation of a complex between an agent and an antibody. Any method of detection or assay for detecting the presence and/or formation of a complex is contemplated by the present invention. Such assays include but are not limited to heterogeneous- or homogeneous-phase assays, such as sandwich assays, radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), fluorescent immunoassays, protein A immunoassays, nephelometric assays, etc. In a preferred embodiment, the method of detection is Western blotting.

In another embodiment, the complex between an agent and an antibody present in the urine of a patient may be detected by the specific binding of a second antibody that specifically binds the urine antibody. The second antibody may contain a detectable label. This label may be attached directly or indirectly through a linker or conjugating agent such as glutaraldehyde, periodate, and the like. A wide variety of different conjugation techniques exist, which are well known to the skilled artisan. Such detectable labels include, but are not limited to: radioactive moieties, substrate-converting enzymes, such as horseradish

peroxidase, glucose oxidase, .beta.-galactosidase, alkaline phosphatase, and the like, fluorophores, biotin, proteins, magnetic beads, metals, and the like. *See, e.g., Antibodies: A Laboratory Manual*, Harlow *et al.* (1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; incorporated herein by reference.

In a preferred embodiment, the method of detection of the agent-antibody complexes is magnetic separation using antibody-coated or agent-coated magnetic beads. Kemmner *et al.* (1992) *J. Immunol. Methods* **147**: 197-200; Racila *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**: 4589-4594. The antibody used to coat the magnetic bead specifically binds the antibody isolated from patient urine or serum.

As used herein the term “a differentiation antigen” refers to any cell-surface antigen—a molecule that stimulates the production of antibodies specific to that molecule—wherein the expression of that molecule varies during successive developmental stages of a particular cell type. Differentiation antigens include tyrosinase, brain glycogen phosphorylase, Melan-A, NY-BR-1 (GenBank Accession Nos. XM165546 and XM166711 (both version 1 and submitted May 13, 2002); Cross *et al.* (2000) *Mamm. Genome* **11**(5): 373-383; all incorporated herein by reference), clone 18CGI1F2 (GenBank Accession No. AJ236609 (May 2, 2000; version 1) and the UniGene designation Hs.339651; both incorporated herein by reference), MART-1, gp100, TRP-1, TRP-2, etc., and antibody-binding fragments thereof.

As used herein the term “disease-associated antigen or disease-associated marker” refers to any specific molecule that corresponds to a disease or disorder, and that may be detected by a genetic or biochemical test.

As used herein the term “a disorder characterized by aberrant expression of a naturally occurring gene” refers to a disease or disorder produced by altered expression of one or more genes or the presence of one or more proteins. These genes and proteins may be naturally occurring, mutated, autologous or derived from a different organism.

As used herein, the term “high serum-antibody titer” refers to a serum sample that produces a strong band on a Western blot, when the serum is diluted 1000-fold.

As used herein, “high urine-antibody titer” refers to a urine sample that produces a strong band on a Western blot, when the urine is 100-fold diluted.

As used herein the term “immobilized” refers to attachment of an agent or antibody of the present invention to a solid matrix. Covalent attachment includes attachment through the N- or C-terminus or any other amino acid residue, carbohydrate or other moiety. The

antigens or antibodies of the present invention may be attached directly or through a linker or conjugating agent. The term also refers to the inability of the immobilized agent or antibody to diffuse into and through the solvent, fluid or reagent.

As used herein, the term "immunoreactive substance" refers to any substance that binds to the antigen-binding domain of an antibody or stimulates a humoral or cellular immune response.

As used herein the term "label" refers to a molecule that directly or indirectly mediates the production of a signal that is indicative of the presence or absence of a specific antibody in the urine sample. A label may be attached directly or indirectly through a linker or conjugating agent such as glutaraldehyde, periodate, and the like. A wide variety of different conjugation techniques exist, which are well known to the skilled artisan. Labels may include enzymes, fluorescent molecules, metal sols, colored latex spheres, colloidal metals, magnetic beads and those disclosed in U.S. Patent No. 4,954,452; U.S. Patent No. 4,313,734; U.S. Patent No. 5,252,459; U.S. Patent No. 4,373,932; and U.S. Patent No. 4,703,013; each incorporated by reference herein in their entirety. *See, e.g., Antibodies: A Laboratory Manual*, Harlow *et al.* (1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Colored latex particles are described in U.S. Patent No. 4,703,017, which is incorporated herein by reference in its entirety. The label may also be an inducible property of the particles, such as colorable latex particles. *See, e.g.,* U.S. Patent No. 4,373,932; U.S. Patent No. 4,837,168; both incorporated herein by reference in their entirety. Metal labels include metal or metal compounds such as metal oxides, metal hydroxides, metal salts or polymer nuclei coated with a metal or metal compound. These metal labels may include dry forms of any of the above-named metals or metal compounds, and include colloidal gold in dry form. For example, the metal label can be composed of a metal sol, a selenium sol or a carbon sol. *See, e.g.,* U.S. Patent No. 4,313,734; U.S. Patent No. 4,775,636; U.S. Patent No. 4,954,452; and U.S. Patent No. 5,559,041; each incorporated herein by reference in their entirety.

In a preferred embodiment, the agent-antibody complex is detected by an ELISA assay. The ELISA assays of the present invention may use any number of different enzymes, such as horseradish peroxidase, glucose oxidase, beta.-galactosidase, alkaline phosphatase, and the like. Enzymes suitable for ELISA assays are well known in the art. Suitable substrates corresponding to a specific enzyme is generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a product that is either colored and detectable by

visible spectrometry, or is colorless and detectable by UV spectrometry. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine or tetramethylbenzidine are commonly used. Fluorogenic substrates that produce a fluorescent product may also be used. These fluorogenic substrates include substrates labeled directly or indirectly with fluorescein or rhodamine. In all cases, the enzyme-labeled second antibody is added to the agent-antibody complex. A solution containing the appropriate substrate is then added to the tertiary complex of antibody-antigen-labeled second antibody complex. The substrate reacts with the enzyme linked to the antibody, giving a signal that correlates to the amount of antibody present in the patient's urine.

As used herein the term "marker" refers to any specific molecule that corresponds to a specific characteristic, state or quality, and that may be detected by a genetic or biochemical test.

As used herein the term "a mutated normal gene" refers to a gene and its corresponding product, wherein the normally-occurring gene has been mutated to alter the expression of the mutated gene or another gene, alter the differentiation state of the cell, or that leads to the development of unregulated growth or division of the cell. Mutated normal genes and proteins include MUM-1, CDK4, NAG, β -catenin, gp-100-in4, p15, etc., and antibody-binding fragments thereof.

As used herein the term "naturally-occurring gene" or "naturally-occurring protein" refers to a gene or protein that is endogenous to a cell. Naturally-occurring genes and proteins include mutated forms that lose function or biological activity, or retain function or biological activity.

As used herein the term "an overexpressed self antigen" refers to an antigen that is present in a subject's own cells or tissues and is overexpressed. As used herein, this term also refers to self antigens that are expressed at normal levels or below normal levels where autologous antibodies are produced against the antigen.

As used herein the term "solid matrix" refers to any solid material of any size, shape, composition or construction that is suitable as an attachment material for any agent or antibody of the present invention. The solid matrix is preferably poly(vinyl-chloride), but may be other polymers such as cellulose, polyacrylamide, nylon, polystyrene or polypropylene. The solid matrix may be in the form of tubes, beads, discs or micro plates, or

any other surfaces suitable for conducting an assay. Any agent or antibody of the present invention may be covalently or non-covalently attached to the solid matrix. Covalent attachment includes attachment through the N- or C-terminus or any other amino acid residue, carbohydrate or other moiety. The antigens or antibodies of the present invention may be attached directly or through a linker or conjugating agent. Methods of attaching molecules to a solid matrix are well known in the art and include treatment of the molecule or the solid matrix with CNBr, carbonyldimidazole, tresyl chloride and the like.

In a preferred embodiment, the solid-matrix is contained in a dip-stick device that allows for the direct assay of a patient's urine. This device may be placed into a urine stream or dipped into a previously collected urine sample. In known devices, the test fluid wicks up through an absorbent membrane that is contact with the reagents that detect an analyte in the fluid. An example of such a device that is designed to reduce artifacts, such as flow and leading edge effects, is described in U.S. Patent No. 6,372,514, which is incorporated herein by reference in its entirety. *See also*, U.S. Patent Nos. 5,656,503 and 4,200,690; both incorporated herein by reference in its entirety. Reagents suitable in the detection of an agent-antibody complex in such an assay format are described in detail in: U.S. Patent Nos. 4,861,711; 4,271,140; 5,622,871; 5,656,503; 5,602,040; and 5,591,645; each incorporated by reference herein in their entirety.

As used herein, the term "specifically binds" refers to the ability of an antibody to bind an agent, wherein that binding depends on the specific structure and properties of the antibody and the agent. "Specific binding" excludes all non-specific binding which does not depend on the particular structure and/or properties of the antibody and agent and the specific relationship between the agent and antibody.

As used herein, the term "specifically binds the antibody present in the urine of the patient" refers to the ability of an antibody to specifically bind any portion of the antibody present in urine, when it is complexed with an agent of the present invention. Preferably, this antibody binds the F_c portion of the antibody of the described complex. "Specific binding" excludes all non-specific binding which does not depend on the particular structure and/or properties of the antibody and agent and the specific relationship between the agent and antibody.

As used herein the term "tumor-associated antigen" refers to an antigen that is differentially expressed on cancerous cells and tissues and not on the corresponding non-

cancerous cell or tissue. The tumor-associated antigens of the present invention include antigens identified by SEREX. The tumor-associated antigen of the present invention preferably induces the production of a cellular-immune response and/or a humoral-immune response. Tumor-associated antigens include members of the cancer-testis (CT) antigen family; differentiation antigens; mutated normal genes; overexpressed self antigens; and viral antigens.

As used herein, the term “a viral antigen” refers to an antigen that is encoded by a viral genome and includes those virally encoded proteins that are post-translationally modified by the infected-host cell. The “viral antigen” or “viral antigens” of the present invention are limited to those antigens produced by oncogenic viruses, such as human papilloma virus (HPV), Epstein-Barr Virus (EBV), *etc.* As used herein, “viral antigen” specifically excludes any antigen produced by HIV.

EXAMPLES

The terms and expressions employed in the following Examples are used as terms of description and not of limitation. There is no intention of excluding any equivalents of the features shown and described. It is recognized that various modifications are possible within the scope of the invention.

EXAMPLE 1: Immunoblot Analysis

Serum and urine antibody responses against the recombinant NY-ESO-1 protein were assayed by standard Western blot analysis (Towbin *et al.* (1979) *Proc. Natl. Acad. Sci. USA* 76: 4350-4). Briefly, 1 µg of purified recombinant NY-ESO-1 protein was diluted in SDS, and electrophoresed on a 15% SDS gel. In all assays, the recombinant NY-ESO-1 ‘short’ protein—corresponding to amino acid positions 10-121 and a molecular weight of 14 kDa—was used. Full length and recombinant NY-ESO-1 ‘short’ protein are both recognized by serum antibodies. After overnight blotting on a nitrocellulose filter (0.45 µm) and blocking with 3% BSA, blots were incubated with patient sera at 1:250, 1:1000, 1:5000, 1:10.000, 1:50.000, and 1:100.000 dilution. Dilutions with patient urine were 1:1 (undiluted), 1:250, and 1:1.000. The E978 mouse monoclonal antibody against NY-ESO-1 was used as a positive control. Binding of recombinant NY-ESO-1 ‘short’ protein by serum and urine antibodies correlates with the binding of the naturally-expressed NY-ESO-1 protein (26 kDa)(expressed in the melanoma cell line NW-MEL-38) and the full-length recombinant NY-ESO-1 expressed in COS-7-transfected cells. Serum and urine antibodies binding to NY-

ESO-1 were detected by incubation with goat anti-human IgG (Fc-spec.) 1:10.000, or goat anti-mouse IgG 1:3000, and visualized with NBT/X-phosphate. Serum and urine samples were considered positive for NY-ESO-1 antibody with a detectable 14 kDa protein band (recombinant NY-ESO-1 'short' protein) and a 26 kDa protein band (naturally-expressed NY-ESO-1 and full-length recombinant NY-ESO-1).

EXAMPLE 2: ELISA

Recombinant NY-ESO-1 'short' protein at a concentration of 1 µg/ml in coating buffer (15 mM Na₂CO₃, 30 mM NaHCO₃, pH 9.6, with 0.02% NaN₃) was adsorbed to microwell plates 60 x 10 at 10 µl/well overnight at 4°C. Plates were washed with PBS, and blocked overnight at 4°C with 10 µl/well of 2 % BSA/PBS. After washing, 10 µl/well of serum and urine dilutions in 2 % BSA was added and incubated for 2 h at room temperature. Plates were washed and 10µl/well diluted secondary antibody in 2% BSA was added (Goat anti-human IgG-AP). The second antibody was incubated for 1 hour at room temperature. Plates were washed, incubated with 10 µl/well substrate solution for 25 minutes at room temperature, and immediately read. Serum and urine were tested over a four-fold range of dilutions—1:1, 1:100 to 1:100.000. (Stockert *et al.* (1998) *J. Exp. Med.* **187**: 1349-54).

NY-ESO-1 urine antibody was detected in cancer patients, who had a high-titer serum reactivity. Nephropathy as a possible reason for increased renal protein loss was excluded in all patients, because none of the cancer patients exhibited decreased renal function. Strong NY-ESO-1 antibody reactivity that is detectable in patient urine is indicative of NY-ESO-1-specific CD8-positive T-cell reactivity. In contrast, none of the patients with weak or negative NY-ESO-1 serum and negative urine antibody had detectable CD8-positive T-cell reactivity. In the series of patients analyzed, there was no relation between the extent or location of NY-ESO-1 metastatic sites and NY-ESO-1 serum- and urine-antibody status. Changes in baseline NY-ESO-1 antibody reactivity, however, are associated with the clinical evolution of metastatic disease, and may be easily monitored by testing urine samples over time.

EXAMPLE 3: High serum NY-ESO-1-antibody titer correlates with detectable urine NY-ESO-1 antibodies

Urine and serum samples from patients previously diagnosed with NY-ESO-1-positive cancer were assayed for NY-ESO-1-specific antibodies. Patient tissue was typed to

determine if their tumors expressed NY-ESO-1. Tumor typing for NY-ESO-1 is described in US Patent No. 5,804,381, which is incorporated herein by reference. Using this methodology, ten NY-ESO-1-positive patients were identified: three breast cancer patients, two with non-small-cell lung carcinoma, and one each of neuroendocrine carcinoma, melanoma, sarcoma, bladder and stomach. All patients were tested for renal function, and all were normal. No subjects showed any evidence of significant proteinuria.

Serum and urine samples from each patient were examined by Western blotting. Jäger *et al.* (1999) *Int. J. Cancer* **84**: 506-570; Stockert *et al.* (1998) *J. Exp. Med.* **187**: 1349-1354; and U.S. Patent No. 6,251,603 and U.S. Patent Application No. 09/262,422, filed April 17, 1998; all of which are incorporated herein by reference. Each Western blot included full-length recombinant NY-ESO-1 protein, and recombinant SSX protein—used as a negative control. The Western blots—also confirmed by ELISA—indicate that patients with a high serum NY-ESO-1-antibody titer—detectable band at a dilution of 1:1000—excrete NY-ESO-1 antibodies into urine at levels sufficient to produce a strong signal on Western blots. In low-titer serum samples, where NY-ESO-1 antibodies are detectable in urine that has not been diluted more than 250-fold, NY-ESO-1 antibodies were not detected in urine. (Table 1). Thus, these experiments demonstrate that NY-ESO-1 antibodies are present in the urine of patients who show high serum antibody titers (*i.e.*, $\geq 1:1000$). Also, the presence of the antibodies is not associated with reduced renal function, as the antibody was found in the urine of patients with normal renal function.

EXAMPLE 4: Detection of NY-ESO-1 serum and urine antibody

Both Western blot and ELISA assays have been standardized to detect NY-ESO-1 serum antibody. (Jager *et al.* (1999) *Int. J. Cancer* **84**: 506-10; Stockert *et al.* (1998) *J. Exp. Med.* **187**: 1349-54). In this study, NY-ESO-1 serum antibody reactivity was assessed by Western blot analysis, and the results were confirmed by ELISA in a subset of 10 serum samples. To exclude any non-specific reactivity with the recombinant NY-ESO-1 protein, sera were also tested against lysates of the NY-ESO-1-positive melanoma cell line NW-MEL-38.

Forty-three patients with advanced NY-ESO-1-positive malignancies (including melanoma 11, prostate cancer 5, breast cancer 4, stomach cancer 2, non-small cell lung cancer 9, ovarian cancer 3, sarcoma 3, cholangiocellular carcinoma 1, Non-Hodgkin's lymphoma 1, small cell carcinoma 2, multiple myeloma 1, bladder cancer 1) were selected

for the assessment of NY-ESO-1 serum and urine antibody. Of the 43 patients tested, 17 had a strong NY-ESO-1 serum-antibody reactivity detectable at serum dilutions $\geq 1:1,000$. Seven patients had a weak antibody reactivity—faint bands at 1:250 dilutions and negative at 1:1,000 dilutions—and 19 patients had no detectable NY-ESO-1 serum antibody (Table 2). Only those patients with high-titer ($\geq 1:1,000$) serum antibody exhibit detectable urine antibody. In low-titer serum ($\leq 1:250$), even when NY-ESO-1 antibodies are detectable, antibodies were not detected in urine. Thus, these experiments demonstrate that NY-ESO-1 antibodies are present in the urine of patients who show strong titers of serum antibody (*i.e.*, $\geq 1:1,000$).

EXAMPLE 5: Reactivity of serum and urine antibody with recombinant NY-ESO-1

Western blots were conducted using full-length recombinant NY-ESO-1 protein, recombinant SSX protein—used as a negative control—a lysate of NY-ESO-1-positive-tumor cells (NW-MEL-38) cultured in Dulbecco's modified Eagle's medium (10 mM HEPES buffer, L-arginine (84 mg/l), L-glutamine (584 mg/l), penicillin (10 IU/ml), streptomycin (100 ug/ml), and 10% FCS), and a lysate of COS-7 cells which had been transfected with cDNA encoding full-length NY-ESO-1. Serum and urine samples from NY-ESO-1-positive individuals contain antibodies that bind naturally-occurring NY-ESO-1 and COS-7 lysates containing recombinant NY-ESO-1. The serum and urine antibodies from these individuals did not bind SSX protein or cells that do not express NY-ESO-1.

Recombinant NY-ESO-1 was prepared in COS-7 cells and lysed to yield COS-7 lysates. The full-length coding sequence of NY-ESO-1 was cloned into BamHI-HindIII sites of the pcDNA3.1(-) vector, and this construct was used for the transfection of COS-7 cells. (Jager *et al.* (1998) *J. Exp. Med.* **187**: 265-70). Lysates of NY-ESO-1-transformed COS-7 cells were applied to membranes and subsequently contacted with a urine sample from the lung cancer patient positive for antibodies to NY-ESO-1. Patient urine was tested in undiluted form, as well as after being diluted 1:100, and 1:1000. Negative controls were conducted using urine from an NY-ESO-1-negative individual, urine from an NY-ESO-1-positive individual diluted 1:2500, and without urine. Both undiluted and diluted (1:100) urine detect a 22 kDa protein that corresponds to the known molecular weight of the recombinant NY-ESO-1 protein. Detection of this 22 kDa protein indicates the presence of NY-ESO-1-specific antibodies in patient urine and demonstrates that recombinant NY-ESO-1 is a suitable agent for the detection of either serum or urine NY-ESO-1-specific antibodies.

Western blots of recombinant-NY-ESO-1 protein and lysates of NY-ESO-1-positive NW-MEL-38 cells—using serum and urine from the NY-ESO-1-positive, non-small-cell lung-cancer (NSCLC) patient NW900 and the NY-ESO-1-positive, melanoma patient NW29—demonstrate that both serum and urine antibodies bind recombinant and naturally-occurring NY-ESO-1 in Western blot assays.

EXAMPLE 6: Identification of tumor-specific antigens using SEREX and UREX methods

A testicular cDNA expression library was screened using the SEREX method and serum isolated from breast cancer patient NW1189. (Table 2). The antigens identified in this screening are listed in Table 3. Three tumor-associated antigens—NY-BR-1 (GenBank Accession Nos. XM165546 and XM166711 (both version 1 and submitted May 13, 2002); Cross *et al.* (2000) *Mamm. Genome* **11(5)**: 373-383; all incorporated herein by reference), SCP-1 (GenBank Accession No. D67035 (February 2, 1999; version 1); Kondoh *et al.* (1997) *Cytogenet. Cell. Genet.* **78(2)**: 103-104; all incorporated herein by reference), and NW-BR-3 (GenBank Accession No. AJ236609 (May 2, 2000; version 1) and the UniGene designation Hs.339651; both incorporated herein by reference)—were identified. These three tumor-associated clones expressing the antigens NY-BR-1 (GenBank Accession Nos. XM165546 and XM166711 (both version 1 and submitted May 13, 2002); Cross *et al.* (2000) *Mamm. Genome* **11(5)**: 373-383; all incorporated herein by reference), SCP-1 (GenBank Accession No. D67035 (February 2, 1999; version 1); Kondoh *et al.* (1997) *Cytogenet. Cell. Genet.* **78(2)**: 103-104; all incorporated herein by reference), and NW-BR-3 (GenBank Accession No. AJ236609 (May 2, 2000; version 1) and the UniGene designation Hs.339651; both incorporated herein by reference) were screened using urine isolated from breast cancer patient NW1189. The urine used in these screenings was undiluted and not preabsorbed. The three tumor-associated clones were positive and their identity was confirmed by sequencing.

EXAMPLE 7: Comparison of Western blot and UREX analysis

The detection of NY-ESO-1-specific antibodies using NY-ESO-1 expressing clones, following the standard SEREX methodology but with the substitution of urine instead of serum, was compared to the detection of NY-ESO-1-specific antibodies using isolated NY-ESO-1. Undiluted urine antibody from patients NW29, NW1716, and NW1622 was used in each assay. Both assays produced to identical results.

Although the present invention has been described in detail with reference to the examples above, it is understood that various modifications can be made without departing from the spirit of the invention. All cited patents, patent applications and publications referred to in this application are herein incorporated by reference in their entirety.

We Claim:

1. A method for determining that a patient suffers from a disorder characterized by aberrant expression of a naturally occurring gene, comprising:
 - i) contacting a urine containing sample from a patient that is believed to suffer from said disorder with an agent that specifically binds to an antibody present in said urine and characteristic of said condition, under conditions favoring complex formation between the agent and the antibody; and
 - ii) detecting the complex between the agent and the antibody present in said urine that specifically binds to the tumor-associated antigen,wherein presence of the antibody, as indicated by formation and detection of the complex indicates that the patient suffers from said disorder.
2. The method of claim 1, wherein the agent is a tumor associated antigen.
3. The method of claim 2, wherein the tumor-associated antigen is selected from the group consisting of: a cancer-testis (CT) antigen; a differentiation antigen; an expressed product of a mutated normal gene; an overexpressed self antigen; and a viral antigen.
4. The method of claim 3 wherein the tumor-associated antigen is NY-ESO-1, NY-BR-1, SCP-1 or clone 18CGI1F2.
5. The method of claim 4, wherein the tumor-associated antigen is NY-ESO-1.
6. The method of claim 1, wherein the complex between the tumor-associated antigen and the antibody is detected by a Western Blot.
7. The method of claim 1, wherein said agent is a cell that presents a binding partner for said antibody on its surface.
8. The method of claim 1, wherein the complex between the agent and the antibody is detected by the specific binding of an antibody to the antibody present in the urine of a patient.

9. The method of claim 8, wherein the agent or the antibody that specifically binds the antibody present in the urine of a patient is immobilized on a solid matrix.
10. The method of claim 9, wherein the solid matrix is selected from the group consisting of: plastic, glass, paper, nitrocellulose, nylon, poly(vinyl-chloride), cellulose, polyacrylamide, polystyrene, and polypropylene.
11. The method of claim 9, wherein the solid matrix is a magnetic bead.
12. The method of claim 8, wherein the complex between the tumor-associated antigen and the antibody is detected by an enzyme-linked immunosorbent assay.
13. The method of claim 12, wherein the antibody that specifically binds the antibody present in the urine of a patient contains a detectable label.
14. The method of claim 13, wherein the detectable label is: a fluorescent molecule, a radioactive molecule, a protein, a magnetic bead, or a metal.
15. A method for monitoring the status of a disorder in a patient characterized by aberrant expression of a naturally occurring gene, comprising:
 - i) contacting a sample containing urine from a patient that is believed to suffer from said disorder with an agent that specifically binds to an antibody present in said urine and characteristic of said condition, under conditions favoring complex formation between the agent and the antibody; and
 - ii) detecting the complex between the agent and the antibody present in said urine that specifically binds to the tumor-associated antigen; and
 - iii) comparing a value obtained to a prior value obtained following assay of a prior sample taken from said patient,wherein any difference is indicative of a change in status of said disorder.
16. A kit comprising:
 - i) an agent that binds an antibody present in urine of a patient suffering from a disorder characterized by aberrant expression of a naturally occurring gene; and

- ii) a second component selected from the group consisting of:
 - a) a control antibody;
 - b) a control antigen;
 - c) a positive control sample containing urine;
 - d) a negative control sample containing urine;
 - e) an antibody that specifically binds the antibody present in the urine of said patient; and
 - f) instructions for use of the agent for the detection of an antibody that is present in urine and specifically binds to the agent.
- 17. The kit of claim 16, wherein the agent is a tumor-associated antigen.
- 18. The kit of claim 17, wherein the tumor-associated antigen is NY-ESO-1, SCP-1, NY-BR-1 or clone 18CGI1F2.
- 19. The kit of claim 18, wherein the tumor-associated antigen is NY-ESO-1.
- 20. The kit of claim 16, further comprising an antibody that specifically binds the antibody present in the urine of a patient suffering from a disorder.
- 21. The kit of claim 20, wherein the antibody that specifically binds the antibody present in the urine of a patient suffering from a disorder contains a detectable label.
- 22. The kit of claim 21, wherein the label is: a fluorescent molecule, a radioactive molecule, a protein, a magnetic bead, or a metal.
- 23. The kit of claim 20, wherein the agent or the antibody that specifically binds the antibody present in the urine of a patient suffering from a disorder is immobilized on a solid matrix.
- 24. The kit of claim 23, wherein the solid matrix is selected from the group consisting of: plastic, glass, paper, nitrocellulose, nylon, poly(vinyl-chloride), cellulose, polyacrylamide, polystyrene, and polypropylene.

25. The kit of claim 23, wherein the solid matrix is a magnetic bead.
26. A method for determining an immunoreactive substance produced by a subject, wherein said subject is capable of eliciting an immune response, comprising:
- i) producing a cDNA library from a cell taken from said subject;
 - ii) inserting said cDNA library into an expression vector;
 - iii) transfecting said vector into a host cell to produce a transfected host cell;
 - iv) culturing said transfected host cell to express said immunoreactive substance;
 - v) contacting a urine sample which contains an antibody specific to said immunoreactive substance with a sample of non-transfected host cells, to remove an immunologic binding partner from said sample which is specific for said non-transfected host cell, to produce a stripped urine sample;
 - vi) contacting said stripped urine sample with a sample of host cells transfected with the same vector into which said cDNA library has been inserted, wherein said vector does not contain any library cDNA, to remove any immunologic binding partners specific for vector produced antigens thereby producing a twice stripped urine sample;
 - vii) contacting said twice stripped urine sample to the transfected host cell of (iv), thereby any immunologic binding partners specific for said immunoreactive substance bind thereto; and
 - viii) determining binding in (vii) to determine said immunoreactive substance.
27. The method of claim 26, wherein the cultured transfected cell of step (iv) is lysed to produce cell lysate and said cell lysate is contacted with urine as in step (v).
28. The method of claim 26, wherein said immunoreactive substance is a tumor-associated antigen.

29. The method of claim 26, wherein said immunoreactive substance is an autoimmune-disease-associated antigen.