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(56) Related Art
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**Reynolds et al (2000) Journal of Immunological Methods, 244:59-67**
**Kessler et al (2001) Journal of Experimental Medicine, 193(1):73-88**
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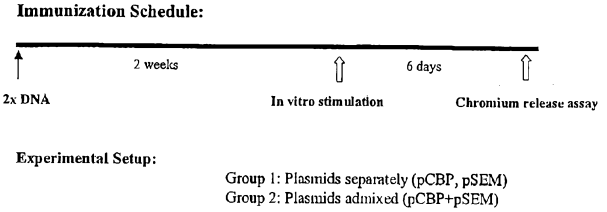
*[Continued on next page]*

(54) Title: USE OF COMPOSITIONS COMPRISING VARIOUS TUMOR-ASSOCIATED ANTIGENS AS ANTI-CANCER VACCINES

Schedule of immunization with plasmids (pCBP expressing SSX2 41-49; and pSEM expressing Melan A)

**Schedule of immunization**




(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.

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

## Cross Reference to Related Applications

**[0001]** This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/640,598, filed on December 29, 2004, entitled COMBINATIONS OF TUMOR-ASSOCIATED ANTIGENS IN COMPOSITIONS FOR VARIOUS TYPES OF CANCERS; the disclosure of which is hereby expressly incorporated by reference in its entirety.

## Background of the Invention

### Field of the Invention

**[0002]** 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

**[0003]** 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.

**[0004]** 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 typically begin with out-of-control growth of abnormal cells.

**[0005]** 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.

**[0006]** 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 immunotherapies 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.

**[0007]** A continuing need exists for additional treatment options.

## Summary of the Invention

**[0008]** Embodiments of the invention disclosed herein are directed to the use of effective combinations of tumor-associated antigens (TuAAs) for the immunotherapy of patients with various types of cancer. In some embodiments, the TuAAs are antigens expressed by the cancer cell itself. In some embodiments, the TuAAs are antigens associated with non-cancerous components of the tumor, such as tumor-associated neovasculature or other stroma. In some embodiments, the combinations further include a tumor growth factor and/or a signal transduction protein. Both immunogenic compositions for inducing an immune response to these combinations of antigens and methods for their use are disclosed.

**[0009]** 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 in preferred embodiments to administer to the patient some portion of the antigen, or some other immunogenic product that is capable of inducing a specific immune response directed against the antigen. Accordingly, in some embodiments, immunizing against PRAME includes administering a complete and intact PRAME antigen to the patient. In some embodiments, immunizing against PRAME includes 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 as a representative antigen for the purpose of discussion, any antigen against which a patient can be immunized can be used.

**[0010]** Some embodiments relate to methods of treating ovarian or colorectal cancer. The methods can include the step of immunizing a patient against, for example, PRAME and/or PSMA and at least one other tumor associated antigen. In some embodiments, the at least one other tumor associated antigen can include, for example,

SSX-2, NY-ESO-1, PSMA, PRAME, mesothelin, a MAGE protein, MAGE-3, Melan-A, PLK1, VEGF-A, VEGFR2, Tie-2, and the like, or subsets thereof. Preferably, the methods include immunizing against PRAME, NY-ESO-1 and/or SSX-2, for example. More preferably, the methods include immunizing against PRAME, NY-ESO-1, SSX-2, and PSMA, for example. The methods 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/or Tie-2. The methods can further include immunizing against a growth factor, such as VEGF-A, and/or a signal transduction protein, such as PLK1. In preferred embodiments, the methods induce a cytolytic T cell response.

**[0011]** In some embodiments, methods of inducing an anti-cancer immune response in the treatment of ovarian or colorectal cancer are disclosed. The methods can include, for example, the step of immunizing a patient against PRAME and/or PSMA and at least one tumor-associated antigen. In some embodiments, the at least one other tumor-associated antigen can be, for example, SSX-2, NY-ESO-1, PSMA, PRAME, mesothelin, a MAGE protein, MAGE-3, Melan-A, PLK1, VEGF-A, VEGFR2, Tie-2, and the like, or subsets thereof. For example, in some embodiments, the methods can include immunization against PRAME, NY-ESO-1, and/or SSX-2. The methods can further include immunizing against PSMA. The methods 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/or Tie-2. The methods can further include immunizing against a growth factor, such as VEGF-A, and/or a signal transduction protein, such as PLK1. In preferred embodiments, the anti-cancer immune response is a CTL response.

**[0012]** Other embodiments relate to methods of treating pancreatic cancer. The methods can include the step of immunizing against, for example, PRAME and/or PSMA and at least one other tumor-associated antigen. In an embodiment, the methods further include immunizing against at least one antigen selected from an antigen associated with tumor neovasculature, a growth factor and a signal transduction protein. The at least one other tumor-associated antigen can be, for example, PRAME, PSMA, mesothelin, SSX-2 and/or NY-ESO-1, a MAGE protein, MAGE-3, Melan-A, PLK1, VEGF-A, VEGFR2, Tie-2, and the like, or subsets thereof. For example, the methods can include immunizing against PSMA, NY-ESO-1, and/or SSX-2. In some embodiments,

the methods can include immunizing against PRAME, NY-ESO-1, and/or SSX-2. In some embodiments, the methods can include immunizing against PRAME, PSMA, NY-ESO-1, and SSX-2. The methods 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/or Tie-2. In some embodiments, the growth factor is VEGF-A. In some embodiments, the signal transduction protein is PLK1. In a preferred embodiment, the methods induce a cytolytic T cell response.

**[0013]** Further, in some embodiments, methods of inducing an anti-cancer immune response in the treatment of pancreatic cancer are disclosed. The methods can include, for example, the step of immunizing a patient against PRAME and/or PSMA and at least one tumor-associated antigen. In an embodiment, the methods further include immunizing against at least one antigen selected from an antigen associated with tumor neovasculature, a growth factor and a signal transduction protein. In some embodiments, the at least one other tumor-associated antigen can be, for example, PRAME, PSMA, SSX-2, NY-ESO-1, mesothelin, a MAGE protein, MAGE-3, Melan-A, PLK1, VEGF-A, VEGFR2, Tie-2, and the like, or subsets thereof. For example, the methods can include immunization against PSMA and NY-ESO-1, and/or SSX-2. In some embodiments, the methods can include immunizing against PRAME and NY-ESO-1 and/or SSX-2. In some embodiments, the methods can include immunizing against PRAME, PSMA and NY-ESO-1 and/or SSX-2. The methods 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/or Tie-2. In some embodiments, the growth factor is VEGF-A. In some embodiments, the signal transduction protein is PLK1. In preferred embodiments, the anti-cancer immune response is a CTL response.

**[0014]** 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 a MAGE protein, MAGE-3, Melan-A, mesothelin, SSX-2, NY-ESO-1, PRAME, PSMA, VEGF-A, PLK1, VEGFR2, Tie-2, and the like, or subsets thereof. Preferably, the methods can include immunizing against PSMA, NY-ESO-1 and/or SSX-2. More preferably, the methods can include immunization against PSMA,

NY-ESO-1, SSX-2, and/or MAGE-3, for example. The methods 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/or Tie-2. The methods can further include immunizing against a growth factor, such as VEGF-A, and/or a signal transduction protein, such as PLK1. In a preferred embodiment, the methods can induce a cytolytic T cell response.

**[0015]** In a further embodiment, methods of inducing an anti-cancer immune response in the treatment of non-small cell lung cancer are disclosed. The methods can include, for example, the step of immunizing a patient against PSMA and at least one tumor-associated antigen. The at least one other tumor-associated antigen can be, for example, a MAGE protein, MAGE-3, Melan-A, mesothelin, SSX-2, NY-ESO-1, PRAME, PSMA, VEGF-A, PLK1, VEGFR2, Tie-2, and the like, or subsets thereof. For example, the methods can include immunization against PSMA, NY-ESO-1, and/or SSX-2. The methods can further include immunization against PSMA, NY-ESO-1, SSX-2, and/or MAGE-3. In an embodiment, the methods 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/or Tie-2. The methods can further include immunizing against a growth factor, such as VEGF-A, and/or a signal transduction protein, such as PLK1. In preferred embodiments, the anti-cancer immune response is a CTL response.

**[0016]** Other embodiments relate to methods of treating renal cell carcinoma. The methods can include the step of immunizing a patient against, for example, PSMA and/or PRAME and at least one other tumor associated antigen. The at least one other tumor-associated antigen can be, for example, PRAME, PSMA, SSX-2, NY-ESO-1, a MAGE protien, MAGE-3, Melan-A, mesothelin, VEGF-A, VEGFR2, Tie-2, and the like, or subsets thereof. Preferably, the methods can include immunizing against PSMA, PRAME, and/or SSX-2, for example. The methods 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/or Tie-2. In a preferred embodiment, the methods induce a cytolytic T cell response.

**[0017]** Further, in some embodiments, methods of inducing an anti-cancer immune response in the treatment of renal cell carcinoma are disclosed. The methods 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, PSMA, SSX-2, NY-ESO-1, a MAGE protien, MAGE-3, Melan-A, mesothelin, VEGF-A, VEGFR2, Tie-2, and the like, or subsets thereof. For example, the methods can include immunization against PSMA, PRAME, and/or SSX-2. The methods 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/or Tie-2. In preferred embodiments, the anti-cancer immune response is a CTL response.

**[0018]** 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. In some embodiments, the methods can include immunizing against Melan-A, SSX-2, and/or NY-ESO. In some embodiments, the methods can include immunization against Melan-A, SSX-2, and/or tyrosinase, for example. The methods 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/or Tie-2. The methods can further include immunizing against a growth factor, such as VEGF-A, and/or a signal transduction protein, such as PLK1. In a preferred embodiment, the methods induce a cytolytic T cell response.

**[0019]** In some embodiments, methods of inducing an anti-cancer immune response in the treatment of melanoma are disclosed. The methods 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/or NY-ESO. Alternatively, the method can include, for example, immunization against Melan-A, SSX-2, and/or 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/or Tie-2. The methods can further include immunizing against a growth factor, such

as VEGF-A, and/or a signal transduction protein, such as PLK1. In preferred embodiments, the anti-cancer immune response is a CTL response.

**[0020]** Some embodiments relate to the use of a composition comprising, for example, PRAME and/or PSMA and at least one other tumor associated antigen, in the preparation of a medicament for the treatment of ovarian or colorectal cancer. In some embodiments the at least one other tumor associated antigen can include, for example, SSX-2, NY-ESO-1, PSMA, PRAME, mesothelin, a MAGE protein, MAGE-3, Melan-A, PLK1, VEGF-A, VEGFR2, Tie-2, and the like, or subsets thereof. Thus, the composition can comprise NY-ESO-1 and/or SSX-2, for example. In some embodiments, the composition can comprise NY-ESO-1, SSX-2, and/or PSMA, for example. The composition can further include at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2, and/or Tie-2. In a preferred embodiment, the medicament induces a cytolytic T cell response.

**[0021]** Other embodiments relate to the use of a composition comprising, for example, PRAME and/or PSMA and at least one other tumor-associated antigen in the preparation of medicament for the treatment of pancreatic cancer. The composition can further include an antigen associated with tumor neovasculature, a growth factor, or a signal transduction protein. The at least one other tumor-associated antigen can be, for example, PRAME, PSMA, SSX-2 and/or NY-ESO-1, mesothelin, a MAGE protein, MAGE-3, Melan-A, PLK1, VEGF-A, VEGFR2, Tie-2, and the like, or subsets thereof. In an embodiment, the composition comprises PSMA and NY-ESO-1, and/or SSX-2. In an embodiment, the composition comprises PRAME and NY-ESO-1, and/or SSX-2. The composition can further include at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2 and/or Tie-2. The growth factor can be VEGF-A, for example. The signal transduction protein can be, for example, PLK1. In a preferred embodiment, the medicament can induce a cytolytic T cell response. In some embodiments, the medicament is for use in combination with at least one additional medicament comprising a tumor-associated antigen.

**[0022]** 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 a MAGE protein, MAGE-3, Melan-A, mesothelin, SSX-2, NY-ESO-1, PRAME, PSMA, VEGF-A, PLK1, VEGFR2, Tie-2, and the like, or subsets thereof. Preferably, the composition comprises PSMA, NY-ESO-1 and/or SSX-2. More preferably, the composition comprises PSMA, NY-ESO-1, SSX-2, and/or MAGE-3, for example. The composition can further include at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2 and/or Tie-2. In a preferred embodiment, the medicament can induce a cytolytic T cell response.

**[0023]** Alternative embodiments relate to the use of a composition comprising PRAME and/or PSMA and at least one other tumor associated antigen in the preparation of a medicament for the treatment of renal cell carcinoma. The at least one other tumor-associated antigen can be, for example, PSMA, PRAME, SSX-2, NY-ESO-1, a MAGE protien, MAGE-3, Melan-A, mesothelin, VEGF-A, VEGFR2, Tie-2, and the like, or subsets thereof. Preferably, the composition comprises PSMA, PRAME, and/or SSX-2, for example. The composition can further include at least one antigen associated with tumor neovasculature. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2 and/or Tie-2. In a preferred embodiment, the medicament can induce a cytolytic T cell response.

**[0024]** 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/or NY-ESO. More preferably, the composition comprises Melan-A, SSX-2, and/or 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/or Tie-2. The composition can further include a growth factor, such as VEGF-A and/or a signal transduction protein, such as PLK1. In a preferred embodiment, the medicament can induce a cytolytic T cell response.

**[0025]** Other embodiments relate to immunogenic compositions for inducing an anti-cancer immune response, comprising, 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 comprise a first antigen selected from PRAME, PSMA, and/or tyrosinase; and at least one other tumor-associated antigen. The cancer treated by the immunogenic compositions can be, for example, ovarian cancer, colorectal cancer, pancreatic cancer, non-small cell lung cancer, melanoma, renal cell carcinoma, and the like. In a preferred embodiment, the at least one other tumor-associated antigen is selected from NY-ESO-1, SSX-2, a MAGE protein, MAGE-3, mesothelin, Melan-A, VEGFR2, Tie-2, and the like, or subsets thereof. In some embodiments, the compositions can further include a neovasculature antigen or other stromal cell antigen. In some embodiments, the compositions can also include an extra-cellular factor. In some embodiments, the compositions further include a non-target antigen. In some embodiments, the compositions can include a means for inducing immunity to a factor that promotes the growth, survival, invasiveness, or metastasis of a tumor. The compositions can also include a means for inducing bystander help for the tumor-associated antigens. In some embodiments, the compositions can include a means for causing inflammation in a tumor lesion. The compositions can further include a growth factor and/or a signal transduction protein. The growth factor can be, for example, VEGF-A. The signal transduction factor can be, for example, PLK1.

**[0026]** Still other embodiments relate to immunogenic compositions for the treatment of 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 PRAME and/or PSMA and/or tyrosinase and at least one other tumor-associated antigen. In a preferred embodiment, the at least one other tumor-associated antigen can be selected from SSX-2 and/or NY-ESO-1, a MAGE protein, MAGE-3, mesothelin, Melan-A, VEGFR2, Tie-2, and the like, or subsets thereof. The cancer treated can be, for example, ovarian cancer, colorectal cancer, pancreatic cancer, non-small cell lung cancer, melanoma, renal cell carcinoma, and the like. The compositions can further include at least one antigen associated with tumor neovasculature or stroma. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2 and/or Tie-2. Thus, in some embodiments, immunogenic compositions comprising a PSMA antigen and further comprising at least one additional antigen selected from PRAME, NY-ESO and SSX-2 are disclosed. In some embodiments, immunogenic compositions comprising a PRAME antigen and further

comprising at least additional antigen selected from PRAME, NY-ESO and SSX-2 are disclosed. In some embodiments, the compositions further include an extra-cellular factor. In some embodiments, the compositions further include a non-target antigen. In some embodiments, the compositions further include a means for inducing immunity to a factor that promotes the growth, survival, invasiveness, or metastasis of a tumor. In some embodiments, the compositions further comprise a means for inducing bystander help for the tumor-associated antigens. In some embodiments, the compositions further comprise a means for causing inflammation in a tumor lesion. The compositions can further include a growth factor and/or a signal transduction protein. The growth factor can be, for example, VEGF-A. The signal transduction factor can be, for example, PLK1.

**[0027]** Other embodiments relate to immunogenic compositions for the treatment of or for inducing an anti-cancer 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 a first antigen selected from PRAME, PSMA, and/or tyrosinase and at least one other tumor-associated antigen. The at least one other tumor-associated antigen can include, for example, SSX-2, NY-ESO-1, a MAGE protein, MAGE-3, Melan-A, mesothelin, VEGF-A, PLK1, PRAME, PSMA, VEGFR2, Tie-2, and the like, or subsets thereof. The compositions can further include at least one antigen associated with tumor neovasculature or stroma. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2 and/or Tie-2. The compositions can further include a growth factor and/or a signal transduction protein. The growth factor can be, for example, VEGF-A. The signal transduction factor can be, for example, PLK1.

**[0028]** Other embodiments relate to immunogenic compositions for the treatment of, or for inducing an anti-cancer 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 are PRAME and/or PSMA and at least one other tumor-associated antigen. In a preferred embodiment, the at least one other tumor-associated antigen can be selected from PSMA, PRAME, mesothelin, SSX-2, NY-ESO-1, a MAGE protein, MAGE-3, Melan-A, VEGF-

A, PLK1, VEGFR2, Tie-2, and the like, or subsets thereof. In some embodiments, the composition comprises PRAME and NY-ESO-1 and/or SSX-2. In some embodiments, the composition comprises PSMA and NY-ESO-1 and/or SSX-2. In some embodiments, the composition comprises PRAME, PSMA and NY-ESO-1 and/or SSX-2. In some embodiments, the compositions can further include a neovasculature antigen or other stromal cell antigen. The antigen associated with tumor neovasculature can be, PSMA, VEGFR2 and/or Tie-2. In some embodiments, the compositions can also include an extra-cellular factor. In some embodiments, the compositions further include a non-target antigen. In other embodiments, the compositions can include a means for inducing immunity to a factor that promotes the growth, survival, invasiveness, or metastasis of a tumor. The compositions can also include a means for inducing bystander help for the tumor-associated antigens. In some embodiments, the compositions can include a means for causing inflammation in a tumor lesion. The compositions can further include a growth factor and/or a signal transduction protein. The growth factor can be, for example, VEGF-A. The signal transduction factor can be, for example, PLK1.

**[0029]** Some embodiments relate to immunogenic compositions for the treatment of or for inducing an anti-cancer 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 a first antigen selected from PRAME, PSMA, and/or tyrosinase and at least one other tumor-associated antigen. The at least one other tumor associated antigen can include, for example, a MAGE protein, MAGE-3, Melan-A, PLK1, VEGF-A, SSX-2, NY-ESO-1, mesothelin, PRAME, PSMA, VEGFR2, Tie-2, and the like, or subsets thereof. The compositions can further include at least one antigen associated with tumor neovasculature or stroma. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2 and/or Tie-2. The compositions can further include a growth factor and/or a signal transduction protein. The growth factor can be, for example, VEGF-A. The signal transduction factor can be, for example, PLK1.

**[0030]** Some embodiments relate to immunogenic compositions for the treatment of or for inducing an anti-cancer 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 a first antigen selected from PRAME, PSMA, and/or tyrosinase and at least one other tumor-associated antigen. In some embodiments, the at least one other tumor associated antigen can be a MAGE protein, MAGE-3, Melan-A, mesothelin, NY-ESO-1, SSX-2, PSMA, PRAME, VEGF-A, VEGFR2, Tie-2, and the like, or subsets thereof. The compositions can further include at least one antigen associated with tumor neovasculature or stroma. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2 and/or Tie-2.

**[0031]** Still other embodiments relate to immunogenic compositions for the treatment of or for inducing an anti-cancer response against melanoma. 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 be selected from each of two groups; wherein the first group includes, for example, PRAME, PSMAE and/or tyrosinase; and wherein the second group includes a MAGE protein, MAGE-3, Melan-A, SSX-2 and/or NY-ESO-1, and the like. The compositions can further include at least one antigen associated with tumor neovasculature or stroma. The antigen associated with tumor neovasculature can be, for example, PSMA, VEGFR2 and/or Tie-2. The compositions can further include a growth factor and/or a signal transduction protein. The growth factor can be, for example, VEGF-A. The signal transduction factor can be, for example, PLK1.

**[0032]** Further embodiments relate to compositions and methods for inducing an anti-cancer response using combinations of tumor-associated antigens, including fragments of tumor-associated antigens, clusters and epitopes. The compositions can include nucleic acid constructs, for example, a single construct that encodes all of the desired antigens. 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.

**[0033]** 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.

[0034] In some embodiments, methods of inducing an anti-cancer immune response are disclosed. The cancer can be, for example, ovarian cancer, colorectal cancer, pancreatic cancer, non-small cell lung cancer, melanoma, and renal cell carcinoma, and the like, or any subset thereof. The methods can include, for example, the step of immunizing against a first antigen and at least one tumor associated antigen. The first antigen can be, for example, PRAME, PSMA, tyrosinase, and the like. For example, in some embodiments, the methods include the step of immunizing against PRAME and at least one tumor-associated antigen and the cancers treated can be ovarian cancer, colorectal cancer, pancreatic cancer, melanoma, and renal cell carcinoma. In some embodiments, the methods include the step of immunizing against PSMA and at least one tumor-associated antigen, and the cancer can be non-small cell lung cancer, ovarian cancer, colorectal cancer, pancreatic cancer, and renal cell carcinoma. The at least one tumor-associated antigen can be, for example, PRAME, PSMA, VEGFR-2, NY-ESO 1, SSX-2, a MAGE protein, MAGE-3, mesothelin, PLK-1, VEGF-A, Melan A, VEGFR2, Tie-2, and the like, or subsets thereof.

[0035] In some embodiments, the methods can further include the step of immunizing against a stromal cell antigen, such as by active immunotherapies, passive immunotherapies, or the like. In some embodiments, the methods can further include a step for causing inflammation in a tumor lesion. In some embodiments, the methods further include immunizing against an extra-cellular factor. The extra-cellular factor can be, for example, an autocrine factor, a paracrine factor, a growth factor, chorionic gonadatropin, gastrin, an NF-κB activating factor, VEGF-A CXCL1, CXCL8, CCL2 and the like. In some embodiments, the methods can further include immunizing against a factor that promotes the growth, survival, invasiveness, or metastasis of a tumor.

[0036] In another embodiment, the methods can include immunizing against a non-self antigen, such as a B cell epitope or a Th epitope. The methods can also include a step for co-inducing a helper response, such as a B cell response or a Th cell response.

[0037] In some embodiments, the methods can further include administering a treatment such as, for example, chemotherapy, radiotherapy, chemotherapy, biotherapy, passive immunotherapy, antibody therapy, surgery, and the like. In some embodiments, the methods further include a step for tumor debulking. In some embodiments, the methods can include a step for inducing tissue damage, necrosis, or apoptosis within a

tumor. In some embodiments, the methods can include a step for inducing inflammation within a tumor.

## Brief Description of the Drawings

**[0038]** Figure 1 is a timeline depicting the schedule of immunization with two plasmid (pCBP expressing SSX2 41-49 and pSEM expressing Melan A).

**[0039]** Figure 2 is a bar graph that shows CTL activity obtained using the protocol in Figure 1.

**[0040]** Figure 3 is a timeline depicting the schedule of immunization of an entrain-and-amplify immunization protocol using plasmids and peptides representing two epitopes.

**[0041]** Figure 4 is a table showing *in vivo* clearance of epitope-pulsed cells in mice immunized according to the protocol of Figure 3.

**[0042]** Figures 5A and 5B are timelines depicting preferred immunization protocols for inducing strong multivalent responses. Figure 5A shows the use of peptides for boosting restores multivalent immune responses even if plasmids and peptides are used as mixtures. Figure 5B shows segregation of plasmid and peptide components allows induction of multivalent immune responses.

## Detailed Description of the Preferred Embodiment

**[0043]** 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.

**[0044]** 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 is maximized. Secondly, some 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 loss of expression of the TuAAs. In preferred embodiments, this technique is employed when the tumor is positive for two, three, or more of the TuAAs of the combination used.

**[0045]** Multivalent attack can offer another advantage in increasing the sensitivity of the tumor to attack. If more than a single antigen on a tumor cell is targeted, the effective concentration of anti-tumor agent is increased. In addition, attack on stroma associated with the tumor, such as vasculature, can increase the accessibility of the tumor cells to the agent(s) targeting them. Thus, even an antigen that is also expressed on some normal tissue can receive greater consideration as a target antigen, if the other antigens to be targeted in a multivalent attack are not also expressed by that tissue.

**[0046]** In some embodiments, practice of the methods includes use of at least two different compositions and, especially when there is more than a single target antigen, can involve several compositions to be administered together and/or at different times. Thus, embodiments of the invention include sets and subsets of immunogenic compositions and individual doses thereof. Multivalency can be achieved using compositions comprising multivalent immunogens, combinations of monovalent immunogens, coordinated use of compositions comprising one or more monovalent immunogens or various combinations thereof. Multiple compositions, manufactured for use in a particular treatment regimen or protocol according to such methods, define an immunotherapeutic product. In some embodiments all or a subset of the compositions of the product are packaged together in a kit.

Definitions

**[0047]** 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.

[0048] PROFESSIONAL ANTIGEN-PRESENTING CELL (pAPC) – a cell that possesses T cell co-stimulatory molecules and is able to induce a T cell response. Well characterized pAPCs include dendritic cells, B cells, and macrophages.

[0049] PERIPHERAL CELL – a cell that is not a pAPC.

[0050] HOUSEKEEPING PROTEASOME – a proteasome normally active in peripheral cells, and generally not present or not strongly active in pAPCs.

[0051] IMMUNOPROTEASOME – a proteasome normally active in pAPCs; the immunoproteasome is also active in some peripheral cells in infected tissues or following exposure to interferon.

[0052] 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.

[0053] MHC EPITOPE – a polypeptide having a known or predicted binding affinity for a mammalian class I or class II major histocompatibility complex (MHC) molecule.

[0054] 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. Patent Application Nos. 10/117,937, filed on April 4, 2002 (Pub. No. 20030220239 A1), 11/067,159 (Pub. No. 2005-0221440 A1), filed February 25, 2005, 11/067,064 (Pub. No. 2005-0142144 A1), filed February 25, 2005, and 10/657,022 (Pub. No. 2004-0180354 A1), filed September 5, 2003, and in PCT Application No. PCT/US2003/027706 (Pub. No. WO 2004/022709 A2), 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; 60/363,210 filed March 7, 2002; and 60/409,123, filed on September 6, 2002. Each of the listed applications is entitled "EPITOPE SEQUENCES." Each of the applications mentioned in this paragraph is incorporated herein by reference in its entirety.

**[0055]** 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.

**[0056]** TARGET CELL – In a preferred embodiment, a target cell 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.

**[0057]** TARGET-ASSOCIATED ANTIGEN (TAA) – a protein or polypeptide present in a target cell.

**[0058]** TUMOR-ASSOCIATED ANTIGEN (TuAA) – a TAA, wherein the target cell is a neoplastic cell. In alternate embodiments, a TuAA is an antigen associated with non-cancerous cells of the tumor such as tumor neovasculature or other stromal cells within the tumor microenvironment.

**[0059]** HLA EPITOPE – a polypeptide having a known or predicted binding affinity for a human class I or class II HLA complex molecule.

**[0060]** 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.

**[0061]** 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.

**[0062]** 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.

**[0063]** 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.

**[0064]** 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.

**[0065]** MATURE EPITOPE – a peptide with no additional sequence beyond that present when the epitope is bound in the MHC peptide-binding cleft.

**[0066]** 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, filed April 28, 2000, entitled "EPITOPE CLUSTERS," which is incorporated herein by reference in its entirety.

**[0067]** 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.

**[0068]** 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*.

**[0069]** 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 $CD62L^{-}$ $CD44^{hi}$ $CD107\alpha^{-}$ $IGN\text{-}\gamma^{-}$ $LT\beta^{-}$ $TNF\text{-}\alpha^{-}$ and is in G0 of the cell cycle.

**[0070]** 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.

**[0071]** EFFECTOR FUNCTION – Generally, T cell activation generally, including acquisition of cytolytic activity and/or cytokine secretion.

**[0072]** 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.

**[0073]** AMPLIFYING a T cell response – Includes in many embodiments, the 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.

**[0074]** ENTRAINMENT – Includes in many embodiments an induction that confers particular stability on the immune profile of the induced lineage of T cells. In various embodiments, the term "entrain" can correspond to "induce," and/or "initiate."

**[0075]** 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.

**[0076]** TOLL-LIKE RECEPTOR (TLR) LIGAND – Any molecule capable of binding and activating a toll-like receptor. Examples include, without limitation: poly IC A synthetic, double-stranded RNA known 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.

**[0077]** 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, which is incorporated herein by reference in its entirety.

**[0078]** 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.

**[0079]** 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.

**[0080]** 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.

**[0081]** 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.

**[0082]** COVERAGE – the fraction or proportion of tumor cells expressing a particular TuAA or at least one TuAA from a set of selected TuAAs.

**[0083]** 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.

**[0084]** CO-TARGETING – in preferred embodiments, co-targeting involves inducing and/or amplifying an immune response against a target cell, while also inducing an immune response against at least one other agent in the vicinity and/or *milieu* of a tumor. In some embodiments, agents within the vicinity and/or *milieu* of the tumor include, but are not limited to, cancer cells, stromal cells, including those associated with neovasculature, endothelial cells, fibroblasts, inflammatory cells, epithelial cells, autocrine factors, and paracrine factors. In some embodiments, tumor cells and stromal cells are specifically targeted. In other embodiments, an immune response is induced and/or amplified against neovasculature and other non-transformed, non-lymphoid cells within the tumor microenvironment. In still other embodiments, an immune response is induced against cancer cells and autocrine and/or paracrine factors produced by cells in the tumor microenvironment.

Tumor Associated Antigens

**[0085]** 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), Her2/Neu (SEQ. ID NO. 9), mesothelin (SEQ. ID NOs. 10 and 11), VEGF-A (SEQ. ID NO. 12), and PLK1 (SEQ. ID NO. 13). The natural coding sequences for these proteins, or any segments within them, can be determined from their cDNA or complete coding (cds) sequences, SEQ. ID NOS. 14-26, respectively. The protein and cDNA sequences are identified by accession number and provided in the sequence listing filed herewith.

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 | Mesothelin, isoform 1, protein | NP005814 |
| 11 | Mesothelin, isoform 2, protein | NP037536 |
| 12 | VEGF-A protein | P15692 |
| 13 | PLK1 protein | P53350 |
| 14 | Tyrosinase cDNA | NM_000372 |
| 15 | Melan-A cDNA | U06452 |
| 16 | SSX-2 cDNA | NM_003147 |
| 17 | PSMA cDNA | NM_004476 |
| 18 | MAGE-1 cds | M77481 |
| 19 | MAGE-3 cds | U03735 |
| 20 | NY-ESO-1 cDNA | U87459 |
| 21 | PRAME cDNA | NM_006115 |
| 22 | Her2/Neu cDNA | M11730 |
| 23 | Mesothelin, isoform 1, cDNA | NM005823 |
| 24 | Mesothelin, isoform 2, cDNA | NM013404 |
| 25 | VEGF-A cDNA | NM_001025366 |
| 26 | Plk1 cDNA | NM_005030 |

**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.

**[0086]** 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 No. 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" which is hereby incorporated by reference in its entirety.

**[0087]** GP100, also known as PMel17, is another melanin biosynthetic protein expressed at high levels in melanomas. GP100 as a TuAA is disclosed in U.S. Patent No. 5,844,075, entitled "MELANOMA ANTIGENS AND THEIR USE IN DIAGNOSTIC

AND THERAPEUTIC METHODS," which is hereby incorporated by reference in its entirety.

**[0088]** Melan-A, also known as 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 entitled "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," each of which is hereby incorporated by reference in its entirety.

**[0089]** SSX-2, also known as Hom-Mel-40, is a member of a family of highly conserved cancer-testis (CT) antigens (Gure, A.O. *et al.*, *Int. J. Cancer* 72:965-971, 1997, which is hereby incorporated by reference in its entirety). Its identification as a TuAA is taught in U.S. Patent No. 6,025,191, entitled "ISOLATED NUCLEIC ACID MOLECULES WHICH ENCODE A MELANOMA SPECIFIC ANTIGEN AND USES THEREOF," which is hereby incorporated by reference in its entirety. 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 MHC molecule, so that some vaccines directed against one member of this family can cross-react and be effective against other members of this family.

**[0090]** 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 No. 5,342,774, entitled "NUCLEOTIDE SEQUENCE ENCODING THE TUMOR REJECTION ANTIGEN PRECURSOR, MAGE-1," which is hereby incorporated by reference in its entirety, 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.

**[0091]** 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, each of which is hereby incorporated by reference in its entirety). 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.

**[0092]** 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, entitled "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 of which is hereby incorporated by reference in its entirety.

**[0093]** 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," which is hereby incorporated by reference in its entirety. 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. 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).

**[0094]** PRAME, also known 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 No. 5,830,753, entitled "ISOLATED NUCLEIC ACID MOLECULES CODING FOR TUMOR REJECTION ANTIGEN PRECURSOR DAGE AND USES THEREOF," which is hereby incorporated by reference in its entirety.

**[0095]** PSMA (prostate-specific membranes antigen), a TuAA described in U.S. Patent No. 5,538,866 entitled, "PROSTATE-SPECIFIC MEMBRANES ANTIGEN," which is hereby incorporated by reference in its entirety, 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. Provisional Patent Application No. 60/274,063, and U.S. Patent Application Nos. 10/094,699 (Pub. No. 20030046714 A1), filed on March 7, 2002, and 11/073,347 (Pub. No. _____), filed June 30, 2005, each entitled "ANTI-NEOVASCULATURE PREPARATIONS FOR CANCER," each of which is hereby incorporated by reference in its entirety. 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.

**[0096]** 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 No. 5,935,818, entitled "ISOLATED NUCLEIC ACID MOLECULE ENCODING ALTERNATIVELY SPLICED PROSTATE-SPECIFIC MEMBRANES ANTIGEN AND USES THEREOF," which is hereby incorporated by reference in its entirety. 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.

**[0097]** 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.

**[0098]** 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 No. 5,856,136, entitled "HUMAN STEM CELL ANTIGENS," which is hereby incorporated by reference in its entirety.

**[0099]** 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, which is hereby incorporated by reference in its entirety). 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.

**[0100]** 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, each of which is hereby incorporated by reference in its entirety).

**[0101]** 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, each of which is hereby incorporated by reference in its entirety). 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. Provisional Patent Application No. 60/274,063, filed on March 7, 2001, and U.S. Patent Application Nos. 10/094,699, (Pub. No. 20030046714 A1), filed March 7, 2002, and 11/073,347 (Pub. No. _____), filed June 30, 2005, all entitled "ANTI-NEOVASCULATURE PREPARATIONS FOR CANCER," filed on March 7, 2002, each of which is hereby incorporated by reference in its entirety. A tumor cell line is disclosed in U.S. Provisional Application No. 60/363,131, filed on March 7, 2002, entitled "HLA-TRANSGENIC

MURINE TUMOR CELL LINE," which is hereby incorporated by reference in its entirety.

**[0102]** Carcinoembryonic antigen (CEA) is a paradigmatic oncofetal protein first described in 1965 (Gold and Freedman, *J. Exp. Med.* 121: 439-462, 1965, which is hereby incorporated by reference in its entirety). Fuller references can be found in the Online Mendelian 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.

**[0103]** 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, which is hereby incorporated by reference in its entirety) and its function as an inhibitor of apoptosis is believed to contribute to the malignant phenotype.

**[0104]** 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, which is hereby incorporated by reference in its entirety), and apparently identical to the c-ERBB2 oncogene (Di Fiore, *et al.*, *Science* 237: 178-182, 1987, which is hereby incorporated by reference in its entirety). 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, which is hereby incorporated by reference in its entirety). A more detailed description is available in the Online Mendelian Inheritance in Man; record *164870.

**[0105]** MESOTHELIN is an antigen originally found in mesotheliomas but also known to be upregulated in many pancreatic and ovarian cancers. Its use as a vaccine target and useful epitopes are described in Thomas, A.M. *et al.*, *J. Exp. Med.* 200:297-306, 2004, which is hereby incorporated by reference in its entirety.

**[0106]** Vascular Endothelial Growth Factor (VEGF-A or VEGF) is a mitogenic protein structurally related to platelet-derived growth factor, but with narrower

mitogenic activity focused on vascular endothelial cells. The protein and its receptors are important to tumor growth and their potential as targets of cancer therapies has been noted (Folkman, *J. Nature Med.* 1: 27-31, 1995, which is hereby incorporated by reference in its entirety). A more detailed description is available in the Online Mendelian Inheritance in Man; record *192240.

**[0107]** PLK1 is an intracellular serine/threonine kinase that plays a critical role in cell cycle regulation and in DNA damage responses. Mutation or knock down of PLK1 result in abnormal mitosis, cell cycle arrest and apoptosis (Reagan-Shaw S. and Ahmad N., *FASEB J.* 19:611, 2005). PLK1 belongs to the Polo-like kinase family of structurally conserved kinases. The PLK1 family contains two conserved regions, an N-terminal kinase domain and a C-terminal non-catalytic Polo box region (Lowery DM *et al.*, *Oncogene* 24:248.2005) responsible for subcellular localization. PLK1 is predominantly found in the cytoplasm during interphase and localizes to the nucleus during mitosis (Takai N *et al.*, *Oncogene* 24:2872005). Nuclear localization of PLK1 is essential for its biological function (Lee KS *et al.*, *Proc Natl Acad Sci U S A* 95:9301, 1998). While most classical tumor associated antigens are not known to participate in tumor initiation or disease progression, PLK1 has been shown to drive tumor growth. Thus, in some embodiments, PLK1 can be considered a tumor-associated antigen that can be used as a target for cancer immunotherapy. Based on its expression profile, PLK1 is expressed in proliferating cells, with elevated expression found in most solid tumors. This expression pattern renders PLK1 a valid target for active and passive immunotherapy.

**[0108]** 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.

**[0109]** Additional tumor-associated antigens are described in Chen, YT, "Identification of human tumor antigens by serological 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), each of which is hereby incorporated by reference in its entirety.

**[0110]** Table 2, adapted from Scanlan *et al.*, "The cancer/testis genes: Review, standardization, and commentary," *Cancer Immunity* 4:1 (January 23, 2004), which is hereby incorporated by reference in its entirety, 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), which is hereby incorporated by reference in its entirety.

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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bla d | Br n | Brst | Co l | Eso | Gas | H/N | Live r | Leuk/ Lymph | Lung (NSCLC) | Me l | O v | Panc r | Pros | Rena l | Sar c | |
| 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 | - | - | - | - | - | - | - | - | - | 8 | - | - | - | 22 | 47 |
| HAGE/CT13 | 24 | 37 | 5 | 31 | 27 | - | - | 20 | 9 | 32 | 17 | - | - | 22 | 6 | 20 | 13 |
| SAGE/CT14 | 12 | 0 | 5 | 0 | 20 | - | 17 | - | 4 | 22 | 4 | - | - | 0 | 5 | 5 | 13 |
| ADAM2/CT15 | - | - | 0 | 0 | - | - | - | - | - | 0 | 0 | 0 | - | - | 12 | - | 17 |
| PAGE-5/CT16 | - | - | 5 | 11 | - | - | - | - | - | 39 | 22 | 0 | - | - | 44 | - | 17 |
| LIP1/CT17 | - | - | 5 | 0 | - | - | - | - | - | 0 | 0 | 0 | - | - | 25 | - | 17 |
| NA88/CT18 | - | - | - | - | - | - | - | - | - | - | 11 | - | - | - | - | - | 48 |
| TSP50/CT20 | - | - | 28 | - | - | - | - | - | - | - | - | - | - | - | - | - | 49 |
| CTAGE-1/CT21.1 | - | - | - | - | - | - | - | - | 35 | - | - | - | - | - | - | - | 50 |
| SPA17/CT22 | - | - | - | - | - | - | - | - | 26 | - | - | - | - | - | - | - | 51 |
| OYTES1/CT23 | 28 | - | 40 | 15 | - | 0 | - | 40 | - | 20 | - | - | - | - | 0 | - | 52 |
| MMA1a/CT25.1a | - | - | 0 | 0 | 0 | - | - | - | - | 40 | 26 | - | 0 | - | - | 18 | 15 |
| CAGE/CT26 | - | - | - | - | - | 89 | - | - | - | 100 | - | - | - | - | - | - | 53 |
| HOMTES85/CT28 | - | 35 | 0 | 10 | - | - | - | 19 | - | 28 | 36 | 32 | - | 0 | - | - | 54 |

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

Many antigens listed in the tables above have no documented role in maintaining the transformed phenotype of a cell, and thus, a transformed cell may lose expression of such antigens without affecting the viability of the cell or the malignancy of the disease. Although some CT antigens, such as the SSX proteins, are known to be transcriptional regulators, their role, if any, in tumorigenicity remains obscure. It has also recently emerged that PRAME can repress signaling through the retinoic acid receptor to inhibit retinoic acid-induced differentiation, growth arrest, and apoptosis, thereby suggesting that PRAME over-expression can impart tumor cells with a growth or survival advantage. It would be advantageous to target an antigen, the loss of which necessarily would affect viability of the cell or the malignancy of the disease. Such antigens are included in preferred embodiments of the combinations of tumor and/or tumor and stromal antigens disclosed herein.

However, many genes involved in the regulation of proliferation are important in oncogenesis primarily in mutated form so that specific immune attack on the mutated form depends on the mutation being appropriately placed within an epitope. Furthermore, epitopes present in the wild type molecule (whether or not the targeted molecule is wild type or mutant) will generally only be considered if there is a substantial difference in expression level between transformed and normal cells, or at least the normal cells of vital organs. Thus appropriate antigens have been difficult to recognize.

The expression of PLK1 is regulated during cell cycle progression as well as throughout the various disease stages. PLK1 expression is minimal during the initial phases of cell division, begins to increase during G2 and peaks at M phase. PLK1 is targeted for degradation by the proteasome pathway after cells exit from mitosis. In normal tissues, PLK1 is expressed in adult organs containing highly proliferative cells, such as in the spleen, placenta, ovary, and testis. Its expression is undetected in vital organs such as heart, lung, liver, brain, intestines, smooth muscle, and skin. Wild type PLK1 is overexpressed in tumor tissues, including breast, prostate, ovarian, non-small cell lung, head/neck, colon, pancreatic, endometrial, and esophageal carcinomas (Table 4). Importantly, the level of PLK1 expression often correlates with more advanced stages of tumor progression and poor prognosis (Wolf G *et al*, *Oncogene* 14:543, 1997; Takai N *et al*, *Cancer Lett.* 164:41, 2001). This has been specifically demonstrated in the case of NSCLC, esophageal, and head/neck carcinomas. Thus, PLK1 is a viable target for cancer immunotherapy for those tumors in which it is overexpressed.

**Table 4. Plk-1 Expression Profile**

| Tumor | Penetrance | Expression Level | Reference |
|---|---|---|---|
| Lung (NSCLC) | > 90 % | Med/strong | Wolf G *et al*, *Oncogene* 14:543, 1997. |
| Ovarian | > 85 % | Low/strong | Takai N *et al*, *Cancer Lett.* 164:41, 2001 |
| Breast | 43 % | Med/strong | Weichert W *et al*, *Virchows Arch*, 446: 442, 2005. |
| Prostate | 53 % | Med/strong | Weichert W *et al*, *Curr. Biol.* 101:4419, 2004. |
| Colorectal | 73 % | Med/strong | Takahashi T *et al*, *Cancer Sci.* 94:148 2003. |
| Pancreatic | 48 % | Med/strong | Gray JP *et al*, *Mol Cancer Ther.* 3:641,2004. |
| Head & Neck | 72 % | Med/strong | Knecht R *et al*, *Cancer Res.* 59:2794, 1999. |
| Melanoma | 53 % | Med | Strebhardt K *et al*, *JAMA.* 283:479, 2000. |
| Esophageal | 96 % | Med | Tokumitsu Y *et al*, *Int J Oncol.* 15:687, 1999. |

**[0111]** Each reference listed in this table is incorporated herein by reference in its entirety.

**[0112]** Additional antigens associated with tumor neovasculature are VEGFR2 (vascular endothelial growth factor receptor 2) described in U.S. Patent No. 6,342,221, which is hereby incorporated by reference in its entirety; and Tie-2, an endothelium specific receptor tyrosine kinase, which is described in WO 99/43801, and which is hereby incorporated by reference in its entirety.

**[0113]** In addition to disrupting blood flow to tumors that can be achieved using anti-neovasculature agents such as those recited above, co-targeting molecules expressed on cancer cells as well as molecules expressed on underlying non-transformed stromal cells (including neovasculature as well as interstitial tissue, for example) can also improve the

effectiveness of the disclosed methods and compositions in limiting tumor growth and promoting cancer regression by other mechanisms. Stroma encompasses neovasculature as well as fibroblasts, and in general, all non-transformed, non-lymphoid cells within a tumor microenvironment. For example, immune mediated attack of the endothelial cells (via cytotoxic T lymphocytes (CTLs) or antibody dependent cytotoxic cells (ADCC) can result in neovasculature permeabilization and initiation of inflammatory events that result in recruitment and translocation of immune effectors, such as CTLs, targeting the neoplastic cells within primary tumor and metastatic lesions. Moreover, as attacks based, for example, on T cell recognition of endothelial cell MHC-peptide complexes occur in the luminal environment, any immune suppressive influence of the tumoral environment is minimized. Compared to strategies targeting only cancer cells, methods to co-target associated stromal tissue thus improve the efficacy of the former. In some embodiments, the efficacy is synergistically enhanced. Similarly, compared to strategies targeting neovasculature only, methods to co-target cancer cells improve the overall therapeutic effect by attacking lesions, including those of limited size and vascularization, especially those adversely located within vital organs. With regard to neovasculature, co-targeting VEGFRs (such as II), CD55 and PSMA as well as other molecules expressed by neovasculature, can be accomplished by generating CTL or antibodies with capability to initiate ADCC or complement activated cell injury. Alternatively, initial endothelial injury can be brought about though passive immunotherapy using available anti-angiogenic antibodies.

**[0114]** In addition or alternatively, co-targeting target-associated antigens, together with growth, metastasis, or survival promoting factors produced by cancer cells or non-transformed cells that are found in the extracellular compartment (diffusing or associated with the extracellular matrix), can also result in a more substantial therapeutic effect. By co-targeting antigens expressed within or on cancer cells as well as factors that exert autocrine or paracrine effects (growth, survival, and/or invasiveness), the pathogenic process can be slowed or disrupted to a significant degree. Co-targeting autocrine or paracrine factors (such as, but not limited to, NF-κB activating molecules - CXCL1, CXCL8, CCL2; or growth factors such as, but not limited to, chorionic-gonadotropic hormone gastrin, and VEGF-A) can be carried out by co-induction of neutralizing antibodies or secondarily, by CTLs recognizing cells that produce such factors.

**[0115]** The interaction between transformed and stromal cells is mediated by VEGF-A. VEGF-A has been shown to play a key role in the establishment and functionality of tumor neovasculature; thus, deprival of VEGF-A by using specific passive immunotherapy (via anti-VEGF-A antibodies such as bevacizumab (AVASTIN®) resulted in control of tumor progression and metastatic disease for colorectal, lung, breast, ovarian carcinoma and other cancers. The mechanism of action likely involves direct neutralization of VEGF-A, thereby slowing down the establishment and progression of neovasculature. Neutralization of VEGF-A by passive immunotherapy can be combined with the active immunotherapies disclosed herein. VEGF-A can also be used as the target antigen for active or T-cell based immunotherapy directed against cells that produce VEGF-A in excess (most likely a subpopulation of tumoral cells, and most often the transformed cancer cells within the tumor environment). Such a strategy can be more effective in controlling a tumoral process both by mediating immune damage of cancer cells that express VEGF-A and by depriving the tumor neovasculature of an essential growth factor.

**[0116]** Overall, co-targeting multiple elements of biological importance for tumor growth and metastasis can limit progression of the malignant process by impacting the processes of clonal selection, immune evasion and escape. Thus, co-targeting stroma-associated antigens provides an additional mode of attack in that such activities are inhibited and/or disrupted.

**[0117]** One of skill in the art will appreciate that any other antigen or protein associated with vascular or other tumor-associated stromal cells can be a target for the immunogenic compositions, including those that are presently known and those yet to be identified.

Compositions

**[0118]** 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.

**[0119]** The immunogenic compositions can also include adjuvants or other biological response modifiers (BRMs). Particularly advantageous methods of using adjuvants and BRMs are disclosed in U.S. provisional patent application 60/640,727, filed December 29, 2004 and U.S. Application No. __/_____, (Pub. No. _____) (Attorney Docket No. MANNK.046A), filed on even date with this application, both entitled, "METHODS TO TRIGGER, MAINTAIN AND MANIPULATE IMMUNE RESPONSES BY TARGETED ADMINISTRATION OF BIOLOGICAL RESPONSE MODIFIERS INTO LYMPHOID ORGANS," each of which is hereby incorporated by reference in its entirety.

**[0120]** An antigen can be delivered to an animal's system either directly or indirectly. For example, a polypeptide can be delivered directly as the polypeptide, or it can 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.

**[0121]** 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 Nos. 09/561,572, filed April 28, 2000, 10/225,568 (Pub No. 2003-0138808), filed August 20, 2002; and PCT Application No. PCT/US2003/026231 (Pub. No. WO 2004/018666); all entitled "EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS;" U.S. Patent Application Nos. 10/292,413 (Pub. No. 2003-0228634 A1), filed November 7, 2002, 10/777,053 (Pub. No. 2004-0132088 A1), filed February 10, 2004, and 10/837,217 (Pub. No. _____), filed April 30, 2004, all entitled "EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS AND METHODS FOR THEIR DESIGN;" U.S. Patent No. 6,709,844, and U.S. Patent Application No. 10/437,830 (Pub. No. 2003-0180949 A1), filed May 13, 2003, both entitled "AVOIDANCE OF UNDESIRABLE REPLICATION INTERMEDIATES IN PLASMIND PROPAGATION," and in U.S. Patent Application Nos. 10/026,066 (Pub. No. 2003-0215425 A1), filed December 7, 2001, 10/895,523 (Pub.

No. 2005-0130920 A1), filed July 20, 2004, 10/896,325 (Pub. No. _____), filed July 20, 2004, all entitled "EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS," each of which is hereby incorporated by reference in its entirety. Additional methodology, compositions, peptides, and peptide analogues are disclosed in U.S. Provisional Application No. 60/581,001, filed June 17, 2004, and U.S. Patent Application No. 11/156,253 (Pub. No. _____), filed on June 17, 2005, both entitled "SSX-2 PEPTIDE ANALOGS;" U.S. Provisional Patent Application No. 60/580,962, filed June 17, 2004, and U.S. Patent Application No. 11/155,929 (Pub. No. _____), filed on June 17, 2005, both entitled "NY-ESO PEPTIDE ANALOGS;" U.S. Patent Application No. 09/999,186, filed November 7, 2001, entitled "METHODS OF COMMERCIALIZING AN ANTIGEN"; U.S. Provisional Patent Application No. 60/640,402, filed on December 29, 2004 and U.S. Patent Application No.__/_____ (Pub. No. _____), (Attorney Docket No. MANNK.047A), filed on even date as the instant application, both entitled, "METHODS TO ELICIT, ENHANCE AND SUSTAIN IMMUNE RESPONSES AGAINST MHC CLASS I- RESTRICTED EPITOPES, FOR PROPHYLACTIC OR THERAPEUTIC PURPOSES"; and U.S. Provisional Patent Application No. 60/640,821, filed on December 29, 2004 and U.S. Patent Application No. __/_____ (Pub. No. _____), (Attorney Docket No. MANNK.048A), filed on even date as the instant application, both entitled "METHODS TO BYPASS CD4+ CELLS IN THE INDUCTION OF AN IMMUNE RESPONSE," and U.S. Provisional Patent Application Nos. 60/691,579, filed June 17, 2005, entitled "METHODS AND COMPOSITIONS TO ELICIT MULTIVALENT IMMUNE RESPONSES AGAINST DOMINANT AND SUBDOMINANT EPITOPES EXPRESSED ON CANCER CELLS AND TUMOR STROMA," and 60/691,581, filed June 17, 2005, entitled "MULTIVALENT ENTRAIN-AND-AMPLIFY IMMUNOTHERAPEUTICS FOR CARCINOMA," each of which is hereby incorporated by reference in its entirety. 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," each of which is hereby incorporated by reference in its entirety. The former

teaches only intramuscular or intradermal injection while the latter teaches only administration to skin or mucosa.

**[0122]** 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 No. 09/380,534, filed September 1, 1999, and U.S. Patent No. 6,977,074, and in PCT Application No. PCTUS98/14289 (Pub. No. WO 99/02183 A2) each entitled "METHOD OF INDUCING A CTL RESPONSE," each of which is hereby incorporated by reference in its entirety. 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, filed June 17, 2003, entitled "METHODS TO CONTROL MHC CLASS I-RESTRICTED IMMUNE RESPONSE," and in U.S. Patent Application No. 10/871,707, (Pub. No. 20050079152 A1), filed June 17, 2004, entitled "METHODS TO ELICIT, ENHANCE AND SUSTAIN IMMUNE RESPONSES AGAINST MHC CLASS I-RESTRICTED EPITOPES, FOR PROPHYLACTIC OR THERAPEUTIC PURPOSE," each of which is hereby incorporated by reference in its entirety.

**[0123]** 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 10/026,066 (Pub. No. 2003-0215425 A1), filed on December 7, 2001, 10/895,523 (Pub No. 2005-0130920 A1), filed July 20, 2004, 10/896,325 (Pub. No. _____), filed July 20, 2004, all entitled "EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS," and U.S.

Patent No. 6,861,234 and U.S. Patent Application No. 10/956,401 (Pub. No. 2005-0069982 A1), filed October 1, 2004, both entitled "METHOD OF EPITOPE DISCOVERY," each of which is hereby incorporated by reference in its entirety.

**[0124]** 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; 60/363,210 filed March 7, 2002; and 60/409,123, filed on September 6, 2002; U.S. Patent Application Nos. 10/117,937 (Publication No. 20030220239 A1), filed on April 4, 2002; and 10/657,022 (Pub. No. 2004-0180354), filed September 5, 2003, and PCT Application No. PCT/US2003/027706 (Pub. No. WO 04/022709 A2), filed September 5, 2003, all entitled "EPITOPE SEQUENCES," each of which is hereby incorporated by reference in its entirety.

**[0125]** 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.

**[0126]** 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, filed on November 7, 2001; 10/026,066 (Pub. No. 2003-0215425 A1), filed on December 7, 2001; 10/895,523 (Pub. No. 2005-0130920 A1), filed July 20, 2004, and 10/896,325 (Pub. No. _____), filed July 20, 2004, all entitled "EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS," each of which is hereby incorporated by reference in its entirety.

**[0127]** 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.

**[0128]** 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, which is hereby incorporated by reference in its entirety). *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, each of which is hereby incorporated by reference in its entirety).

**[0129]** 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 intra 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.

**[0130]** 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, in preferred embodiments, the term vector as used herein is intended to encompass all such possibilities.

**[0131]** 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 protozoa. 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.

**[0132]** 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 can 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.

**[0133]** Immunogenic compositions can additionally contain non-target antigens in order to improve the response to the target antigen. Thus, co-induction of a helper response, such as Th and/or B cell immunity against non-self or foreign antigens not expressed within the tumoral process or in the body, can result in a substantial

improvement in the magnitude and quality of the immune response to the "self" or "self-modified" target antigens expressed within the tumor or underlying stroma. For example, co-initiating a Th immune response against a non-target antigen such as tetanus toxoid can result in the generation of helper cells with bystander effect relative to generation of CTL or B cell responses against the target tumor or self antigens. Any defined sequence expressing or encompassing peptide motifs that bind to at least one class II MHC protein expressed by recipient, where such sequences are non-homologous or contain non-homologous segments relative to self antigens, can be used. Preferably, such sequences are of microbial origin and shown to be immunogenic in HLA-defined or broader populations. In addition to tetanus toxoid (whole or portions, including, but not limited to, portions that are 90%, 80%, 75%, 70%, 60%, 50%, 40%, 30%, 25%, 20%, 15%, 10%, or 5% of a whole toxoid), further examples include, but are not limited to, sequences derived from HBVcore, influenza hemagglutinin, Plasmodium circumsporozoite antigen, and HTLV-1 envelope protein, and fragments of these sequences that are 90%, 80%, 75%, 70%, 60%, 50%, 40%, 30%, 25%, 20%, 15%, 10%, or 5% of the respective full-length sequences. In some embodiments, the tetanus toxoid portion is 5% to 90% of a whole toxoid, in other embodiments, the portion is 15% to 80% of a whole toxoid, in still other embodiments, the toxoid portion is 25% to 70% of a whole toxoid, in yet other embodiments, the toxoid portion is 35% to 60% of a whole toxoid, in still other embodiments, the toxoid portion is 45% to 55% of a whole toxoid. Similarly, co-administration of a strongly immunogenic B cell epitope (a non-self antigen) with or without a Th epitope (a non-self antigen) with target epitopes (self, tumoral) in a cognate fashion (that is, within the same molecule), can result in improved immune response, or even break of tolerance (T cell) against the therapeutic target, via immune antibody-antigen complexes and bystander T cell help.

Delivery of the Antigen

**[0134]** 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 No. 6,977,074, entitled

"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, filed June 17, 2003, entitled "METHODS TO CONTROL MHC CLASS I-RESTRICTED IMMUNE RESPONSE," and in U.S. Patent Application No. 10/871,707 (Pub. No. 20050079152 A1), filed June 17, 2004, both entitled "METHODS TO ELICIT, ENHANCE AND SUSTAIN IMMUNE RESPONSES AGAINST MHC CLASS I-RESTRICTED EPITOPES, FOR PROPHYLACTIC OR THERAPEUTIC PURPOSE," each of which is hereby incorporated by reference in its entirety. 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.

**[0135]** 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 can 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.

**[0136]** 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.

**[0137]** 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.

**[0138]** 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.

**[0139]** 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.

**[0140]** 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, which is hereby incorporated by reference in its entirety). For antigens that are less soluble, a suitable cosolvent or surfactant can 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 can 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), which is hereby incorporated by reference in its entirety.

**[0141]** 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.

**[0142]** 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.

**[0143]** 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 lymphatic 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

**[0144]** 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.

**[0145]** 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. This flexibility and customizability improves the success rate of immunotherapy because antigen redundancy can be achieved for each patient. However, the procedure of treating the patient can be more complex. For example, delivery using this approach can 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.

**[0146]** 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.

**[0147]** 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. 60/580,969, filed June 17, 2004, U.S. Patent Application No. 11/155,288 (Publication No. _____), filed June 17, 2005, and U.S. Patent Application No. __/___,___ (Publication No. _____) (Attorney Docket No. MANNK.050CP1), filed on the same date as the instant application, all entitled "COMBINATIONS OF TUMOR-ASSOCIATED ANTIGENS IN DIAGNOTISTICS FOR VARIOUS TYPES OF CANCERS," and which is hereby incorporated by reference in its entirety. Specific antigenic combinations of particular benefit in directing an immune response against particular cancers are disclosed in U.S. Provisional Patent Application No. 60/479,554, filed on June 17, 2003, U.S. Patent Application No. 10/871,708 (Publication No. 2005-0118186 A1), filed on June 17, 2004, both entitled "COMBINATIONS OF TUMOR-ASSOCIATED ANTIGENS IN COMPOSITIONS FOR VARIOUS TYPES OF," and PCT Patent Application No. PCT/US2004/019571 (Pub. No. WO 2004/112825 A1), filed June 17, 2004, each of which is hereby incorporated by reference in its entirety.

**[0148]** 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 U.S. Provisional Patent Application No. 60/580,964, filed on June

17, 2004, and U.S. Patent Application No. 11/155,928 (Pub. No. _____), filed on June 17, 2005, both entitled "IMPROVED EFFICACY OF ACTIVE IMMUNOTHERAPY BY INTEGRATING DIAGNOSTIC WITH THERAPEUTIC METHODS," each of which is hereby incorporated by reference in its entirety.

**[0149]** Combination of active immunotherapies, as disclosed herein, with other treatment modalities can increase the susceptibility of tumoral processes to the elicited immune response and thereby result in increased therapeutic benefit. In some embodiments, the therapeutic benefit is synergistically enhanced. Tumor debulking prior to or during active immunotherapy increases the potential for any particular level of immune response to slow or halt disease progression or to bring about tumor regression or elimination. Additionally, tissue damage, necrosis, or apoptosis initiated with antibody therapy, radiotherapy, biotherapy, chemotherapy, passive immunotherapy (including treatment with mono- and/or polyclonal antibodies, recombinant TCR, and/or adoptive transfer of CTL or other cells of the immune system) or surgery, can facilitate the active immunotherapeutic approach via general inflammation resulting in recruitment of immune effector cells including antigen-specific effectors. In general, any method to induce a transient or more permanent general inflammation within one or multiple tumors / metastatic lesions can facilitate the active immunotherapy. Alternatively or in addition to enabling recruitment of effectors, general inflammation can also increase the susceptibility of target cells to immune mediated attack (*e.g.*, as interferons increase expression of target molecules on cancer cells and underlying stroma). Still other strategies to increase susceptibility of tumor cells to immune mediated attack - by providing factors that interfere with the "stress response" or increase target molecules on cancer cells or stromal cells – can synergize with active immunotherapy.

**[0150]** 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 variations or elements.

**[0151]** In some embodiments, the numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical

parameters set forth in the written description and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

**[0152]** In some embodiments, the terms "a" and "an" and "the" and similar referents used in the context of describing a particular embodiment of the invention (especially in the context of certain of the following claims) may be construed to cover both the singular and the plural. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g. "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

**[0153]** Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

**[0154]** Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations on those preferred embodiments will become apparent to those of ordinary skill in the art upon

reading the foregoing description. It is contemplated that skilled artisans may employ such variations as appropriate, and the invention may be practiced otherwise than specifically described herein. Accordingly, many embodiments of this invention include all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

**[0155]** Furthermore, numerous references have been made to patents and printed publications throughout this specification. Each of the above cited references and printed publications are herein individually incorporated by reference in their entirety.

**[0156]** In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed may be within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

**[0157]** Each of the references cited herein is hereby incorporated herein by reference in its entirety.

**[0158]** 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

**[0159]** 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 5 below.

Table 5

| | 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

**[0160]** 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.

**[0161]** In order to achieve antigen redundancy and improve coverage in a large population, combinations of other antigens in addition to PRAME were considered. 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.

**[0162]** 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 preferred antigen redundancy, and thus, lower possibility of tumor escape.

Example 2

Colorectal cancer

**[0163]** 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

**[0164]** Real-Time PCR (RT-PCR) was utilized to determine the presence of PRAME, SSX2, NY-ESO-1, and PSMA. Briefly, total RNA was isolated from 5 pancreatic 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.

**[0165]** In the pancreatic cancer specimens, the presence of PRAME, NYESO-1, SSX-2, and PSMA was detected in 100%, 40%, 20%, and 100% of the specimens, respectively (see Table 6). Elsewhere, 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, each of which is hereby incorporated by reference in its entirety). 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, plus PRAME or PSMA provides excellent coverage and some redundancy for treating pancreatic cancer. Inclusion of both PRAME and PSMA significantly improves redundancy.

Table 6

| TAA | PRAME | SSX2 | NY-ESO-1 | PSMA |
|---|---|---|---|---|
| Detection Freq. | 5/5 | 1/5 | 2/5 | 5/5 |
| % positive | 100 | 20 | 40 | 100 |

Example 4

Non-small cell lung cancer

**[0166]** 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, each of which is

hereby incorporated by reference in its entirety). 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

**[0167]** 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, each of which is hereby incorporated by reference in its entirety). 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

**[0168]** 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

**[0169]** Further studies involving the foregoing tumor types confirmed the observed expression patterns and preferred panels of TuAA. A total of 34 ovarian, 44 colon, 18 renal, and 13 pancreatic tissue samples obtained from various vendors were analyzed for tumor-associated antigen expression using qRTPCR. The results of these assays demonstrated that PRAME and PSMA were expressed frequently (ranging from 68% to 100%) in all four types of tumors studied. NY-ESO-1 and SSX2 were expressed in 20% to 40% of ovarian and pancreatic tumors.

Table 7:

Overall Expression Profiles for Tumor Associated Antigens

From RTPCR analysis of Primary Tumors and Metastases

| Tumor-Associated Antigen | % Samples Expressing a Given Antigen | | | |
|---|---|---|---|---|
| | Ovarian[a] | Renal[b] | Pancreatic[c] | Colorectal[d] |
| SSX2 | 36 | 6 | 20 | 8 |
| NY-ESO-1 | 30 | 6 | 40 | 12 |
| PRAME | 97 | 83 | 80 | 76 |
| PSMA | 91 | 100 | 100 | 68 |
| MAGE-1 | 27 | 6 | 33 | 8 |
| MAGE-3 | 30 | 22 | 42 | 20 |
| SCP-1 | 30 | 11 | 0 | 0 |
| CEA | 30 | 0 | 58 | 92 |

[a] 33 samples (27 primary tumors and 6 metastases)

[b] 18 samples (18 primary tumors)

[c] 15 samples (14 primary tumors and 1 metastasis; PSMA on 10 samples)

[d] 25 samples (13 primary tumors and 12 metastases)

Example 8

Schedule of immunization with plasmids expressing epitopes from two antigens

**[0170]** Two groups of HHD mice (n=4) were immunized via intra lymph node injection with either pSEM expressing Melan-A $_{26\text{-}35}$A27L (ELA) and pCBP expressing SSX-$2_{41\text{-}49}$ 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 9

Co-administration of different vectors carrying distinct antigens

**[0171]** The animals immunized as described in Example 8, were sacrificed and splenocytes from each group pooled and stimulated with the two peptides (ELA or SSX-$2_{41\text{-}49}$) 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.

**[0172]** 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 10

### Rescue of Multivalent Response by Addition of Peptide Boost Steps

**[0173]** 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 11

### Peptide amplification rescues the immunogenicity of the less dominant epitope

**[0174]** Mice were immunized as described in Example 10 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.

**[0175]** 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 12

### Clinical practice for entrain-and–amplify immunization

**[0176]** 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.

## Example 13

### MKC1207: an Entrain-and-Amplify Therapeutic for Melanoma

**[0177]** MKC1207 comprises the plasmid pSEM (described in U.S. Patent Application No. 10/292,413 (Pub. No. 2003-0228634 A1), filed November 7, 2002, 10/777,053 (Pub. No. 2004-0132088 A1), filed February 10, 2004, 10/837,217 (Pub. No. _____), filed April 30, 2004, each of which is hereby incorporated by reference in its entirety, in which it is referred to as pMA2M) and peptides corresponding to Melan-A 26-35 and tyrosinase 369-377. The plasmid encodes the A27L analogue of the Melan-A epitope and the native tyrosinase epitope sequence. The plasmids encode both of these epitopes in such a manner that they can be expressed and presented by pAPC. In alternate embodiments of the therapeutic, the peptides can comprise the native sequence or be analogues such as those disclosed in U.S. Patent Application No. 11/156,369 (Pub. No. _____), entitled EPITOPE ANALOGUES, filed on June 17, 2005, and incorporated herein by reference in its entirety.

**[0178]** Briefly, the plasmid is administered intranodally to the inguinal lymph nodes as an entraining immunogen. Subsequently, the peptides are administered intranodally, one to the left node, the other to the right as amplifying immunogens. The entrain-and-amplify protocol is described in greater detail in U.S. Patent Application Nos.

10/871,707 (Pub. No. 2005-0079152 A1), filed on June 17, 2004 and 60/479,393, filed on June 17, 2003, each of which is hereby incorporated by reference in its entirety.

**[0179]** Melanoma patients can be screened according to the methods disclosed herein and MKC1207 administered to patients whose tumor antigen profile includes Melan-A and/or tyrosinase. In a preferred embodiment, the patient's tumor tissue also expresses HLA-A2, particularly HLA-A*0201.

Example 14

MKC1106: a Tetravalent Entrain-and-Amplify Therapeutic for Carcinoma

**[0180]** MKC1106 comprises the plasmids pCBP (described in U.S. Patent Application No. 10/292,413 (Pub. No. 2003/0228634 A1), filed November 7, 2002, 10/777,053 (Pub. No. 2004-0132088 A1), filed February 10, 2004, 10/837,217 (Pub. No. _____), filed April 30, 2004, each of which is hereby incorporated by reference in its entirety) and pRP12 (described in U.S. Provisional Application No. 60/691,579, entitled METHODS AND COMPOSITIONS TO ELICIT MULTIVALENT IMMUNE RESPONSES AGAINST DOMINANT AND SUBDOMINANT EPITOPES, EXPRESSED ON CANCER CELLS AND TUMOR STROMA, filed on June 17, 2005, and incorporated herein by reference in its entirety; and peptides corresponding to NY-ESO-1 157-165, SSX-2 41-49, PRAME 425-433 and PSMA 288-297. The plasmids encode both of these epitopes in such a manner that they can be expressed and presented by pAPC. In alternate embodiments of the therapeutic, the peptides can comprise the native sequence or be analogues such as those disclosed in U.S. Patent Application Nos. 11/156,253 (Pub. No. _____), entitled SSX-2 PEPTIDE ANALOGS, and _11/155,929 (Pub No. _____), entitled NY-ESO-1 PEPTIDE ANALOGS, and _11/156,369 (Pub. No. _____), entitled EPITOPE ANALOGS, and U.S. Provisional Patent Application No. 60/691,889, entitled EPITOPE ANALOGS, each of which was filed on June 17, 2005, and each of which is expressly incorporated by reference in its entirety.

**[0181]** Briefly, the plasmids are administered intranodally to the inguinal lymph nodes, one to the left side and one to the right, as entraining immunogens. Subsequently the peptides are sequentially administered intranodally, two on separate days to the left node, the other two on separate days to the right as amplifying immunogens. Preferably, the peptides are administered to the same lymph node that received the plasmid encoding the corresponding epitopes. The entrain-and-amplify protocol is described in greater detail

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in U.S. Patent Application Nos. 10/871,707 (Pub. No. 2005-0079152 A1), filed on June 17, 2004 and 60/479,393, filed on June 17, 2003, each of which is expressly incorporated by reference in its entirety.

**[0182]** Carcinoma patients, especially those with ovarian, colorectal, pancreatic, or renal cell carcinoma, can be screened according to the methods disclosed herein and MKC1106 administered to patients whose tumor profile includes PRAME, PSMA, NY-ESO-1, and/or SSX-2. The NY-ESO-1 epitope targeted by MKC1106 is also found in LAGE 1a/s, so the presence of this antigen in a profile would also be considered a match. As tumor antigen expression tends to be heterogeneous, any particular tissue sample is likely not to give a complete indication of all the antigens expressed. Thus, it is not necessary that a patient's profile contain all four of the antigens for that patient to be a candidate for treatment with MKC1106. However, preferably the profile contains 2, 3, or 4 of the antigens.

**[0183]** 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.

**[0184]** 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.

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**THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:**

1. Use of a PSMA antigen and a PRAME antigen for the manufacture of a medicament for the treatment of pancreatic cancer, wherein the medicament is suitable for inducing an immune response.

2. The use of Claim 1, wherein the medicament further comprises at least one other tumor-associated antigen selected from the group consisting of NY-ESO-I, SSX-2, a MAGE protein, MAGE-3, and Melan-A.

3. A method for the treatment of pancreatic cancer by inducing an immune response, which comprises immunizing with a composition comprising a PSMA antigen and a PRAME antigen.

4. The method of Claim 3, wherein the composition further comprises at least one other tumor-associated antigen selected from the group consisting of NY-ESO-I, SSX-2, a MAGE protein, MAGE-3, and Melan-A.

5. The use of Claim 1 or the method of Claim 3, wherein a cytolytic T cell response is induced.

6. The use of Claim 1 or the method of Claim 3, further comprising the use of at least one antigen selected from the group consisting of an antigen associated with tumor neovasculature, a growth factor, and a signal transduction protein for inducing an immune response.

7. The use or method of Claim 6, wherein the antigen associated with tumor neovasculature is selected from the group consisting of VEGFR2 and Tie-2.

8. The use or method of Claim 6, wherein said growth factor is VEGF-A.

9. The use or method of Claim 6, wherein said signal transduction protein is PLKI.

10. The use of Claim 1 or the method of Claim 3, further comprising the use of a stromal cell antigen, an extracellular factor antigen, or a non-self antigen for inducing an immune response, or the use of means for inducing immunity to a factor that promotes the growth, survival, invasiveness, or metastasis of a tumor.

11. The use or method of Claim 10, wherein the extra-cellular factor is selected from the group consisting of an autocrine factor, a paracrine factor, a growth factor,



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chorionic gonadatropin, gastrin, an NF-kB activating factor, VEGF-A, CXCL1, CXCL8, and CCL2.

12. The use or method of Claim 10, wherein the non-self antigen comprises a B cell epitope, or a Th epitope.

13. The use of Claim 1 or the method of Claim 3, wherein the use is combined with a treatment selected from the group consisting of chemotherapy, radiotherapy, biotherapy, passive immunotherapy, antibody therapy, and surgery.

14. The use of Claim 1 or the method of Claim 3, wherein the medicament further comprises a NY-ESO-1 antigen.

15. The use of Claim 1 or the method of Claim 3, further comprising a step for co-inducing a helper response, tumor debulking, inducing tissue damage, necrosis, or apoptosis within a tumor, or inducing inflammation within a tumor.

16. The use or method of Claim 15, wherein the helper response comprises a B cell response, or a Th cell response.

17. An immunogenic composition for the treatment of pancreatic cancer comprising a PSMA antigen and a PRAME antigen.

18. The immunogenic composition of Claim 17, wherein the antigens are provided in the form of 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.

19. The composition of claim 17, further comprising at least one other tumor-associated antigen selected from the group consisting of NY-ESO-1, SSX-2, a MAGE protein, MAGE-3, and Melan-A.

20. The composition of Claim 19, wherein the at least one other tumor-associated antigen is selected from NY-ESO and SSX-2.

21. The composition of Claim 20, further comprising at least one antigen selected from the group consisting of an antigen associated with tumor neovasculature, a growth factor, and a signal transduction protein.

22. The composition of Claim 21, wherein the antigen associated with tumor neovasculature is selected from the group consisting of VEGFR2 and Tie-2.

23. The composition of Claim 21, wherein said growth factor is VEGF-A.



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24. The composition of Claim 21, wherein said signal transduction protein is PLK1.

25. The composition of Claim 17, further comprising a neovasculature or other stromal antigen, an extra-cellular factor antigen, or a non-target antigen, or means for inducing immunity to a factor that promotes the growth, survival, invasiveness, or metastasis of a tumor, inducing bystander help for the tumor-associated antigens, or causing inflammation in a tumor lesion.

26. The composition of claim 17, further comprising a NY-ESO-1 antigen.

27. The composition of any one of Claims 19-24, wherein the at least one other tumor-associated antigen is an antigen associated with a non-cancerous component of the tumor, or a cell.

28. The use of Claim 1, the immunogenic composition of Claim 17, or the method of Claim 3, substantially as hereinbefore described.



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**Figure 1.** Schedule of immunization with plasmids (pCBP expressing SSX2 41-49; and pSEM expressing Melan A)

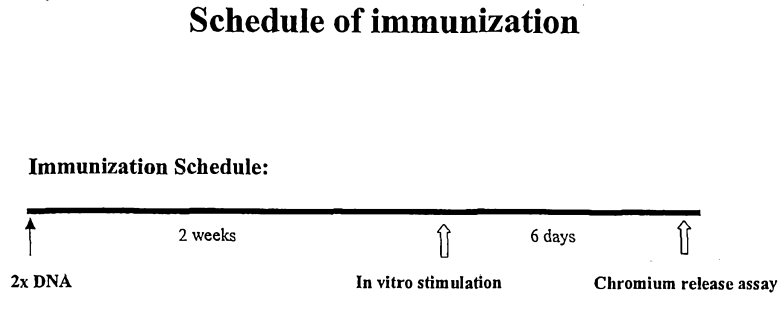


**Figure 2.** Co-administration of different vectors carrying distinct antigens results in establishment of a hierarchy in regard to immunogenicity. Vector segregation rescues the immunogenicity of the less dominant component, resulting in a multivalent response.

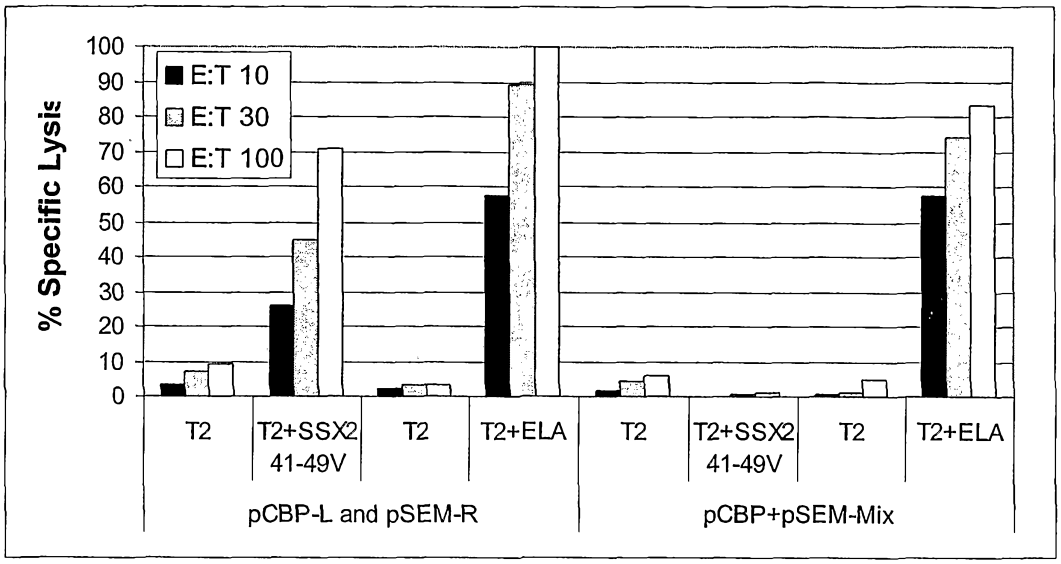
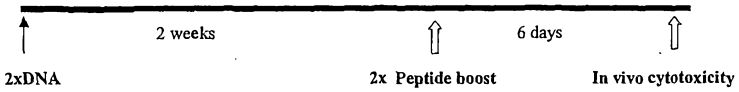


**Figure 3.** Addition of peptide boost steps to the immunization protocol described in Fig. 1.

## Schedule of immunization

**Immunization Schedule:**




**Experimental Setup:**

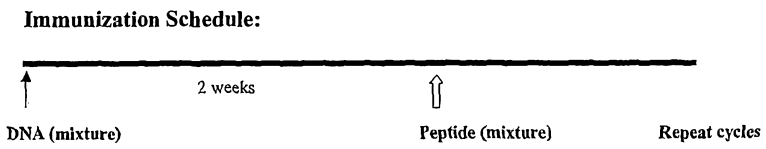
Group 1: Naïve controls
Group 2: Mixture of plasmids followed by mixture of peptides
Group 3: Plasmids and peptides into different lymph nodes
Group 4: pSEM plasmid and melan A peptide only
Group 5: pCBP plasmid and SSX2 peptide only

**Figure 4.** Peptide boost rescues the immunogenicity of a less dominant epitope even when the vectors and peptides respectively, are used as a mixture.

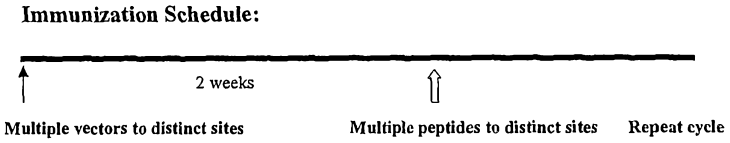
| | Spleen | Specific Lysis (%) | |
|---|---|---|---|
| Animal # | Treatment | SSX2 | Melan A |
| #3505 | Control | Control | Control |
| #3473 | Control | 6 | 4 |
| #3481 | Control | 8 | 6 |
| #3951 | Control | Control | Control |
| #3474 | Control | -4 | -8 |
| #3513 | Control | -3 | -4 |
| | **Mean** | **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** |

**Figure 5.** Methodology to induce strong, multivalent responses: reduction to practice in clinic

## A. Schedule of immunization




## B. Schedule of immunization



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SEQUENCE LISTING

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Simard, John J.L.
Diamond, David C.
Bot, Adrian Ion
Liu , Xiping

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

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        35                  40                  45
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    50                  55                  60
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65                  70                  75                  80
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        35                  40                  45
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    50                  55                  60
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Pro Gln Gly Ala Ser Ser Leu Pro Thr Thr Met Asn Tyr Pro Leu Trp
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                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> Homo sapiens

<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
```

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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> Homo sapiens

<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

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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> Homo sapiens

<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
```

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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> 622
<212> PRT
<213> Homo sapiens

<400> 10

```
Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro
 1               5                  10                  15
Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln
            20                  25                  30
Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
        35                  40                  45
Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg
    50                  55                  60
Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65                  70                  75                  80
Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
                85                  90                  95
Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
            100                 105                 110
Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
        115                 120                 125
Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile
    130                 135                 140
Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145                 150                 155                 160
Arg Leu Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu
                165                 170                 175
Leu Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu
            180                 185                 190
Pro Gly Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu
        195                 200                 205
Val Ser Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg
    210                 215                 220
Ala Ala Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp
225                 230                 235                 240
Ser Val Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly
                245                 250                 255
Gln Pro Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg
            260                 265                 270
Gln Arg Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile
        275                 280                 285
Leu Arg Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser
    290                 295                 300
Gly Lys Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys
305                 310                 315                 320
Trp Glu Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met
                325                 330                 335
Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu
            340                 345                 350
Lys His Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val
        355                 360                 365
Ile Gln His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile
    370                 375                 380
Arg Lys Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu
385                 390                 395                 400
Val Asn Lys Gly His Glu Met Ser Pro Gln Val Ala Thr Leu Ile Asp
                405                 410                 415
Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr
            420                 425                 430
Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu
        435                 440                 445
Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln Asp
    450                 455                 460
Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala
465                 470                 475                 480
Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile
```

485 490 495
Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser
500 505 510
Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr
515 520 525
Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu Gly
530 535 540
Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg
545 550 555 560
Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu
565 570 575
Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu Ser
580 585 590
Met Gln Glu Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro
595 600 605
Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
610 615 620

<210> 11
<211> 630
<212> PRT
<213> Homo sapiens

<400> 11
Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro
1 5 10 15
Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln
20 25 30
Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
35 40 45
Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg
50 55 60
Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65 70 75 80
Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
85 90 95
Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
100 105 110
Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
115 120 125
Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile
130 135 140
Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145 150 155 160
Arg Leu Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu
165 170 175
Leu Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu
180 185 190
Pro Gly Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu
195 200 205
Val Ser Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg
210 215 220
Ala Ala Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp
225 230 235 240
Ser Val Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly
245 250 255
Gln Pro Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg
260 265 270
Gln Arg Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile
275 280 285
Leu Arg Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser
290 295 300

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

<210> 12
<211> 232
<212> PRT
<213> Homo sapiens

<400> 12

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

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100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
130 135 140
Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145 150 155 160
Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys Leu Met Pro Trp
165 170 175
Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys
180 185 190
His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn
195 200 205
Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr
210 215 220
Cys Arg Cys Asp Lys Pro Arg Arg
225 230

<210> 13
<211> 603
<212> PRT
<213> Homo sapiens

<400> 13
Met Ser Ala Ala Val Thr Ala Gly Lys Leu Ala Arg Ala Pro Ala Asp
1 5 10 15
Pro Gly Lys Ala Gly Val Pro Gly Val Ala Ala Pro Gly Ala Pro Ala
20 25 30
Ala Ala Pro Pro Ala Lys Glu Ile Pro Glu Val Leu Val Asp Pro Arg
35 40 45
Ser Arg Arg Arg Tyr Val Arg Gly Arg Phe Leu Gly Lys Gly Gly Phe
50 55 60
Ala Lys Cys Phe Glu Ile Ser Asp Ala Asp Thr Lys Glu Val Phe Ala
65 70 75 80
Gly Lys Ile Val Pro Lys Ser Leu Leu Leu Lys Pro His Gln Arg Glu
85 90 95
Lys Met Ser Met Glu Ile Ser Ile His Arg Ser Leu Ala His Gln His
100 105 110
Val Val Gly Phe His Gly Phe Phe Glu Asp Asn Asp Phe Val Phe Val
115 120 125
Val Leu Glu Leu Cys Arg Arg Arg Ser Leu Leu Glu Leu His Lys Arg
130 135 140
Arg Lys Ala Leu Thr Glu Pro Glu Ala Arg Tyr Tyr Leu Arg Gln Ile
145 150 155 160
Val Leu Gly Cys Gln Tyr Leu His Arg Asn Arg Val Ile His Arg Asp
165 170 175
Leu Lys Leu Gly Asn Leu Phe Leu Asn Glu Asp Leu Glu Val Lys Ile
180 185 190
Gly Asp Phe Gly Leu Ala Thr Lys Val Glu Tyr Asp Gly Glu Arg Lys
195 200 205
Lys Thr Leu Cys Gly Thr Pro Asn Tyr Ile Ala Pro Glu Val Leu Ser
210 215 220
Lys Lys Gly His Ser Phe Glu Val Asp Val Trp Ser Ile Gly Cys Ile
225 230 235 240
Met Tyr Thr Leu Leu Val Gly Lys Pro Pro Phe Glu Thr Ser Cys Leu
245 250 255
Lys Glu Thr Tyr Leu Arg Ile Lys Lys Asn Glu Tyr Ser Ile Pro Lys
260 265 270
His Ile Asn Pro Val Ala Ala Ser Leu Ile Gln Lys Met Leu Gln Thr
275 280 285
Asp Pro Thr Ala Arg Pro Thr Ile Asn Glu Leu Leu Asn Asp Glu Phe
290 295 300

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```
Phe Thr Ser Gly Tyr Ile Pro Ala Arg Leu Pro Ile Thr Cys Leu Thr
305                 310                 315                 320
Ile Pro Pro Arg Phe Ser Ile Ala Pro Ser Ser Leu Asp Pro Ser Asn
                325                 330                 335
Arg Lys Pro Leu Thr Val Leu Asn Lys Gly Leu Glu Asn Pro Leu Pro
            340                 345                 350
Glu Arg Pro Arg Glu Lys Glu Glu Pro Val Val Arg Glu Thr Gly Glu
        355                 360                 365
Val Val Asp Cys His Leu Ser Asp Met Leu Gln Gln Leu His Ser Val
    370                 375                 380
Asn Ala Ser Lys Pro Ser Glu Arg Gly Leu Val Arg Gln Glu Glu Ala
385                 390                 395                 400
Glu Asp Pro Ala Cys Ile Pro Ile Phe Trp Val Ser Lys Trp Val Asp
                405                 410                 415
Tyr Ser Asp Lys Tyr Gly Leu Gly Tyr Gln Leu Cys Asp Asn Ser Val
            420                 425                 430
Gly Val Leu Phe Asn Asp Ser Thr Arg Leu Ile Leu Tyr Asn Asp Gly
        435                 440                 445
Asp Ser Leu Gln Tyr Ile Glu Arg Asp Gly Thr Glu Ser Tyr Leu Thr
    450                 455                 460
Val Ser Ser His Pro Asn Ser Leu Met Lys Lys Ile Thr Leu Leu Lys
465                 470                 475                 480
Tyr Phe Arg Asn Tyr Met Ser Glu His Leu Leu Lys Ala Gly Ala Asn
                485                 490                 495
Ile Thr Pro Arg Glu Gly Asp Glu Leu Ala Arg Leu Pro Tyr Leu Arg
            500                 505                 510
Thr Trp Phe Arg Thr Arg Ser Ala Ile Ile Leu His Leu Ser Asn Gly
        515                 520                 525
Ser Val Gln Ile Asn Phe Phe Gln Asp His Thr Lys Leu Ile Leu Cys
    530                 535                 540
Pro Leu Met Ala Ala Val Thr Tyr Ile Asp Glu Lys Arg Asp Phe Arg
545                 550                 555                 560
Thr Tyr Arg Leu Ser Leu Leu Glu Glu Tyr Gly Cys Cys Lys Glu Leu
                565                 570                 575
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2005321898 23 Aug 2007



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