(11) Application No. AU 2004249254 B2

(19) AUSTRALIAN PATENT OFFICE

(54) Title

Combinations of tumor-associated antigens for the treatment of various types of cancers

(51)[6] International Patent Classification(s)

A61K 39/00 (2006.01) 8BMEP **A61K**

A61K 48/00 (2006.01) 48/00

A61K 39/00 20060101ALN200707

20060101AFI2005100 21BMEP

PCT/US2004/019571

(21) Application No: 2004249254 (22) Application Date: 2004.06.17

(87) WIPO No: WO04/112825

(30) Priority Data

(31) Number	(32) Date	(33) Country
60/479,554	2003.06.17	US

(43) Publication Date : 2004.12.29

(71) Applicant(s)

MannKind Corporation

(72) Inventor(s)

Simard, John J. L.; Chiang, Chih-Sheng; Diamond, David C.; Bot, Adrian

(74) Agent/Attorney

Davies Collison Cave, 1 Nicholson Street, Melbourne, VIC, 3000

(56) Related Art

Moingeon, P., Vaccine, Vol.19, pages 1305-1326, 2001

Chang, S.S., Cancer Research, Vol.59, pages 3192-3198, July 1999

WO 2003/075952 A1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 December 2004 (29.12.2004)

PCT

(10) International Publication Number
WO 2004/112825 A3

(51) International Patent Classification[7]: A61K 38/17, 39/00, 31/7088, 48/00

(21) International Application Number: PCT/US2004/019571

(22) International Filing Date: 17 June 2004 (17.06.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/479,551 17 June 2003 (17.06.2003) US

(71) Applicant *(for all designated States except US)*: **MANNKIND CORPORATION** [US/US]; 28903 North Avenue Paine, Valencia, CA 91355 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CHIANG, Chih-Sheng** [US/US]; 10339 Oakdale Avenue, Chatsworth, CA 91311 (US). **BOT, Adrian** [RO/US]; 27003 Carmelita Dr., Valencia, CA 91355 (US). **SIMARD, John, J., L.** [CA/CA]; Suite #7, 1684 Alberni Street, Vancouver, British Colombia Z6G1A6 (CA). **DIAMOND, David, C.** [US/US]; 23135 Schoenborn Street, West Hills, CA 91304 (US).

(74) Agent: **MORLEY, Marc, T.**; Knobbe Martens Olson & Bear LLP, 2040 Main Street, 14th Floor, Irvine, CA 92614 (US).

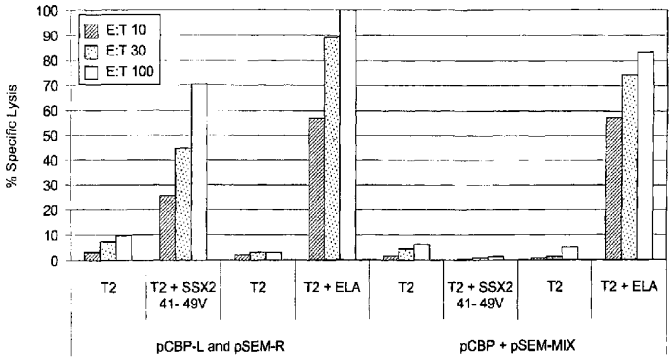
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
of inventorship (Rule 4.17(iv)) for US only

[Continued on next page]

(54) Title: COMBINATIONS OF TUMOR-ASSOCIATED ANTIGENS FOR THE TREATMENT OF VARIOUS TYPES OF CANCERS



(57) Abstract: Disclosed herein are methods and compositions for inducing an immune response against various combinations of tumor-associated antigens, which can promote effective immunologic intervention in pathogenic processes. Embodiments of the invention disclosed herein are directed to the use of effective combinations of TuAAs for the immunotherapy of patients with various types of cancer. Both immunogenic compositions for inducing an immune response to these combinations of antigens and methods for their use are disclosed.

WO 2004/112825 A3

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
6 May 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

COMBINATIONS OF TUMOR-ASSOCIATED ANTIGENS IN COMPOSITIONS FOR VARIOUS TYPES OF CANCERS

Background of the Invention

Field of the Invention

[0001] Disclosed herein are methods and compositions for inducing an immune response against various combinations of tumor-associated antigens, which can promote effective immunologic intervention in pathogenic processes.

Description of the Related Art

[0002] The American Cancer Society has estimated that over one million people get cancer each year, and that approximately one out of every two American men and one out of every three American women will have some type of cancer at some point during their lifetime.

[0003] Cancer generally develops when cells in a part of the body begin to grow out of control. Although there are many kinds of cancer, they usually start because of out-of-control growth of abnormal cells.

[0004] Normal body cells grow, divide, and die in an orderly fashion. Cancer cells are different in that they continue to grow and divide. Instead of dying, they outlive normal cells and continue to form new abnormal cells.

[0005] Usual treatment options for cancer include surgery, radiation therapy, and chemotherapy. A fourth branch of treatment is developing, which is referred to as immunotherapy. Immunotherapies attempt to help the immune system recognize cancer cells, and/or to strengthen a response against cancer cells in order to destroy the cancer. Immunotherapies include active and passive immunotherapies. Active immuotherapies attempt to stimulate the body's own immune system to fight the disease. Passive immunotherapies generally do not rely on the body to attack the disease; instead, they use immune system components (such as antibodies) created outside of the body.

[0006] Despite the various types of treatments, a continuing need exists for additional treatment options.

Summary of the Invention

[0007] Embodiments of the invention disclosed herein are directed to the use of effective combinations of TuAAs for the immunotherapy of patients with various types of

cancer. Both immunogenic compositions for inducing an immune response to these combinations of antigens and methods for their use are disclosed.

[0008] Some embodiments relate to methods of treating neoplastic diseases. The methods can include the step of immunizing a patient against, for example, PRAME and at least one other tumor associated antigen. To immunize a patient against an antigen such as, for example, PRAME, means to administer to the patient some portion of the antigen, or some other immunogenic agent, that is capable of inducing a specific immune response directed to the antigen. Accordingly, in some embodiments, immunizing against PRAME can include administering a complete and intact PRAME antigen to the patient, while in other embodiments it can include administering one or more epitopes, one or more epitope clusters, one or more fragments, and the like, of PRAME, and/or administering, for example, a nucleic acid encoding any of the foregoing epitope(s), cluster(s), fragment(s), and the like. Although PRAME is used in an exemplary way for convenience in this discussion, the principle is applicable to any antigen against which a patient may be immunized.

[0009] One embodiment relates to methods of treating ovarian or colorectal cancer. The methods can include the step of immunizing a patient against, for example, PRAME and at least one other tumor associated antigen. In some embodiments the tumor associated antigen can include, for example, SSX-2, NY-ESO-1, PSMA, and the like. Preferably, the methods can include immunizing against PRAME, NY-ESO-1 and SSX-2, for example. More preferably, the methods can include immunization against PRAME, NY-ESO-1, SSX-2, and PSMA, for example. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2, and Tie-2. In a preferred embodiment, the methods can induce a cytolytic T cell response.

[0010] In one embodiment, a method of inducing a cytolytic T-cell response in the treatment of ovarian or colorectal cancer is disclosed. The method can include, for example, the step of immunizing a pateint against PRAME and at least one of the tumor-associated antigens. In some embodiments, the tumor-associated antigen can be, for example, SSX-2, NY-ESO-1, PSMA, and the like. For example, the method can include immunization against PRAME, NY-ESO-1, and SSX-2. The method can further include immunization against PSMA. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2, and Tie-2.

[0011] Other embodiments relate to methods of treating pancreatic cancer. The methods can include the step of immunizing against, for example, PSMA and at least one other tumor-associated antigen. The tumor-associated antigen can be, for example, SSX-2 and NY-ESO-1. Preferably, the methods can include immunizing against PSMA, NY-ESO-1, and SSX-2. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2. In a preferred embodiment, the methods can induce a cytolytic T cell response.

[0012] Further, in one embodiment, a method of inducing a cytolytic T-cell response in the treatment of pancreatic cancer is disclosed. The method can include, for example, the step of immunizing a patient against PSMA and at least one of the tumor-associated antigens. In some embodiments, the tumor-associated antigen can be, for example, SSX-2, NY-ESO-1, and the like. For example, the method can include immunization against PSMA, NY-ESO-1, and SSX-2. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2.

[0013] Still other embodiments relate to methods of treating non-small cell lung cancer. The methods can include the step of immunizing a patient against, for example, PSMA and at least one other tumor associated antigen. For example, the tumor-associated antigen can be MAGE-3, SSX-2, NY-ESO-1, and the like. Preferably, the methods can include immunizing against PSMA, NY-ESO-1 and SSX-2. More preferably, the methods can include immunization against PSMA, NY-ESO-1, SSX-2, and MAGE-3, for example. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2. In a preferred embodiment, the methods can induce a cytolytic T cell response.

[0014] In a further embodiment, a method of inducing a cytolytic T-cell response in the treatment of non-small cell lung cancer is disclosed. The method can include, for example, the step of immunizing a patient against PSMA and at least one of tumor-associated antigen. The tumor-associated antigen can be, for example, MAGE-3, SSX-2, NY-ESO-1, and the like. For example, the method can include immunization against PSMA, NY-ESO-1, and SSX-2. The method can further include immunization against PSMA, NY-ESO-1, SSX-2, and MAGE-3. The method can further include the step of immunizing against at least one antigen

associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2.

[0015] Alternative embodiments relate to methods of treating renal cell carcinoma. The methods can include the step of immunizing a patient against, for example, PSMA and at least one other tumor associated antigen. The tumor-associated antigen can be, for example, PRAME, SSX-2, and the like. Preferably, the methods can include immunizing against PSMA, PRAME, and SSX-2, for example. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2. In a preferred embodiment, the methods can induce a cytolytic T cell response.

[0016] Further, in one embodiment, a method of inducing a cytolytic T-cell response in the treatment of renal cell carcinoma is disclosed. The method can include, for example, the step of immunizing a patient against PSMA and at least one other tumor-associated antigen. In some embodiments, the tumor-associated antigen can be PRAME, SSX-2, and the like. For example, the method can include immunization against PSMA, PRAME, and SSX-2. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2.

[0017] Some embodiments relate to methods of treating melanoma. The methods can include the step of immunizing a patient against, for example, at least one tumor-associated antigen selected from each of two groups. The first group can include, for example, tyrosinase, Melan-A, and the like. The second group can include, for example, SSX-2, NY-ESO-1, and the like. Preferably, the method can include immunizing against Melan-A, SSX-2, and NY-ESO. More preferably, the method can include immunization against Melan-A, SSX-2, and tyrosinase, for example. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2, and Tie-2. In a preferred embodiment, the methods can induce a cytolytic T cell response.

[0018] In one embodiment, a method of inducing a cytolytic T-cell response in the treatment of melanoma is disclosed. The method can include, for example, the step of immunizing a patient against at least one tumor-associated antigen selected from each of two groups. The first group can include, for example, tyrosinase, Melan-A, and the like. The second group can include, for example, SSX-2, NY-ESO-1, and the like. For example, the method can include immunization against Melan-A, SSX-2, and NY-ESO. Alternatively, the

method can include, for example, immunization against Melan-A, SSX-2, and tyrosinase. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2, and Tie-2.

[0019] Some embodiments relate to the use of a composition comprising, for example, PRAME and at least one other tumor associated antigen, in the preparation of a medicament for the treatment of treating ovarian or colorectal cancer. In some embodiments the tumor associated antigen can include, for example, SSX-2, NY-ESO-1, PSMA, and the like. Preferably, the tumor-associated antigens can be NY-ESO-1 and SSX-2, for example. More preferably, the tumor-associated antigens can be NY-ESO-1, SSX-2, and PSMA, for example. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2, and Tie-2. In a preferred embodiment, the medicament can induce a cytolytic T cell response.

[0020] Other embodiments relate to the use of a composition comprising, for example, PSMA and at least one other tumor-associated antigen in the preparation of medicament for the treatment of pancreatic cancer. The tumor-associated antigen can be, for example, SSX-2 and NY-ESO-1. Preferably, the medicament comprises PSMA, NY-ESO-1, and SSX-2. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2. In a preferred embodiment, the medicament can induce a cytolytic T cell response.

[0021] Still other embodiments relate to the use of a composition comprising PSMA and at least one other tumor associated antigen in the preparation of a medicament for the treatment of non-small cell lung cancer. In some embodiments, the tumor-associated antigen can be MAGE-3, SSX-2, NY-ESO-1, and the like. Preferably, the composition comprises PSMA, NY-ESO-1 and SSX-2. More preferably, the composition comprises PSMA, NY-ESO-1, SSX-2, and MAGE-3, for example. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2. In a preferred embodiment, the medicament can induce a cytolytic T cell response.

[0022] Alternative embodiments relate to the use of a composition comprising PSMA and at least one other tumor associated antigen in the preparation of a medicament for the treatment of renal cell carcinoma. The tumor-associated antigen can be, for example,

PRAME, SSX-2, and the like. Preferably, the composition comprises PSMA, PRAME, and SSX-2, for example. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2. In a preferred embodiment, the medicament can induce a cytolytic T cell response.

[0023] Still other embodiments relate to the use of a composition comprising at least one tumor-associated antigen selected from each of two groups in the preparation of a medicament for the treatment of melanoma. The first group can include, for example, tyrosinase, Melan-A, and the like. The second group can include, for example, SSX-2, NY-ESO-1, and the like. Preferably, the composition comprises Melan-A, SSX-2, and NY-ESO. More preferably, the composition comprises Melan-A, SSX-2, and tyrosinase, for example. The method can further include the step of immunizing against at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2, and Tie-2. In a preferred embodiment, the medicament can induce a cytolytic T cell response.

[0024] Other embodiments relate to immunogenic compositions for the treatment of or for inducing a T cell response against ovarian or colorectal cancer, for example. The compositions can include, for example, individually or in combination, 1) whole antigens, 2) fragments of antigens, 3) epitope clusters derived from antigens, 4) epitopes derived from antigens, 5) nucleic acids encoding any of 1 to 4, and the like; wherein the antigens can include PRAME and a tumor-associated antigen, including, for example, SSX-2, NY-ESO-1, PSMA, and the like. The compositions can further include at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2 and Tie-2.

[0025] Still other embodiments relate to immunogenic compositions for the treatment of or for inducing a T cell response against pancreatic cancer. The compositions can include, for example, individually or in combination, 1) whole antigens, 2) fragments of antigens, 3) epitope clusters derived from antigens, 4) epitopes derived from antigens, or 5) nucleic acids encoding any of 1 to 4; wherein the antigens can include PSMA and at least one tumor-associated antigen. In a preferred embodiment, the tumor-associated antigen can be seleced from SSX-2 and NY-ESO-1, for example. The compositions can further include at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2.

[0026] Alternative embodiments relate to immunogenic compositions for the treatment of or for inducing a T cell response against non-small cell lung cancer. The compositions can include, for example, individually or in combination, 1) whole antigens, 2) fragments of antigens, 3) epitope clusters derived from antigens, 4) epitopes derived from antigens, or 5) nucleic acids encoding any of 1 to 4; wherein the antigens can include PSMA and at least one tumor-associated antigen, including, for example, MAGE-3, SSX-2 and NY-ESO-1. The compositions can further include at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2.

[0027] Some embodiments relate to immunogenic compositions for the treatment of or for inducing a T cell response against renal cell carcinoma. The compositions can include, for example, individually or in combination, 1) whole antigens, 2) fragments of antigens, 3) epitope clusters derived from antigens, 4) epitopes derived from antigens, or 5) nucleic acids encoding any of 1 to 4; wherein the antigens can include PSMA and at least one tumor-associated antigen. In a preferred embodiment the tumor associated antigen can be PRAME or SSX-2. The compositions can further include at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, VEGFR2 and Tie-2.

[0028] Still other embodiments relate to an immunogenic composition for the treatment of or for inducing a T cell response against melanoma. The composition can include, for example, individually or in combination, 1) whole antigens, 2) fragments of antigens, 3) epitope clusters derived from antigens, 4) epitopes derived from antigens, or 5) nucleic acids encoding any of 1 to 4; wherein the antigens can be selected from each of two groups; wherein the first group includes, for example, tyrosinase and melan-A; and wherein the second group includes SSX-2 and NY-ESO-1, and the like. The compositions can further include at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2 and Tie-2.

[0029] Further embodiments relate to compositions and methods for inducing a CTL response using combinations of tumor-associated antigens, including fragments of tumor-associated antigens, clusters and epitopes. The compositons can include nucleic acid constructs, for example, a single construct that encodes all of the desired angitens. In other embodiments a single construct encodes a single antigen, while in other embodiments one construct can have a combination of epitopes with similar immunogenicity and another construct can have epitopes with similar immunogenicity.

7

[0030] Still other embodiments relate to methods of designing and preparing immunogenic compositions, which methods can include the steps of determining the presence of one or more antigens on a tumor type, and obtaining the one or more antigens for inclusion in a composition that induces CTL.

Brief Description of the Drawings

[0031] Figure 1 shows the schedule of immunization with two plasmid (pCBP expressing SSX2 41-49 and pSEM expressing Melan A).

[0032] Figure 2 is a bar graph that shows CTL activity obtained using the protocol in Figure 1.

[0033] Figure 3 shows the schedule of immunization of an entrain-and-amplify immunization protocol using plasmids and peptides representing two epitopes.

[0034] Figure 4 is a table showing *in vivo* clearance of epitope-pulsed cells in mice immunized according to the protocol of Figure 3.

[0035] Figure 5 shows the preferred immunization protocols for inducing multivalent responses.

Detailed Description of the Preferred Embodiment

[0036] The frequency of expression of many tumor-associated antigens (TuAAs) in various types of cancers is known. However, the frequency of appearance of some antigens, and especially certain combinations of TuAAs in various types of cancers has not been reported. Accurate measurement of the presence of TuAAs in tumor tissues aids in determining which TuAAs will be useful for the treatment of a particular type of cancer.

[0037] Many attempts to develop active immunotherapies for cancer have utilized a single antigen. This can be problematic for two distinct reasons. Firstly, the expression of any particular TuAA in cancer can be mosaic with the antigen expression ranging from high in some cells within a tumor mass to completely absent in others. Moreover, the TuAA may be expressed in some lesions but not others. By directing an immune response against more than a single antigen, if properly selected, the number of tumor cells that can be recognized can be maximized. Secondly, it has been observed in some cases that tumors lose expression of a TuAA following immunization, giving rise to a resistant population. If the immune response is directed against more than one TuAA it becomes much more difficult for a resistant tumor to arise because it must then simultaneously lose expression of each of the antigens in order to escape. Thus, in treating cancer with immunotherapy, it can be advantageous to use a combination of TuAAs both due to more complete coverage of the population of tumor cells,

and because there will be less chance of tumor escape through antigen loss of expression of the TuAAs, provided the tumor is positive for at least two of the TuAAs used.

Definitions:

[0038] Unless otherwise clear from the context of the use of a term herein, the following listed terms shall generally have the indicated meanings for purposes of this description.

[0039] PROFESSIONAL ANTIGEN-PRESENTING CELL (pAPC) – a cell that possesses T cell costimulatory molecules and is able to induce a T cell response. Well characterized pAPCs include dendritic cells, B cells, and macrophages.

[0040] PERIPHERAL CELL – a cell that is not a pAPC.

[0041] HOUSEKEEPING PROTEASOME – a proteasome normally active in peripheral cells, and generally not present or not strongly active in pAPCs.

[0042] IMMUNOPROTEASOME – a proteasome normally active in pAPCs; the immunoproteasome is also active in some peripheral cells in infected tissues or following exposure to interferon.

[0043] EPITOPE – a molecule or substance capable of stimulating an immune response. In preferred embodiments, epitopes according to this definition include but are not necessarily limited to a polypeptide and a nucleic acid encoding a polypeptide, wherein the polypeptide is capable of stimulating an immune response. In other preferred embodiments, epitopes according to this definition include but are not necessarily limited to peptides presented on the surface of cells, the peptides being non-covalently bound to the binding cleft of class I MHC, such that they can interact with T cell receptors (TCR). Epitopes presented by class I MHC may be in immature or mature form. "Mature" refers to an MHC epitope in distinction to any precursor ("immature") that may include or consist essentially of a housekeeping epitope, but also includes other sequences in a primary translation product that are removed by processing, including without limitation, alone or in any combination, proteasomal digestion, N-terminal trimming, or the action of exogenous enzymatic activities. Thus, a mature epitope may be provided embedded in a somewhat longer polypeptide, the immunological potential of which is due, at least in part, to the embedded epitope; likewise, the mature epitope can be provided in its ultimate form that can bind in the MHC binding cleft to be recognized by TCR.

[0044] MHC EPITOPE – a polypeptide having a known or predicted binding affinity for a mammalian class I or class II major histocompatibility complex (MHC) molecule.

[0045] HOUSEKEEPING EPITOPE – In a preferred embodiment, a housekeeping epitope is defined as a polypeptide fragment that is an MHC epitope, and that is displayed on a cell in which housekeeping proteasomes are predominantly active. In another preferred embodiment, a housekeeping epitope is defined as a polypeptide containing a housekeeping epitope according to the foregoing definition, that is flanked by one to several additional amino acids. In another preferred embodiment, a housekeeping epitope is defined as a nucleic acid that encodes a housekeeping epitope according to the foregoing definitions. Exemplary housekeeping epitopes are provided in U.S. Application Nos. 10/117,937, filed on April 4, 2002 (Pub. No. 20030220239 A1), and 10/657,022, and in PCT Application No. PCT/US2003/027706 (Pub. No. WO04022709A2), filed 9/5/2003; and U.S. Provisional Application Nos. 60/282,211, filed on April 6, 2001; 60/337,017, filed on November 7, 2001; 60/363210 filed 3/7/02; and 60/409,123, filed on September 5, 2002. Each of the listed applications is entitled EPITOPE SEQUENCES.

[0046] IMMUNE EPITOPE – In a preferred embodiment, an immune epitope is defined as a polypeptide fragment that is an MHC epitope, and that is displayed on a cell in which immunoproteasomes are predominantly active. In another preferred embodiment, an immune epitope is defined as a polypeptide containing an immune epitope according to the foregoing definition, that is flanked by one to several additional amino acids. In another preferred embodiment, an immune epitope is defined as a polypeptide including an epitope cluster sequence, having at least two polypeptide sequences having a known or predicted affinity for a class I MHC. In yet another preferred embodiment, an immune epitope is defined as a nucleic acid that encodes an immune epitope according to any of the foregoing definitions.

[0047] TARGET CELL – In a preferred embodiment, a target cells is a cell associated with a pathogenic condition that can be acted upon by the components of the immune system, for example, a cell infected with a virus or other intracellular parasite, or a neoplastic cell. In another embodiment, a target cell is a cell to be targeted by the vaccines and methods of the invention. Examples of target cells according to this definition include but are not necessarily limited to: a neoplastic cell and a cell harboring an intracellular parasite, such as, for example, a virus, a bacterium, or a protozoan. Target cells can also include cells that are targeted by CTL as a part of an assay to determine or confirm proper epitope liberation and processing by a cell expressing immunoproteasome, to determine T cell specificity or immunogenicity for a desired epitope. Such cells can be transformed to express the liberation sequence, or the cells can simply be pulsed with peptide/epitope.

10

[0048] TARGET-ASSOCIATED ANTIGEN (TAA) – a protein or polypeptide present in a target cell.

[0049] TUMOR-ASSOCIATED ANTIGENS (TuAA) – a TAA, wherein the target cell is a neoplastic cell.

[0050] HLA EPITOPE – a polypeptide having a known or predicted binding affinity for a human class I or class II HLA complex molecule.

[0051] ANTIBODY – a natural immunoglobulin (Ig), poly- or monoclonal, or any molecule composed in whole or in part of an Ig binding domain, whether derived biochemically, or by use of recombinant DNA, or by any other means. Examples include *inter alia,* F(ab), single chain Fv, and Ig variable region-phage coat protein fusions.

[0052] SUBSTANTIAL SIMILARITY – this term is used to refer to sequences that differ from a reference sequence in an inconsequential way as judged by examination of the sequence. Nucleic acid sequences encoding the same amino acid sequence are substantially similar despite differences in degenerate positions or minor differences in length or composition of any non-coding regions. Amino acid sequences differing only by conservative substitution or minor length variations are substantially similar. Additionally, amino acid sequences comprising housekeeping epitopes that differ in the number of N-terminal flanking residues, or immune epitopes and epitope clusters that differ in the number of flanking residues at either terminus, are substantially similar. Nucleic acids that encode substantially similar amino acid sequences are themselves also substantially similar.

[0053] FUNCTIONAL SIMILARITY – this term is used to refer to sequences that differ from a reference sequence in an inconsequential way as judged by examination of a biological or biochemical property, although the sequences may not be substantially similar. For example, two nucleic acids can be useful as hybridization probes for the same sequence but encode differing amino acid sequences. Two peptides that induce cross-reactive CTL responses are functionally similar even if they differ by non-conservative amino acid substitutions (and thus may not be within the substantial similarity definition). Pairs of antibodies, or TCRs, that recognize the same epitope can be functionally similar to each other despite whatever structural differences exist. Testing for functional similarity of immunogenicity can be conducted by immunizing with the "altered" antigen and testing the ability of an elicited response, including but not limited to an antibody response, a CTL response, cytokine production, and the like, to recognize the target antigen. Accordingly, two sequences may be designed to differ in certain respects while retaining the same function. Such designed

sequence variants of disclosed or claimed sequences are among the embodiments of the present invention.

[0054] EXPRESSION CASSETTE – a polynucleotide sequence encoding a polypeptide, operably linked to a promoter and other transcription and translation control elements, including but not limited to enhancers, termination codons, internal ribosome entry sites, and polyadenylation sites. The cassette can also include sequences that facilitate moving it from one host molecule to another.

[0055] EMBEDDED EPITOPE – in some embodiments, an embedded epitope is an epitope that is wholly contained within a longer polypeptide; in other embodiments, the term also can include an epitope in which only the N-terminus or the C-terminus is embedded such that the epitope is not wholly in an interior position with respect to the longer polypeptide.

[0056] MATURE EPITOPE – a peptide with no additional sequence beyond that present when the epitope is bound in the MHC peptide-binding cleft.

[0057] EPITOPE CLUSTER – a polypeptide, or a nucleic acid sequence encoding it, that is a segment of a protein sequence, including a native protein sequence, comprising two or more known or predicted epitopes with binding affinity for a shared MHC restriction element. In preferred embodiments, the density of epitopes within the cluster is greater than the density of all known or predicted epitopes with binding affinity for the shared MHC restriction element within the complete protein sequence. Epitope clusters are disclosed and more fully defined in U.S. Patent Application No. 09/561,571 entitled EPITOPE CLUSTERS.

[0058] LIBERATION SEQUENCE – a designed or engineered sequence comprising or encoding a housekeeping epitope embedded in a larger sequence that provides a context allowing the housekeeping epitope to be liberated by processing activities including, for example, immunoproteasome activity, N terminal trimming, and/or other processes or activities, alone or in any combination.

[0059] CTLp – CTL precursors are T cells that can be induced to exhibit cytolytic activity. Secondary *in vitro* lytic activity, by which CTLp are generally observed, can arise from any combination of naïve, effector, and memory CTL *in vivo*.

[0060] MEMORY T CELL – A T cell, regardless of its location in the body, that has been previously activated by antigen, but is in a quiescent physiologic state requiring re-exposure to antigen in order to gain effector function. Phenotypically they are generally $CD62L^{-}$ $CD44^{hi}$ $CD107\alpha^{-}$ IGN-γ^{-} $LT\beta^{-}$ TNF-α^{-} and is in G0 of the cell cycle.

[0061] EFFECTOR T CELL – A T cell that, upon encountering antigen antigen, readily exhibits effector function. Effector T cells are generally capable of exiting the

lymphatic system and entering the immunological periphery. Phenotypically they are generally $CD62L^{-}$ $CD44^{hi}$ $CD107\alpha^{+}$ $IGN\text{-}\gamma^{+}$ $LT\beta^{+}$ $TNF\text{-}\alpha^{+}$ and actively cycling.

[0062] EFFECTOR FUNCTION – Generally, T cell activation generally, including acquisition of cytolytic activity and/or cytokine secretion.

[0063] INDUCING a T cell response – Includes in many embodiments the process of generating a T cell response from naïve, or in some contexts, quiescent cells; activating T cells.

[0064] AMPLIFYING a T cell response – Includes in many embodimentsthe process or increasing the number of cells, the number of activated cells, the level of activity, rate of proliferation, or similar parameter of T cells involved in a specific response.

[0065] ENTRAINMENT – Includes in many embodiments an induction that confers particular stability on the immune profile of the induced lineage of T cells.

[0066] TOLL-LIKE RECEPTOR (TLR) – Toll-like receptors (TLRs) are a family of pattern recognition receptors that are activated by specific components of microbes and certain host molecules. As part of the innate immune system, they contribute to the first line of defense against many pathogens, but also play a role in adaptive immunity.

[0067] TOLL-LIKE RECEPTOR (TLR) LIGAND – Any molecule capable of binding and activating a toll-like recepetor. Examples include, without limitation: poly IC A synthetic, double-stranded RNA know for inducing interferon. The polymer is made of one strand each of polyinosinic acid and polycytidylic acid, double-stranded RNA, unmethylated CpG oligodeoxyribonucleotide or other immunostimulatory sequences (ISSs), lipopolysacharide (LPS) , β-glucans, and imidazoquinolines, as well as derivatives and analogues thereof.

[0068] IMMUNOPOTENTIATING ADJUVANTS – Adjuvants that activate pAPC or T cells including, for example: TLR ligands, endocytic-Pattern Recognition Receptor (PRR) ligands, quillaja saponins, tucaresol, cytokines, and the like. Some preferred adjuvants are disclosed in Marciani, D.J. *Drug Discovery Today* 8:934-943, 2003.

[0069] IMMUNOSTIMULATORY SEQUENCE (ISS) – Generally an oligodeoxyribonucleotide containing an unmethlylated CpG sequence. The CpG may also be embedded in bacterially produced DNA, particularly plasmids. Further embodiments include various analogues; among preferred embodiments are molecules with one or more phosphorothioate bonds or non-physiologic bases.

[0070] VACCINE – In preferred embodiments a vaccine can be an immunogenic composition providing or aiding in prevention of disease. In other embodiments, a vaccine is a

composition that can provide or aid in a cure of a disease. In others, a vaccine composition can provide or aid in amelioration of a disease. Further embodiments of a vaccine immunogenic composition can be used as therapeutic and/or prophylactic agents.

[0071] IMMUNIZATION – a process to induce partial or complete protection against a disease. Alternatively, a process to induce or amplify an immune system response to an antigen. In the second definition it can connote a protective immune response, particularly proinflammatory or active immunity, but can also include a regulatory response. Thus in some embodiments immunization is distinguished from tolerization (a process by which the immune system avoids producing proinflammatory or active immunity) while in other embodiments this term includes tolerization.

[0072] ENCODE – an open-ended term such that a nucleic acid encoding a particular amino acid sequence can consist of codons specifying that (poly)peptide, but can also comprise additional sequences either translatable, or for the control of transcription, translation, or replication, or to facilitate manipulation of some host nucleic acid construct.

[0073] COVERAGE – the fraction or proportion of tumor cells expressing a particular TuAA or at least one TuAA from a set of selected TuAAs.

[0074] REDUNDANCY – the degree to which a population of tumor cells, or some subset of them, express more than one of a selected set of TuAAs.

Tumor Associated Antigens

[0075] Examples of TuAAs useful in embodiments disclosed herein include tyrosinase (SEQ. ID NO. 1), melan-A, (SEQ. ID NO. 2), SSX-2, (SEQ. ID NO.3), PSMA (prostate-specific membrane antigen) (SEQ. ID NO. 4), MAGE-1, (SEQ. ID NO. 5), MAGE-3 (SEQ. ID NO. 6), NY-ESO-1, (SEQ. ID NO. 7), PRAME, (SEQ. ID NO.8), and Her2/Neu (SEQ. ID NO. 9). The natural coding sequences for these nine proteins, or any segments within them, can be determined from their cDNA or complete coding (cds) sequences, SEQ. ID NOS. 10-18, respectively.

Table 1. SEQ. ID NOS.

SEQ. ID NO.	IDENTITY	ACCESSION NUMBER**
1	Tyrosinase protein	P14679
2	Melan-A protein	Q16655
3	SSX-2 protein	NP_003138
4	PSMA protein	NP_004467
5	MAGE-1 protein	P43355
6	MAGE-3 protein	P43357
7	NY-ESO-1 protein	P78358

8	PRAME protein	NP 006106
9	Her2/Neu protein	P04626
10	Tyrosinase cDNA	NM_000372
11	Melan-A cDNA	U06452
12	SSX-2 cDNA	NM_003147
13	PSMA cDNA	NM_004476
14	MAGE-1 cds	M77481
15	MAGE-3 cds	U03735
16	NY-ESO-1 cDNA	U87459
17	PRAME cDNA	NM_006115
18	Her2/Neu cDNA	M11730

**All accession numbers used here and throughout can be accessed through the NCBI databases, for example, through the Entrez seek and retrieval system on the world wide web.

[0076] Tyrosinase is a melanin biosynthetic enzyme that is considered one of the most specific markers of melanocytic differentiation. Tyrosinase is expressed in few cell types, primarily in melanocytes, and high levels are often found in melanomas. The usefulness of tyrosinase as a TuAA is taught in U.S. Patent 5,747,271 entitled "METHOD FOR IDENTIFYING INDIVIDUALS SUFFERING FROM A CELLULAR ABNORMALITY SOME OF WHOSE ABNORMAL CELLS PRESENT COMPLEXES OF HLA-A2/TYROSINASE DERIVED PEPTIDES, AND METHODS FOR TREATING SAID INDIVIDUALS".

[0077] GP100, also known as PMel17, also is a melanin biosynthetic protein expressed at high levels in melanomas. GP100 as a TuAA is disclosed in U.S. Patent 5,844,075 entitled "MELANOMA ANTIGENS AND THEIR USE IN DIAGNOSTIC AND THERAPEUTIC METHODS,".

[0078] Melan-A, also called MART-1 (Melanoma Antigen Recognized by T cells), is another melanin biosynthetic protein expressed at high levels in melanomas. The usefulness of Melan-A/MART-1 as a TuAA is taught in U.S. Patent Nos. 5,874,560 and 5,994,523 both entitiled "MELANOMA ANTIGENS AND THEIR USE IN DIAGNOSTIC AND THERAPEUTIC METHODS," as well as U.S. Patent No. 5,620,886, entitled "ISOLATED NUCLEIC ACID SEQUENCE CODING FOR A TUMOR REJECTION ANTIGEN PRECURSOR PROCESSED TO AT LEAST ONE TUMOR REJECTION ANTIGEN PRESENTED BY HLA-A2".

[0079] SSX-2, also know as Hom-Mel-40, is a member of a family of highly conserved cancer-testis antigens (Gure, A.O. et al. *Int. J. Cancer* 72:965-971, 1997). Its identification as a TuAA is taught in U.S. Patent 6,025,191 entitled "ISOLATED NUCLEIC

ACID MOLECULES WHICH ENCODE A MELANOMA SPECIFIC ANTIGEN AND USES THEREOF,". Cancer-testis antigens are found in a variety of tumors, but are generally absent from normal adult tissues except testis. Expression of different members of the SSX family has been found in various tumor cell lines. Due to the high degree of sequence identity among SSX family members, similar epitopes from more than one member of the family will be generated and able to bind to an MIIC molecule, so that some vaccines directed against one member of this family can cross-react and be effective against other members of this family.

[0080] MAGE-1 (melanoma-associated antigen-1), MAGE-2 (melanoma-associated antigen-2), and MAGE-3 (melanoma-associated antigen-3) are members of another family of cancer-testis antigens originally discovered in melanoma but found in a variety of tumors. The identification of MAGE proteins as TuAAs is taught in U.S. Patent 5,342,774 entitled NUCLEOTIDE SEQUENCE ENCODING THE TUMOR REJECTION ANTIGEN PRECURSOR, MAGE-1, and in numerous subsequent patents. Currently there are 17 entries for (human) MAGE in the SWISS Protein database. There is extensive similarity among these proteins, such that in many cases, an epitope from one can induce a cross-reactive response to other members of the family. A few members of the MAGE family have not been observed in tumors, most notably MAGE-H1 and MAGE-D1, which are expressed in testes and brain, and bone marrow stromal cells, respectively. The possibility of cross-reactivity on normal tissue is ameliorated by the fact that they are among the least similar to the other MAGE proteins.

[0081] GAGE-1 is a member of the GAGE family of cancer testis antigens (Van den Eynde, B., et al., *J. Exp. Med.* 182: 689-698, 1995; U.S Patent Nos. 5,610,013; 5648226; 5,858,689; 6,013,481; and 6,069,001). The PubGene database currently lists 12 distinct accessible members, some of which are synonymously known as PAGE or XAGE. GAGE–1 through GAGE–8 have a very high degree of sequence identity, so most epitopes can be shared among multiple members of the family.

[0082] BAGE is a cancer-testis antigen commonly expressed in melanoma, particularly metastatic melanoma, as well as in carcinomas of the lung, breast, bladder, and squamous cells of the head and neck. Its usefulness as a TuAA is taught in U.S. Patent Nos. 5,683,88 entiltled "TUMOR REJECTION ANTIGENS WHICH CORRESPOND TO AMINO ACID SEQUENCES IN TUMOR REJECTION ANTIGEN PRECURSOR BAGE, AND USES THEREOF" and 5,571,711 entitled "ISOLATED NUCLEIC ACID MOLECULES CODING FOR BAGE TUMOR REJECTION ANTIGEN PRECURSORS," each.

[0083] NY-ESO-1, also known as CTAG-1 (Cancer-Testis Antigen-1) and CAG-3 (Cancer Antigen-3), is a cancer-testis antigen found in a wide variety of tumors. NY-ESO-1 as

a TuAA is disclosed in U.S. Patent 5,804,381 entitled ISOLATED NUCLEIC ACID MOLECULE ENCODING AN ESOPHAGEAL CANCER ASSOCIATED ANTIGEN, THE ANTIGEN ITSELF, AND USES THEREOF. A paralogous locus encoding antigens with extensive sequence identity, LAGE-1a/s and LAGE-1b/L, has been disclosed in publicly available assemblies of the human genome, and has been concluded to arise through alternate splicing. Additionally, CT-2 (or CTAG-2, Cancer-Testis Antigen-2) appears to be either an allele, a mutant, or a sequencing discrepancy of LAGE-1b/L. Due to the extensive sequence identity, many epitopes from NY-ESO-1 can also induce immunity to tumors expressing these other antigens. See Figure 1. NY-ESO-1 and LAGE are virtually identical through amino acid 70. From amino acid 71 through 134 the longest run of identity between the two proteins is 6 residues, but potentially cross-reactive sequences are present. From amino acid 135 through 180, NY-ESO and LAGE-1a/s are identical except for a single residue, but LAGE-1b/L is unrelated due to the alternate splice. The CAMEL and LAGE-2 antigens appear to derive from the LAGE-1 mRNA, but from alternate reading frames, thus giving rise to unrelated protein sequences. More recently, GenBank Accession AF277315.5, Homo sapiens chromosome X clone RP5-865E18, RP5-1087L19, complete sequence, reports three independent loci in this region which are labeled as LAGE1 (corresponding to CTAG-2 in the genome assemblies), LAGE2-A and LAGE2-B (both corresponding to CTAG-1 in the genome assemblies).

[0084] PRAME, also know as MAPE, DAGE, and OIP4, was originally observed as a melanoma antigen. Subsequently, it has been recognized as a cancer-testis (CT) antigen, but unlike many CT antigens, such as, MAGE, GAGE and BAGE, PRAME is expressed in acute myeloid leukemias. PRAME is a member of the MAPE family, which consists largely of hypothetical proteins with which it shares limited sequence similarity. The usefulness of PRAME as a TuAA is taught in U.S. Patent 5,830,753 entitled "ISOLATED NUCLEIC ACID MOLECULES CODING FOR TUMOR REJECTION ANTIGEN PRECURSOR DAGE AND USES THEREOF,".

[0085] PSMA (prostate-specific membranes antigen), a TuAA described in U.S. Patent 5,538,866 entitled "PROSTATE-SPECIFIC MEMBRANES ANTIGEN", is expressed by normal prostate epithelium and, at a higher level, in prostatic cancer. It has also been found in the neovasculature of non-prostatic tumors. PSMA can thus form the basis for vaccines directed to both prostate cancer and to the neovasculature of other tumors. This later concept is more fully described in a provisional U.S. Patent Application No. 60/274,063 entitled "ANTI-NEOVASCULAR VACCINES FOR CANCER," filed March 7, 2001, and U.S. Application No. 10/094,699 (Pub. No. 20030046714 A1), filed on March 7, 2002, entitled

"ANTI-NEOVASCULAR PREPARATIONS FOR CANCER,". Briefly, as tumors grow they recruit ingrowth of new blood vessels. This is understood to be necessary to sustain growth as the centers of unvascularized tumors are generally necrotic and angiogenesis inhibitors have been reported to cause tumor regression. Such new blood vessels, or neovasculature, express antigens not found in established vessels, and thus can be specifically targeted. By inducing CTL against neovascular antigens the vessels can be disrupted, interrupting the flow of nutrients to, and removal of wastes from, tumors, leading to regression.

[0086] Alternate splicing of the PSMA mRNA leads to a protein with an apparent start at Met_{58}, thereby deleting the putative membrane anchor region of PSMA as described in U.S. Patent 5,935,818 entitled "ISOLATED NUCLEIC ACID MOLECULE ENCODING ALTERNATIVELY SPLICED PROSTATE-SPECIFIC MEMBRANES ANTIGEN AND USES THEREOF,". A protein termed PSMA-like protein, Genbank accession number AF261715, is nearly identical to amino acids 309-750 of PSMA, but has a different expression profile. Thus the most preferred epitopes are those with an N-terminus located from amino acid 58 to 308.

[0087] PSA, prostate specific antigen, is a peptidase of the kallikrein family and a differentiation antigen of the prostate. Expression in breast tissue has also been reported. Alternate names include gamma-seminoprotein, kallikrein 3, seminogelase, seminin, and P-30 antigen. PSA has a high degree of sequence identity with the various alternate splicing products prostatic/glandular kallikrein–1 and –2, as well as kalikrein 4, which is also expressed in prostate and breast tissue. Other kallikreins generally share less sequence identity and have different expression profiles. Nonetheless, cross-reactivity that might be provoked by any particular epitope, along with the likelihood that that epitope would be liberated by processing in non-target tissues (most generally by the housekeeping proteasome), should be considered in designing a vaccine.

[0088] PSCA, prostate stem cell antigen, and also known as SCAH-2, is a differentiation antigen preferentially expressed in prostate epithelial cells, and overexpresssed in prostate cancers. Lower level expression is seen in some normal tissues including neuroendocrine cells of the digestive tract and collecting ducts of the kidney. PSCA is described in U.S. Patent 5,856,136 entitled "HUMAN STEM CELL ANTIGENS,".

[0089] Synaptonemal complex protein 1 (SCP-1), also known as HOM-TES-14, is a meiosis-associated protein and also a cancer-testis antigen (Tureci, O., et al. Proc. Natl. Acad. Sci. USA 95:5211-5216, 1998). As a cancer antigen its expression is not cell-cycle regulated and it is found frequently in gliomas, breast, renal cell, and ovarian carcinomas. It

has some similarity to myosins, but with few enough identities that cross-reactive epitopes are not an immediate prospect.

[0090] The ED-B domain of fibronectin is also a potential target. Fibronectin is subject to developmentally regulated alternative splicing, with the ED-B domain being encoded by a single exon that is used primarily in oncofetal tissues (Matsuura, H. and S. Hakomori *Proc. Natl. Acad. Sci. USA* 82:6517-6521, 1985; Carnemolla, B. et al. *J. Cell Biol.* 108:1139-1148, 1989; Loridon-Rosa, B. et al. *Cancer Res.*50:1608-1612, 1990; Nicolo, G. et al. *Cell Differ. Dev.* 32:401-408, 1990; Borsi, L. et al. *Exp. Cell Res.* 199:98-105, 1992; Oyama, F. et al. *Cancer Res.* 53:2005-2011, 1993; Mandel, U. et al. *APMIS* 102:695-702, 1994; Farnoud, M.R. et al. *Int. J. Cancer* 61:27-34, 1995; Pujuguet, P. et al. Am. J. Pathol. 148:579-592, 1996; Gabler, U. et al. *Heart* 75:358-362, 1996;Chevalier, X. *Br. J. Rheumatol.* 35:407-415, 1996; Midulla, M. *Cancer Res.* 60:164-169, 2000).

[0091] The ED-B domain is also expressed in fibronectin of the neovasculature (Kaczmarek, J. et al. *Int. J. Cancer* 59:11-16, 1994; Castellani, P. et al. *Int. J. Cancer* 59:612-618, 1994; Neri, D. et al. *Nat. Biotech.* 15:1271-1275, 1997; Karelina, T.V. and A.Z. Eisen *Cancer Detect. Prev.* 22:438-444, 1998; Tarli, L. et al. *Blood* 94:192-198, 1999; Castellani, P. et al. *Acta Neurochir. (Wien)* 142:277-282, 2000). As an oncofetal domain, the ED-B domain is commonly found in the fibronectin expressed by neoplastic cells in addition to being expressed by the neovasculature. Thus, CTL-inducing vaccines targeting the ED-B domain can exhibit two mechanisms of action: direct lysis of tumor cells, and disruption of the tumor's blood supply through destruction of the tumor-associated neovasculature. As CTL activity can decay rapidly after withdrawal of vaccine, interference with normal angiogenesis can be minimal. The design and testing of vaccines targeted to neovasculature is described in Provisional U.S. Patent Application No. 60/274,063 entitled "ANTI-NEOVASCULATURE VACCINES FOR CANCER," filed on March 7, 2001, and in U.S. Patent Application No. 10/094,699, (Pub. No. 20030046714 A1), entitled "ANTI-NEOVASCULATURE PREPARATIONS FOR CANCER," filed on March 7, 2002. A tumor cell line is disclosed in Provisional U.S. Application No. 60/363,131, filed on March 7, 2002, entitled "HLA-TRANSGENIC MURINE TUMOR CELL LINE,".

[0092] Carcinoembryonic antigen (CEA) is a paradigmatic oncofetal protein first described in 1965 (Gold and Freedman, J. Exp. Med. 121: 439-462, 1965. Fuller references can be found in the Online Medelian Inheritance in Man; record *114890. It has officially been renamed carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5). Its expression is most strongly associated with adenocarcinomas of the epithelial lining of the

digestive tract and in fetal colon. CEA is a member of the immunoglobulin supergene family and the defining member of the CEA subfamily.

[0093] Survivin, also known as Baculoviral IAP Repeat-Containing Protein 5 (BIRC5), is another protein with an oncofetal pattern of expression. It is a member of the inhibitor of apoptosis protein (IAP) gene family. It is widely over-expressed in cancers (Ambrosini, G. et al., *Nat. Med.* 3:917-921, 1997; Velculiscu V.E. et al., *Nat. Genet.* 23:387-388, 1999) and its function as an inhibitor of apoptosis is believed to contribute to the malignant phenotype.

[0094] HER2/NEU is an oncogene related to the epidermal growth factor receptor (van de Vijver, et al., *New Eng. J. Med.* 319:1239-1245, 1988), and apparently identical to the c-ERBB2 oncogene (Di Fiore, et al., Science 237: 178-182, 1987). The over-expression of ERBB2 has been implicated in the neoplastic transformation of prostate cancer. As with HER2, it is amplified and over-expressed in 25-30% of breast cancers among other tumors where expression level is correlated with the aggressiveness of the tumor (Slamon, et al., *New Eng. J. Med.* 344:783-792, 2001). A more detailed description is available in the Online Medelian Inheritance in Man; record *164870.

[0095] Further examples of tumor-associated antigens include MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15(58), CEA, RAGE, NY-ESO (LAGE), SCP-1, Hom/Mel-40, PRAME, p53, H-Ras, HER-2/neu, BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, β-Catenin, CDK4, Mum-1, p16, TAGE, PSMA, PSCA, CT7, telomerase, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, ß-HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, and the like.

[0096] Additional tumor-associated antigens are described in Chen, YT, "Identification of human tumor antigens by scrological expression cloning: an online review on SEREX" *Cancer Immun.* 2004 [updated 2004 Mar 10; cited 2004 Apr 1] *at* world wide web cancerimmunotherapy.org/SEREX/; and Renkvist, N. et al., "A listing of tumor antigens recognized by T cells," *Cancer Immunology Immunotherapy*, 50:3-15 (2001).

[0097] Table 2, adapted from Scanlan et al., "The cancer/testis genes: Review, standardization, and commentary," *Cancer Immunity* 4:1 (January 23, 2004), provides a listing of CT Antigens. Table 3 provides the frequency of mRNA expression in various tumor types for the CT antigens in Table 2. Scanlan et al., "The cancer/testis genes: Review, standardization, and commentary," *Cancer Immunity* 4:1 (January 23, 2004).

Table 2

Listing of CT genes

CT Identifier	Transcript/Transcript family	Family Members/CT Identifier (Synonyms)
CT1	MAGEA	MAGEA1/CT1.1, MAGEA2/CT1.2, MAGEA3/CT1.3, MAGEA4/CT1.4, MAGEA5/CT1.5, MAGEA6/CT1.6, MAGEA7/CT1.7, MAGEA8/CT1.8, MAGEA9/CT.9, MAGEA10/CT1.10, MAGEA11/CT1.11, MAGEA12/CT1.12
CT2	BAGE	BAGE/CT2.1, BAGE2/CT2.2, BAGE3/CT2.3, BAGE4/CT2.4, BAGE5/CT2.5
CT3	MAGEB	MAGEB1/CT3.1, MAGEB2/CT3.2, MAGEB5/CT3.3, MAGEB6/CT3.4
CT4	GAGE1	GAGE1/CT4.1, GAGE2/CT4.2, GAGE3/CT4.3, GAGE4/CT4.4, GAGE5/CT4.5, GAGE6/CT4.6, GAGE7/CT4.7, GAGE8/CT4.8
CT5	SSX	SSX1/CT5.1, SSX2/CT5.2a, SSX2/CT5.2b, SSX3/CT5.3, SSX4/CT5.4
CT6	NY-ESO-1	NY-ESO-1/CT6.1, LAGE-1a/CT6.2a, LAGE-1b/CT6.2b
CT7	MAGEC1	MAGEC1/CT7.1, MAGEC3/CT7.2
CT8	SYCP1	SYCP1/CT8

CT9	BRDT	BRDT/CT9
CT10	MAGEE1	MAGEE1/CT10
CT11	CTp11/SPANX	SPANXA1/CT11.1, SPANXB1/CT11.2, SPANXC/CT11.3, SPANXD/CT11.4
CT12	XAGE-1/GAGED	XAGE-1a/CT12.1a, XAGE-1b/CT12.1b, XAGE-1c/CT12.1c, XAGE-1d/CT12.1d, XAGE-2/CT12.2, XAGE-3a/CT12.3a, XAGE-3b/CT12.3b, XAGE-4/CT12.4
CT13	HAGE	HAGE/CT13
CT14	SAGE	SAGE/CT14
CT15	ADAM2	ADAM2/CT15
CT16	PAGE-5	PAGE-5/CT16.1, CT16.2
CT17	LIP1	LIP1/CT17
CT18	NA88	NA88/CT12
CT19	IL13RA1	IL13RA1/CT19
CT20	TSP50	TSP50/CT20
CT21	CTAGE-1	CTAGE-1/CT21.1, CTAGE-2/CT21.2
CT22	SPA17	SPA17/CT22
CT23	OY-TES-1	OY-TES-1/CT23
CT24	CSAGE	CSAGE/CT24.1, TRAG3/CT24.2
CT25	MMA1/DSCR8	MMA-1a/CT25.1a, MMA-1b/CT25.1b

CT26	CAGE	CAGE/CT26
CT27	BORIS	BORIS/CT27
CT28	HOM-TES-85	HOM-TES-85/CT28
CT29	AF15q14/ D40	D40/CT29
CT30	E2F-like/HCA661	HCA661/CT30
CT31	PLU-1	PLU-1/CT31
CT32	LDHC	LDHC/CT32
CT33	MORC	MORC/CT33
CT34	SGY-1	SGY-1/CT34
CT35	SPO11	SPO11/CT35
CT36	TPX1	TPX-1/CT36
CT37	NY-SAR-35	NY-SAR-35/CT37
CT38	FTHL17	FTHL17/CT38
CT39	NXF2	NXF2/CT39
CT40	TAF7L	TAF7L/CT40
CT41	TDRD1	TDRD1/CT41.1, NY-CO-45/CT41.2
CT42	TEX15	TEX15/CT42
CT43	FATE	FATE/CT43
CT44	TPTE	TPTE/CT44

---	PRAME	(MAPE, DAGE)

Table 3.

CT Family (Member)	Frequency (%) of Expression in Tumor Type																		Ref
	Blad	Brn	Brst	Col	Eso	Gas	H/N	Liver	Leuk/ Lymph	Lung (NSCLC)	Mel	Ov	Panc	Pros	Renal	Sarc	
MAGEA1/CT1.1	22	-	18	2	53	29	28	80	0	49	48	28	-	15	0	14	44
BAGE1/CT2.1	15	-	10	0	-	-	8	-	0	4	26	15	-	0	0	6	44
MAGEB1/CT3.1	0	0	17	0	-	0	0	-	0	14	22	-	-	0	0	9	45
GAGE/CT4.1	12	-	9	0	-	-	19	38[b]	1	19	28	31	-	10	0	25	44
SSX2/CT5.2	44	6	7	12	-	-	35	9[b]	36	16	35	-	-	40	5	50	46
NY-ESO-1/CT6.1	80	0	30	0	-	0	-	29	0	17	34	25	0	25	9	0	8
MAGEC1/CT7.1	44	-	30	10	-	-	36	-	-	33	70	-	-	-	-	60	20
SYCP1/CT8	-	47	20	0	-	7	-	28[b]	0	7	14	0	-	0	8	0	9
BRDT/CT9	0	-	0	0	8	-	8	-	-	25	0	-	-	-	0	-	16
MAGEE1/CT10	44	-	38	0	-	-	36	-	-	24	50	-	-	-	-	0	12
SPANXC/CT11.3	9	-	25	22	0	-	-	-	-	33	70	-	0	-	-	-	14

XAGE-1a/CT12.1a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22	42
HAGE/CT13	24	37	5	31	27	-	20	-	-	9	32	17	-	20	-	-	-	-	-	6	20	6	43
SAGE/CT14	12	0	5	0	20	-	17	-	-	4	22	4	-	-	-	-	-	-	0	5	5	5	43
ADAM2/CT15	-	-	0	0	-	-	-	-	-	-	0	0	0	-	-	-	-	-	-	12	-	-	47
PAGE-5/CT16	-	-	5	11	-	-	-	-	-	-	39	22	0	-	-	-	-	-	-	44	-	-	47
LIP1/CT17	-	-	5	0	-	-	-	-	-	-	0	0	0	-	-	-	-	-	-	25	-	-	47
NA88/CT18	-	-	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-	-	-	-	-	-	48
TSP50/CT20	-	-	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	49
CTAGE-1/CT21.1	-	-	-	-	-	-	-	-	-	35	-	-	-	-	-	-	-	-	-	-	-	-	50
SPA17/CT22	-	-	-	-	-	-	-	-	-	26	-	-	-	-	-	-	-	-	-	-	-	-	51
OY-TES1/CT23	28	-	40	15	-	-	-	-	-	-	-	20	-	40	-	-	-	-	-	0	-	-	52
MMA1a/CT25.1a	-	-	0	0	0	-	-	-	-	-	-	40	26	-	-	-	-	-	0	-	18	-	45
CAGE/CT26	-	-	-	-	-	-	89	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	53
HOM-TES-85/CT28	-	35	0	10	-	-	-	-	-	19	-	28	36	32	-	-	-	-	0	-	-	-	54

D40/CT29	-	20	-	13	-	0	-	-	41	-	20	36	27	-	-	-	-	55
HCA661/CT30	0	-	-	-	-	0	0	29	-	-	20	-	-	-	-	-	-	56
PLU-1/CT31	-	-	86	-	-	-	-	-	-	-	-	-	-	37	57	0	-	27
LDHC/CT32	-	-	35	15	-	-	-	-	47	-	44	42	-	37	57	0	-	18
MORC/CT33	-	-	0	0	-	-	-	-	18	-	18	14	-	0	0	0	-	18
SGY-1/CT34	-	-	20	0	-	-	-	-	12	-	25	57	-	12	0	0	-	18
SPO11/CT35	-	-	0	0	-	-	-	-	0	-	6	0	-	0	0	0	-	18
TPX1/CT36	-	-	15	0	-	-	-	-	-	-	6	14	-	37	14	14	-	18
NYSAR35/CT37	42	-	23	0	8	-	-	-	17	-	6	8	-	-	0	8	8	57
FTHL17/CT38	22	-	14	0	0	-	10	-	25	0	0	-	-	0	0	0	0	58
NXF2/CT39	19	-	0	11	12	-	5	-	15	0	55	-	-	14	0	27	27	58
TAF7L/CT40	10	-	0	0	0	-	10	-	9	0	21	-	-	0	0	12	12	58
TDRD1/CT41.1	28	-	37	0	10	-	22	-	5	5	0	-	-	38	0	0	0	58
TEX15/CT42	21	-	0	0	20	-	11	-	21	0	27	-	-	12	33	28	28	58

FATE/CT43	-	-	-	21	-	7	-	66	-	0	-	-	-	-	-	19
TPTE/CT44	-	-	-	0	-	0	-	39	-	36	-	-	-	-	-	19

[a]Abbreviations: Blad, bladder; Brn, brain; Brst, breast; Col, colon; Gas, gastric; H/N, head and neck; Leuk, leukemia; Lymph, lymphoma, NSCLC, non-small cell lung carcinoma; Mel, melanoma; Ov, ovarian; Pancr, pancreatic; Pros, prostate; Sarc, sarcoma; Ref, reference. [b]Reference 59.

[0098] Additional antigens associated with tumor neovasculature are VEGFR2 (vascular endothelial growth factor receptor 2) described in U.S. Patent No. 6,342,221; and Tie-2, an endothelium specific receptor tyrosine kinase which is described in WO9943801.

[0099] One of skill in the art will appreciate that any other antigen or protein associated with vascular cells can be a target for the immunogenic compositions, including those that are presently known and those yet to be identified.

Compositions

[0100] Immunogenic compositions, including, for example, vaccines, can be prepared using whole antigen or an epitopic peptide. Peptide immunogens can be readily prepared using standard peptide synthesis means known in the art, for example. Immunogens can be prepared commercially by one of numerous companies that do chemical synthesis. An example such a company is American Peptides, Inc., where the distributor is CLINALFA AG (Laufelfingen, Switzerland). The antigens or immunogens can be prepared in accordance with GMP standards and purity can be assessed by analytical HPLC. The product can be characterized by amino-acid analysis and tested for sterility and the absence of pyrogens.

[0101] An antigen may be delivered to an animal's system either directly or indirectly. For example, a polypeptide may be delivered directly as the polypeptide, or it may be delivered indirectly, for example, using a DNA construct or vector, or a recombinant virus that codes for the desired antigen. Any vector driving expression in a professional antigen presenting cell can be suitable for this purpose. In indirect delivery, the antigen is expressed in the cell, then presented by the MHC Class I on the surface of the cell to stimulate a CTL response. Expression of a secreted form of the antigen can be useful to induce an antibody response recognizing antigens that are membrane proteins.

[0102] In a preferred embodiment, an encoded antigen can be delivered in the form of a naked plasmid expression vector. Particularly useful constructs are disclosed in U.S. Patent Application No. 09/561,572, entitled "EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS;" U.S. Patent Application No. 10/292,413 (Pub. No. 20030228634 A1), entitled "EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS AND METHODS FOR THEIR DESIGN;" U.S. Patent Application No. 10/225,568 (Pub No. 2003-0138808); PCT Application No. PCT/US2003/026231 (Pub. No. WO 2004/018666); U.S. Patent No. 6,709,844, entitled AVOIDANCE OF UNDESIRABLE REPLICATION INTERMEDIATES IN PLASMIND PROPAGATION, and in U.S. Patent Application No. 10/026,066 (Pub. No. 20030215425 A1), entitled EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING

CELLS. Additional methodology, compositions, peptides, and peptide analogues are disclosed in U.S. Provisional Application No. __/___,___ (Attorney Docket MANNK.038PR), filed on the same date as the instant application, entitled "SSX-2 PEPTIDE ANALOGS;". Further methodology, compositions, peptides, and peptide analogues are disclosed in U.S. Provisional Application No. __/___,___ (Attorney Docket MANNK.039PR), filed on the same date as the instant application, entitled "NY-ESO PEPTIDE ANALOGS;". The feasibility of and general procedures related to the use of naked DNA for immunization are described in U.S. Patent No. 5,589,466, entitled "INDUCTION OF A PROTECTIVE IMMUNE RESPONSE IN A MAMMAL BY INJECTING A DNA SEQUENCE" and in U.S. Patent No. 5,679,647, entitled "METHODS AND DEVICES FOR IMMUNIZING A HOST AGAINST TUMOR-ASSOCIATED ANTIGENS THROUGH ADMINISTRATIONS OF NAKED POLYNUCLEOTIDES WHICH ENCODE TUMOR-ASSOCIATED ANTIGENIC PEPTIDES,". The former teaches only intramuscular or intradermal injection while the latter teaches only administration to skin or mucosa.

[0103] In a preferred embodiment, the antigen can be administered directly to the lymphatic system. Intranodal administration for the generation of CTL is taught in U.S. Patent Application Nos. 09/380,534 and 09/776,232 (Pub. No.20020007173 A1), and in PCT Application No. PCTUS98/14289 (Pub. No. WO9902183A2) each entitled "A METHOD OF INDUCING A CTL RESPONSE,". Single bolus injection intra lymph node (i.ln.) required only 0.1% of the dose required in order to obtain a similar level of CTL response by intramuscular (i.m.) injection. Therefore a protective response can be established against systemic viral infection with a single bolus delivered i.ln., but not with a dose nearing the practical limit delivered i.m. Repeated bolus injections i.m. failed to establish a protective response against a peripheral virus infection or transplanted tumor, whereas lower doses administered i.ln. were completely effective. Particularly useful intranodal immunization protocols are taught in Provisional U.S. Patent Application No. 60/479,393, entitled "METHODS TO CONTROL MAGNITUDE AND QUALITY THE MHC CLASS I-RESTRICTED IMMUNE RESPONSE," and in U.S. Patent Application No. __/_____ entitled "METHODS TO ELICIT, ENHANCE AND SUSTAIN IMMUNE RESPONSES AGAINST MHC CLASS I-RESTRICTED EPITOPES, FOR PROPHYLACTIC OR THERAPEUTIC PURPOSE" (Attorney Docket No. MANNK.034A) (Pub. No. _____), filed on date even with the instant Application.

[0104] A class of epitopes that can be advantageous in anti-cancer immunogenic compositions are housekeeping epitopes. These are produced through the action of the

housekeeping (or standard) proteasome. Housekeeping epitopes can be liberated from the translation product of expression vectors through proteolytic processing by the immunoproteasome of professional antigen presenting cells (pAPC). In one embodiment of the invention, sequences flanking the housekeeping epitope(s) can be altered to promote cleavage by the immunoproteasome at the desired location(s). Housekeeping epitopes, their uses, and identification are described in U.S. Patent Application Nos. 09/560,465 filed on April 28, 2000, and U.S. Patent Application No. 10/026,066 (Pub. No. 20030215425 A1), filed on December 7, 2001, entitled "EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS," and U.S. Patent Application No. 09/561,074, filed on April 28, 2000, entitled "METHOD OF EPITOPE DISCOVERY," .

[0105] Examples of housekeeping epitopes are disclosed in Provisional U.S. Patent Applications Nos. 60/282,211, filed on April 6, 2001; 60/337,017, filed on November 7, 2001; 60/363210 filed March 7, 2002; and 60/409,123, filed on September 5, 2002; U.S. Patent Application No. 10/117,937 (Publication No. 20030220239A1), filed on April 4, 2002; and U.S. Patent Application No. 10/657,022, and PCT Application No. PCT/US2003/027706 (Pub. No. WO04022709A2) both entitled EPITOPE SEQUENCES.

[0106] In other embodiments of the invention, the housekeeping epitope(s) can be flanked by arbitrary sequences or by sequences incorporating residues known to be favored in immunoproteasome cleavage sites. As used herein the term "arbitrary sequences" refers to sequences chosen without reference to the native sequence context of the epitope, their ability to promote processing, or immunological function. In further embodiments of the invention multiple epitopes can be arrayed head-to-tail. These arrays can be made up entirely of housekeeping epitopes. Likewise, the arrays can include alternating housekeeping and immune epitopes. Alternatively, the arrays can include housekeeping epitopes flanked by immune epitopes, whether complete or distally truncated. Further, the arrays can be of any other similar arrangement. There is no restriction on placing a housekeeping epitope at the terminal positions of the array. The vectors can additionally contain authentic protein coding sequences or segments thereof containing epitope clusters as a source of immune epitopes. The term "authentic" refers to natural protein sequences.

[0107] Epitope clusters and their uses are described in U.S. Patent Application Nos. 09/561,571, entitled "EPITOPE CLUSTERS," filed on April 28, 2000; 10/005,905, entitled "EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS," filed on November 7, 2001; and 10/026,066, filed on December 7, 2001, also entitled "EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS,".

[0108] In another embodiment of the invention an encoded antigen can be delivered in the form of a viral vector. A wide array of viruses with modified genomes adapted to express interposed reading frames but often no, or at least a reduced number of, viral proteins are known in the art, including without limitation, retroviruses including lentiviruses, adenoviruses, parvoviruses including adeno-associated virus, herpesviruses, and poxviruses including vaccinia virus. Such viral vectors facilitate delivery of the nucleic acid component into the cell allowing for expression. A subset of these vectors, such as retroviruses and parvoviruses, promote integration of their nucleic acid component into the host genome, whereas others do not.

[0109] Bacteria can also serve as vectors, that is they can be used to deliver a nucleic acid molecule capable of causing expression of an antigen. For example, a strain of *Listeria monocytogenes* has been devised that effects its own lysis upon entering the cytosol of macrophages (its normal target), thereby releasing plasmid from which antigen is subsequently expressed (Dietrich, G. et al., *Biotechnology* 16:181-185, 1998). *Shigela flexneri* and *Escherichia coli* have been similarly used (Sizemore, D.R. et al., *Science* 270:299-302, 1995, and Courvalin, P. et al., *Life Sci.* 318:1207-1212, 1995, respectively,).

[0110] The use of microbial vectors for nucleic acid delivery can be complicated by the immune reactions the vectors themselves provoke. When prolonged or repeated administration is required, antibody elicited by the earlier treatment can prevent useful quantities of the vector from ever reaching its intended host. However, by direct administration intoa lymph node, for example, the combination of proximity to host cells and the much reduced effective dose makes it possible to administer a dose capable of evading or overwhelming an existing antibody titer.

[0111] The word vector has been used, here and elsewhere, in reference to several modalities and variously modified (e.g., expression vector, viral vector, delivery vector, etc.). The underlying principle is that a nucleic acid capable of causing expression of an antigen, rather than the antigen itself, ultimately arrives in an APC. Unless modified, explicitly or by local context, the term vector as used herein is intended to encompass all such possibilities.

[0112] The techniques discussed above are distinct from the approach of modifying the microbial genome, including extra-chromosomal DNA, such that the antigen is produced as a component of the microbe, which is then itself administered as the immunogen. Examples of microbes used in the genomic modification approach include viruses, bacteria, fungi, and protazoa. In embodiments of the invention described herein, the compositions, including the vaccines, can include the already synthesized antigen or a nucleic acid capable of

causing an APC to express the antigen *in vivo*. In alternative embodiments, combinations of these two techniques are used. For example, one embodiment contemplates the use of a virus vector as discussed above that also incorporates a target epitope into a capsid or envelope protein.

[0113] Antigens may be used alone or may be delivered in combination with other antigens or with other compounds such as cytokines. Cytokines that are known to enhance immune stimulation of CTL responses, include, for example, GM-CSF, IL- 12, IL-2, TNF, IFN, IL-18, IL-3, IL-4, IL-8, IL-9, IL-13, IL-10, IL-14, IL-15, G-SCF, IFN alpha, IFN beta, IFN gamma, TGF alpha, TGF beta, and the like. Cytokines are known in the art and are readily available in the literature or commercially. Many animal and human tumors have been shown to produce cytokines, such as IL-4, IL-10, TGF-B, that are potent modulators of the immune response and that protect tumors from immune-mediated destruction. The production of IL-4, IL-10 or TGF-B by tumors may achieve this protective effect by suppressing the induction of cellular immunity, including the elaboration of CTL responses. Alternatively, cytokines that support CTL responses can be exogenously added to help in the balance between induction of anti-tumor cell mediated and non-tumor-destructive humoral responses. Several such exogenous cytokines show utility in experimental mouse vaccination models which are known to enhance CTL responses, including GM-CSF, IFN and IL-2. An example of an effective exogenous cytokine that may be used is GM-CSF. GM-CSF is reported to enhance the expression of the so called "co-stimulatory" molecules, such as B7-1 or B7-2 on antigen presenting cells (APC). These co-stimulatory molecules are important players in the variety of interactions that occur during stimulation of CTL by APC. Moreover, GM-CSF is known to induce activation of APCs and to facilitate growth and differentiation of APCs, thereby making these APCs important CTL stimulating cells available both in greater numbers and potency.

Delivery of the Antigen

[0114] While not wanting to be bound by any particular theory, it is thought that T cells do not have a functional memory that is long-lived. Antibody-mediated B-cell memory, on the other hand, appears to have a long-lived effector memory. Thus, delivering an antigen that induces a CTL response is most preferably done over time to keep the patient's immune system appropriately stimulated to attack the target cells. In one approach the presence of antigen is maintained virtually continuously within the lymphatic system to maintain effector CTL function as disclosed in U.S. Patent Application No. 09/776,232 (Pub. No.20020007173 A1), entitled "A METHOD OF INDUCING A CTL RESPONSE," which is hereby expressly

incorporated by reference. In another approach T cell memory is repeatedly induced, and re-amplified and reactivated as described in Provisional U.S. Patent Application No. 60/479,393, entitled "METHODS TO CONTROL MAGNITUDE AND QUALITY THE MHC CLASS I-RESTRICTED IMMUNE RESPONSE," and in U.S. Patent Application No. __/_____ entitled "METHODS TO ELICIT, ENHANCE AND SUSTAIN IMMUNE RESPONSES AGAINST MHC CLASS I-RESTRICTED EPITOPES, FOR PROPHYLACTIC OR THERAPEUTIC PURPOSE" (Attorney Docket No. MANNK.034A) (Pub. No. _____), filed on date even with the instant Application. While it has been suggested that antigens and adjuvants can be prepared as biodegradable microspheres or liposomes, none of these preparations have thus far provided a CTL response that is useful for attacking cancer cells or pathogens on a long term basis. Preferably, delivery of the antigen is sustained over the desired period of time at a level sufficient to maintain the antigen level to obtain the desired response. In one embodiment, a reservoir having fluid antigen composition can be used to deliver the antigen such that it reaches the animal's lymphatic system. While much of the following discussion focuses on the use of infusion to deliver the antigen it is also possible to use bolus injections directly into the lymphatic system, the number and frequency of which will depend on the persistence of antigen conferred by the particular form and formulation of antigen used.

[0115] Ultimately antigen finds its way into the lymphatic system in order to most efficiently stimulate CTL. Delivery of antigen can involve infusion into various compartments of the body, including but not limited to subcutaneous, intravenous, intraperitoneal and intralymphatic, the latter being preferred. While each of these points of infusion results in antigen uptake into the lymphatic system, the relative amounts of antigen needed to induce a beneficial CTL response varies according to the site of infusion. In general, direct infusion of antigen into the lymph system is deemed to be the most efficient means of inducing a CTL response, however, any delivery route may be used. Pump systems are capable of delivering material quantities of antigen in a range that is suitable for inducing a CTL response through delivery to all compartments of the body. CTL stimulation following delivery of antigen via the various routes will vary depending on the properties of different antigens, including factors that influence antigen behavior in the body and its rate of equilibration to (or longevity in) the lymph, such as antigen stability in the body fluid, solubility of antigen in body fluid, binding affinity for HLA and potency as a stimulator of CTL.

[0116] In a preferred embodiment, introduction of the antigen is done as directly as possible to the lymphatic system to avoid the destruction of the antigen by metabolism in the

body. When introduction of a fluid antigen composition occurs subcutaneously, larger quantities of antigen are needed to assure enough antigen reaches the lymphatic system. Such subcutaneous injection is contemplated by the invention disclosed herein, depending on factors such as cost, stability of the antigen, how quickly the antigen gets to the lymph system, how well it equilibrates with the lymph, and other factors that the attending doctor or specialist will recognize. Subcutaneous delivery generally can require 100 to 1000 times more antigen than direct delivery to the lymph system. It is preferable, therefore, that the antigen composition is introduced through a device for local administration to the lymphatic system, *e.g.*, the spleen, a lymph node, or a lymph vessel. The device for local administration can be positioned outside the patient or implanted into the patient. In either case, the device can have a reservoir to hold the fluid antigen-containing composition, a pump to transfer the composition, and a transmission channel leading from the reservoir to be directed to the preferred region of administration in the patient's body. In either case it is preferably portable.

[0117] For the device positioned outside the patient's body (the external device), there are numerous devices used for delivering insulin to diabetic patients that are useful in delivering antigen according to the embodiments described herein. Generally these devices can be comprised of a reservoir for holding the antigen composition (instead of insulin), a programmable pump to pump the composition out of the reservoir, a transmission channel or line for transmitting the composition, and a means to introduce the composition into the animal's body to ultimately reach the lymphatic system.

[0118] Preferably, the reservoir for the antigen composition should be large enough for delivery of the desired amount of antigen over time and easily refillable or replaceable without requiring the user to reinsert the means for introducing the antigen composition to the lymph system.

[0119] In preparing the antigen compositions of embodiments of the invention disclosed herein, a composition (preferably aqueous) can be prepared to be compatible with the lymph system and physiologically acceptable to the animal being treated. Relevant considerations include, for example, the physicochemical properties of the antigen, such as the isoelectric point, molecular weight, glycosylation or other post-translational modification, and overall amino acid composition. These properties along with any known behavior of the drug in different solutions (*e.g.*, different buffers, cofactors, etc.) as well as its *in vivo* behavior can help guide the choice of formulation components. One parameter that impacts all the major degradation pathways is the solution pH. Thus, the initial formulations also assess the pH dependence of the degradation reactions and the mechanism for degradation, which can often

be determined from the pH dependence to determine the stability of the protein in each solution. Rapid screening methods usually involve the use of accelerated stability at elevated temperatures (e.g., 40° C) using techniques known in the art.

[0120] In general the antigen compositions useful in embodiments described herein can be suitable for parenteral injection, in very small quantities. As such a composition should be free of contamination and have a pH compatible with the lymphatic system. However, because very small quantities of the antigenic composition will be delivered it need not be the same pH as blood or lymph, and it need not be aqueous-based. The preferable pH range that is compatible is from about 6.7 - 7.3 and can be prepared using water for injection to meet USP specifications (see Remington: The Science and Practice of Pharmacy, Nineteenth Edition; Chapters 86-88). For antigens that are less soluble, a suitable cosolvent or surfactant may be used, such as dimethyl sulfoxide (DMSO) or PLURONIC brand surfactants. Generally, a standard saline solution that is buffered with a physiologically acceptable weak acid and its base conjugate, *e.g.*, a phosphate or citrate buffering system, will be the basis of the antigen composition. In some cases, a small amount of an antioxidant may be useful to stabilize the composition and prevent oxidation. Factors to consider in preparing the antigen compositions may be found in the 1994 American Chemical Society book entitled "Formulation and Delivery of Proteins and Peptides" (Acs Symposium Series, No. 567) by Jeffery L. Cleland and Robert Langer (Editor)).

[0121] For nucleic acid encoded antigens similar considerations can apply, although the variety of physico-chemical properties encountered with polypeptides is absent, so that acceptable formulations will have nearly universal applicability. As seen in Examples 6-10, plasmid DNA in standard phosphate buffered saline (PBS) is an acceptable and effective formulation. In some embodiments of the invention, DNA is administered continuously or intermittently at short intervals, from a reservoir worn on, or implanted in, the patient's body. It is preferable that the DNA be maintained in a soluble, stable form at or near body temperature over a period of time measured minimally in days. In such applications where the formulated nucleic acid will be delivered from a reservoir over a period of several days or longer, the stability of the nucleic acid at room or body temperature for that period of time, as well as its continued sterility, take on increased importance. The addition of bacteriostatic agents (*e.g.*, benzyl or ethyl alcohol) and chelating agents (e.g. EDTA) is useful toward these ends. Formulations containing about 0.5-2 % ethyl alcohol, 0.25-0.5mM EDTA generally perform well. Such formulations are also appropriate for bolus injections.

[0122] Generally the amount of the antigen in the antigen composition will vary from patient to patient and from antigen to antigen, depending on such factors as the activity of the antigen in inducing a response and the flow rate of the lymph through the patient's system. In general the antigen composition may be delivered at a rate of from about 1 to about 500 microliters/hour or about 24 to about 12000 microliters/day. The concentration of the antigen is such that about 0.1 micrograms to about 10,000 micrograms of the antigen will be delivered during 24 hours. The flow rate is based on the knowledge that each minute approximately about 100 to about 1000 microliters of lymph fluid flows through an adult inguinal lymph node. The objective is to maximize local concentration of vaccine formulation in the lymph system. A certain amount of empirical investigation on patients will be necessary to determine the most efficacious level of infusion for a given vaccine preparation in humans.

[0123] To introduce the antigen composition into the lymphatic system of the patient the composition is preferably directed to a lymph vessel, lymph node, the spleen, or other appropriate portion of the lymph system. Preferably, the composition is directed to a lymph node such as an inguinal or axillary node by inserting a catheter or needle to the node and maintaining the catheter or needle throughout the delivery. Suitable needles or catheters are available made of metal or plastic (e.g., polyurethane, polyvinyl chloride [PVC], TEFLON, polyethylene, and the like). In inserting the catheter or needle into the inguinal node for example, the inguinal node is punctured under ultrasonographic control using a Vialon™ Insyte-W™ cannula and catheter of 24G3/4 (Becton Dickinson, USA) which is fixed using Tegaderm™ transparent dressing (Tegaderm™ 1624, 3M, St. Paul, MN 55144, USA). This procedure is generally done by an experienced radiologist. The location of the catheter tip inside the inguinal lymph node is confirmed by injection of a minimal volume of saline, which immediately and visibly increases the size of the lymph node. The latter procedure allows confirmation that the tip is inside the node. This procedure can be performed to ensure that the tip does not slip out of the lymph node and can be repeated on various days after implantation of the catheter. In the event that the tip does slip out of location inside the lymph node, a new catheter can be implanted.

Formulation and Treatment protocol

[0124] There are several approaches to utilizing the combination of TuAAs with DNA vaccines. A first approach is to include all the antigens or epitopes from all the antigens in a given combination into a single DNA expression vector. This approach has the advantages of simplicity for manufacturing and administration to patients. However, in some instances, epitope competition can limit the usefulness of this approach. That is, it is possible that only

the most immunogenic epitope will elicit an immune response when a vaccine with several epitopes representing all TuAAs in the combination is given to patients. It is also more difficult to design and construct a DNA vaccine in which all epitopes are expressed at high efficiencies. Nevertheless, because the procedure for treating patients is simple and uniform within each type of cancer, the cost is likely to be lower than for the other approaches described below.

[0125] An alternate approach is to include only one antigen or epitopes of one antigen in a DNA expression vector. This approach has the advantages of simplicity in designing and constructing the DNA vector, flexibility, and customized administration to patients. If a large number of individual TuAA vaccines are available, then one can customize treatment for each individual patient based on the TuAA expression profile of his or her tumor. For example, if the standard combination for treating a given type of cancer is TuAA A, B, and C (where A, B, and C designate different tumor associated antigens), but a patient's tumor expresses TuAA A, C, and Z (but not B), then the patient can be treated with separate vaccines for each of A, C, and Z. It is expected that this flexibility and customizability will improve the success rate of immunotherapy because antigen redundancy can be achieved for each patient. However, the procedure of treating the patient may be more complex. For example, delivery using this approach may include a sequential administration scheme (one antigen at a time), or injection into multiple, anatomically separate sites of the patient at about the same time.

[0126] Still another approach is to combine epitopes from multiple TuAAs that have similar immunogenicity into a DNA expression vector (more than one vector may be used for some combinations). This approach can have some of the advantages of the above two approaches but also can suffer from the disadvantages of the previous two.

[0127] A profile of the antigen expression of a particular tumor can be used to determine which antigen or combination of antigens to use. Exemplary methodology is found in U.S. Provisional Application No. __/___,___ (Attorney Docket: MANNK.035PR2), filed on even date herewith, entitled "COMBINATIONS OF TUMOR-ASSOCIATED ANTIGENS IN DIAGNOTISTICS FOR VARIOUS TYPES OF CANCERS;".

[0128] Patients that can benefit from such methods of immunization can be recruited using methods to define their MHC protein expression profile and general level of immune responsiveness. In addition, their level of immunity can be monitored using standard techniques in conjunction with access to peripheral blood. Finally, treatment protocols can be adjusted based on the responsiveness to induction or amplification phases and variation in antigen expression. For example, repeated entrainment doses preferably can be administered

until a detectable response is obtained, and then administering the amplifying peptide dose(s), rather than amplifying after some set number of entrainment doses. Similarly, scheduled amplifying or maintenance doses of peptide can be discontinued if their effectiveness wanes, antigen-specific regulatory T cell numbers rise, or some other evidence of tolerization is observed, and further entrainment can be administered before resuming amplification with the peptide. The integration of diagnostic techniques to assess and monitor immune responsiveness with methods of immunization is discussed more fully in Provisional U.S. Patent Application No. __/_____ (Atty. Docket No. MANNK.040PR), entitled IMPROVED EFFICACY OF ACTIVE IMMUNOTHERAPY BY INTEGRATING DIAGNOSTIC WITH THERAPEUTIC METHODS, which was filed on date even with the present application.

[0129] Many variations and alternative elements of the invention have been disclosed. Still further variations and alternate elements will be apparent to one of skill in the art. Various embodiments of the invention can specifically include or exclude any of these variation or elements.

[0130] The following examples are for illustrative purposes only and are not intended to limit the scope of the embodiments in any way.

Examples

TuAA analysis and selection of combinations

[0131] The presence of TuAAs was measured by Real-Time PCR (RT-PCR). Briefly, total RNA was isolated from tumor specimens by standard methods and cDNA was made with standard reverse transcription procedures. Complementary DNA (cDNA) was amplified with specially designed, gene specific, primers that anneal only to cDNA but not genomic DNA. TuAA expression patterns of 12 ovarian and 7 colorectal tumor specimens were analyzed by RT-PCR. The results are summarized in the Table 4 below.

Table 4

	Total #	PRAME	NY-ESO-1	SSX-2	PSMA	MAGE1	MAGE3
Ovarian	12	12	5	6	6	4	3
Colorectal	7	5	1	2	5	0	1

Example 1

Ovarian Cancer

[0132] In the case of ovarian cancer, all samples analyzed were positive for PRAME. Thus the inclusion of PRAME in the combination improves coverage of the cases with ovarian cancer.

[0133] In order to achieve antigen redundancy and improve coverage in a large population, combinations of other antigens were considered in addition to PRAME. SSX-2 as well as PSMA were present in 6 of the 12 cases individually, but the combination of SSX-2 and PSMA provided coverage in 9 of 12 cases. Although NY-ESO-1 and SSX-2 were only present in 5 and 6 of the 12 cases, respectively, either NYESO-1 or SSX-2 was detected in 7 of the 12 cases.

[0134] Thus, the combination of PRAME, SSX-2, and PSMA or PRAME, NY-ESO-1, and SSX-2 provided preferable coverage and redundancy compared to the combination of PRAME and PSMA or the combination of PRAME and SSX-2. The combination of PRAME, SSX-2, and PSMA provided excellent coverage of cases and good antigen redundancy because the majority of ovarian tumor samples analyzed had at least two of the four TuAA in the combination present. The combination of PRAME, SSX-2, PSMA, and NY-ESO-1 provided more prefered antigen redundancy, and thus, lower possibility of tumor escape.

Example 2

Colorectal cancer

[0135] In the case of colorectal cancer, PRAME and PSMA were each detected in 5 of the 7 samples analyzed. In 6 of the 7 cases, either PRAME or PSMA was detected. Although SSX-2 was only detected in 2 of 7 cases, both SSX-2-PRAME and SSX2-PSMA combinations increased coverage to 6 of 7. Similarly, although NYESO-1 was detected in only 1 of 7 cases, the combination of NY-ESO-1-PRAME as well as the NYESO-1-PSMA combination increased coverage to 6 of 7. The addition of SSX-2 or NYESO-1 to the PRAME and PSMA combination improved coverage to 7 of 7. Thus, the combination of PRAME, PSMA, and NYESO-1, or the combination of PRAME, PSMA, and SSX-2 provided good coverage of cases and redundancy of antigens for a majority of patients. The combination of PRAME, PSMA, NY-ESO-1, and SSX-2 provided further redundancy.

Example 3

Pancreatic Cancer

[0136] In pancreatic cancer specimens, the presence of NYESO-1 and SSX-2 was detected in 40% and 20% of the specimens, respectively. PSMA and over-expression of HER2-/neu were reported to be present in 100% and 21% of pancreatic tumors, respectively (Chang SS et al, Cancer Res 1999, 59:3192; Safran H et al, Am J Clin Oncol. 2001, 24:496). Although over-expression of HER2/neu may render the cancer tissue a preferred target, thus providing some specificity for immunotherapy, low level expression of HER2/neu in normal tissues remains a concern. Thus, the combination of NYESO-1, SSX-2, and PSMA provides excellent coverage and some redundancy for treating pancreatic cancer.

Example 4

Non-small cell lung cancer

[0137] For non-small cell lung cancer, the reported presence of NYESO-1, SSX-2, MAGE-3, BAGE, over-expression of Her2/neu, and PSMA was 21, 15, 60, 6, 16, and 100%, respectively (Scanlan MJ et al, Cancer lett 2000, 150:155; Chang SS et al, Cancer Res 1999, 59:3192; Selvaggi G et al, Cancer 2002, 94:2669). Thus, the combination of NYESO-1, SSX-2, MAGE-3, and PSMA provides coverage and antigen redundancy for the immunotherapy of non-small cell lung cancer.

Example 5

Renal cell carcinoma

[0138] For renal cell carcinoma, SSX-2, PSMA and PRAME were detected with frequencies of 5, 100 and 40%, respectively (Sahin, U et al, Clin Cancer Res. 2000, 6:3916; Chang SS et al, Urology 2001, 57:801; Neumann E et al, Cancer Res. 1998, 58:4090). Thus, the combination of PSMA and PRAME provides excellent coverage and redundancy for renal cell carcinoma. Adding SSX-2 to the combination of PSMA and PRAME improves redundancy.

Example 6

Melanoma

[0139] For melanoma, Melan A, Tyrosinase, NYESO-1, and SSX-2 were reported to be present in 92, 92, 41, and 35% of tumor specimens, respectively (Fetsch PA, et al, Cancer 1999, 87:37; Fetsch PA, et al, Cancer 2000, 90:252; Schultz-Thater E et al, Br J Cancer 2000, 83:204; Sahin, U et al, Clin Cancer Res. 2000, 6:3916). Therefore, the combination of Melan A, Tyrosinase, NYESO-1, and SSX-2 provides excellent coverage and antigen redundancy for the immunotherapy of melanoma. Significant redundancy is achieved using tyrosinase and

melan-A together, or by combining NY-ESO-1 and SSX-2 with either of tyrosinase or melan-A.

Example 7

Schedule of immunization with plasmids expressing epitopes from two antigens

[0140] Two groups of HHD mice (n=4) were immunized via intra lymph node injection with either pSEM expressing Melan-A $_{26-35}$A27L (ELA) and pCBP expressing SSX-2_{41-49} as a mixture; or with pSEM in the left inguinal lymph node and pCBP in the right inguinal lymph node, twice, at day 0 and 4 as shown in Figure 1. The amount of the plasmid was 25μg/plasmid/dose. Two weeks later, the animals were sacrificed, and cytotoxicity was measured against T2 cells pulsed or not with peptide.

Example 8

Co-administration of different vectors carrying distinct antigens

[0141] The animals immunized as described in Example 7, were sacrificed and splenocytes from each group pooled and stimulated with the two peptides (ELA or SSX-2_{41-49}) in parallel. The cytotoxicity was measured by incubation with Cr^{51}-tagged, peptide loaded T2 target cells. Data in Figure 2 show mean of specific cytotoxicity (n=4/group) against various target cells.

[0142] The results show that use of plasmid mixture interferes with the response elicited by pCBP plasmid; however, segregating the two plasmids relative to site of administration rescues the activity of pCBP. Thus, the co-administration of different vectors carrying distinct antigens can result in establishment of a hierarchy with regard to immunogenicity. Vector segregation can rescue the immunogenicity of the less dominant component, resulting in a multivalent response.

Example 9

Rescue of Multivalent Response by Addition of Peptide Boost Steps

[0143] Four groups of HHD mice (n=6) were immunized via intra lymph node injection with either pSEM and pCBP as a mixture; or with pSEM in the left inguinal lymph node and pCBP in the right inguinal lymph node, twice, at day 0 and 4 as shown in Figure 3. As a control, mice were immunized with either pSEM or pCBP plasmid. The amount of the plasmid was 25μg/plasmid/dose. Two weeks later, the animals were boosted with melan A and/or SSX-2 peptides, mirroring the plasmid immunization dose and combination. Two weeks later, the animals were challenged with splenocytes stained with CFSE and loaded or not with Melan A or SSX-2 peptide, for evaluation of *in vivo* cytotoxicity.

Example 10

Peptide amplification rescues the immunogenicity of the less dominant epitope

[0144] Mice were immunized as described in Example 9 and challenged with HHD littermate splenocytes coated with ELA or SSX-2 peptide, employing a triple peak CFSE *in vivo* cytotoxicity assay that allows the assessment of the specific lysis of two antigen targets simultaneously. Equal numbers of control-CFSE^{lo}, SSX-2_{41-49}-CFSE^{med}, and ELA-CFSE^{hi} cells were intravenously infused into immunized mice and 18 hours later the mice were sacrificed and target cell elimination was measured in the spleen (Figure 4) by CFSE fluorescence using a flow cytometry. Figure 4 shows the percent specific lysis of the SSX2 and Melan-A antigen targets from individual mice, as well as the mean and SEM for each group.

[0145] The results show that immunizing the animals with a mixture of the two vaccines comprising plasmids followed by peptides generated immunity to both antigens and resulted in the highest immune response, representing an average SSX-2 percent specific lysis in the spleen of 30+/-11, and an average Melan-A percent specific lysis of 97+/-1.

Example 11

Clinical practice for entrain-and–amplify immunization

[0146] The data in figures 2 and 4 suggest two scenarios for achieving a strong multivalent response in the clinic, shown in Figure 5. In the first scenario (A), use of peptides for boosting restores multivalent immune responses even if plasmids and peptides are used as mixtures. In the second scenario (B), segregation of plasmid and peptide components respectively, allows induction of multivalent immune responses.

[0147] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0148] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Example 12

Antigen sequences

[0147] Below are sequences of the antigens listed in Table 1.

SEQ. ID NO. 1
SIZE: 529 aa
DEFINITION Tyrosinase precursor
ACCESSION NO.: P14679

mllavlycll wsfqtsaghf pracvssknl mekeccppws gdrspcgqls
grgscqnill snaplgpqfp ftgvddresw psvfynrtcq csgnfmgfnc
gnckfgfwgp ncterrllvr rnifdlsape kdkffayltl akhtissdyv
ipigtygqmk ngstpmfndi niydlfvwmh yyvsmdallg gseiwrdidf
aheapaflpw hrlfllrweq eiqkltgden ftipywdwrd aekcdictde
ymggqhptnp nllspasffs swqivcsrle eynshqslcn gtpegplrrn
pgnhdksrtp rlpssadvef clsltqyesg smdkaanfsf rntlegfasp
ltgiadasqs smhnalhiym ngtmsqvqgs andpifllhh afvdsifeqw
lrrhrplqev ypeanapigh nresymvpfi plyrngdffi sskdlgydys
ylqdsdpdsf qdyiksyleq asriwswllg aamvgavlta llaglvsllc
rhkrkqlpee kqpllmeked yhslyqshl

SEQ. ID NO. 2
SIZE: 118 aa
DEFINITION Melanoma antigen recognized by T-cells 1 (MART-1) (Melan-A protein)
ACCESSION NO.: Q16655

mpredahfiy gypkkghghs yttaeeaagi giltvilgvl lligcwycrr
rngyralmdk slhvgtqcal trrcpqegfd hrdskvslqe kncepvvpna
ppayeklsae qspppysp

SEQ. ID NO. 3
SIZE: 223 aa
DEFINITION: synovial sarcoma, X breakpoint 2 isoform a; sarcoma, synovial, X-chromosome-related 2; SSX2 protein [Homo sapiens].
ACCESSION NO.: NP_003138

mngddafarr ptvgaqipek iqkafddiak yfskeewekm kasekifyvy
mkrkyeamtk lgfkatlppf mcnkraedfq gndldndpnr gnqverpqmt
fgrlqgispk impkkpaeeg ndseevpeas gpqndgkelc ppgkpttsek
ihersgnrea qekeerrgta hrwssqnthn igrfslstsm gavhgtpkti
thnrdpkggn mpgptdcvre nsw

SEQ. ID NO. 4
SIZE: 750 aa
DEFINITION folate hydrolase (prostate-specific membrane antigen) [Homo sapiens].
ACCESSION NO.: NP_004467

mwnllhetds avatarrprw lcagalvlag gffllgflfg wfikssneat
nitpkhnmka fldelkaeni kkflynftqi phlagteqnf qlakqiqsqw
kefgldsvel ahydvllsyp nkthpnyisi inedgneifn tslfeppppg
yenvsdivpp fsafspqgmp egdlvyvnya rtedffkler dmkincsgki
viarygkvfr gnkvknaqla gakgvilysd padyfapgvk sypdgwnlpg
ggvqrgniln lngagdpltp gypaneyayr rgiaeavglp sipvhpigyy
daqkllekmg gsappdsswr gsikvpynvg pgftgnfstq kvkmhihstn
evtriynvig tlrgavepdr yvilgghrds wvfggidpqs gaavvheivr
sfgtlkkegw rprrtilfas wdaeefgllg stewaeensr llqergvayi
nadssiegny tlrvdctplm yslvhnltke lkspdegfeg kslyeswtkk
spspefsgmp risklgsgnd fevffqrlgi asgrarytkn wetnkfsgyp
lyhsvyetye lvekfydpmf kyhltvaqvr ggmvfelans ivlpfdcrdy
avvlrkyadk iysismkhpq emktysvsfd slfsavknft eiaskfserl
qdfdksnpiv lrmmndqlmf lerafidplg lpdrpfyrhv iyapsshnky
agesfpgiyd alfdieskvd pskawgevkr qiyvaaftvq aaaetlseva

SEQ. ID NO. 5
SIZE: 309 aa
DEFINITION Melanoma-associated antigen 1 (MAGE-1 antigen) (Antigen MZ2-E).
ACCESSION NO.: P43355

msleqrslhc kpeealeaqq ealglvcvqa atssssplvl gtleevptag
stdppqspqg asafpttinf trqrqpsegs ssreeegpst scileslfra
vitkkvadlv gflllkyrar epvtkaemle sviknykhcf peifgkases
lqlvfgidvk eadptghsyv lvtclglsyd gllgdnqimp ktgfliivlv
miamegghap eeeiweelsv mevydgrehs aygeprkllt qdlvqekyle
yrqvpdsdpa ryeflwgpra laetsyvkvl eyvikvsarv rfffpslrea
alreeeegv

SEQ. ID NO. 6
SIZE: 314 aa
DEFINITION Melanoma-associated antigen 3 (MAGE-3 antigen) (Antigen MZ2-D).
ACCESSION NO.: P43357

mpleqrsqhc kpeeglearg ealglvgaqa pateeqeaas ssstlvevtl
gevpaaespd ppqspqgass lpttmnyplw sqsyedssnq eeegpstfpd
lesefqaals rkvaelvhfl llkyrarepv tkaemlgsvv gnwqyffpvi
fskassslql vfgielmevd pighlyifat clglsydgll gdnqimpkag
lliivlaiia regdcapeek iweelsvlev fegredsilg dpkklltqhf
vqenyleyrq vpgsdpacye flwgpralve tsyvkvlhhm vkisggphis
ypplhewvlr egee

SEQ. ID NO. 7
SIZE: 180 aa
DEFINITION Cancer/testis antigen 1 (Autoimmunogenic cancer/testis antigen NY-ESO-1).
ACCESSION NO.: P78358

mqaegrgtgg stgdadgpgg pgipdgpggn aggpgeagat ggrgprgaga
arasgpggga prgphggaas glngccrcga rgpesrllef ylampfatpm
eaelarrsla qdapplpvpg vllkeftvsg niltirltaa dhrqlqlsis
sclqqlsllm witqcflpvf laqppsgqrr

SEQ. ID NO. 8
SIZE: 509 aa
DEFINITION preferentially expressed antigen in melanoma; melanoma antigen preferentially expressed in tumors; Opa-interacting protein OIP4; preferentially expressed antigen of melanoma [Homo sapiens].
ACCESSION NO.: NP_006106

merrrlwgsi qsryismsvw tsprrlvela gqsllkdeal aiaalellpr
elfpplfmaa fdgrhsqtlk amvqawpftc lplgvlmkgq hlhletfkav
ldgldvllaq evrprrwklq vldlrknshq dfwtvwsgnr aslysfpepe
aaqpmtkkrk vdglsteaeq pfipvevlvd lflkegacde lfsyliekvk
rkknvlrlcc kklkifampm qdikmilkmv qldsiedlev tctwklptla
kfspylgqmi nlrrlllshi hassyispek eeqyiaqfts qflslqclqa
lyvdslfflr grldqllrhv mnpletlsit ncrlsegdvm hlsqspsvsq
lsvlslsgvm ltdvspeplq allerasatl qdlvfdecgi tddqllallp
slshcsqltt lsfygnsisi salqsllqhl iglsnlthvl ypvplesyed
ihgtlhlerl aylharlrel lcelgrpsmv wlsanpcphc gdrtfydpep
ilcpcfmpn

SEQ. ID NO. 9
SIZE: 1255 aa3
DEFINITION Receptor protein-tyrosine kinase erbB-2 precursor (p185erbB2) (NEU proto-oncogene) (C-erbB-2) (Tyrosine kinase-type cell surface receptor HER2) (MLN 19).
ACCESSION NO.: P04626

melaalcrwg lllallppga astqvctgtd mklrlpaspe thldmlrhly
qgcqvvqgnl eltylptnas lsflqdiqev qgyvliahnq vrqvplqrlr
ivrgtqlfed nyalavldng dplnnttpvt gaspgglrel qlrslteilk
ggvliqrnpq lcyqdtilwk difhknnqla ltlidtnrsr achpcspmck
gsrcwgesse dcqsltrtvc aggcarckgp lptdccheqc aagctgpkhs
dclaclhfnh sgicelhcpa lvtyntdtfe smpnpegryt fgascvtacp
ynylstdvgs ctlvcplhnq evtaedgtqr cekcskpcar vcyglgmehl
revravtsan iqefagckki fgslaflpes fdgdpasnta plqpeqlqvf
etleeitgyl yisawpdslp dlsvfqnlqv irgrilhnga ysltlqglgi
swlglrslre lgsglalihh nthlcfvhtv pwdqlfrnph qallhtanrp
edecvgegla chqlcarghc wgpgptqcvn csqflrgqec veecrvlqgl
preyvnarhc lpchpecqpq ngsvtcfgpe adqcvacahy kdppfcvarc
psgvkpdlsy mpiwkfpdee gacqpcpinc thscvdlddk gcpaeqrasp
ltsiisavvg illvvvlgvv fgilikrrqq kirkytmrrl lqetelvepl
tpsgampnqa qmrilketel rkvkvlgsga fgtvykgiwi pdgenvkipv
aikvlrents pkankeilde ayvmagvgsp yvsrllgicl tstvqlvtql

mpygclldhv renrgrlgsq dllnwcmqia kgmsyledvr lvhrdlaarn
vlvkspnhvk itdfglarll dideteyhad ggkvpikwma lesilrrrft
hqsdvwsygv tvwelmtfga kpydgipare ipdllekger lpqppictid
vymimvkcwm idsecrprfr elvsefsrma rdpqrfvviq nedlgpaspl
dstfyrslle ddámgdlvda eeylvpqqgf fcpdpapgag gmvhhrhrss
strsgggdlt lglepseeea prsplapseg agsdvfdgdl gmgaakglqs
lpthdpsplq rysedptvpl psetdgyvap ltcspqpeyv nqpdvrpqpp
spregplpaa rpagatlerp ktlspgkngv vkdvfafgga venpeyltpq
ggaapqphpp pafspafdnl yywdqdpper gappstfkgt ptaenpeylg
ldvpv

SEQ. ID NO. 10
SIZE: 2384 bp
DEFINITION Homo sapiens tyrosinase (oculocutaneous albinism IA) (TYR), mRNA.
ACCESSION NO.: NM_000372

tattgagttc ttcaaacatt gtagcctctt tatggtctct gagaaataac
taccttaaac ccataatctt taatacttcc taaactttct taataagaga
agctctattc ctgacactac ctctcatttg caaggtcaaa tcatcattag
ttttgtagtc tattaactgg gtttgcttag gtcaggcatt attattacta
accttattgt taatattcta accataagaa ttaaactatt aatggtgaat
agagtttttc actttaacat aggcctatcc cactggtggg atacgagcca
attcgaaaga aaagtcagtc atgtgctttt cagaggatga aagcttaaga
taaagactaa aagtgtttga tgctggaggt gggagtggta ttatataggt
ctcagccaag acatgtgata atcactgtag tagtagctgg aaagagaaat
ctgtgactcc aattagccag ttcctgcaga ccttgtgagg actagaggaa
gaatgctcct ggctgttttg tactgcctgc tgtggagttt ccagacctcc
gctggccatt tccctagagc ctgtgtctcc tctaagaacc tgatggagaa
ggaatgctgt ccaccgtgga gcggggacag gagtccctgt ggccagcttt
caggcagagg ttcctgtcag aatatccttc tgtccaatgc accacttggg
cctcaatttc ccttcacagg ggtggatgac cgggagtcgt ggccttccgt
cttttataat aggacctgcc agtgctctgg caacttcatg ggattcaact
gtggaaactg caagtttggc ttttggggac caaactgcac agagagacga
ctcttggtga gaagaaacat cttcgatttg agtgccccag agaaggacaa
atttttgcc tacctcactt tagcaaagca taccatcagc tcagactatg
tcatccccat agggacctat ggccaaatga aaaatggatc aacacccatg
tttaacgaca tcaatattta tgacctcttt gtctggatgc attattatgt
gtcaatggat gcactgcttg ggggatctga aatctggaga gacattgatt
ttgcccatga agcaccagct tttctgcctt ggcatagact cttcttgttg
cggtgggaac aagaaatcca gaagctgaca ggagatgaaa acttcactat
tccatattgg gactggcggg atgcagaaaa gtgtgacatt tgcacagatg
agtacatggg aggtcagcac cccacaaatc ctaacttact cagcccagca
tcattcttct cctcttggca gattgtctgt agccgattgg aggagtacaa
cagccatcag tctttatgca atggaacgcc cgagggacct ttacggcgta
atcctggaaa ccatgacaaa tccagaaccc caaggctccc ctcttcagct
gatgtagaat tttgcctgag tttgacccaa tatgaatctg gttccatgga
taaagctgcc aatttcagct ttagaaatac actggaagga tttgctagtc
cacttactgg gatagcggat gcctctcaaa gcagcatgca caatgccttg
cacatctata tgaatggaac aatgtcccag gtacagggat ctgccaacga
tcctatcttc cttcttcacc atgcatttgt tgacagtatt tttgagcagt
ggctccgaag gcaccgtcct cttcaagaag tttatccaga agccaatgca
cccattggac ataaccggga atcctacatg gttcctttta taccactgta
cagaaatggt gatttcttta tttcatccaa agatctgggc tatgactata
gctatctaca agattcagac ccagactctt ttcaagacta cattaagtcc

tatttggaac aagcgagtcg gatctggtca tggctccttg gggcggcgat
ggtaggggcc gtcctcactg ccctgctggc agggcttgtg agcttgctgt
gtcgtcacaa gagaaagcag cttcctgaag aaaagcagcc actcctcatg
gagaaagagg attaccacag cttgtatcag agccatttat aaaaggctta
ggcaatagag tagggccaaa aagcctgacc tcactctaac tcaaagtaat
gtccaggttc ccagagaata tctgctggta ttttttctgta aagaccattt
gcaaaattgt aacctaatac aaagtgtagc cttcttccaa ctcaggtaga
acacacctgt ctttgtcttg ctgttttcac tcagcccttt taacatttte
ccctaagccc atatgtctaa ggaaaggatg ctatttggta atgaggaact
gttatttgta tgtgaattaa agtgctctta tttt

SEQ. ID NO. 11
SIZE: 1524 bp
DEFINITION Human melanoma antigen recognized by T-cells (MART-1) mRNA.
ACCESSION NO.: U06452

agcagacaga ggactctcat taaggaaggt gtcctgtgcc ctgaccctac
aagatgccaa gagaagatgc tcacttcatc tatggttacc ccaagaaggg
gcacggccac tcttacacca cggctgaaga ggccgctggg atcggcatcc
tgacagtgat cctgggagtc ttactgctca tcggctgttg gtattgtaga
agacgaaatg gatacagagc cttgatggat aaaagtcttc atgttggcac
tcaatgtgcc ttaacaagaa gatgcccaca agaagggttt gatcatcggg
acagcaaagt gtctcttcaa gagaaaaact gtgaacctgt ggttcccaat
gctccacctg cttatgagaa actctctgca gaacagtcac caccacctta
ttcaccttaa gagccagcga gacacctgag acatgctgaa attatttctc
tcacactttt gcttgaattt aatacagaca tctaatgttc tcctttggaa
tggtgtagga aaaatgcaag ccatctctaa taataagtca gtgttaaaat
tttagtaggt ccgctagcag tactaatcat gtgaggaaat gatgagaaat
attaaattgg gaaaactcca tcaataaatg ttgcaatgca tgatactatc
tgtgccagag gtaatgttag taaatccatg gtgttatttt ctgagagaca
gaattcaagt gggtattctg gggccatcca atttctcttt acttgaaatt
tggctaataa caaactagtc aggttttcga accttgaccg acatgaactg
tacacagaat tgttccagta ctatggagtg ctcacaaagg atacttttac
aggttaagac aaagggttga ctggcctatt tatctgatca agaacatgtc
agcaatgtct ctttgtgctc taaaattcta ttatactaca ataatatatt
gtaaagatcc tatagctctt ttttttgag atggagtttc gcttttgttg
cccaggctgg agtgcaatgg cgcgatcttg gctcaccata acctccgcct
cccaggttca agcaattctc ctgccttagc ctcctgagta gctgggatta
caggcgtgcg ccactatgcc tgactaattt tgtagtttta gtagagacgg
ggtttctcca tgttggtcag gctggtctca aactcctgac ctcaggtgat
ctgcccgcct cagcctccca aagtgctgga attacaggcg tgagccacca
cgcctggctg gatcctatat cttaggtaag acatataacg cagtctaatt
acatttcact tcaaggctca atgctattct aactaatgac aagtattttc
tactaaacca gaaattggta gaaggattta ataagtaaa agctactatg
tactgcctta gtgctgatgc ctgtgtactg ccttaaatgt acctatggca
atttagctct cttgggttcc caaatccctc tcacaagaat gtgcagaaga
aatcataaag gatcagagat tctg

SEQ. ID NO. 12
SIZE: 1466 bp
DEFINITION Homo sapiens synovial sarcoma, X breakpoint 2 (SSX2), transcript variant 1, mRNA.
ACCESSION NO.: NM_003147

gcatgctctg actttctctc tctttcgatt cttccatact cagagtacgc
acggtctgat tttctctttg gattcttcca aaatcagagt cagactgctc
ccggtgccat gaacggagac gacgcctttg caaggagacc cacggttggt
gctcaaatac cagagaagat ccaaaaggcc ttcgatgata ttgccaaata
cttctctaag gaagagtggg aaaagatgaa agcctcggag aaaatcttct
atgtgtatat gaagagaaag tatgaggcta tgactaaact aggttttcaag
gccaccctcc caccttttcat gtgtaataaa cgggccgaag acttccaggg
gaatgatttg gataatgacc ctaaccgtgg gaatcaggtt gaacgtcctc
agatgacttt cggcaggctc cagggaatct ccccgaagat catgcccaag
aagccagcag aggaaggaaa tgattcggag gaagtgccag aagcatctgg
cccacaaaat gatgggaaag agctgtgccc cccgggaaaa ccaactacct
ctgagaagat tcacgagaga tctggaaata gggaggccca agaaaaggaa
gagagacgcg gaacagctca tcggtggagc agtcagaaca cacacaacat
tggtcgattc agtttgtcaa cttctatggg tgcagttcat ggtaccccca
aaacaattac acacaacagg gacccaaaag gggggaacat gcctggaccc
acagactgcg tgagagaaaa cagctggtga tttatgaaga gatcagcgac
cctgaggaag atgacgagta actcccctca gggatacgac acatgcccat
gatgagaagc agaacgtggt gacctttcac gaacatgggc atggctgcgg
acccctcgtc atcaggtgca tagcaagtga aagcaagtgt tcacaacagt
gaaaagttga gcgtcatttt tcttagtgtg ccaagagttc gatgttagcg
tttacgttgt attttcttac actgtgtcat tctgttagat actaacattt
tcattgatga gcaagacata cttaatgcat attttggttt gtgtatccat
gcacctacct tagaaaacaa gtattgtcgg ttacctctgc atggaacagc
attaccctcc tctctcccca gatgtgacta ctgagggcag ttctgagtgt
ttaatttcag atttttttcct ctgcatttac acacacacgc acacaaacca
caccacacac acacacacac acacacacac acacacacac acacaccaag
taccagtata agcatctgcc atctgctttt cccattgcca tgcgtcctgg
tcaagctccc ctcactctgt ttcctggtca gcatgtactc ccctcatccg
attcccctgt agcagtcact gacagttaat aaaccttttgc aaacgttcaa
aaaaaaaaaa aaaaaa

SEQ. ID NO. 13
SIZE: 2653 bp
DEFINITION Homo sapiens folate hydrolase (prostate-specific membrane antigen) 1 (FOLH1), mRNA.
ACCESSION NO.: NM_004476

ctcaaaaggg gccggatttc cttctcctgg aggcagatgt tgcctctctc
tctcgctcgg attggttcag tgcactctag aaacactgct gtggtggaga
aactggaccc caggtctgga gcgaattcca gcctgcaggg ctgataagcg
aggcattagt gagattgaga gagactttac cccgccgtgg tggttggagg
gcgcgcagta gagcagcagc acaggcgcgg gtcccgggag gccggctctg
ctcgcgccga gatgtggaat ctccttcacg aaaccgactc ggctgtggcc
accgcgcgcc gcccgcgctg gctgtgcgct ggggcgctgg tgctggcggg
tggcttcttt ctcctcggct tcctcttcgg gtggtttata aaatcctcca
atgaagctac taacattact ccaaagcata atatgaaagc atttttggat
gaattgaaag ctgagaacat caagaagttc ttatataatt ttacacagat
accacattta gcaggaacag aacaaaactt tcagcttgca aagcaaattc
aatcccagtg gaaagaattt ggcctggatt ctgttgagct agcacattat

gatgtcctgt tgtcctaccc aaataagact catcccaact acatctcaat
aattaatgaa gatggaaatg agattttcaa cacatcatta tttgaaccac
ctcctccagg atatgaaaat gtttcggata ttgtaccacc tttcagtgct
ttctctcctc aaggaatgcc agagggcgat ctagtgtatg ttaactatgc
acgaactgaa gacttctttа aattggaacg ggacatgaaa atcaattgct
ctgggaaaat tgtaattgcc agatatggga aagttttcag aggaaataag
gttaaaaatg cccagctggc aggggccaaa ggagtcattc tctactccga
ccctgctgac tactttgctc ctggggtgaa gtcctatcca gatggttgga
atcttcctgg aggtggtgtc cagcgtggaa atatcctaaa tctgaatggt
gcaggagacc ctctcacacc aggttaccca gcaaatgaat atgcttatag
gcgtggaatt gcagaggctg ttggtcttcc aagtattcct gttcatccaa
ttggatacta tgatgcacag aagctcctag aaaaaatggg tggctcagca
ccaccagata gcagctggag aggaagtctc aaagtgccct acaatgttgg
acctggcttt actggaaact tttctacaca aaaagtcaag atgcacatcc
actctaccaa tgaagtgaca agaatttaca atgtgatagg tactctcaga
ggagcagtgg aaccagacag atatgtcatt ctgggaggtc accgggactc
atgggtgttt ggtggtattg accctcagag tggagcagct gttgttcatg
aaattgtgag gagctttgga acactgaaaa aggaagggtg gagacctaga
agaacaattt tgtttgcaag ctgggatgca gaagaatttg gtcttcttgg
ttctactgag tgggcagagg agaattcaag actccttcaa gagcgtggcg
tggcttatat taatgctgac tcatctatag aaggaaacta cactctgaga
gttgattgta caccgctgat gtacagcttg gtacacaacc taacaaaaga
gctgaaaagc cctgatgaag gctttgaagg caaatctctt tatgaaagtt
ggactaaaaa aagtccttcc ccagagttca gtggcatgcc caggataagc
aaattgggat ctggaaatga ttttgaggtg ttcttccaac gacttggaat
tgcttcaggc agagcacggt atactaaaaa ttgggaaaca aacaaattca
gcggctatcc actgtatcac agtgtctatg aaacatatga gttggtggaa
aagttttatg atccaatgtt taaatatcac ctcactgtgg cccaggttcg
aggagggatg gtgtttgagc tagccaattc catagtgctc ccttttgatt
gtcgagatta tgctgtagtt ttaagaaagt atgctgacaa aatctacagt
atttctatga aacatccaca ggaaatgaag acatacagtg tatcatttga
ttcacttttt tctgcagtaa agaattttac agaaattgct tccaagttca
gtgagagact ccaggacttt gacaaaagca acccaatagt attaagaatg
atgaatgatc aactcatgtt tctggaaaga gcatttattg atccattagg
gttaccagac aggccttttt ataggcatgt catctatgct ccaagcagcc
acaacaagta tgcaggggag tcattcccag gaatttatga tgctctgttt
gatattgaaa gcaaagtgga cccttccaag gcctggggag aagtgaagag
acagatttat gttgcagcct tcacagtgca ggcagctgca gagactttga
gtgaagtagc ctaagaggat tctttagaga atccgtattg aatttgtgtg
gtatgtcact cagaaagaat cgtaatgggt atattgataa attttaaaat
tggtatattt gaaataaagt tgaatattat atataaaaaa aaaaaaaaaa aaa

SEQ. ID NO. 14
SIZE: 2420 bp
DEFINITION Human antigen (MAGE-1) gene, complete cds.
ACCESSION NO.: M77481

ggatccaggc cctgccagga aaaatataag ggccctgcgt gagaacagag
ggggtcatcc actgcatgag agtggggatg tcacagagtc cagcccaccc
tcctggtagc actgagaagc cagggctgtg cttgcggtct gcaccctgag
ggcccgtgga ttcctcttcc tggagctcca ggaaccaggc agtgaggcct
tggtctgaga cagtatcctc aggtcacaga gcagaggatg cacagggtgt
gccagcagtg aatgtttgcc ctgaatgcac accaagggcc ccacctgcca
caggacacat aggactccac agagtctggc ctcacctccc tactgtcagt
cctgtagaat cgacctctgc tggccggctg taccctgagt accctctcac

ttcctccttc aggttttcag gggacaggcc aacccagagg acaggattcc
ctggaggcca cagaggagca ccaaggagaa gatctgtaag taggcctttg
ttagagtctc caaggttcag ttctcagctg aggcctctca cacactccct
ctctccccag gcctgtgggt cttcattgcc cagctcctgc ccacactcct
gcctgctgcc ctgacgagag tcatcatgtc tcttgagcag aggagtctgc
actgcaagcc tgaggaagcc cttgaggccc aacaagaggc cctgggcctg
gtgtgtgtgc aggctgccac ctcctcctcc tctcctctgg tcctgggcac
cctggaggag gtgcccactg ctgggtcaac agatcctccc cagagtcctc
agggagcctc cgcctttccc actaccatca acttcactcg acagaggcaa
cccagtgagg gttccagcag ccgtgaagag gaggggccaa gcacctcttg
tatcctggag tccttgttcc gagcagtaat cactaagaag gtggctgatt
tggttggttt tctgctcctc aaatatcgag ccagggagcc agtcacaaag
gcagaaatgc tggagagtgt catcaaaaat tacaagcact gttttcctga
gatcttcggc aaagcctctg agtccttgca gctggtcttt ggcattgacg
tgaaggaagc agaccccacc ggccactcct atgtccttgt cacctgccta
ggtctctcct atgatggcct gctgggtgat aatcagatca tgcccaagac
aggcttcctg ataattgtcc tggtcatgat tgcaatggag ggcggccatg
ctcctgagga ggaaatctgg gaggagctga gtgtgatgga ggtgtatgat
gggagggagc acagtgccta tggggagccc aggaagctgc tcacccaaga
tttggtgcag gaaaagtacc tggagtaccg gcaggtgccg gacagtgatc
ccgcacgcta tgagttcctg tggggtccaa gggccctcgc tgaaaccagc
tatgtgaaag tccttgagta tgtgatcaag gtcagtgcaa gagttcgctt
tttcttccca tccctgcgtg aagcagcttt gagagaggag gaagagggag
tctgagcatg agttgcagcc aaggccagtg ggagggggac tgggccagtg
caccttccag ggccgcgtcc agcagcttcc cctgcctcgt gtgacatgag
gcccattctt cactctgaag agagcggtca gtgttctcag tagtaggttt
ctgttctatt gggtgacttg gagatttatc tttgttctct tttggaattg
ttcaaatgtt ttttttaag ggatggttga atgaacttca gcatccaagt
ttatgaatga cagcagtcac acagttctgt gtatatagtt taagggtaag
agtcttgtgt tttattcaga ttgggaaatc cattctattt tgtgaattgg
gataataaca gcagtggaat aagtacttag aaatgtgaaa aatgagcagt
aaaatagatg agataaagaa ctaaagaaat taagagatag tcaattcttg
ccttatacct cagtctattc tgtaaaattt ttaaagatat atgcatacct
ggatttcctt ggcttctttg agaatgtaag agaaattaaa tctgaataaa
gaattcttcc tgttcactgg ctcttttctt ctccatgcac tgagcatctg
ctttttggaa ggccctgggt tagtagtgga gatgctaagg taagccagac
tcatacccac ccatagggtc gtagagtcta ggagctgcag tcacgtaatc
gaggtggcaa gatgtcctct aaagatgtag ggaaaagtga gagaggggtg
agggtgtggg gctccgggtg agagtggtgg agtgtcaatg ccctgagctg
gggcattttg ggctttggga aactgcagtt ccttctgggg gagctgattg
taatgatctt gggtggatcc

SEQ. ID NO. 15
SIZE: 4204 bp DNA linear PRI 07-APR-1994
DEFINITION Human MAGE-3 antigen (MAGE-3) gene, complete cds.
ACCESSION NO.: U03735

acgcaggcag tgatgtcacc cagaccacac cccttccccc aatgccactt
caggggtac tcagagtcag agacttggtc tgaggggagc agaagcaatc
tgcagaggat ggcggtccag gctcagccag gcatcaactt caggaccctg
agggatgacc gaaggccccg cccacccacc cccaactccc ccgaccccac
caggatctac agcctcagga cccccgtccc aatccttacc ccttgcccca
tcaccatctt catgcttacc tccacccccа tccgatcccc atccaggcag
aatccagttc caccctgcc cggaacccag ggtagtaccg ttgccaggat
gtgacgccac tgacttgcgc attggaggtc agaagaccgc gagattctcg

ccctgagcaa cgagcgacgg cctgacgtcg gcggagggaa gccggcccag
gctcggtgag gaggcaaggt aagacgctga gggaggactg aggcgggcct
cacctcagac agagggcctc aaataatcca gtgctgcctc tgctgccggg
cctgggccac cccgcagggg aagacttcca ggctgggtcg ccactacctc
accccgccga ccccgccgc tttagccacg gggaactctg gggacagagc
ttaatgtggc cagggcaggg ctggttagaa gaggtcaggg cccacgctgt
ggcaggaatc aaggtcagga cccgagagg gaactgaggg cagcctaacc
accaccctca ccaccattcc cgtcccccaa cacccaaccc caccccatc
ccccattccc atccccaccc ccaccccctat cctggcagaa tccgggcttt
gcccctggta tcaagtcacg gaagctccgg gaatggcggc caggcacgtg
agtcctgagg ttcacatcta cggctaaggg agggaagggg ttcggtatcg
cgagtatggc cgttgggagg cagcgaaagg gcccaggcct cctggaagac
agtggagtcc tgaggggacc cagcatgcca ggacaggggg cccactgtac
ccctgtctca aaccgaggca ccttttcatt cggctacggg aatcctaggg
atgcagaccc acttcagcag ggggttgggg cccagccctg cgaggagtca
tggggaggaa gaagagggag gactgagggg accttggagt ccagatcagt
ggcaaccttg ggctgggggа tgctgggcac agtggccaaa tgtgctctgt
gctcattgcg ccttcagggt gaccagagag ttgagggctg tggtctgaag
agtgggactt caggtcagca gagggaggaa tcccaggatc tgcagggccc
aaggtgtacc cccaaggggc ccctatgtgg tggacagatg cagtggtcct
aggatctgcc aagcatccag gtgaagagac tgagggagga ttgagggtac
ccctgggaca gaatgcggac tgggggcccc ataaaaatct gccctgctcc
tgctgttacc tcagagagcc tgggcagggc tgtcagctga ggtccctcca
ttatcctagg atcactgatg tcagggaagg ggaagccttg gtctgagggg
gctgcactca gggcagtaga gggaggctct cagaccctac taggagtgga
ggtgaggacc aagcagtctc ctcacccagg gtacatggac ttcaataaat
ttggacatct ctcgttgtcc tttccgggag gacctgggaa tgtatggcca
gatgtgggtc ccctcatgtt tttctgtacc atatcaggta tgtgagttct
tgacatgaga gattctcagg ccagcagaag ggagggatta ggccctataa
ggagaaaggt gagggccctg agtgagcaca gaggggatcc tccaccccag
tagagtgggg acctcacaga gtctggccaa ccctcctgac agttctggga
atccgtggct gcgtttgctg tctgcacatt gggggcccgt ggattcctct
cccaggaatc aggagctcca ggaacaaggc agtgaggact tggtctgagg
cagtgtcctc aggtcacaga gtagaggggg ctcagatagt gccaacggtg
aaggtttgcc ttggattcaa accaagggcc ccacctgccc cagaacacat
ggactccaga gcgcctggcc tcaccctcaa tactttcagt cctgcagcct
cagcatgcgc tggccggatg taccctgagg tgccctctca cttcctcctt
caggttctga ggggacaggc tgacctggag gaccagaggc ccccggagga
gcactgaagg agaagatctg taagtaagcc tttgttagag cctccaaggt
tccattcagt actcagctga ggtctctcac atgctccctc tctccccagg
ccagtgggtc tccattgccc agctcctgcc cacactcccg cctgttgccc
tgaccagagt catcatgcct cttgagcaga ggagtcagca ctgcaagcct
gaagaaggcc ttgaggcccg aggagaggcc ctgggcctgg tgggtgcgca
ggctcctgct actgaggagc aggaggctgc ctcctcctct tctactctag
ttgaagtcac cctgggggag gtgcctgctg ccgagtcacc agatcctccc
cagagtcctc agggagcctc cagcctcccc actaccatga actaccctct
ctggagccaa tcctatgagg actccagcaa ccaagaagag gaggggccaa
gcaccttccc tgacctggag tccgagttcc aagcagcact cagtaggaag
gtggccgagt tggttcattt tctgctcctc aagtatcgag ccagggagcc
ggtcacaaag gcagaaatgc tggggagtgt cgtcggaaat tggcagtatt
tctttcctgt gatcttcagc aaagcttcca gttccttgca gctggtcttt
ggcatcgagc tgatggaagt ggaccccatc ggccacttgt acatctttgc
cacctgcctg ggcctctcct acgatggcct gctgggtgac aatcagatca
tgcccaaggc aggcctcctg ataatcgtcc tggccataat cgcaagagag
ggcgactgtg cccctgagga gaaaatctgg gaggagctga gtgtgttaga
ggtgtttgag gggagggaag acagtatctt gggggatccc aagaagctgc

tcacccaaca tttcgtgcag gaaaactacc tggagtaccg gcaggtcccc
ggcagtgatc ctgcatgtta tgaattcctg tggggtccaa gggccctcgt
tgaaaccagc tatgtgaaag tcctgcacca tatggtaaag atcagtggag
gacctcacat ttcctaccca cccctgcatg agtgggtttt gagagagggg
gaagagtgag tctgagcacg agttgcagcc agggccagtg ggagggggtc
tgggccagtg caccttccgg ggccgcatcc cttagtttcc actgcctcct
gtgacgtgag gcccattctt cactctttga agcgagcagt cagcattctt
agtagtgggt ttctgttctg ttggatgact ttgagattat tctttgtttc
ctgttggagt tgttcaaatg ttcctttaa cggatggttg aatgagcgtc
agcatccagg tttatgaatg acagtagtca cacatagtgc tgtttatata
gtttaggagt aagagtcttg ttttttactc aaattgggaa atccattcca
ttttgtgaat tgtgacataa taatagcagt ggtaaaagta tttgcttaaa
attgtgagcg aattagcaat aacatacatg agataactca agaaatcaaa
agatagttga ttcttgcctt gtacctcaat ctattctgta aaattaaaca
aatatgcaaa ccaggatttc cttgacttct ttgagaatgc aagcgaaatt
aaatctgaat aaataattct tcctcttcac tggctcgttt cttttccgtt
cactcagcat ctgctctgtg ggaggccctg ggttagtagt ggggatgcta
aggtaagcca gactcacgcc tacccatagg gctgtagagc ctaggacctg
cagtcatata attaaggtgg tgagaagtcc tgtaagatgt agaggaaatg
taagagaggg gtgagggtgt ggcgctccgg gtgagagtag tggagtgtca gtgc

SEQ. ID NO. 16
SIZE: 752 bp
DEFINITION Human autoimmunogenic cancer/testis antigen NY-ESO-1 mRNA, complete cds.
ACCESSION NO.: U87459

atcctcgtgg gccctgacct tctctctgag agccgggcag aggctccgga
gccatgcagg ccgaaggccg gggcacaggg ggttcgacgg gcgatgctga
tggcccagga ggccctggca ttcctgatgg cccagggggc aatgctggcg
gcccaggaga ggcgggtgcc acgggcggca gaggtccccg gggcgcaggg
gcagcaaggg cctcggggcc gggaggaggc gccccgcggg gtccgcatgg
cggcgcggct tcagggctga atggatgctg cagatgcggg gccagggggc
cggagagccg cctgcttgag ttctacctcg ccatgccttt cgcgacaccc
atggaagcag agctggcccg caggagcctg gcccaggatg cccaccgct
tcccgtgcca ggggtgcttc tgaaggagtt cactgtgtcc ggcaacatac
tgactatccg actgactgct gcagaccacc gccaactgca gctctccatc
agctcctgtc tccagcagct ttccctgttg atgtggatca cgcagtgctt
tctgcccgtg tttttggctc agcctccctc agggcagagg cgctaagccc
agcctggcgc ccattcctag gtcatgcctc ctcccctagg gaatggtccc
agcacgagtg gccagttcat tgtgggggcc tgattgtttg tcgctggagg
aggacggctt acatgtttgt ttctgtagaa aataaaactg agctacgaaa aa

SEQ. ID NO. 17
SIZE: 2148 bp
DEFINITION Homo sapiens preferentially expressed antigen in melanoma (PRAME), mRNA.
ACCESSION NO.: NM_006115

gcttcagggt acagctcccc cgcagccaga agccgggcct gcagcgcctc
agcaccgctc cgggacaccc cacccgcttc ccaggcgtga cctgtcaaca
gcaacttcgc ggtgtggtga actctctgag gaaaaaccat tttgattatt
actctcagac gtgcgtggca acaagtgact gagacctaga aatccaagcg
ttggaggtcc tgaggccagc ctaagtcgct tcaaaatgga acgaaggcgt

ttgtggggtt ccattcagag ccgatacatc agcatgagtg tgtggacaag
cccacggaga cttgtggagc tggcagggca gagcctgctg aaggatgagg
ccctggccat tgccgccctg gagttgctgc ccagggagct cttcccgcca
ctcttcatgg cagcctttga cgggagacac agccagaccc tgaaggcaat
ggtgcaggcc tggcccttca cctgcctccc tctgggagtg ctgatgaagg
gacaacatct tcacctggag accttcaaag ctgtgcttga tggacttgat
gtgctccttg cccaggaggt tcgccccagg aggtggaaac ttcaagtgct
ggatttacgg aagaactctc atcaggactt ctggactgta tggtctggaa
acagggccag tctgtactca tttccagagc cagaagcagc tcagcccatg
acaaagaagc gaaaagtaga tggtttgagc acagaggcag agcagccctt
cattccagta gaggtgctcg tagacctgtt cctcaaggaa ggtgcctgtg
atgaattgtt ctcctacctc attgagaaag tgaagcgaaa gaaaaatgta
ctacgcctgt gctgtaagaa gctgaagatt tttgcaatgc ccatgcagga
tatcaagatg atcctgaaaa tggtgcagct ggactctatt gaagatttgg
aagtgacttg tacctggaag ctacccacct tggcgaaatt ttctccttac
ctgggccaga tgattaatct gcgtagactc ctcctctccc acatccatgc
atcttcctac atttccccgg agaaggaaga gcagtatatc gcccagttca
cctctcagtt cctcagtctg cagtgcctgc aggctctcta tgtggactct
ttatttttcc ttagaggccg cctggatcag ttgctcaggc acgtgatgaa
ccccttggaa accctctcaa taactaactg ccggctttcg gaaggggatg
tgatgcatct gtcccagagt cccagcgtca gtcagctaag tgtcctgagt
ctaagtgggg tcatgctgac cgatgtaagt cccgagcccc tccaagctct
gctggagaga gcctctgcca ccctccagga cctggtcttt gatgagtgtg
ggatcacgga tgatcagctc cttgccctcc tgccttccct gagccactgc
tcccagctta caaccttaag cttctacggg aattccatct ccatatctgc
cttgcagagt ctcctgcagc acctcatcgg gctgagcaat ctgacccacg
tgctgtatcc tgtcccctg gagagttatg aggacatcca tggtaccctc
cacctggaga ggcttgccta tctgcatgcc aggctcaggg agttgctgtg
tgagttgggg cggcccagca tggtctggct tagtgccaac ccctgtcctc
actgtgggga cagaaccttc tatgacccgg agcccatcct gtgcccctgt
ttcatgccta actagctggg tgcacatatc aaatgcttca ttctgcatac
ttggacacta aagccaggat gtgcatgcat cttgaagcaa caaagcagcc
acagtttcag acaaatgttc agtgtgagtg aggaaaacat gttcagtgag
gaaaaaacat tcagacaaat gttcagtgag gaaaaaaagg ggaagttggg
gataggcaga tgttgacttg aggagttaat gtgatctttg gggagataca
tcttatagag ttagaaatag aatctgaatt tctaaaggga gattctggct
tgggaagtac atgtaggagt taatccctgt gtagactgtt gtaaagaaac
tgttgaaaat aaagagaagc aatgtgaagc aaaaaaaaaa aaaaaaaa

SEQ. ID NO. 18
SIZE: 4530 bp
DEFINITION Human tyrosine kinase-type receptor (HER2) mRNA, complete cds.
ACCESSION NO.: M11730

aattctcgag ctcgtcgacc ggtcgacgag ctcgagggtc gacgagctcg
agggcgcgcg cccggccccc acccctcgca gcaccccgcg ccccgcgccc
tcccagccgg gtccagccgg agccatgggg ccggagccgc agtgagcacc
atggagctgg cggccttgtg ccgctggggg ctcctcctcg ccctcttgcc
ccccggagcc gcgagcaccc aagtgtgcac cggcacagac atgaagctgc
ggctcccctgc cagtcccgag acccacctgg acatgctccg ccacctctac
cagggctgcc aggtggtgca gggaaacctg gaactcacct acctgcccac
caatgccagc ctgtccttcc tgcaggatat ccaggaggtg cagggctacg
tgctcatcgc tcacaaccaa gtgaggcagg tcccactgca gaggctgcgg
attgtgcgag gcacccagct ctttgaggac aactatgccc tggccgtgct

agacaatgga gacccgctga acaataccac ccctgtcaca ggggcctccc
caggaggcct gcgggagctg cagcttcgaa gcctcacaga gatcttgaaa
ggaggggtct tgatccagcg gaacccccag ctctgctacc aggacacgat
tttgtggaag gacatcttcc acaagaacaa ccagctggct ctcacactga
tagacaccaa ccgctctcgg gcctgccacc cctgttctcc gatgtgtaag
ggctcccgct gctggggaga gagttctgag gattgtcaga gcctgacgcg
cactgtctgt gccggtggct gtgcccgctg caaggggcca ctgcccactg
actgctgcca tgagcagtgt gctgccggct gcacgggccc caagcactct
gactgcctgg cctgcctcca cttcaaccac agtggcatct gtgagctgca
ctgcccagcc ctggtcacct acaacacaga cacgtttgag tccatgccca
atcccgaggg ccggtatacа ttcggcgcca gctgtgtgac tgcctgtccc
tacaactacc tttctacgga cgtgggatcc tgcaccctcg tctgcccccc
gcacaaccaa gaggtgacag cagaggatgg aacacagcgg tgtgagaagt
gcagcaagcc ctgtgcccga gtgtgctatg gtctgggcat ggagcacttg
cgagaggtga gggcagttac cagtgccaat atccaggagt ttgctggctg
caagaagatc tttgggagcc tggcatttct gccggagagc tttgatgggg
acccagcctc caacactgcc ccgctccagc cagagcagct ccaagtgttt
gagactctgg aagagatcac aggttaccta tacatctcag catggccgga
cagcctgcct gacctcagcg tcttccagaa cctgcaagta atccggggac
gaattctgca caatggcgcc tactcgctga ccctgcaagg gctgggcatc
agctggctgg ggctgcgctc actgagggaa ctgggcagtg gactggccct
catccaccat aacacccacc tctgcttcgt gcacacggtg ccctgggacc
agctctttcg gaacccgcac caagctctgc tccacactgc caaccggcca
gaggacgagt gtgtgggcga gggcctggcc tgccaccagc tgtgcgcccg
agggcactgc tggggtccag ggcccaccca gtgtgtcaac tgcagccagt
tccttcgggg ccaggagtgc gtggaggaat gccgagtact gcaggggctc
cccagggagt atgtgaatgc caggcactgt ttgccgtgcc accctgagtg
tcagccccag aatggctcag tgacctgttt tggaccggag gctgaccagt
gtgtggcctg tgcccactat aaggaccctc ccttctgcgt ggcccgctgc
cccagcggtg tgaaacctga cctctcctac atgcccatct ggaagtttcc
agatgaggag ggcgcatgcc agccttgccc catcaactgc acccactcct
gtgtggacct ggatgacaag ggctgccccg ccgagcagag agccagccct
ctgacgtcca tcgtctctgc ggtggttggc attctgctgg tcgtggtctt
gggggtggtc tttgggatcc tcatcaagcg acggcagcag aagatccgga
agtacacgat gcggagactg ctgcaggaaa cggagctggt ggagccgctg
acacctagcg gagcgatgcc caaccaggcg cagatgcgga tcctgaaaga
gacggagctg aggaaggtga aggtgcttgg atctggcgct tttggcacag
tctacaaggg catctggatc cctgatgggg agaatgtgaa aattccagtg
gccatcaaag tgttgaggga aaacacatcc cccaaagcca acaaagaaat
cttagacgaa gcatacgtga tggctggtgt gggctcccca tatgtctccc
gccttctggg catctgcctg acatccacgg tgcagctggt gacacagctt
atgccctatg gctgcctctt agaccatgtc cgggaaaacc gcggacgcct
gggctcccag gacctgctga actggtgtat gcagattgcc aaggggatga
gctacctgga ggatgtgcgg ctcgtacaca gggacttggc cgctcggaac
gtgctggtca agagtcccaa ccatgtcaaa attacagact tcgggctggc
tcggctgctg gacattgacg agacagagta ccatgcagat gggggcaagg
tgcccatcaa gtggatggcg ctggagtcca ttctccgccg gcggttcacc
caccagagtg atgtgtggag ttatggtgtg actgtgtggg agctgatgac
ttttggggcc aaaccttacg atgggatccc agcccgggag atcctgacc
tgctggaaaa gggggagcgg ctgccccagc cccccatctg caccattgat
gtctacatga tcatggtcaa atgttggatg attgactctg aatgtcggcc
aagattccgg gagttggtgt ctgaattctc ccgcatggcc agggacccc
agcgctttgt ggtcatccag aatgaggact tgggcccagc cagtccttg
gacagcacct tctaccgctc actgctggag gacgatgaca tgggggacct
ggtggatgct gaggagtatc tggtacccca gcagggcttc ttctgtccag
accctgcccc gggcgctggg ggcatggtcc accacaggca ccgcagctca

tctaccagga gtggcggtgg ggacctgaca ctagggctgg agccctctga
agaggaggcc cccaggtctc cactggcacc ctccgaaggg gctggctccg
atgtatttga tggtgacctg ggaatggggg cagccaaggg gctgcaaagc
ctccccacac atgaccccag ccctctacag cggtacagtg aggaccccac
agtacccctg ccctctgaga ctgatggcta cgttgccccc ctgacctgca
gcccccagcc tgaatatgtg aaccagccag atgttcggcc ccagcccctt
tcgccccgag agggccctct gcctgctgcc cgacctgctg gtgccactct
ggaaagggcc aagactctct ccccagggaa gaatggggtc gtcaaagacg
tttttgcctt tgggggtgcc gtggagaacc ccgagtactt gacaccccag
ggaggagctg cccctcagcc ccaccctcct cctgccttca gcccagcctt
cgacaacctc tattactggg accaggaccc accagagcgg ggggctccac
ccagcacctt caaagggaca cctacggcag agaacccaga gtacctgggt
ctggacgtgc cagtgtgaac cagaaggcca agtccgcaga agccctgatg
tgtcctcagg gagcagggaa ggcctgactt ctgctggcat caagaggtgg
gagggccctc cgaccacttc caggggaacc tgccatgcca ggaacctgtc
ctaaggaacc ttccttcctg cttgagttcc cagatggctg gaaggggtcc
agcctcgttg gaagaggaac agcactgggg agtctttgtg gattctgagg
ccctgcccaa tgagactcta gggtccagtg gatgccacag cccagcttgg
ccctttcctt ccagatcctg ggtactgaaa gccttaggga agctggcctg
agaggggaag cggccctaag ggagtgtcta agaacaaaag cgacccattc
agagactgtc cctgaaacct agtactgccc cccatgagga aggaacagca
atggtgtcag tatccaggct ttgtacagag tgcttttctg tttagttttt
acttttttg ttttgttttt ttaaagacga aataaagacc caggggagaa
tgggtgttgt atggggaggc aagtgtgggg ggtccttctc cacacccact
ttgtccattt gcaaatatat tttggaaaac

2004249254 11 Jun 2010

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Use of an immunogenic composition which immunizes against PRAME and at least one other tumor-associated antigen for the manufacture of a medicament for the treatment of ovarian or colorectal cancer, wherein the at least one other tumor-associated antigen is selected from the group consisting of SSX-2, NY-ESO-1, and PSMA.

2. Use of claim 1, wherein the at least one other tumor-associated antigen comprises NY-ESO-1 and SSX-2.

3. Use of claim 1, wherein the at least one other tumor-associated antigen comprises NY-ESO-1, SSX-2, and PSMA.

4. Use of claim 1, wherein a cytolytic T cell response is induced.

5. Use of claim 1, wherein the medicament further comprises at least one antigen associated with tumor neovasculature.

6. Use of claim 5, wherein the antigen associated with tumor neovasculature is selected from the group consisting of PSMA, VEGFR2, and Tie-2.

7. An immunogenic composition for use in the treatment of ovarian or colorectal cancer comprising the antigen PRAME and at least one other tumor-associated antigen, wherein each antigen is 1) a whole antigen, 2) a fragment of an antigen, 3) an epitope cluster derived from an antigen, 4) an epitope derived from an antigen, or 5) a nucleic acid encoding any of 1) to 4), wherein the at least one other tumor-associated antigen is selected from the group consisting of SSX-2, NY-ESO-1 and PSMA.

8. The composition of claim 7, further comprising at least one antigen associated with tumor neovasculature.

9. The composition of claim 8, wherein the antigen associated with tumor neovasculature is selected from the group consisting of PSMA, VEGFR2, and Tie-2.

10. A kit used for the treatment of ovarian or colorectal cancer comprising, separately or in combination, the antigen PRAME and at least one other tumor-associated antigen, wherein each antigen is 1) a whole antigen, 2) a fragment of an antigen, 3) an epitope cluster derived from an antigen, 4) an epitope derived from an antigen, or 5) a nucleic acid encoding any of 1) to 4), wherein the at least one other tumor-associated antigen is selected from the group consisting of SSX-2, NY-ESO-1 and PSMA.

11. The kit of claim 10, further comprising at least one antigen associated with tumor neovasculature.

12. The kit of claim 11, wherein the antigen associated with tumor neovasculature is selected from the group consisting of PSMA, VEGFR2, and Tie-2.

13. A method of treating ovarian or colorectal cancer in a subject in need thereof, the method comprising immunizing the subject against PRAME and at least one other tumor-associated antigen, wherein the at least one other tumor-associated antigen is selected from the group consisting of SSX-2, NY-ESO-1, and PSMA.

14. Method of claim 13, wherein the at least one other tumor-associated antigen comprises NY-ESO-1 and SSX-2.

15. Method of claim 13, wherein the at least one other tumor-associated antigen comprises NY-ESO-1, SSX-2, and PSMA.

16. Method of claim 13, wherein a cytolytic T cell response is induced.

17. Method of claim 13, further comprising immunizing the subject against at least one antigen associated with tumor neovasculature.

18. Method of claim 17, wherein the antigen associated with tumor neovasculature is selected from the group consisting of PSMA, VEGFR2, and Tie-2.

19. Use of any one of claims 1-6, immunogenic composition of any one of claims 7-9, kit of any one of claims 10-12, or method of any one of claims 13-18, substantially as herein before described with reference to the figures and/or examples.

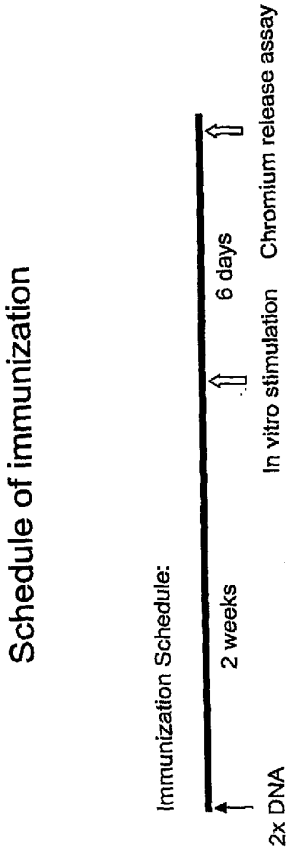

Schedule of immunization
Immunization Schedule:
2 weeks
6 days
2x DNA
In vitro stimulation
Chromium release assay
Experimental Setup:
Group 1: Plasmids separately (pCBP, pSEM)
Group 2: Plasmids admixed (pCBP+pSEM)

FIG. 1

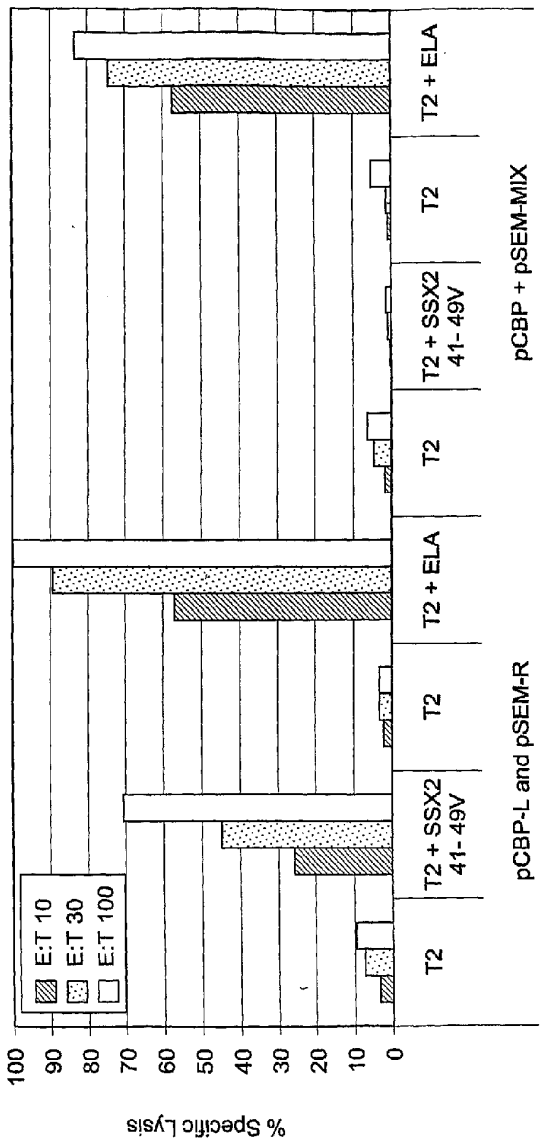

% Specific Lysis
100
90
80
70
60
50
40
30
20
10
0
E:T 10
E:T 30
E:T 100
T2
T2 + SSX2 41- 49V
T2
T2 + ELA
pCBP-L and pSEM-R
T2
T2 + SSX2 41- 49V
T2
T2 + ELA
pCBP + pSEM-MIX

FIG. 2

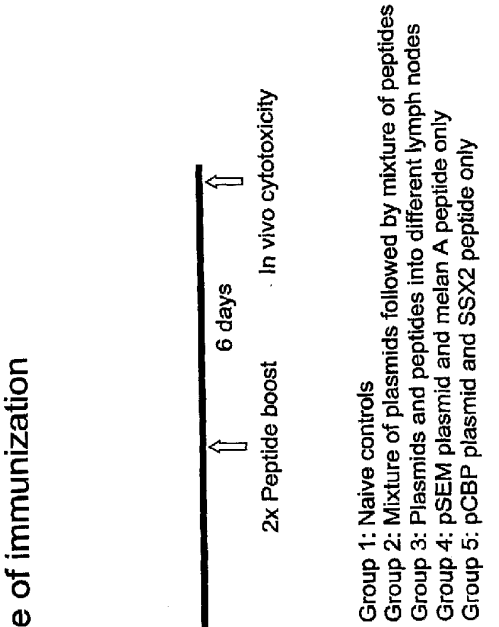

Schedule of immunization
Immunization Schedule:
2 weeks
6 days
2x DNA
2x Peptide boost
In vivo cytotoxicity
Experimental Setup:
Group 1: Naive controls
Group 2: Mixture of plasmids followed by mixture of peptides
Group 3: Plasmids and peptides into different lymph nodes
Group 4: pSEM plasmid and melan A peptide only
Group 5: pCBP plasmid and SSX2 peptide only

FIG. 3

Peptide boost rescues the immunogenicity of a less dominant epitope even when the vectors and peptides respectively, are used as a mixture.

	Spleen	Specific Lysis (%)	
Animal #	Treatment	SSX2	Melan A
#3505	Control	Control	Control
#3473	Control	6	4
#3481	Control	8	6
#3951	Control	Control	Control
#3474	Control	-4	-8
#3513	Control	-3	-4
	Mean	2	-1
	SEM	4	4
#3125	pSEM + pCBP	71	95
#3075	pSEM + pCBP	26	93
#3088	pSEM + pCBP	10	94
#3081	pSEM + pCBP	22	100
#3078	pSEM + pCBP	44	100
#3102	pSEM + pCBP	9	100
	Mean	30	97
	SEM	11	1
#3076	L=pSEM, R=pCBP	16	37
#3112	L=pSEM, R=pCBP	15	88
#3068	L=pSEM, R=pCBP	7	93
#3051	L=pSEM, R=pCBP	46	100
#3146	L=pSEM, R=pCBP	4	100
#3103	L=pSEM, R=pCBP	13	100
	Mean	17	86
	SEM	7	11
#3111	pSEM	3	57
#3036	pSEM	6	94
#3052	pSEM	-1	95
#3067	pSEM		5
#3049	pSEM	1	100
#3039	pSEM		38
	Mean	2	65
	SEM	1	17
#3069	pCBP	26	-3
#3037	pCBP	15	3
#3042	pCBP	6	7
#3070	pCBP	11	11
#3046	pCBP	18	17
#3053	pCBP	33	15
	Mean	18	8
	SEM	4	3

FIG. 4

Schedule of immunization

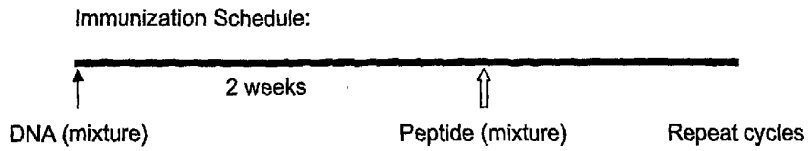


FIG. 5A

Schedule of immunization

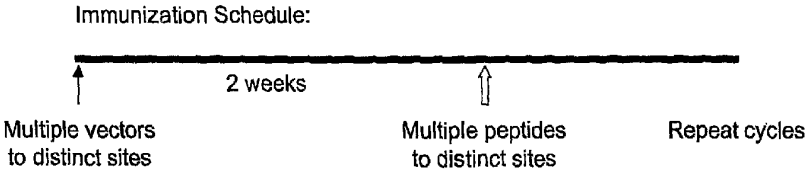


FIG. 5B

SEQUENCE LISTING

<110> Chiang, Chih-Sheng

<120> COMBINATIONS OF TUMOR-ASSOCIATED ANTIGENS IN COMPOSITIONS FOR VARIOUS TYPES OF CANCERS

<130> MANNK.035A

<140> Not Assigned
<141>

<150> 60/479,554
<151> 2003-06-17

<160> 18

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 529
<212> PRT
<213> Tyrosinase Precursor

<400> 1

```
Met Leu Leu Ala Val Leu Tyr Cys Leu Leu Trp Ser Phe Gln Thr Ser
 1               5                  10                  15
Ala Gly His Phe Pro Arg Ala Cys Val Ser Ser Lys Asn Leu Met Glu
            20                  25                  30
Lys Glu Cys Cys Pro Pro Trp Ser Gly Asp Arg Ser Pro Cys Gly Gln
        35                  40                  45
Leu Ser Gly Arg Gly Ser Cys Gln Asn Ile Leu Leu Ser Asn Ala Pro
    50                  55                  60
Leu Gly Pro Gln Phe Pro Phe Thr Gly Val Asp Asp Arg Glu Ser Trp
65                  70                  75                  80
Pro Ser Val Phe Tyr Asn Arg Thr Cys Gln Cys Ser Gly Asn Phe Met
                85                  90                  95
Gly Phe Asn Cys Gly Asn Cys Lys Phe Gly Phe Trp Gly Pro Asn Cys
            100                 105                 110
Thr Glu Arg Arg Leu Leu Val Arg Arg Asn Ile Phe Asp Leu Ser Ala
        115                 120                 125
Pro Glu Lys Asp Lys Phe Phe Ala Tyr Leu Thr Leu Ala Lys His Thr
    130                 135                 140
Ile Ser Ser Asp Tyr Val Ile Pro Ile Gly Thr Tyr Gly Gln Met Lys
145                 150                 155                 160
Asn Gly Ser Thr Pro Met Phe Asn Asp Ile Asn Ile Tyr Asp Leu Phe
                165                 170                 175
Val Trp Met His Tyr Tyr Val Ser Met Asp Ala Leu Leu Gly Gly Ser
            180                 185                 190
Glu Ile Trp Arg Asp Ile Asp Phe Ala His Glu Ala Pro Ala Phe Leu
        195                 200                 205
Pro Trp His Arg Leu Phe Leu Leu Arg Trp Glu Gln Glu Ile Gln Lys
    210                 215                 220
```

```
Leu Thr Gly Asp Glu Asn Phe Thr Ile Pro Tyr Trp Asp Trp Arg Asp
225                 230                 235                 240
Ala Glu Lys Cys Asp Ile Cys Thr Asp Glu Tyr Met Gly Gly Gln His
                245                 250                 255
Pro Thr Asn Pro Asn Leu Leu Ser Pro Ala Ser Phe Phe Ser Ser Trp
            260                 265                 270
Gln Ile Val Cys Ser Arg Leu Glu Glu Tyr Asn Ser His Gln Ser Leu
        275                 280                 285
Cys Asn Gly Thr Pro Glu Gly Pro Leu Arg Arg Asn Pro Gly Asn His
    290                 295                 300
Asp Lys Ser Arg Thr Pro Arg Leu Pro Ser Ser Ala Asp Val Glu Phe
305                 310                 315                 320
Cys Leu Ser Leu Thr Gln Tyr Glu Ser Gly Ser Met Asp Lys Ala Ala
                325                 330                 335
Asn Phe Ser Phe Arg Asn Thr Leu Glu Gly Phe Ala Ser Pro Leu Thr
            340                 345                 350
Gly Ile Ala Asp Ala Ser Gln Ser Ser Met His Asn Ala Leu His Ile
        355                 360                 365
Tyr Met Asn Gly Thr Met Ser Gln Val Gln Gly Ser Ala Asn Asp Pro
    370                 375                 380
Ile Phe Leu Leu His His Ala Phe Val Asp Ser Ile Phe Glu Gln Trp
385                 390                 395                 400
Leu Arg Arg His Arg Pro Leu Gln Glu Val Tyr Pro Glu Ala Asn Ala
                405                 410                 415
Pro Ile Gly His Asn Arg Glu Ser Tyr Met Val Pro Phe Ile Pro Leu
            420                 425                 430
Tyr Arg Asn Gly Asp Phe Phe Ile Ser Ser Lys Asp Leu Gly Tyr Asp
        435                 440                 445
Tyr Ser Tyr Leu Gln Asp Ser Asp Pro Asp Ser Phe Gln Asp Tyr Ile
    450                 455                 460
Lys Ser Tyr Leu Glu Gln Ala Ser Arg Ile Trp Ser Trp Leu Leu Gly
465                 470                 475                 480
Ala Ala Met Val Gly Ala Val Leu Thr Ala Leu Leu Ala Gly Leu Val
                485                 490                 495
Ser Leu Leu Cys Arg His Lys Arg Lys Gln Leu Pro Glu Glu Lys Gln
            500                 505                 510
Pro Leu Leu Met Glu Lys Glu Asp Tyr His Ser Leu Tyr Gln Ser His
        515                 520                 525
Leu
```

<210> 2
<211> 118
<212> PRT
<213> MART-1

```
<400> 2
Met Pro Arg Glu Asp Ala His Phe Ile Tyr Gly Tyr Pro Lys Lys Gly
 1               5                  10                  15
His Gly His Ser Tyr Thr Thr Ala Glu Glu Ala Ala Gly Ile Gly Ile
            20                  25                  30
Leu Thr Val Ile Leu Gly Val Leu Leu Leu Ile Gly Cys Trp Tyr Cys
        35                  40                  45
Arg Arg Arg Asn Gly Tyr Arg Ala Leu Met Asp Lys Ser Leu His Val
```

```
    50                  55                  60
Gly Thr Gln Cys Ala Leu Thr Arg Arg Cys Pro Gln Glu Gly Phe Asp
65                  70                  75                  80
His Arg Asp Ser Lys Val Ser Leu Gln Glu Lys Asn Cys Glu Pro Val
                85                  90                  95
Val Pro Asn Ala Pro Pro Ala Tyr Glu Lys Leu Ser Ala Glu Gln Ser
            100                 105                 110
Pro Pro Pro Tyr Ser Pro
        115
```

<210> 3
<211> 223
<212> PRT
<213> Synovial Sarcoma

<400> 3

```
Met Asn Gly Asp Asp Ala Phe Ala Arg Arg Pro Thr Val Gly Ala Gln
 1               5                  10                  15
Ile Pro Glu Lys Ile Gln Lys Ala Phe Asp Asp Ile Ala Lys Tyr Phe
            20                  25                  30
Ser Lys Glu Glu Trp Glu Lys Met Lys Ala Ser Glu Lys Ile Phe Tyr
        35                  40                  45
Val Tyr Met Lys Arg Lys Tyr Glu Ala Met Thr Lys Leu Gly Phe Lys
    50                  55                  60
Ala Thr Leu Pro Pro Phe Met Cys Asn Lys Arg Ala Glu Asp Phe Gln
65                  70                  75                  80
Gly Asn Asp Leu Asp Asn Asp Pro Asn Arg Gly Asn Gln Val Glu Arg
                85                  90                  95
Pro Gln Met Thr Phe Gly Arg Leu Gln Gly Ile Ser Pro Lys Ile Met
            100                 105                 110
Pro Lys Lys Pro Ala Glu Glu Gly Asn Asp Ser Glu Glu Val Pro Glu
        115                 120                 125
Ala Ser Gly Pro Gln Asn Asp Gly Lys Glu Leu Cys Pro Pro Gly Lys
    130                 135                 140
Pro Thr Thr Ser Glu Lys Ile His Glu Arg Ser Gly Asn Arg Glu Ala
145                 150                 155                 160
Gln Glu Lys Glu Glu Arg Arg Gly Thr Ala His Arg Trp Ser Ser Gln
                165                 170                 175
Asn Thr His Asn Ile Gly Arg Phe Ser Leu Ser Thr Ser Met Gly Ala
            180                 185                 190
Val His Gly Thr Pro Lys Thr Ile Thr His Asn Arg Asp Pro Lys Gly
        195                 200                 205
Gly Asn Met Pro Gly Pro Thr Asp Cys Val Arg Glu Asn Ser Trp
    210                 215                 220
```

<210> 4
<211> 750
<212> PRT
<213> Folate Hydrolase

<400> 4

```
Met Trp Asn Leu Leu His Glu Thr Asp Ser Ala Val Ala Thr Ala Arg
 1               5                  10                  15
```

```
Arg Pro Arg Trp Leu Cys Ala Gly Ala Leu Val Leu Ala Gly Gly Phe
            20                  25                  30
Phe Leu Leu Gly Phe Leu Phe Gly Trp Phe Ile Lys Ser Ser Asn Glu
        35                  40                  45
Ala Thr Asn Ile Thr Pro Lys His Asn Met Lys Ala Phe Leu Asp Glu
    50                  55                  60
Leu Lys Ala Glu Asn Ile Lys Lys Phe Leu Tyr Asn Phe Thr Gln Ile
65                  70                  75                  80
Pro His Leu Ala Gly Thr Glu Gln Asn Phe Gln Leu Ala Lys Gln Ile
                85                  90                  95
Gln Ser Gln Trp Lys Glu Phe Gly Leu Asp Ser Val Glu Leu Ala His
            100                 105                 110
Tyr Asp Val Leu Leu Ser Tyr Pro Asn Lys Thr His Pro Asn Tyr Ile
        115                 120                 125
Ser Ile Ile Asn Glu Asp Gly Asn Glu Ile Phe Asn Thr Ser Leu Phe
    130                 135                 140
Glu Pro Pro Pro Pro Gly Tyr Glu Asn Val Ser Asp Ile Val Pro Pro
145                 150                 155                 160
Phe Ser Ala Phe Ser Pro Gln Gly Met Pro Glu Gly Asp Leu Val Tyr
                165                 170                 175
Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys Leu Glu Arg Asp Met
            180                 185                 190
Lys Ile Asn Cys Ser Gly Lys Ile Val Ile Ala Arg Tyr Gly Lys Val
        195                 200                 205
Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu Ala Gly Ala Lys Gly
    210                 215                 220
Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe Ala Pro Gly Val Lys
225                 230                 235                 240
Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly Gly Val Gln Arg Gly
                245                 250                 255
Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp Pro Leu Thr Pro Gly Tyr
            260                 265                 270
Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly Ile Ala Glu Ala Val Gly
        275                 280                 285
Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr Tyr Asp Ala Gln Lys
    290                 295                 300
Leu Leu Glu Lys Met Gly Gly Ser Ala Pro Pro Asp Ser Ser Trp Arg
305                 310                 315                 320
Gly Ser Leu Lys Val Pro Tyr Asn Val Gly Pro Gly Phe Thr Gly Asn
                325                 330                 335
Phe Ser Thr Gln Lys Val Lys Met His Ile His Ser Thr Asn Glu Val
            340                 345                 350
Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu Arg Gly Ala Val Glu Pro
        355                 360                 365
Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp Ser Trp Val Phe Gly
    370                 375                 380
Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val His Glu Ile Val Arg
385                 390                 395                 400
Ser Phe Gly Thr Leu Lys Lys Glu Gly Trp Arg Pro Arg Arg Thr Ile
                405                 410                 415
Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly Leu Leu Gly Ser Thr
            420                 425                 430
Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu Gln Glu Arg Gly Val Ala
        435                 440                 445
Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn Tyr Thr Leu Arg Val
```

```
    450                 455                 460
Asp Cys Thr Pro Leu Met Tyr Ser Leu Val His Asn Leu Thr Lys Glu
465                 470                 475                 480
Leu Lys Ser Pro Asp Glu Gly Phe Glu Gly Lys Ser Leu Tyr Glu Ser
                485                 490                 495
Trp Thr Lys Lys Ser Pro Ser Pro Glu Phe Ser Gly Met Pro Arg Ile
            500                 505                 510
Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu Val Phe Phe Gln Arg Leu
        515                 520                 525
Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr Lys Asn Trp Glu Thr Asn
    530                 535                 540
Lys Phe Ser Gly Tyr Pro Leu Tyr His Ser Val Tyr Glu Thr Tyr Glu
545                 550                 555                 560
Leu Val Glu Lys Phe Tyr Asp Pro Met Phe Lys Tyr His Leu Thr Val
                565                 570                 575
Ala Gln Val Arg Gly Gly Met Val Phe Glu Leu Ala Asn Ser Ile Val
            580                 585                 590
Leu Pro Phe Asp Cys Arg Asp Tyr Ala Val Val Leu Arg Lys Tyr Ala
        595                 600                 605
Asp Lys Ile Tyr Ser Ile Ser Met Lys His Pro Gln Glu Met Lys Thr
    610                 615                 620
Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser Ala Val Lys Asn Phe Thr
625                 630                 635                 640
Glu Ile Ala Ser Lys Phe Ser Glu Arg Leu Gln Asp Phe Asp Lys Ser
                645                 650                 655
Asn Pro Ile Val Leu Arg Met Met Asn Asp Gln Leu Met Phe Leu Glu
            660                 665                 670
Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro Phe Tyr Arg
        675                 680                 685
His Val Ile Tyr Ala Pro Ser Ser His Asn Lys Tyr Ala Gly Glu Ser
    690                 695                 700
Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile Glu Ser Lys Val Asp
705                 710                 715                 720
Pro Ser Lys Ala Trp Gly Glu Val Lys Arg Gln Ile Tyr Val Ala Ala
                725                 730                 735
Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser Glu Val Ala
            740                 745                 750
```

<210> 5
<211> 309
<212> PRT
<213> MAGE-1

```
<400> 5
Met Ser Leu Glu Gln Arg Ser Leu His Cys Lys Pro Glu Glu Ala Leu
 1               5                  10                  15
Glu Ala Gln Gln Glu Ala Leu Gly Leu Val Cys Val Gln Ala Ala Thr
            20                  25                  30
Ser Ser Ser Ser Pro Leu Val Leu Gly Thr Leu Glu Glu Val Pro Thr
        35                  40                  45
Ala Gly Ser Thr Asp Pro Pro Gln Ser Pro Gln Gly Ala Ser Ala Phe
    50                  55                  60
Pro Thr Thr Ile Asn Phe Thr Arg Gln Arg Gln Pro Ser Glu Gly Ser
65                  70                  75                  80
```

```
Ser Ser Arg Glu Glu Glu Gly Pro Ser Thr Ser Cys Ile Leu Glu Ser
                85                  90                  95
Leu Phe Arg Ala Val Ile Thr Lys Lys Val Ala Asp Leu Val Gly Phe
            100                 105                 110
Leu Leu Leu Lys Tyr Arg Ala Arg Glu Pro Val Thr Lys Ala Glu Met
        115                 120                 125
Leu Glu Ser Val Ile Lys Asn Tyr Lys His Cys Phe Pro Glu Ile Phe
    130                 135                 140
Gly Lys Ala Ser Glu Ser Leu Gln Leu Val Phe Gly Ile Asp Val Lys
145                 150                 155                 160
Glu Ala Asp Pro Thr Gly His Ser Tyr Val Leu Val Thr Cys Leu Gly
                165                 170                 175
Leu Ser Tyr Asp Gly Leu Leu Gly Asp Asn Gln Ile Met Pro Lys Thr
            180                 185                 190
Gly Phe Leu Ile Ile Val Leu Val Met Ile Ala Met Glu Gly Gly His
        195                 200                 205
Ala Pro Glu Glu Glu Ile Trp Glu Glu Leu Ser Val Met Glu Val Tyr
    210                 215                 220
Asp Gly Arg Glu His Ser Ala Tyr Gly Glu Pro Arg Lys Leu Leu Thr
225                 230                 235                 240
Gln Asp Leu Val Gln Glu Lys Tyr Leu Glu Tyr Arg Gln Val Pro Asp
                245                 250                 255
Ser Asp Pro Ala Arg Tyr Glu Phe Leu Trp Gly Pro Arg Ala Leu Ala
            260                 265                 270
Glu Thr Ser Tyr Val Lys Val Leu Glu Tyr Val Ile Lys Val Ser Ala
        275                 280                 285
Arg Val Arg Phe Phe Phe Pro Ser Leu Arg Glu Ala Ala Leu Arg Glu
    290                 295                 300
Glu Glu Glu Gly Val
305
```

<210> 6
<211> 314
<212> PRT
<213> MAGE-3

<400> 6

```
Met Pro Leu Glu Gln Arg Ser Gln His Cys Lys Pro Glu Glu Gly Leu
 1               5                  10                  15
Glu Ala Arg Gly Glu Ala Leu Gly Leu Val Gly Ala Gln Ala Pro Ala
            20                  25                  30
Thr Glu Glu Gln Glu Ala Ala Ser Ser Ser Ser Thr Leu Val Glu Val
        35                  40                  45
Thr Leu Gly Glu Val Pro Ala Ala Glu Ser Pro Asp Pro Pro Gln Ser
    50                  55                  60
Pro Gln Gly Ala Ser Ser Leu Pro Thr Thr Met Asn Tyr Pro Leu Trp
65                  70                  75                  80
Ser Gln Ser Tyr Glu Asp Ser Ser Asn Gln Glu Glu Glu Gly Pro Ser
                85                  90                  95
Thr Phe Pro Asp Leu Glu Ser Glu Phe Gln Ala Ala Leu Ser Arg Lys
            100                 105                 110
Val Ala Glu Leu Val His Phe Leu Leu Leu Lys Tyr Arg Ala Arg Glu
        115                 120                 125
Pro Val Thr Lys Ala Glu Met Leu Gly Ser Val Val Gly Asn Trp Gln
```

```
    130                 135                 140
Tyr Phe Phe Pro Val Ile Phe Ser Lys Ala Ser Ser Ser Leu Gln Leu
145                 150                 155                 160
Val Phe Gly Ile Glu Leu Met Glu Val Asp Pro Ile Gly His Leu Tyr
                165                 170                 175
Ile Phe Ala Thr Cys Leu Gly Leu Ser Tyr Asp Gly Leu Leu Gly Asp
            180                 185                 190
Asn Gln Ile Met Pro Lys Ala Gly Leu Leu Ile Ile Val Leu Ala Ile
        195                 200                 205
Ile Ala Arg Glu Gly Asp Cys Ala Pro Glu Glu Lys Ile Trp Glu Glu
    210                 215                 220
Leu Ser Val Leu Glu Val Phe Glu Gly Arg Glu Asp Ser Ile Leu Gly
225                 230                 235                 240
Asp Pro Lys Lys Leu Leu Thr Gln His Phe Val Gln Glu Asn Tyr Leu
                245                 250                 255
Glu Tyr Arg Gln Val Pro Gly Ser Asp Pro Ala Cys Tyr Glu Phe Leu
            260                 265                 270
Trp Gly Pro Arg Ala Leu Val Glu Thr Ser Tyr Val Lys Val Leu His
        275                 280                 285
His Met Val Lys Ile Ser Gly Gly Pro His Ile Ser Tyr Pro Pro Leu
    290                 295                 300
His Glu Trp Val Leu Arg Glu Gly Glu Glu
305                 310
```

<210> 7
<211> 180
<212> PRT
<213> Autoimmunogenic Cancer

<400> 7

```
Met Gln Ala Glu Gly Arg Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp
 1                5                  10                  15
Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly
            20                  25                  30
Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala
        35                  40                  45
Gly Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro
    50                  55                  60
His Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Cys Arg Cys Gly Ala
65                  70                  75                  80
Arg Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe
                85                  90                  95
Ala Thr Pro Met Glu Ala Glu Leu Ala Arg Arg Ser Leu Ala Gln Asp
            100                 105                 110
Ala Pro Pro Leu Pro Val Pro Gly Val Leu Leu Lys Glu Phe Thr Val
        115                 120                 125
Ser Gly Asn Ile Leu Thr Ile Arg Leu Thr Ala Ala Asp His Arg Gln
    130                 135                 140
Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met
145                 150                 155                 160
Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Pro Pro Ser
                165                 170                 175
Gly Gln Arg Arg
            180
```

<210> 8
<211> 509
<212> PRT
<213> Preferentially expressed antigen in melanoma

<400> 8
Met Glu Arg Arg Arg Leu Trp Gly Ser Ile Gln Ser Arg Tyr Ile Ser
1 5 10 15
Met Ser Val Trp Thr Ser Pro Arg Arg Leu Val Glu Leu Ala Gly Gln
20 25 30
Ser Leu Leu Lys Asp Glu Ala Leu Ala Ile Ala Ala Leu Glu Leu Leu
35 40 45
Pro Arg Glu Leu Phe Pro Pro Leu Phe Met Ala Ala Phe Asp Gly Arg
50 55 60
His Ser Gln Thr Leu Lys Ala Met Val Gln Ala Trp Pro Phe Thr Cys
65 70 75 80
Leu Pro Leu Gly Val Leu Met Lys Gly Gln His Leu His Leu Glu Thr
85 90 95
Phe Lys Ala Val Leu Asp Gly Leu Asp Val Leu Leu Ala Gln Glu Val
100 105 110
Arg Pro Arg Arg Trp Lys Leu Gln Val Leu Asp Leu Arg Lys Asn Ser
115 120 125
His Gln Asp Phe Trp Thr Val Trp Ser Gly Asn Arg Ala Ser Leu Tyr
130 135 140
Ser Phe Pro Glu Pro Glu Ala Ala Gln Pro Met Thr Lys Lys Arg Lys
145 150 155 160
Val Asp Gly Leu Ser Thr Glu Ala Glu Gln Pro Phe Ile Pro Val Glu
165 170 175
Val Leu Val Asp Leu Phe Leu Lys Glu Gly Ala Cys Asp Glu Leu Phe
180 185 190
Ser Tyr Leu Ile Glu Lys Val Lys Arg Lys Lys Asn Val Leu Arg Leu
195 200 205
Cys Cys Lys Lys Leu Lys Ile Phe Ala Met Pro Met Gln Asp Ile Lys
210 215 220
Met Ile Leu Lys Met Val Gln Leu Asp Ser Ile Glu Asp Leu Glu Val
225 230 235 240
Thr Cys Thr Trp Lys Leu Pro Thr Leu Ala Lys Phe Ser Pro Tyr Leu
245 250 255
Gly Gln Met Ile Asn Leu Arg Arg Leu Leu Leu Ser His Ile His Ala
260 265 270
Ser Ser Tyr Ile Ser Pro Glu Lys Glu Glu Gln Tyr Ile Ala Gln Phe
275 280 285
Thr Ser Gln Phe Leu Ser Leu Gln Cys Leu Gln Ala Leu Tyr Val Asp
290 295 300
Ser Leu Phe Phe Leu Arg Gly Arg Leu Asp Gln Leu Leu Arg His Val
305 310 315 320
Met Asn Pro Leu Glu Thr Leu Ser Ile Thr Asn Cys Arg Leu Ser Glu
325 330 335
Gly Asp Val Met His Leu Ser Gln Ser Pro Ser Val Ser Gln Leu Ser
340 345 350
Val Leu Ser Leu Ser Gly Val Met Leu Thr Asp Val Ser Pro Glu Pro
355 360 365
Leu Gln Ala Leu Leu Glu Arg Ala Ser Ala Thr Leu Gln Asp Leu Val

```
    370                 375                 380
Phe Asp Glu Cys Gly Ile Thr Asp Asp Gln Leu Leu Ala Leu Leu Pro
385                 390                 395                 400
Ser Leu Ser His Cys Ser Gln Leu Thr Thr Leu Ser Phe Tyr Gly Asn
                405                 410                 415
Ser Ile Ser Ile Ser Ala Leu Gln Ser Leu Leu Gln His Leu Ile Gly
            420                 425                 430
Leu Ser Asn Leu Thr His Val Leu Tyr Pro Val Pro Leu Glu Ser Tyr
        435                 440                 445
Glu Asp Ile His Gly Thr Leu His Leu Glu Arg Leu Ala Tyr Leu His
    450                 455                 460
Ala Arg Leu Arg Glu Leu Leu Cys Glu Leu Gly Arg Pro Ser Met Val
465                 470                 475                 480
Trp Leu Ser Ala Asn Pro Cys Pro His Cys Gly Asp Arg Thr Phe Tyr
                485                 490                 495
Asp Pro Glu Pro Ile Leu Cys Pro Cys Phe Met Pro Asn
            500                 505
```

<210> 9
<211> 1255
<212> PRT
<213> Receptor protein-tyrosine kinase

<400> 9

```
Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
 1               5                  10                  15
Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
            20                  25                  30
Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
        35                  40                  45
Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
    50                  55                  60
Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65                  70                  75                  80
Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
                85                  90                  95
Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
            100                 105                 110
Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
        115                 120                 125
Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
    130                 135                 140
Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145                 150                 155                 160
Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
                165                 170                 175
Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
            180                 185                 190
His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
        195                 200                 205
Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
    210                 215                 220
Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
225                 230                 235                 240
```

```
Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
                245                 250                 255
His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
            260                 265                 270
Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
        275                 280                 285
Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
    290                 295                 300
Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305                 310                 315                 320
Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
                325                 330                 335
Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
            340                 345                 350
Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
        355                 360                 365
Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
    370                 375                 380
Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385                 390                 395                 400
Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
                405                 410                 415
Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
            420                 425                 430
Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
        435                 440                 445
Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
    450                 455                 460
Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465                 470                 475                 480
Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
                485                 490                 495
Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
            500                 505                 510
Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
        515                 520                 525
Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
    530                 535                 540
Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545                 550                 555                 560
Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
                565                 570                 575
Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
            580                 585                 590
Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
        595                 600                 605
Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
    610                 615                 620
Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
625                 630                 635                 640
Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser
                645                 650                 655
Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
            660                 665                 670
Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
```

```
      675                 680                 685
Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly
    690                 695                 700
Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
705                 710                 715                 720
Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
                725                 730                 735
Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
            740                 745                 750
Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
        755                 760                 765
Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
    770                 775                 780
Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
785                 790                 795                 800
Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
                805                 810                 815
Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
            820                 825                 830
Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
        835                 840                 845
Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
    850                 855                 860
Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
865                 870                 875                 880
Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
                885                 890                 895
Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
            900                 905                 910
Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
        915                 920                 925
Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
    930                 935                 940
Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
945                 950                 955                 960
Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
                965                 970                 975
Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
            980                 985                 990
Asp Leu Gly Pro Ala Ser Pro Leu Asp Ser Thr Phe Tyr Arg Ser Leu
        995                 1000                1005
Leu Glu Asp Asp Asp Met Gly Asp Leu Val Asp Ala Glu Glu Tyr Leu
    1010                1015                1020
Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly Ala Gly
1025                1030                1035                1040
Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg Ser Gly Gly
                1045                1050                1055
Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Ala Pro Arg
            1060                1065                1070
Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser Asp Val Phe Asp Gly
        1075                1080                1085
Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln Ser Leu Pro Thr His
    1090                1095                1100
Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro Leu
1105                1110                1115                1120
```

```
Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu Thr Cys Ser Pro Gln
                1125                1130                1135
Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro Gln Pro Pro Ser Pro
            1140                1145                1150
Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu
        1155                1160                1165
Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val
    1170                1175                1180
Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln
1185                1190                1195                1200
Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser Pro Ala
                1205                1210                1215
Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu Arg Gly Ala
            1220                1225                1230
Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu Asn Pro Glu Tyr
        1235                1240                1245
Leu Gly Leu Asp Val Pro Val
    1250                1255
```

<210> 10
<211> 2384
<212> DNA
<213> Homo sapiens

<400> 10

```
tattgagttc ttcaaacatt gtagcctctt tatggtctct gagaaataac taccttaaac 60
ccataatctt taatacttcc taaactttct taataagaga agctctattc ctgacactac 120
ctctcatttg caaggtcaaa tcatcattag ttttgtagtc tattaactgg gtttgcttag 180
gtcaggcatt attattacta accttattgt taatattcta accataagaa ttaaactatt 240
aatggtgaat agagtttttc actttaacat aggcctatcc cactggtggg atacgagcca 300
attcgaaaga aaagtcagtc atgtgctttt cagaggatga aagcttaaga taaagactaa 360
aagtgtttga tgctggaggt gggagtggta ttatataggt ctcagccaag acatgtgata 420
atcactgtag tagtagctgg aaagagaaat ctgtgactcc aattagccag ttcctgcaga 480
ccttgtgagg actagaggaa gaatgctcct ggctgttttg tactgcctgc tgtggagttt 540
ccagacctcc gctggccatt tccctagagc ctgtgtctcc tctaagaacc tgatggagaa 600
ggaatgctgt ccaccgtgga gcggggacag gagtccctgt ggccagcttt caggcagagg 660
ttcctgtcag aatatccttc tgtccaatgc accacttggg cctcaatttc ccttcacagg 720
ggtggatgac cgggagtcgt ggccttccgt cttttataat aggacctgcc agtgctctgg 780
caacttcatg ggattcaact gtggaaactg caagtttggc ttttggggac caaactgcac 840
agagagacga ctcttggtga gaagaaacat cttcgatttg agtgccccag agaaggacaa 900
atttttgcc tacctcactt tagcaaagca taccatcagc tcagactatg tcatccccat 960
agggacctat ggccaaatga aaaatggatc aacacccatg tttaacgaca tcaatattta 1020
tgacctcttt gtctggatgc attattatgt gtcaatggat gcactgcttg ggggatctga 1080
aatctggaga gacattgatt ttgcccatga agcaccagct tttctgcctt ggcatagact 1140
cttcttgttg cggtgggaac aagaaatcca gaagctgaca ggagatgaaa acttcactat 1200
tccatattgg gactggcggg atgcagaaaa gtgtgacatt tgcacagatg agtacatggg 1260
aggtcagcac cccacaaatc ctaacttact cagcccagca tcattcttct cctcttggca 1320
gattgtctgt agccgattgg aggagtacaa cagccatcag tctttatgca atggaacgcc 1380
cgagggacct ttacggcgta atcctggaaa ccatgacaaa tccagaaccc caaggctccc 1440
ctcttcagct gatgtagaat tttgcctgag tttgacccaa tatgaatctg gttccatgga 1500
taaagctgcc aatttcagct ttagaaatac actggaagga tttgctagtc cacttactgg 1560
gatagcggat gcctctcaaa gcagcatgca caatgccttg cacatctata tgaatggaac 1620
aatgtcccag gtacagggat ctgccaacga tcctatcttc cttcttcacc atgcatttgt 1680
tgacagtatt tttgagcagt ggctccgaag gcaccgtcct cttcaagaag tttatccaga 1740
```

agccaatgca cccattggac ataaccggga atcctacatg gttcctttta taccactgta 1800
cagaaatggt gatttcttta tttcatccaa agatctgggc tatgactata gctatctaca 1860
agattcagac ccagactctt ttcaagacta cattaagtcc tatttggaac aagcgagtcg 1920
gatctggtca tggctccttg gggcggcgat ggtaggggcc gtcctcactg ccctgctggc 1980
agggcttgtg agcttgctgt gtcgtcacaa gagaaagcag cttcctgaag aaaagcagcc 2040
actcctcatg gagaaagagg attaccacag cttgtatcag agccatttat aaaaggctta 2100
ggcaatagag tagggccaaa aagcctgacc tcactctaac tcaaagtaat gtccaggttc 2160
ccagagaata tctgctggta tttttctgta aagaccattt gcaaaattgt aacctaatac 2220
aaagtgtagc cttcttccaa ctcaggtaga acacacctgt ctttgtcttg ctgttttcac 2280
tcagcccttt taacattttc ccctaagccc atatgtctaa ggaaaggatg ctatttggta 2340
atgaggaact gttatttgta tgtgaattaa agtgctctta tttt 2384

<210> 11
<211> 1524
<212> DNA
<213> Human melanoma antigen recognized by T-cells (MARA

<400> 11
agcagacaga ggactctcat taaggaaggt gtcctgtgcc ctgaccctac aagatgccaa 60
gagaagatgc tcacttcatc tatggttacc ccaagaaggg gcacggccac tcttacacca 120
cggctgaaga ggccgctggg atcggcatcc tgacagtgat cctgggagtc ttactgctca 180
tcggctgttg gtattgtaga agacgaaatg gatacagagc cttgatggat aaaagtcttc 240
atgttggcac tcaatgtgcc ttaacaagaa gatgcccaca agaagggttt gatcatcggg 300
acagcaaagt gtctcttcaa gagaaaaact gtgaacctgt ggttcccaat gctccacctg 360
cttatgagaa actctctgca gaacagtcac caccacctta ttcaccttaa gagccagcga 420
gacacctgag acatgctgaa attatttctc tcacactttt gcttgaattt aatacagaca 480
tctaatgttc tcctttggaa tggtgtagga aaaatgcaag ccatctctaa taataagtca 540
gtgttaaaat tttagtaggt ccgctagcag tactaatcat gtgaggaaat gatgagaaat 600
attaaattgg gaaaactcca tcaataaatg ttgcaatgca tgatactatc tgtgccagag 660
gtaatgttag taaatccatg gtgttatttt ctgagagaca gaattcaagt gggtattctg 720
gggccatcca atttctcttt acttgaaatt tggctaataa caaactagtc aggttttcga 780
accttgaccg acatgaactg tacacagaat tgttccagta ctatggagtg ctcacaaagg 840
atacttttac aggttaagac aaagggttga ctggcctatt tatctgatca agaacatgtc 900
agcaatgtct ctttgtgctc taaaattcta ttatactaca ataatatatt gtaaagatcc 960
tatagctctt ttttttgag atggagtttc gcttttgttg cccaggctgg agtgcaatgg 1020
cgcgatcttg gctcaccata acctccgcct cccaggttca agcaattctc ctgccttagc 1080
ctcctgagta gctgggatta caggcgtgcg ccactatgcc tgactaattt tgtagtttta 1140
gtagagacgg ggtttctcca tgttggtcag gctggtctca aactcctgac ctcaggtgat 1200
ctgcccgcct cagcctccca aagtgctgga attacaggcg tgagccacca cgcctggctg 1260
gatcctatat cttaggtaag acatataacg cagtctaatt acatttcact tcaaggctca 1320
atgctattct aactaatgac aagtattttc tactaaacca gaaattggta gaaggattta 1380
aataagtaaa agctactatg tactgcctta gtgctgatgc ctgtgtactg ccttaaatgt 1440
acctatggca atttagctct cttgggttcc caaatccctc tcacaagaat gtgcagaaga 1500
aatcataaag gatcagagat tctg 1524

<210> 12
<211> 1466
<212> DNA
<213> Homo sapiens synovial sarcoma, X breakpoint 2 (SSA

<400> 12
gcatgctctg actttctctc tctttcgatt cttccatact cagagtacgc acggtctgat 60
tttctctttg gattcttcca aaatcagagt cagactgctc ccggtgccat gaacggagac 120
gacgcctttg caaggagacc cacggttggt gctcaaatac cagagaagat ccaaaaggcc 180
ttcgatgata ttgccaaata cttctctaag gaagagtggg aaaagatgaa agcctcggag 240

aaaatcttct atgtgtatat gaagagaaag tatgaggcta tgactaaact aggtttcaag 300
gccaccctcc caccttttcat gtgtaataaa cgggccgaag acttccaggg gaatgatttg 360
gataatgacc ctaaccgtgg gaatcaggtt gaacgtcctc agatgacttt cggcaggctc 420
cagggaatct ccccgaagat catgcccaag aagccagcag aggaaggaaa tgattcggag 480
gaagtgccag aagcatctgg cccacaaaat gatgggaaag agctgtgccc cccgggaaaa 540
ccaactacct ctgagaagat tcacgagaga tctggaaata gggaggccca agaaaaggaa 600
gagagacgcg gaacagctca tcggtggagc agtcagaaca cacacaacat tggtcgattc 660
agtttgtcaa cttctatggg tgcagttcat ggtaccccca aaacaattac acacaacagg 720
gacccaaaag gggggaacat gcctggaccc acagactgcg tgagagaaaa cagctggtga 780
tttatgaaga gatcagcgac cctgaggaag atgacgagta actcccctca gggatacgac 840
acatgcccat gatgagaagc agaacgtggt gacctttcac gaacatgggc atggctgcgg 900
acccctcgtc atcaggtgca tagcaagtga aagcaagtgt tcacaacagt gaaaagttga 960
gcgtcatttt tcttagtgtg ccaagagttc gatgttagcg tttacgttgt attttcttac 1020
actgtgtcat tctgttagat actaacattt tcattgatga gcaagacata cttaatgcat 1080
attttggttt gtgtatccat gcacctacct tagaaaacaa gtattgtcgg ttacctctgc 1140
atggaacagc attaccctcc tctctcccca gatgtgacta ctgagggcag ttctgagtgt 1200
ttaatttcag attttttcct ctgcatttac acacacacgc acacaaacca caccacacac 1260
acacacacac acacacacac acacacacac acacaccaag taccagtata agcatctgcc 1320
atctgctttt cccattgcca tgcgtcctgg tcaagctccc ctcactctgt ttcctggtca 1380
gcatgtactc ccctcatccg attcccctgt agcagtcact gacagttaat aaaccttgc 1440
aaacgttcaa aaaaaaaaa aaaaaa 1466

<210> 13
<211> 2653
<212> DNA
<213> Homo sapiens folate hydrolase (prostate-specific A

<400> 13
ctcaaaaggg gccggatttc cttctcctgg aggcagatgt tgcctctctc tctcgctcgg 60
attggttcag tgcactctag aaacactgct gtggtggaga aactggaccc caggtctgga 120
gcgaattcca gcctgcaggg ctgataagcg aggcattagt gagattgaga gagactttac 180
ccgccgtgg tggttggagg gcgcgcagta gagcagcagc acaggcgcgg gtcccgggag 240
gccggctctg ctcgcgccga gatgtggaat ctccttcacg aaaccgactc ggctgtggcc 300
accgcgcgcc gcccgcgctg gctgtgcgct ggggcgctgg tgctggcggg tggcttcttt 360
ctcctcggct tcctcttcgg gtggtttata aaatcctcca atgaagctac taacattact 420
ccaaagcata atatgaaagc atttttggat gaattgaaag ctgagaacat caagaagttc 480
ttatataatt ttacacagat accacattta gcaggaacag aacaaaactt tcagcttgca 540
aagcaaattc aatcccagtg gaaagaattt ggcctggatt ctgttgagct agcacattat 600
gatgtcctgt tgtcctaccc aaataagact catcccaact acatctcaat aattaatgaa 660
gatggaaatg agattttcaa cacatcatta tttgaaccac ctcctccagg atatgaaaat 720
gtttcggata ttgtaccacc tttcagtgct ttctctcctc aaggaatgcc agagggcgat 780
ctagtgtatg ttaactatgc acgaactgaa gacttcttta aattggaacg ggacatgaaa 840
atcaattgct ctgggaaaat tgtaattgcc agatatggga aagttttcag aggaaataag 900
gttaaaaatg cccagctggc aggggccaaa ggagtcattc tctactccga ccctgctgac 960
tactttgctc ctggggtgaa gtcctatcca gatggttgga atcttcctgg aggtggtgtc 1020
cagcgtggaa atatcctaaa tctgaatggt gcaggagacc ctctcacacc aggttaccca 1080
gcaaatgaat atgcttatag gcgtggaatt gcagaggctg ttggtcttcc aagtattcct 1140
gttcatccaa ttggatacta tgatgcacag aagctcctag aaaaaatggg tggctcagca 1200
ccaccagata gcagctggag aggaagtctc aaagtgccct acaatgttgg acctggcttt 1260
actggaaact tttctacaca aaaagtcaag atgcacatcc actctaccaa tgaagtgaca 1320
agaatttaca atgtgatagg tactctcaga ggagcagtgg aaccagacag atatgtcatt 1380
ctgggaggtc accgggactc atgggtgttt ggtggtattg accctcagag tggagcagct 1440
gttgttcatg aaattgtgag gagctttgga acactgaaaa aggaagggtg gagacctaga 1500
agaacaattt tgtttgcaag ctgggatgca gaagaatttg gtcttcttgg ttctactgag 1560
tgggcagagg agaattcaag actccttcaa gagcgtggcg tggcttatat taatgctgac 1620

tcatctatag aaggaaacta cactctgaga gttgattgta caccgctgat gtacagcttg 1680
gtacacaacc taacaaaaga gctgaaaagc cctgatgaag gctttgaagg caaatctctt 1740
tatgaaagtt ggactaaaaa aagtccttcc ccagagttca gtggcatgcc caggataagc 1800
aaattgggat ctggaaatga ttttgaggtg ttcttccaac gacttggaat tgcttcaggc 1860
agagcacggt atactaaaaa ttgggaaaca aacaaattca gcggctatcc actgtatcac 1920
agtgtctatg aaacatatga gttggtggaa aagttttatg atccaatgtt taaatatcac 1980
ctcactgtgg cccaggttcg aggagggatg gtgtttgagc tagccaattc catagtgctc 2040
ccttttgatt gtcgagatta tgctgtagtt ttaagaaagt atgctgacaa aatctacagt 2100
atttctatga aacatccaca ggaaatgaag acatacagtg tatcatttga ttcacttttt 2160
tctgcagtaa agaattttac agaaattgct tccaagttca gtgagagact ccaggacttt 2220
gacaaaagca acccaatagt attaagaatg atgaatgatc aactcatgtt tctggaaaga 2280
gcatttattg atccattagg gttaccagac aggccttttt ataggcatgt catctatgct 2340
ccaagcagcc acaacaagta tgcaggggag tcattcccag gaatttatga tgctctgttt 2400
gatattgaaa gcaaagtgga cccttccaag gcctggggag aagtgaagag acagatttat 2460
gttgcagcct tcacagtgca ggcagctgca gagactttga gtgaagtagc ctaagaggat 2520
tctttagaga atccgtattg aatttgtgtg gtatgtcact cagaaagaat cgtaatgggt 2580
atattgataa attttaaaat tggtatattt gaaataaagt tgaatattat atataaaaaa 2640
aaaaaaaaaa aaa 2653

<210> 14
<211> 2420
<212> DNA
<213> Human antigen (MAGE-1) gene, complete cds

<400> 14
ggatccaggc cctgccagga aaaatataag ggccctgcgt gagaacagag ggggtcatcc 60
actgcatgag agtggggatg tcacagagtc cagcccaccc tcctggtagc actgagaagc 120
cagggctgtg cttgcggtct gcaccctgag ggcccgtgga ttcctcttcc tggagctcca 180
ggaaccaggc agtgaggcct tggtctgaga cagtatcctc aggtcacaga gcagaggatg 240
cacagggtgt gccagcagtg aatgtttgcc ctgaatgcac accaagggcc ccacctgcca 300
caggacacat aggactccac agagtctggc ctcacctccc tactgtcagt cctgtagaat 360
cgacctctgc tggccggctg taccctgagt accctctcac ttcctccttc aggttttcag 420
gggacaggcc aacccagagg acaggattcc ctggaggcca cagaggagca ccaaggagaa 480
gatctgtaag taggcctttg ttagagtctc caaggttcag ttctcagctg aggcctctca 540
cacactccct ctctccccag gcctgtgggt cttcattgcc cagctcctgc ccacactcct 600
gcctgctgcc ctgacgagag tcatcatgtc tcttgagcag aggagtctgc actgcaagcc 660
tgaggaagcc cttgaggccc aacaagaggc cctgggcctg gtgtgtgtgc aggctgccac 720
ctcctcctcc tctcctctgg tcctgggcac cctggaggag gtgcccactg ctgggtcaac 780
agatcctccc cagagtcctc agggagcctc cgcctttccc actaccatca acttcactcg 840
acagaggcaa cccagtgagg gttccagcag ccgtgaagag gaggggccaa gcacctcttg 900
tatcctggag tccttgttcc gagcagtaat cactaagaag gtggctgatt tggttggttt 960
tctgctcctc aaatatcgag ccagggagcc agtcacaaag gcagaaatgc tggagagtgt 1020
catcaaaaat tacaagcact gttttcctga gatcttcggc aaagcctctg agtccttgca 1080
gctggtcttt ggcattgacg tgaaggaagc agaccccacc ggccactcct atgtccttgt 1140
cacctgccta ggtctctcct atgatggcct gctgggtgat aatcagatca tgcccaagac 1200
aggcttcctg ataattgtcc tggtcatgat tgcaatggag ggcggccatg ctcctgagga 1260
ggaaatctgg gaggagctga gtgtgatgga ggtgtatgat gggagggagc acagtgccta 1320
tggggagccc aggaagctgc tcacccaaga tttggtgcag gaaaagtacc tggagtaccg 1380
gcaggtgccg gacagtgatc ccgcacgcta tgagttcctg tggggtccaa gggccctcgc 1440
tgaaaccagc tatgtgaaag tccttgagta tgtgatcaag gtcagtgcaa gagttcgctt 1500
tttcttccca tccctgcgtg aagcagcttt gagagaggag gaagagggag tctgagcatg 1560
agttgcagcc aaggccagtg ggagggggac tgggccagtg caccttccag ggccgcgtcc 1620
agcagcttcc cctgcctcgt gtgacatgag gcccattctt cactctgaag agagcggtca 1680
gtgttctcag tagtaggttt ctgttctatt gggtgacttg gagatttatc tttgttctct 1740
tttggaattg ttcaaatgtt ttttttaag ggatggttga atgaacttca gcatccaagt 1800

ttatgaatga cagcagtcac acagttctgt gtatatagtt taagggtaag agtcttgtgt 1860
tttattcaga ttgggaaatc cattctattt tgtgaattgg gataataaca gcagtggaat 1920
aagtacttag aaatgtgaaa aatgagcagt aaaatagatg agataaagaa ctaaagaaat 1980
taagagatag tcaattcttg ccttatacct cagtctattc tgtaaaattt ttaaagatat 2040
atgcatacct ggatttcctt ggcttctttg agaatgtaag agaaattaaa tctgaataaa 2100
gaattcttcc tgttcactgg ctcttttctt ctccatgcac tgagcatctg ctttttggaa 2160
ggccctgggt tagtagtgga gatgctaagg taagccagac tcatacccac ccatagggtc 2220
gtagagtcta ggagctgcag tcacgtaatc gaggtggcaa gatgtcctct aaagatgtag 2280
ggaaaagtga gagaggggtg agggtgtggg gctccgggtg agagtggtgg agtgtcaatg 2340
ccctgagctg gggcattttg ggctttggga aactgcagtt ccttctgggg gagctgattg 2400
taatgatctt gggtggatcc 2420

<210> 15
<211> 4204
<212> DNA
<213> Human MAGE-3 antigen (MAGE-3) gene, complete cds

<400> 15
acgcaggcag tgatgtcacc cagaccacac cccttccccc aatgccactt caggggtac 60
tcagagtcag agacttggtc tgaggggagc agaagcaatc tgcagaggat ggcggtccag 120
gctcagccag gcatcaactt caggaccctg agggatgacc gaaggccccg cccacccacc 180
cccaactccc ccgaccccac caggatctac agcctcagga cccccgtccc aatccttacc 240
ccttgcccca tcaccatctt catgcttacc tccaccccca tccgatcccc atccaggcag 300
aatccagttc caccctgcc cggaacccag ggtagtaccg ttgccaggat gtgacgccac 360
tgacttgcgc attggaggtc agaagaccgc gagattctcg ccctgagcaa cgagcgacgg 420
cctgacgtcg gcggagggaa gccggcccag gctcggtgag gaggcaaggt aagacgctga 480
gggaggactg aggcgggcct cacctcagac agagggcctc aaataatcca gtgctgcctc 540
tgctgccggg cctgggccac cccgcagggg aagacttcca ggctgggtcg ccactacctc 600
acccccgccga cccccgccgc tttagccacg gggaactctg gggacagagc ttaatgtggc 660
cagggcaggg ctggttagaa gaggtcaggg cccacgctgt ggcaggaatc aaggtcagga 720
ccccgagagg gaactgaggg cagcctaacc accaccctca ccaccattcc cgtcccccaa 780
cacccaaccc cacccccatc ccccattccc atccccaccc ccaccctat cctggcagaa 840
tccgggcttt gcccctggta tcaagtcacg gaagctccgg gaatggcggc caggcacgtg 900
agtcctgagg ttcacatcta cggctaaggg agggaagggg ttcggtatcg cgagtatggc 960
cgttgggagg cagcgaaagg gcccaggcct cctggaagac agtggagtcc tgaggggacc 1020
cagcatgcca ggacaggggg cccactgtac ccctgtctca aaccgaggca ccttttcatt 1080
cggctacggg aatcctaggg atgcagaccc acttcagcag ggggttgggg cccagccctg 1140
cgaggagtca tggggaggaa gaagagggag gactgagggg accttggagt ccagatcagt 1200
ggcaaccttg ggctgggggа tgctgggcac agtggccaaa tgtgctctgt gctcattgcg 1260
ccttcagggt gaccagagag ttgagggctg tggtctgaag agtgggactt caggtcagca 1320
gagggaggaa tcccaggatc tgcagggccc aaggtgtacc cccaaggggc ccctatgtgg 1380
tggacagatg cagtggtcct aggatctgcc aagcatccag gtgaagagac tgagggagga 1440
ttgagggtac ccctgggaca gaatgcggac tgggggcccc ataaaaatct gccctgctcc 1500
tgctgttacc tcagagagcc tgggcagggc tgtcagctga ggtccctcca ttatcctagg 1560
atcactgatg tcagggaagg ggaagccttg gtctgagggg gctgcactca gggcagtaga 1620
gggaggctct cagaccctac taggagtgga ggtgaggacc aagcagtctc ctcacccagg 1680
gtacatggac ttcaataaat ttggacatct ctcgttgtcc tttccgggag gacctgggaa 1740
tgtatggcca gatgtgggtc ccctcatgtt tttctgtacc atatcaggta tgtgagttct 1800
tgacatgaga gattctcagg ccagcagaag ggagggatta ggccctataa ggagaaaggt 1860
gagggccctg agtgagcaca gaggggatcc tccacccccag tagagtgggg acctcacaga 1920
gtctggccaa ccctcctgac agttctggga atccgtggct gcgtttgctg tctgcacatt 1980
gggggcccgt ggattcctct cccaggaatc aggagctcca ggaacaaggc agtgaggact 2040
tggtctgagg cagtgtcctc aggtcacaga gtagaggggg ctcagatagt gccaacggtg 2100
aaggtttgcc ttggattcaa accaagggcc ccacctgccc cagaacacat ggactccaga 2160
gcgcctggcc tcaccctcaa tactttcagt cctgcagcct cagcatgcgc tggccggatg 2220

taccctgagg tgccctctca cttcctcctt caggttctga ggggacaggc tgacctggag 2280
gaccagaggc ccccggagga gcactgaagg agaagatctg taagtaagcc tttgttagag 2340
cctccaaggt tccattcagt actcagctga ggtctctcac atgctccctc tctccccagg 2400
ccagtgggtc tccattgccc agctcctgcc cacactcccg cctgttgccc tgaccagagt 2460
catcatgcct cttgagcaga ggagtcagca ctgcaagcct gaagaaggcc ttgaggcccg 2520
aggagaggcc ctgggcctgg tgggtgcgca ggctcctgct actgaggagc aggaggctgc 2580
ctcctcctct tctactctag ttgaagtcac cctgggggag gtgcctgctg ccgagtcacc 2640
agatcctccc cagagtcctc agggagcctc cagcctcccc actaccatga actaccctct 2700
ctggagccaa tcctatgagg actccagcaa ccaagaagag gaggggccaa gcaccttccc 2760
tgacctggag tccgagttcc aagcagcact cagtaggaag gtggccgagt tggttcattt 2820
tctgctcctc aagtatcgag ccagggagcc ggtcacaaag gcagaaatgc tggggagtgt 2880
cgtcggaaat tggcagtatt tctttcctgt gatcttcagc aaagcttcca gttccttgca 2940
gctggtcttt ggcatcgagc tgatggaagt ggaccccatc ggccacttgt acatctttgc 3000
cacctgcctg ggcctctcct acgatggcct gctgggtgac aatcagatca tgcccaaggc 3060
aggcctcctg ataatcgtcc tggccataat cgcaagagag ggcgactgtg cccctgagga 3120
gaaaatctgg gaggagctga gtgtgttaga ggtgtttgag gggagggaag acagtatctt 3180
gggggatccc aagaagctgc tcacccaaca tttcgtgcag gaaaactacc tggagtaccg 3240
gcaggtcccc ggcagtgatc ctgcatgtta tgaattcctg tggggtccaa gggccctcgt 3300
tgaaaccagc tatgtgaaag tcctgcacca tatggtaaag atcagtggag gacctcacat 3360
ttcctaccca cccctgcatg agtgggtttt gagagagggg gaagagtgag tctgagcacg 3420
agttgcagcc agggccagtg ggaggggctc tgggccagtg caccttccgg ggccgcatcc 3480
cttagtttcc actgcctcct gtgacgtgag gcccattctt cactctttga agcgagcagt 3540
cagcattctt agtagtgggt ttctgttctg ttggatgact ttgagattat tctttgtttc 3600
ctgttggagt tgttcaaatg ttccttttaa cggatggttg aatgagcgtc agcatccagg 3660
tttatgaatg acagtagtca cacatagtgc tgtttatata gtttaggagt aagagtcttg 3720
ttttttactc aaattgggaa atccattcca ttttgtgaat tgtgacataa taatagcagt 3780
ggtaaaagta tttgcttaaa attgtgagcg aattagcaat aacatacatg agataactca 3840
agaaatcaaa agatagttga ttcttgcctt gtacctcaat ctattctgta aaattaaaca 3900
aatatgcaaa ccaggatttc cttgacttct ttgagaatgc aagcgaaatt aaatctgaat 3960
aaataattct tcctcttcac tggctcgttt cttttccgtt cactcagcat ctgctctgtg 4020
ggaggccctg ggttagtagt ggggatgcta aggtaagcca gactcacgcc tacccatagg 4080
gctgtagagc ctaggacctg cagtcatata attaaggtgg tgagaagtcc tgtaagatgt 4140
agaggaaatg taagagaggg gtgagggtgt ggcgctccgg gtgagagtag tggagtgtca 4200
gtgc 4204

<210> 16
<211> 752
<212> DNA
<213> Human autoimmunogenic cancer/testis antigen NY-ESs

<400> 16
atcctcgtgg gccctgacct tctctctgag agccgggcag aggctccgga gccatgcagg 60
ccgaaggccg gggcacaggg ggttcgacgg gcgatgctga tggcccagga ggccctggca 120
ttcctgatgg cccagggggc aatgctggcg gcccaggaga ggcgggtgcc acgggcggca 180
gaggtccccg gggcgcaggg gcagcaaggg cctcggggcc gggaggaggc gccccgcggg 240
gtccgcatgg cggcgcggct tcagggctga atggatgctg cagatgcggg gccaggggcc 300
cggagagccg cctgcttgag ttctacctcg ccatgccttt cgcgacaccc atggaagcag 360
agctggcccg caggagcctg gcccaggatg ccccaccgct tcccgtgcca ggggtgcttc 420
tgaaggagtt cactgtgtcc ggcaacatac tgactatccg actgactgct gcagaccacc 480
gccaactgca gctctccatc agctcctgtc tccagcagct ttcccctgttg atgtggatca 540
cgcagtgctt tctgcccgtg tttttggctc agcctccctc agggcagagg cgctaagccc 600
agcctggcgc cccttcctag gtcatgcctc ctcccctagg gaatggtccc agcacgagtg 660
gccagttcat tgtgggggcc tgattgtttg tcgctggagg aggacggctt acatgtttgt 720
ttctgtagaa aataaaactg agctacgaaa aa 752

<210> 17
<211> 2148
<212> DNA
<213> Homo sapiens preferentially expressed antigen in A

<400> 17
gcttcagggt acagctcccc cgcagccaga agccgggcct gcagcgcctc agcaccgctc 60
cgggacaccc cacccgcttc ccaggcgtga cctgtcaaca gcaacttcgc ggtgtggtga 120
actctctgag gaaaaaccat tttgattatt actctcagac gtgcgtggca acaagtgact 180
gagacctaga aatccaagcg ttggaggtcc tgaggccagc ctaagtcgct tcaaaatgga 240
acgaaggcgt ttgtggggtt ccattcagag ccgatacatc agcatgagtg tgtggacaag 300
cccacggaga cttgtggagc tggcagggca gagcctgctg aaggatgagg ccctggccat 360
tgccgccctg gagttgctgc ccagggagct cttcccgcca ctcttcatgg cagcctttga 420
cgggagacac agccagaccc tgaaggcaat ggtgcaggcc tggcccttca cctgcctccc 480
tctgggagtg ctgatgaagg gacaacatct tcacctggag accttcaaag ctgtgcttga 540
tggacttgat gtgctccttg cccaggaggt tcgccccagg aggtggaaac ttcaagtgct 600
ggatttacgg aagaactctc atcaggactt ctggactgta tggtctggaa acagggccag 660
tctgtactca tttccagagc cagaagcagc tcagcccatg acaaagaagc gaaaagtaga 720
tggtttgagc acagaggcag agcagccctt cattccagta gaggtgctcg tagacctgtt 780
cctcaaggaa ggtgcctgtg atgaattgtt ctcctacctc attgagaaag tgaagcgaaa 840
gaaaaatgta ctacgcctgt gctgtaagaa gctgaagatt tttgcaatgc ccatgcagga 900
tatcaagatg atcctgaaaa tggtgcagct ggactctatt gaagatttgg aagtgacttg 960
tacctggaag ctacccacct tggcgaaatt ttctccttac ctgggccaga tgattaatct 1020
gcgtagactc ctcctctccc acatccatgc atcttcctac atttccccgg agaaggaaga 1080
gcagtatatc gcccagttca cctctcagtt cctcagtctg cagtgcctgc aggctctcta 1140
tgtggactct ttatttttcc ttagaggccg cctggatcag ttgctcaggc acgtgatgaa 1200
ccccttggaa accctctcaa taactaactg ccggctttcg gaaggggatg tgatgcatct 1260
gtcccagagt cccagcgtca gtcagctaag tgtcctgagt ctaagtgggg tcatgctgac 1320
cgatgtaagt cccgagcccc tccaagctct gctggagaga gcctctgcca ccctccagga 1380
cctggtcttt gatgagtgtg ggatcacgga tgatcagctc cttgccctcc tgccttccct 1440
gagccactgc tcccagctta caaccttaag cttctacggg aattccatct ccatatctgc 1500
cttgcagagt ctcctgcagc acctcatcgg gctgagcaat ctgacccacg tgctgtatcc 1560
tgtccccctg gagagttatg aggacatcca tggtaccctc cacctggaga ggcttgccta 1620
tctgcatgcc aggctcaggg agttgctgtg tgagttgggg cggcccagca tggtctggct 1680
tagtgcccaac ccctgtcctc actgtgggga cagaaccttc tatgacccgg agcccatcct 1740
gtgcccctgt ttcatgccta actagctggg tgcacatatc aaatgcttca ttctgcatac 1800
ttggacacta aagccaggat gtgcatgcat cttgaagcaa caaagcagcc acagtttcag 1860
acaaatgttc agtgtgagtg aggaaaacat gttcagtgag gaaaaaacat tcagacaaat 1920
gttcagtgag gaaaaaaagg ggaagttggg gataggcaga tgttgacttg aggagttaat 1980
gtgatctttg gggagataca tcttatagag ttagaaatag aatctgaatt tctaaaggga 2040
gattctggct tgggaagtac atgtaggagt taatccctgt gtagactgtt gtaaagaaac 2100
tgttgaaaat aaagagaagc aatgtgaagc aaaaaaaaaa aaaaaaaa 2148

<210> 18
<211> 4530
<212> DNA
<213> Human tyrosine kinase-type receptor (HER2) mRNA, s

<400> 18
aattctcgag ctcgtcgacc ggtcgacgag ctcgagggtc gacgagctcg agggcgcgcg 60
cccggccccc acccctcgca gcaccccgcg ccccgcgccc tcccagccgg gtccagccgg 120
agccatgggg ccggagccgc agtgagcacc atggagctgg cggccttgtg ccgctggggg 180
ctcctcctcg ccctcttgcc ccccggagcc gcgagcaccc aagtgtgcac cggcacagac 240
atgaagctgc ggctcctgc cagtcccgag acccacctgg acatgctccg ccacctctac 300
cagggctgcc aggtggtgca gggaaacctg gaactcacct acctgcccac caatgccagc 360

ctgtccttcc tgcaggatat ccaggaggtg cagggctacg tgctcatcgc tcacaaccaa 420
gtgaggcagg tcccactgca gaggctgcgg attgtgcgag gcacccagct ctttgaggac 480
aactatgccc tggccgtgct agacaatgga gacccgctga acaataccac ccctgtcaca 540
ggggcctccc caggaggcct gcgggagctg cagcttcgaa gcctcacaga gatcttgaaa 600
ggaggggtct tgatccagcg gaacccccag ctctgctacc aggacacgat tttgtggaag 660
gacatcttcc acaagaacaa ccagctggct ctcacactga tagacaccaa ccgctctcgg 720
gcctgccacc cctgttctcc gatgtgtaag ggctcccgct gctggggaga gagttctgag 780
gattgtcaga gcctgacgcg cactgtctgt gccggtggct gtgcccgctg caaggggcca 840
ctgcccactg actgctgcca tgagcagtgt gctgccggct gcacgggccc caagcactct 900
gactgcctgg cctgcctcca cttcaaccac agtggcatct gtgagctgca ctgcccagcc 960
ctggtcacct acaacacaga cacgtttgag tccatgccca atcccgaggg ccggtataca 1020
ttcggcgcca gctgtgtgac tgcctgtccc tacaactacc tttctacgga cgtgggatcc 1080
tgcaccctcg tctgcccctt gcacaaccaa gaggtgacag cagaggatgg aacacagcgg 1140
tgtgagaagt gcagcaagcc ctgtgcccga gtgtgctatg gtctgggcat ggagcacttg 1200
cgagaggtga gggcagttac cagtgccaat atccaggagt ttgctggctg caagaagatc 1260
tttgggagcc tggcatttct gccggagagc tttgatgggg acccagcctc caacactgcc 1320
ccgctccagc cagagcagct ccaagtgttt gagactctgg aagagatcac aggttaccta 1380
tacatctcag catggccgga cagcctgcct gacctcagcg tcttccagaa cctgcaagta 1440
atccggggac gaattctgca caatggcgcc tactcgctga ccctgcaagg gctgggcatc 1500
agctggctgg ggctgcgctc actgagggaa ctgggcagtg gactggccct catccaccat 1560
aacacccacc tctgcttcgt gcacacggtg ccctgggacc agctctttcg gaacccgcac 1620
caagctctgc tccacactgc caaccggcca gaggacgagt gtgtgggcga gggcctggcc 1680
tgccaccagc tgtgcgcccg agggcactgc tggggtccag ggcccaccca gtgtgtcaac 1740
tgcagccagt tccttcgggg ccaggagtgc gtggaggaat gccgagtact gcaggggctc 1800
cccagggagt atgtgaatgc caggcactgt ttgccgtgcc accctgagtg tcagccccag 1860
aatggctcag tgacctgttt tggaccggag gctgaccagt gtgtggcctg tgcccactat 1920
aaggaccctc ccttctgcgt ggcccgctgc cccagcggtg tgaaacctga cctctcctac 1980
atgcccatct ggaagtttcc agatgaggag ggcgcatgcc agccttgccc catcaactgc 2040
acccactcct gtgtggacct ggatgacaag ggctgccccg ccgagcagag agccagccct 2100
ctgacgtcca tcgtctctgc ggtggttggc attctgctgg tcgtggtctt gggggtggtc 2160
tttgggatcc tcatcaagcg acggcagcag aagatccgga agtacacgat gcggagactg 2220
ctgcaggaaa cggagctggt ggagccgctg acacctagcg gagcgatgcc caaccaggcg 2280
cagatgcgga tcctgaaaga gacggagctg aggaaggtga aggtgcttgg atctggcgct 2340
tttggcacag tctacaaggg catctggatc cctgatgggg agaatgtgaa aattccagtg 2400
gccatcaaag tgttgaggga aaacacatcc cccaaagcca acaaagaaat cttagacgaa 2460
gcatacgtga tggctggtgt gggctcccca tatgtctccc gccttctggg catctgcctg 2520
acatccacgg tgcagctggt gacacagctt atgccctatg gctgcctctt agaccatgtc 2580
cgggaaaacc gcggacgcct gggctcccag gacctgctga actggtgtat gcagattgcc 2640
aaggggatga gctacctgga ggatgtgcgg ctcgtacaca gggacttggc cgctcggaac 2700
gtgctggtca agagtcccaa ccatgtcaaa attacagact tcgggctggc tcggctgctg 2760
gacattgacg agacagagta ccatgcagat gggggcaagg tgcccatcaa gtggatggcg 2820
ctggagtcca ttctccgccg gcggttcacc caccagagtg atgtgtggag ttatggtgtg 2880
actgtgtggg agctgatgac ttttggggcc aaaccttacg atgggatccc agcccgggag 2940
atccctgacc tgctggaaaa gggggagcgg ctgccccagc cccccatctg caccattgat 3000
gtctacatga tcatggtcaa atgttggatg attgactctg aatgtcggcc aagattccgg 3060
gagttggtgt ctgaattctc ccgcatggcc agggaccccc agcgctttgt ggtcatccag 3120
aatgaggact tgggcccagc cagtcccttg gacagcacct tctaccgctc actgctggag 3180
gacgatgaca tgggggacct ggtggatgct gaggagtatc tggtacccca gcagggcttc 3240
ttctgtccag accctgcccc gggcgctggg ggcatggtcc accacaggca ccgcagctca 3300
tctaccagga gtggcggtgg ggacctgaca ctagggctgg agccctctga agaggaggcc 3360
cccaggtctc cactggcacc ctccgaaggg gctggctccg atgtatttga tggtgacctg 3420
ggaatggggg cagccaaggg gctgcaaagc ctccccacac atgacccccag ccctctacag 3480
cggtacagtg aggaccccac agtacccctg ccctctgaga ctgatggcta cgttgccccc 3540
ctgacctgca gcccccagcc tgaatatgtg aaccagccag atgttcggcc ccagccccct 3600
tcgccccgag agggccctct gcctgctgcc cgacctgctg gtgccactct ggaaagggcc 3660

```
aagactctct ccccagggaa gaatggggtc gtcaaagacg tttttgcctt tgggggtgcc 3720
gtggagaacc ccgagtactt gacaccccag ggaggagctg cccctcagcc ccaccctcct 3780
cctgccttca gcccagcctt cgacaacctc tattactggg accaggaccc accagagcgg 3840
ggggctccac ccagcacctt caaagggaca cctacggcag agaacccaga gtacctgggt 3900
ctggacgtgc cagtgtgaac cagaaggcca agtccgcaga agccctgatg tgtcctcagg 3960
gagcagggaa ggcctgactt ctgctggcat caagaggtgg gagggccctc cgaccacttc 4020
caggggaacc tgccatgcca ggaacctgtc ctaaggaacc ttccttcctg cttgagttcc 4080
cagatggctg gaaggggtcc agcctcgttg gaagaggaac agcactgggg agtctttgtg 4140
gattctgagg ccctgcccaa tgagactcta gggtccagtg gatgccacag cccagcttgg 4200
ccctttcctt ccagatcctg ggtactgaaa gccttaggga agctggcctg agaggggaag 4260
cggccctaag ggagtgtcta agaacaaaag cgacccattc agagactgtc cctgaaacct 4320
agtactgccc cccatgagga aggaacagca atggtgtcag tatccaggct ttgtacagag 4380
tgcttttctg tttagttttt actttttttg ttttgttttt ttaaagacga aataaagacc 4440
caggggagaa tgggtgttgt atggggaggc aagtgtgggg ggtccttctc cacacccact 4500
ttgtccattt gcaaatatat tttggaaaac                                  4530
```