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(54) **PROSTATE CANCER VACCINE**

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This patent is subject to a terminal disclaimer.

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C12N 15/117 (2010.01)

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(52) **U.S. Cl.**

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USPC 424/185.1; 514/44 R; 435/320.1; 530/328

See application file for complete search history.

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(57) **ABSTRACT**

Androgen receptor-based vaccines for eliciting an immune reaction in vivo against cells expressing androgen receptor are disclosed. The vaccines are useful in the treatment of prostate cancer. Also disclosed are methods for inducing immune reaction to androgen receptor or treating prostate cancer in a mammal, using the vaccines and pharmaceutical compositions comprising the vaccines.

6 Claims, 15 Drawing Sheets

Specification includes a Sequence Listing.

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A.

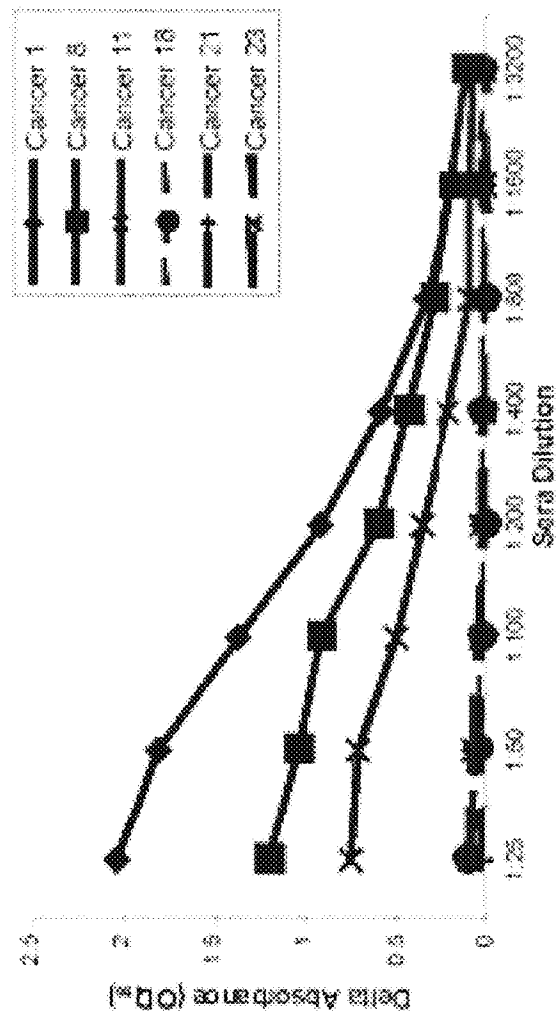


FIG. 1

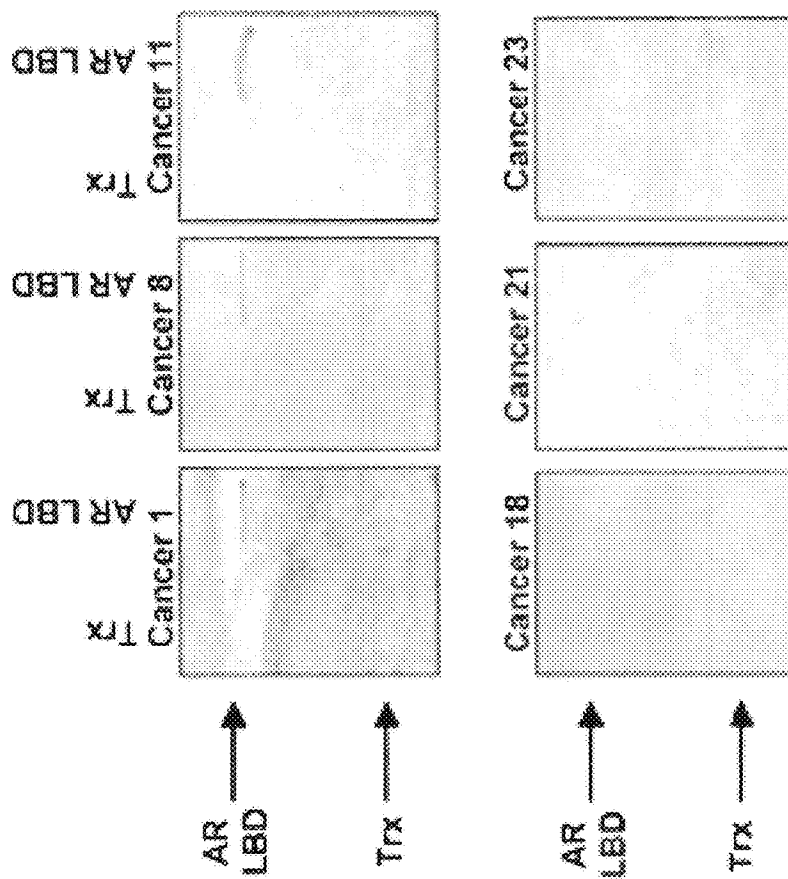


FIG. 1

B.

C.

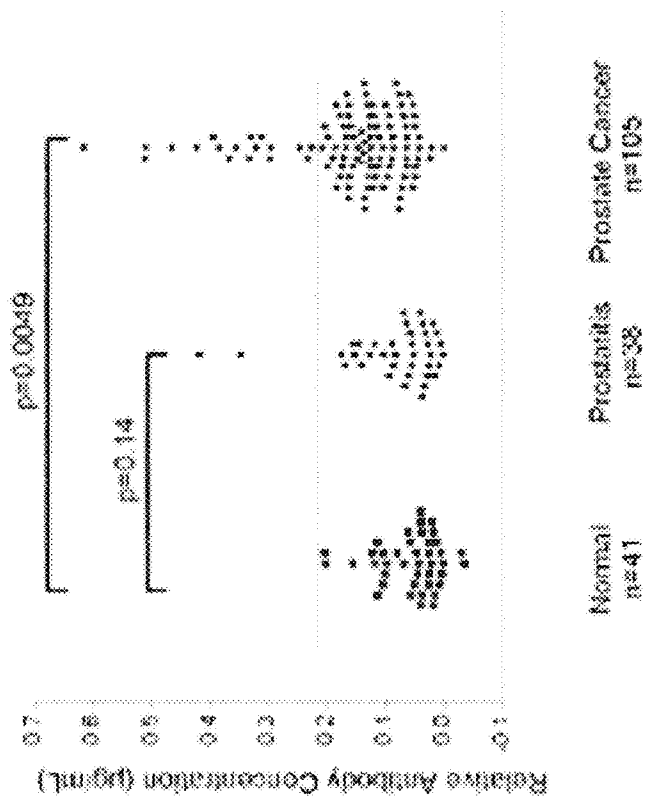


FIG. 1

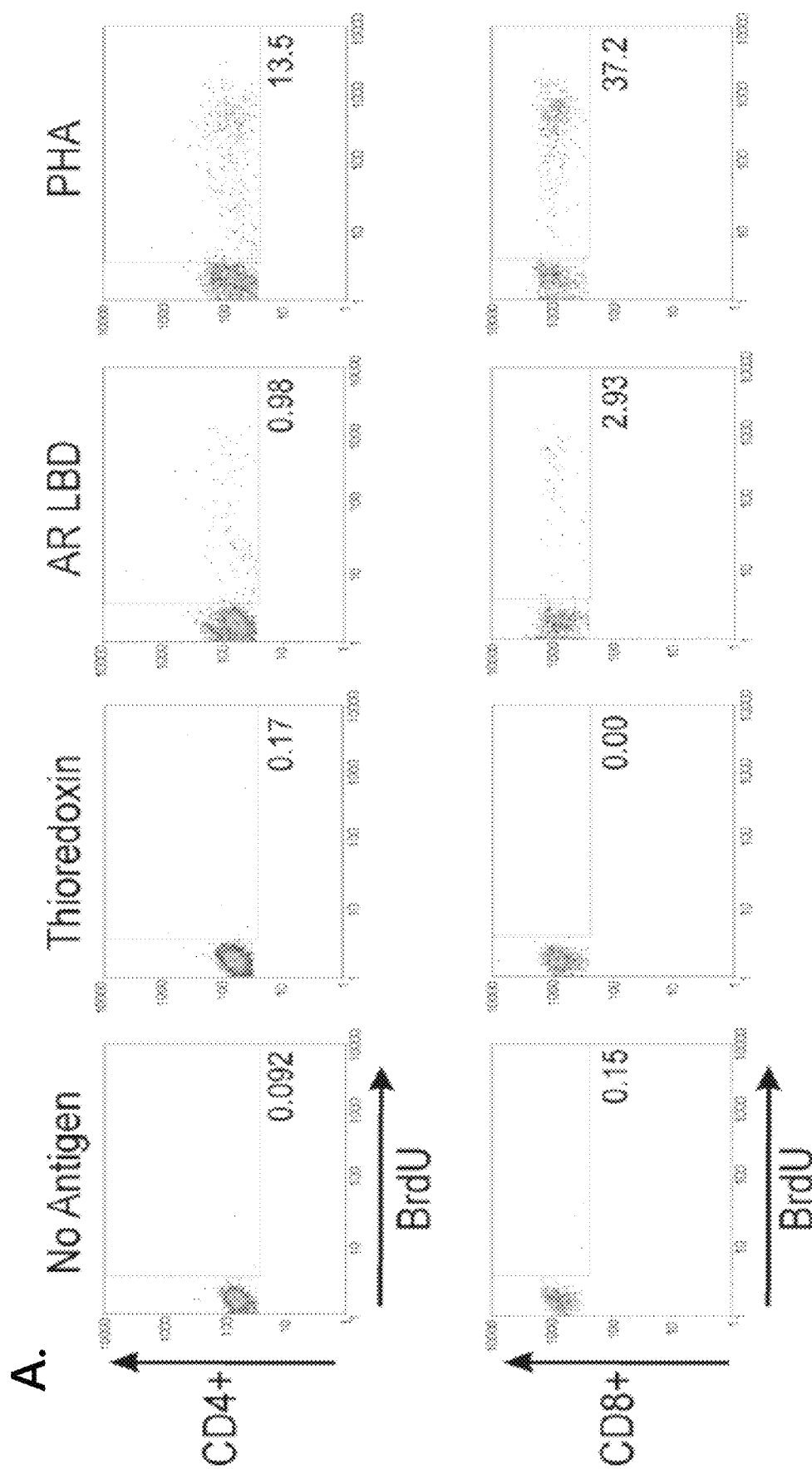


FIG. 2

B.

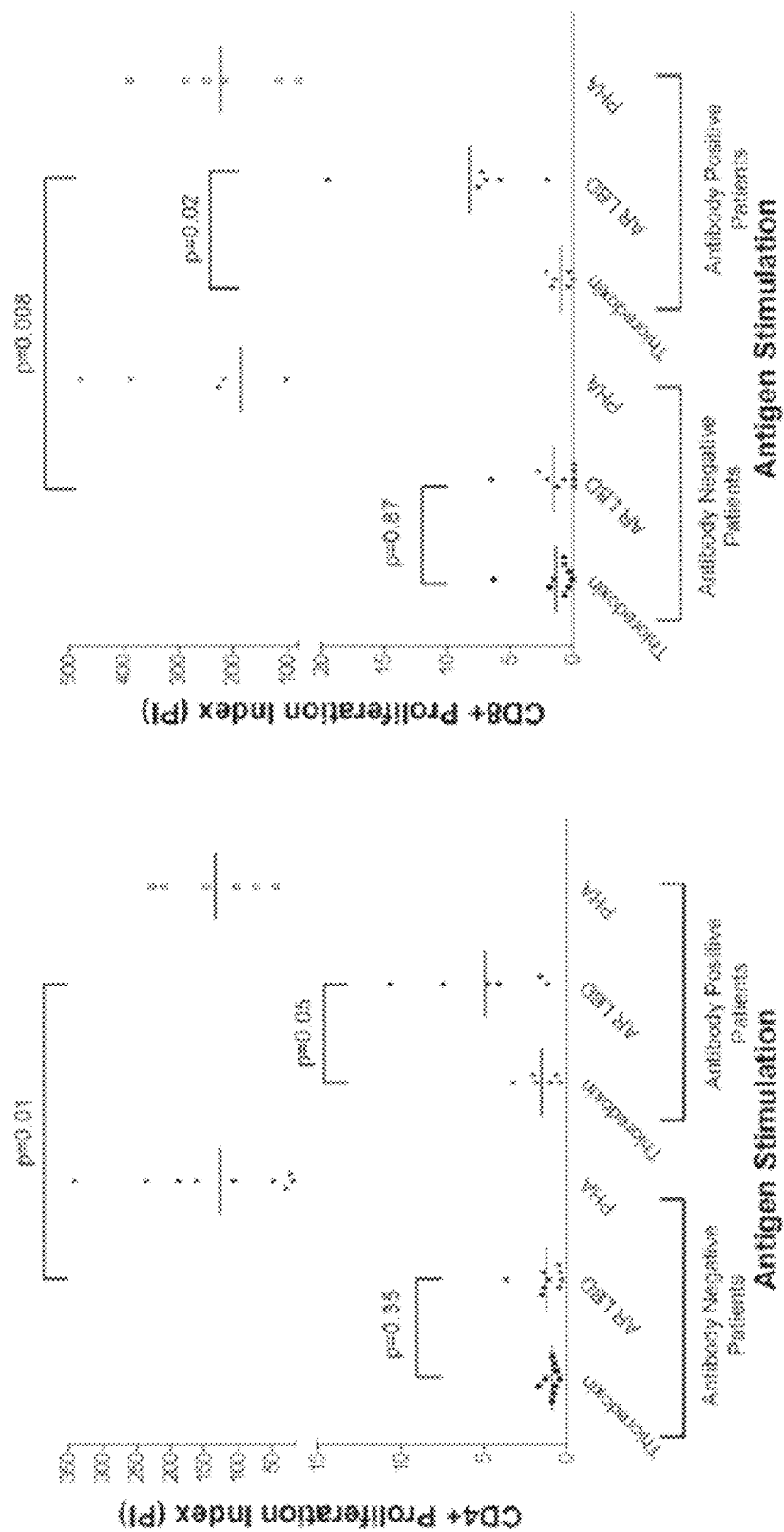


FIG. 2

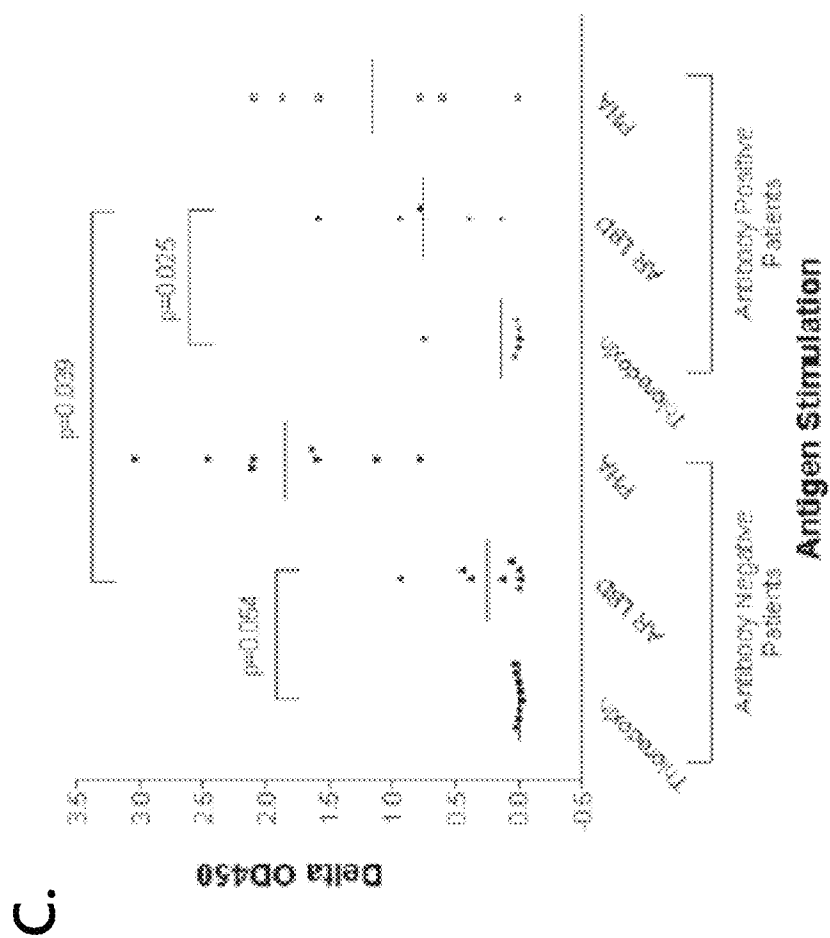


FIG. 2

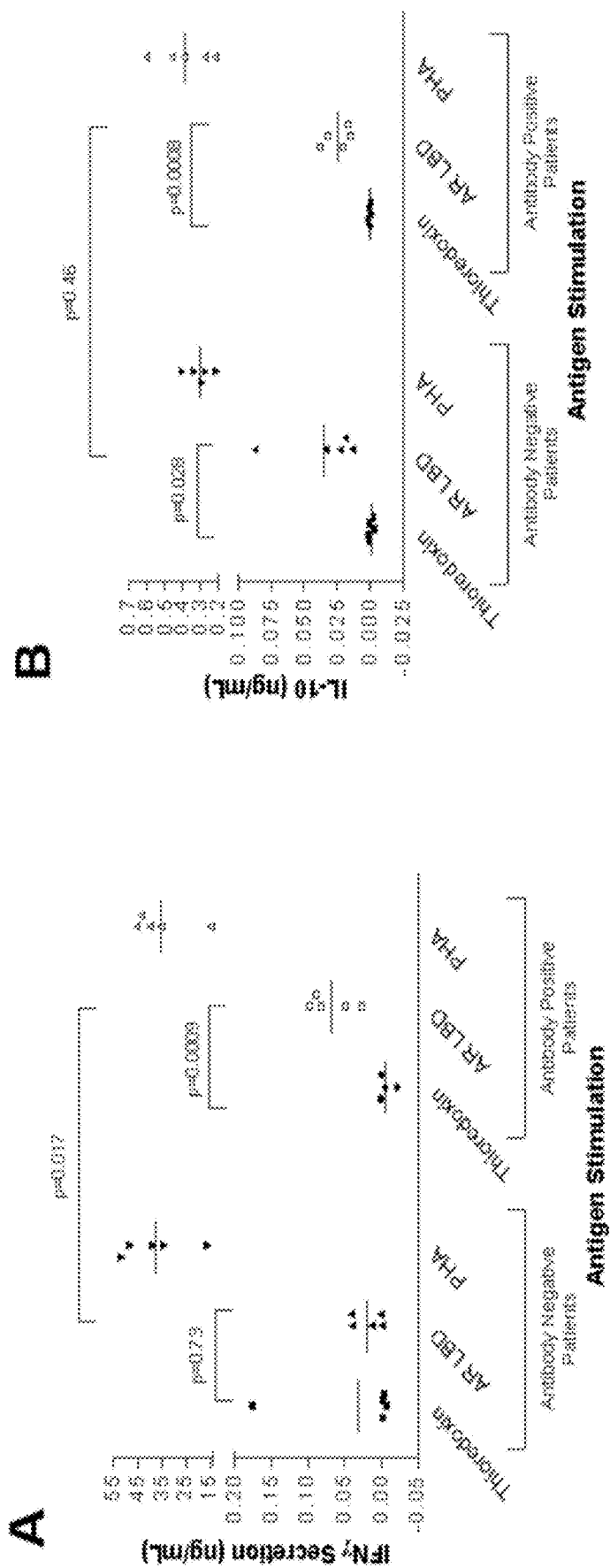


FIG. 3

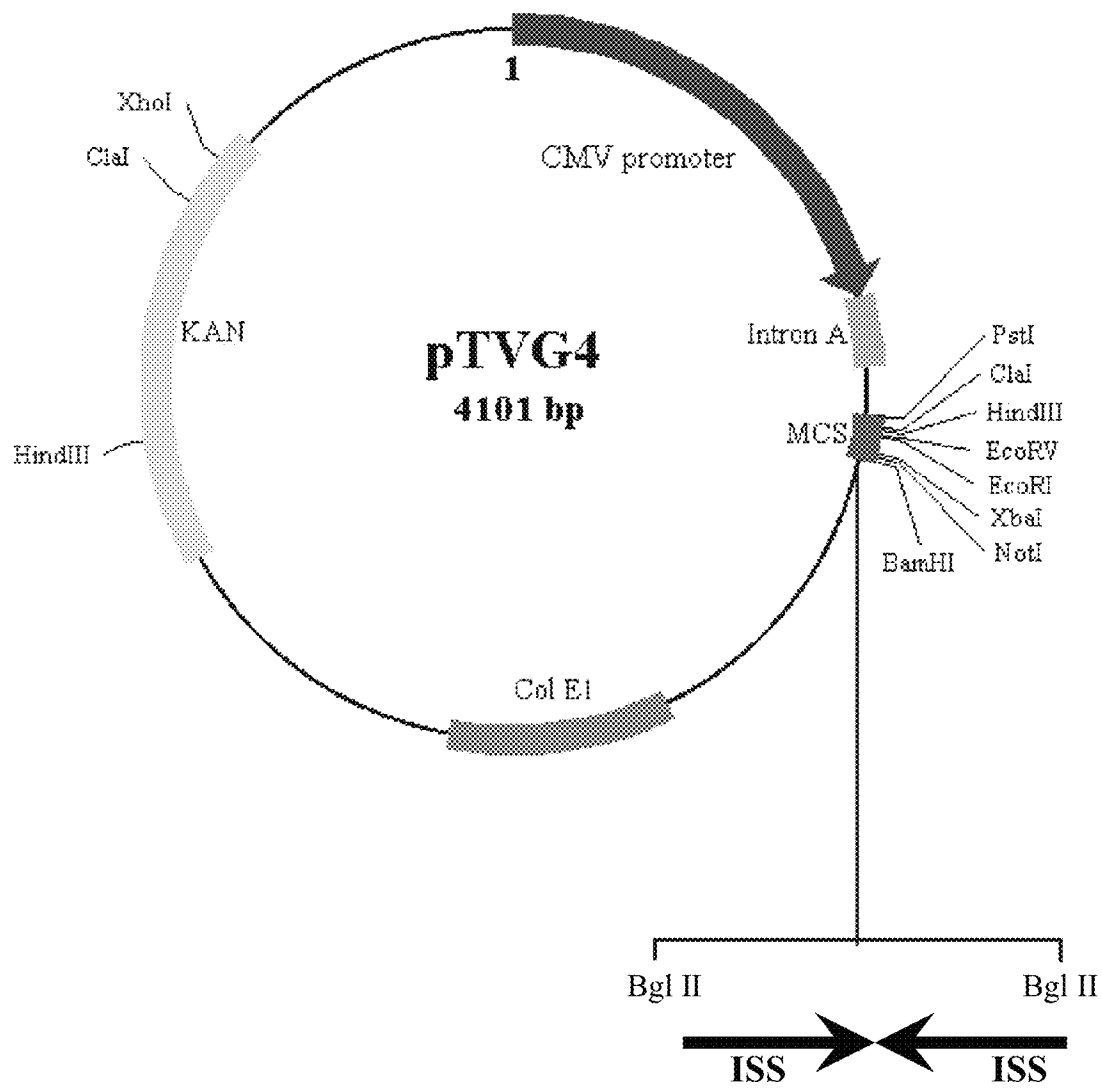


FIG. 4

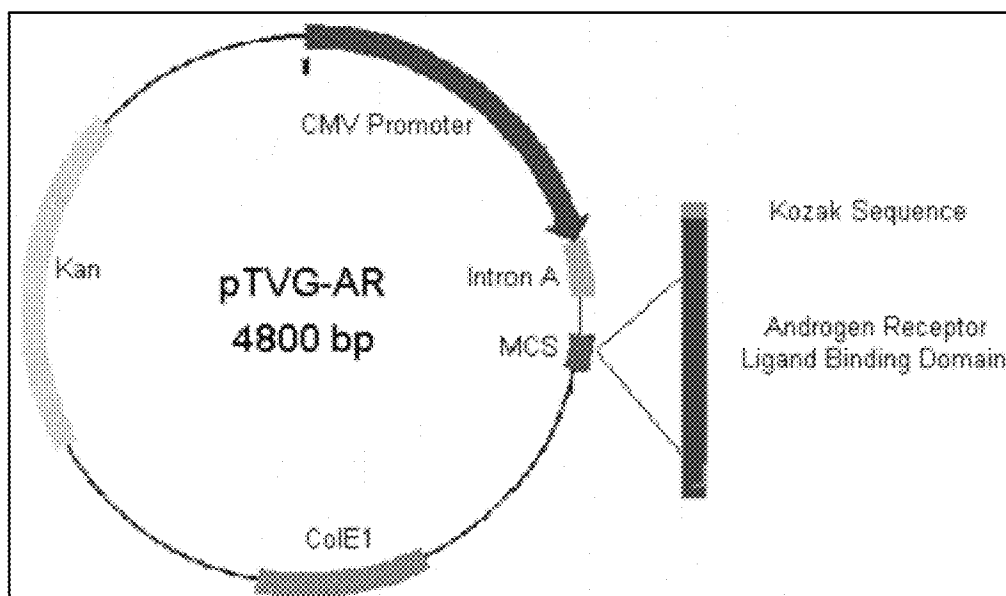


FIG. 5

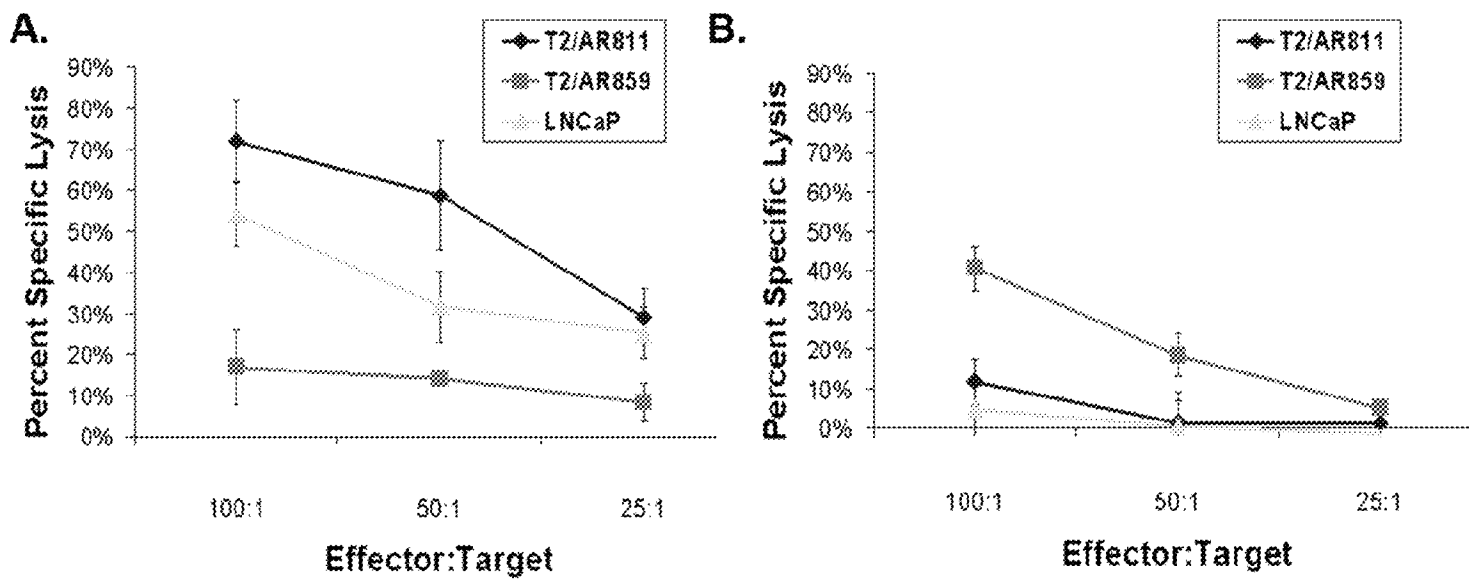


FIG. 6

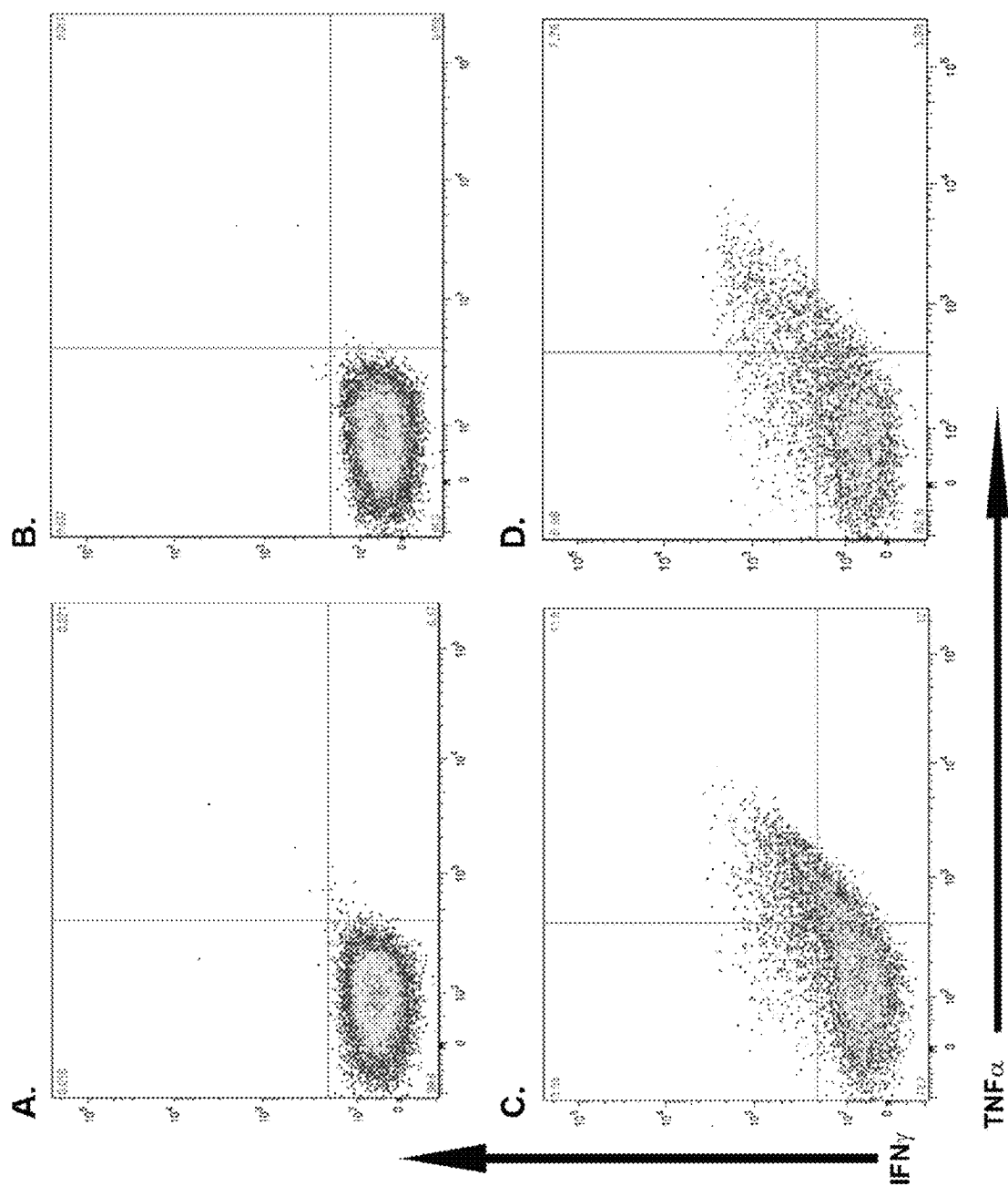


FIG. 7

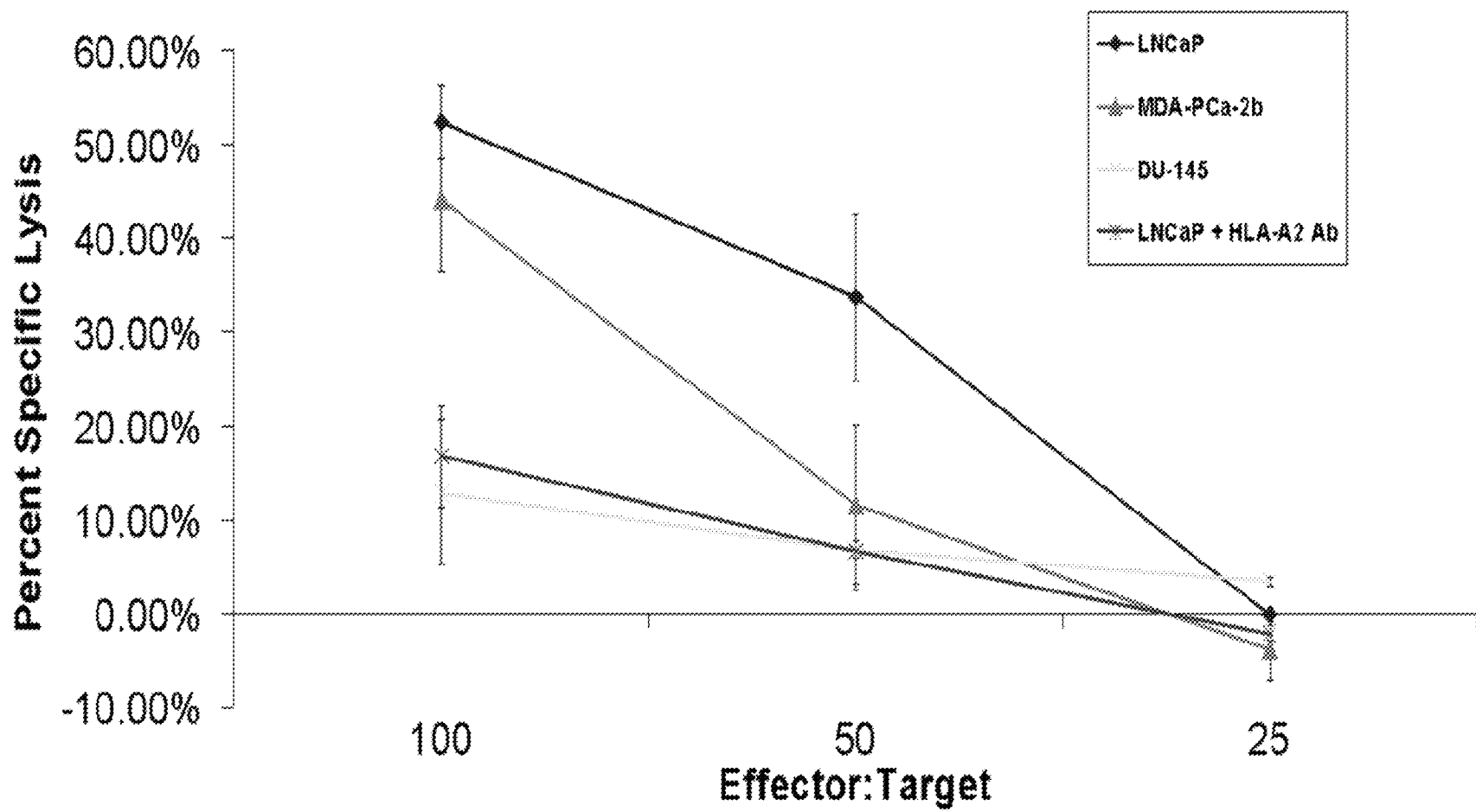


FIG. 8

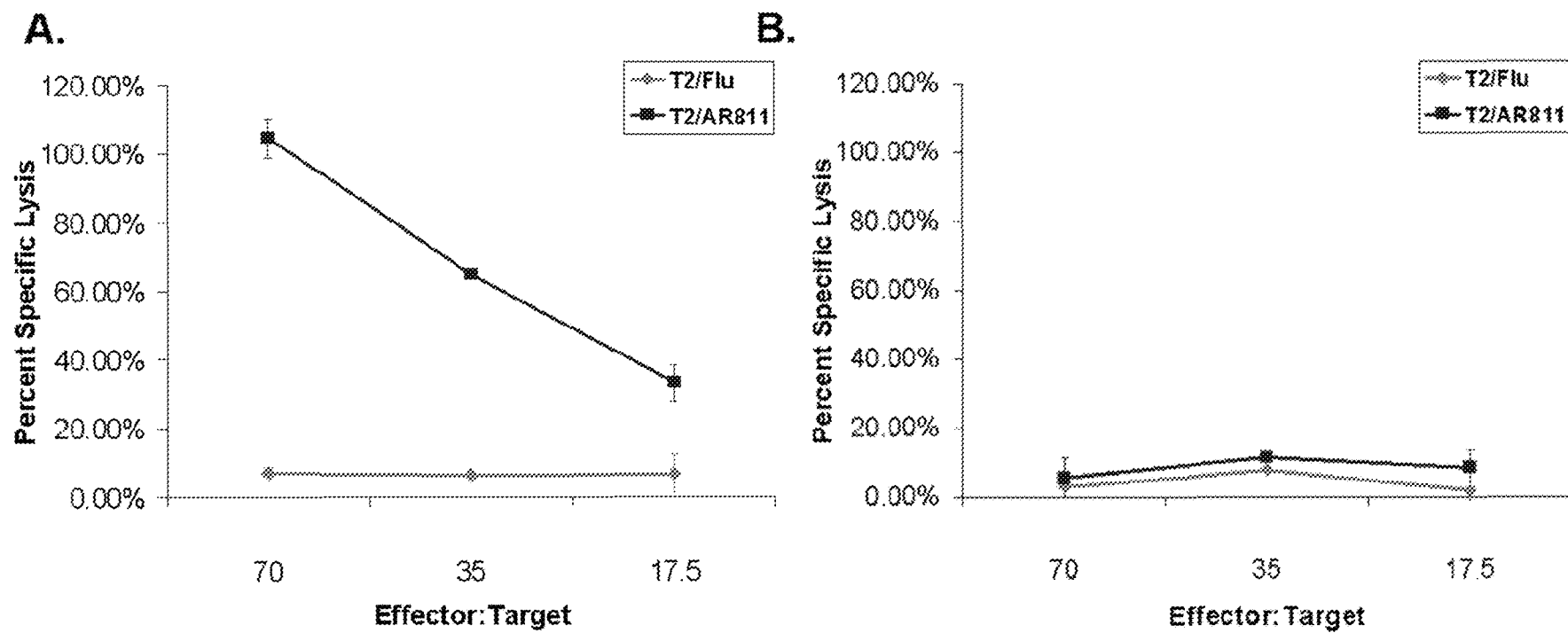


FIG. 9

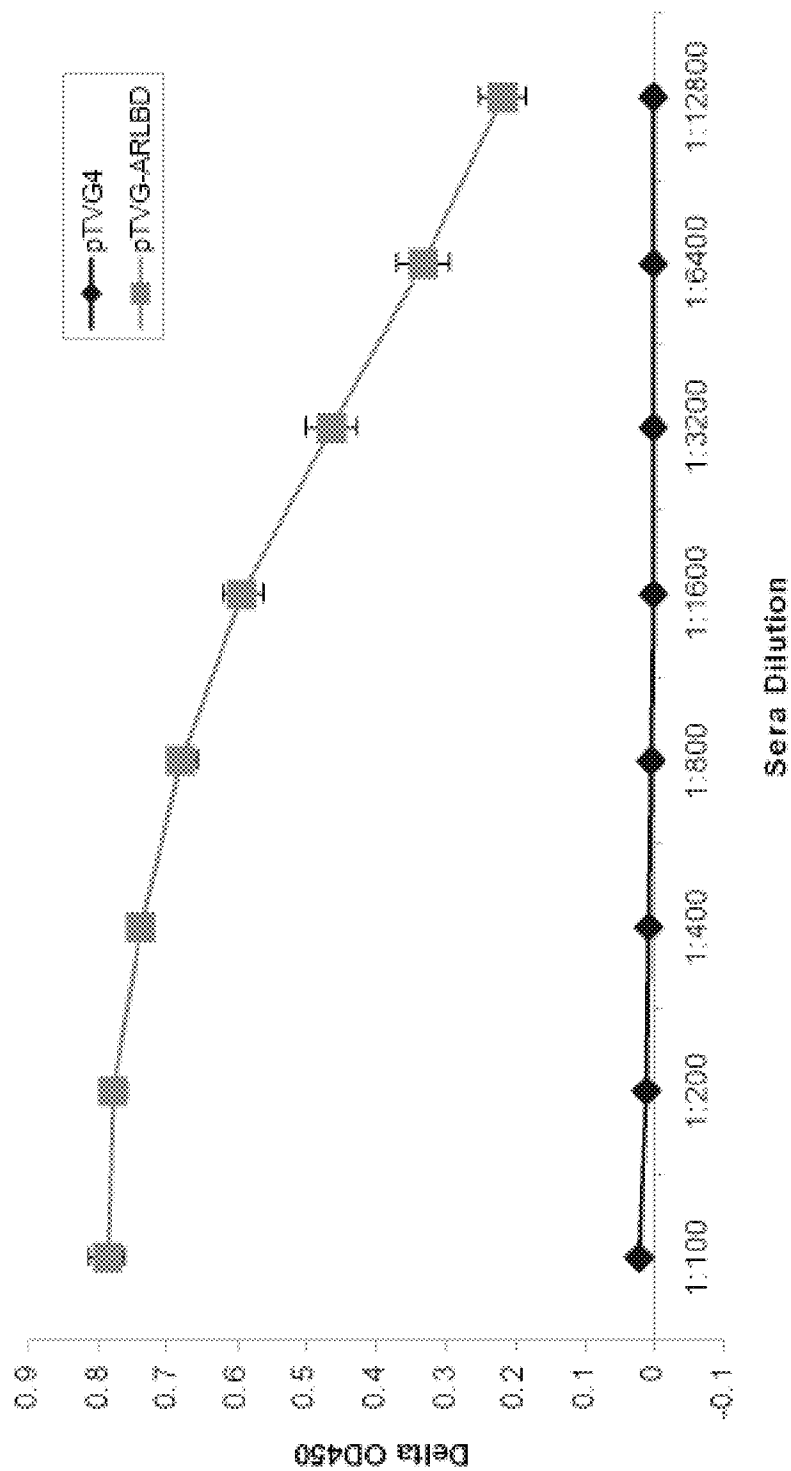


FIG. 10

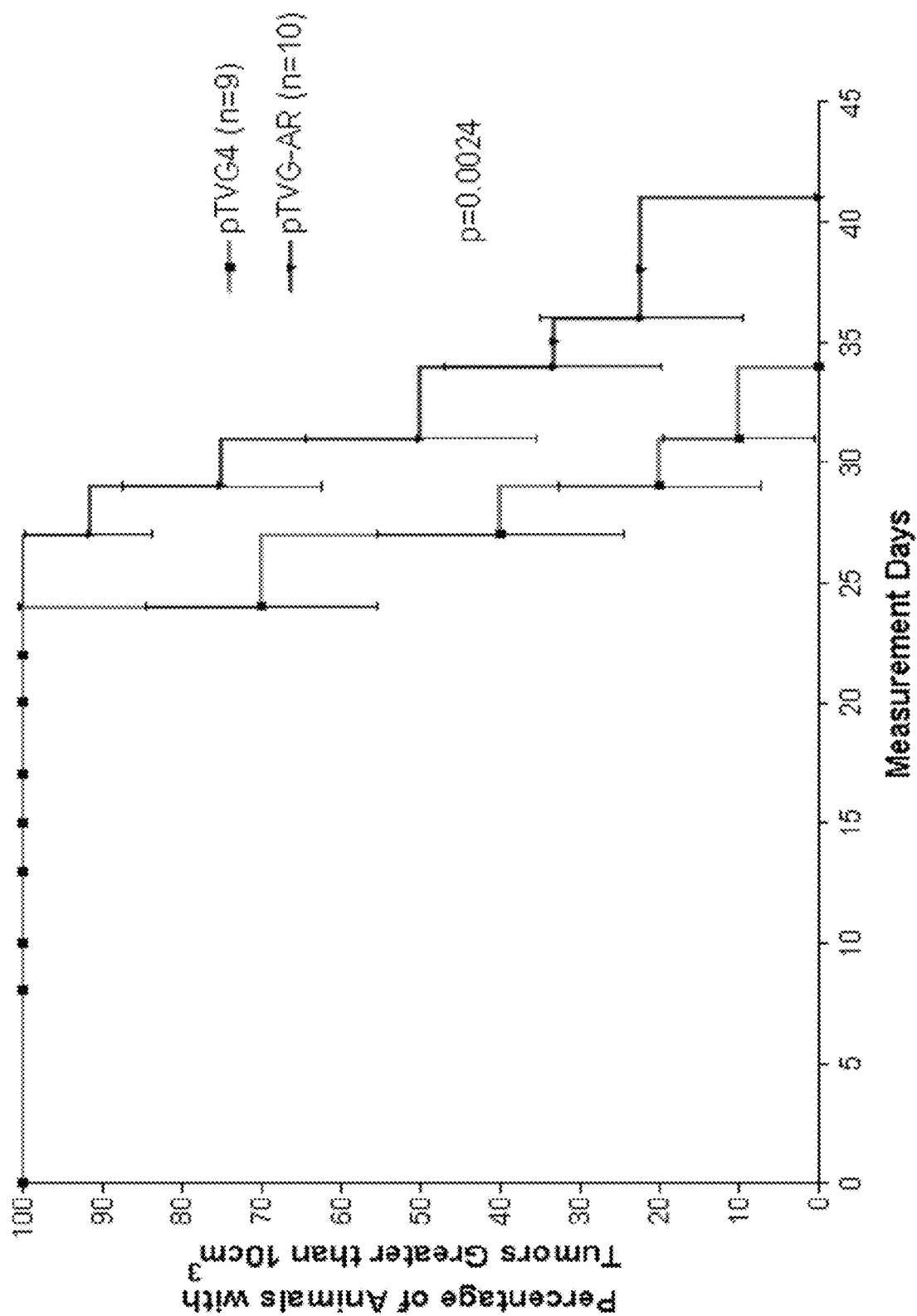


FIG. 11

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PROSTATE CANCER VACCINE**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application is a continuation of U.S. patent application Ser. No. 15/238,016 filed on Aug. 16, 2016 which is a continuation of U.S. patent application Ser. No. 14/614,137, filed on Feb. 4, 2015, which is a continuation of U.S. patent application Ser. No. 13/968,854, filed on Aug. 16, 2013, now U.S. Pat. No. 8,962,590, which is a continuation of U.S. patent application Ser. No. 13/031,396 filed on Feb. 21, 2011, now U.S. Pat. No. 8,513,210, which is a divisional of U.S. patent application Ser. No. 11/848,607 filed on Aug. 31, 2007, now U.S. Pat. No. 7,910,565 and claims the benefit of U.S. provisional application Ser. No. 60/841,769, filed on Sep. 1, 2006, all of which are herein incorporated by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under RR016489 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Prostate cancer is a significant health risk for men over the age of 50, with about 200,000 newly diagnosed cases each year in the United States (Jemal A. et al., *Cancer Statistics*, 2005 (2005) *CA Cancer J Clin*, 55:10-30). It is the most common tumor diagnosed among men and the second leading cause of male cancer-related death in the United States (Jemal et al., *Cancer Statistics*, 2003 (2003) *CA Cancer J Clin*, 53:5-26). Despite advances in screening and early detection, approximately 30% of patients undergoing definitive prostatectomy or ablative radiation therapy will have recurrent disease at 10 years (Oefelein et al., 1997, *J Urol*, 158:1460-1465). At present, there is no accepted adjuvant treatment for patients undergoing radical prostatectomy or ablative radiation therapy that has been shown to prevent the progression to metastatic disease. In addition to new treatments for metastatic disease, new strategies are needed to eradicate microscopic disease to prevent the progression to clinically apparent metastasis.

In patients who have undergone definitive ablative therapy for prostate cancer, the presence of detectable serum levels of prostate-specific antigen (PSA) has provided a valuable indicator of microscopic metastatic disease. In a retrospective review of 1,997 men treated with radical prostatectomy, 15% were found to have evidence of a PSA-only recurrence over a median 5-year follow up, so-called stage D0 disease (Pound et al., 1999, *JAMA* 281: 1591-7). Of these, 34% developed radiographically apparent metastatic disease, with a median time to development of metastatic disease of 8 years. In all patients with metastatic disease, the median time to death was 5 years (Pound et al., 1999, *JAMA* 281:1591-7). These findings suggest that patients with stage D0 disease are at high risk for progressive disease, however with a long window of time to test adjuvant therapies. Similarly, many patients are found to have microscopic pelvic lymph node metastases at the time of radical prostatectomy, so-called stage D1 disease. At present, the best treatment for these patients is controversial, with most treated with androgen deprivation, and others are expectantly observed without specific treatment. In retro-

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spective studies, 10-year disease-specific recurrence and mortality is on the order of 50 to 66% for patients with stage D1 disease (Sgrignoli et al., 1994, *J Urol*, 152:1077-81; and Cadeddu et al., 1997, *Urology*, 50:251-5). This high-risk stage of minimal residual disease also provides an opportunity to test novel adjuvant therapies.

Immunological therapies, and vaccines in particular, are appealing as possible treatment options for prostate cancer for several reasons. Such therapies may be relatively safe and inexpensive treatments compared with chemotherapies for a disease for which no standard adjuvant treatments exist (Kent et al., *Immunity of prostate specific antigens in the clinical expression of prostatic carcinoma* (1976) In: Crispen R G, ed. *Neoplasm immunity: mechanisms*. Chicago, ITR, pp. 85-95; Guinan et al., 1984, *Prostate*, 5:221-230; and McNeel et al., 2000, *Arch. Immunol. Ther. Exp.*, 48:85-93). Moreover, prostate cancer is a slow-growing disease, with typically over five years from the time of diagnosis of organ-confined disease to the development of clinically apparent metastatic disease. Such a slow-growing disease might be more amenable to vaccine-based treatments than a rapidly growing tumor, assuming that microscopic amounts of disease would be easier to treat than bulky or rapidly growing disease by vaccines. In fact, vaccines have already entered clinical trials for prostate cancer targeting a variety of prostate-specific proteins, with at least two dendritic cell-based vaccines suggesting clinical benefit in patients with low-volume metastatic disease (Murphy et al., 1999, *Prostate*, 39:54-59; and Small et al., 2000, *J. Clin. Oncol.* 18:3894-3903).

The use of plasmid DNA alone as a means of in vivo gene delivery by direct injection into muscle tissue was first described by Wolff et al. (Wolff et al., 1990, *Science*, 247:1465-1468). It was subsequently found that intramuscular or intradermal administration of plasmids expressing foreign genes elicited immune responses (Tang, et al., 1992, *Nature*, 356:152-154; Wang et al., 1993, *Proc Natl. Acad. Sci. USA*, 90:4156-4160; and Raz et al., 1994, *Proc Natl. Acad. Sci. USA*, 91:9519-9523). This has quickly led to numerous investigations into the use of plasmid DNA as a means of vaccine antigen delivery, both in animal and human models. DNA vaccines, like peptide-based vaccines, are relatively easy and inexpensive to manufacture, and are not individualized for patients as are dendritic cell-based vaccines. With recombinant protein vaccines, the antigen is taken up by antigen presenting cells and expressed predominantly in the context of MHC class II. DNA in nucleic acid vaccines is taken up and expressed by antigen-presenting cells directly, leading to antigen presentation through naturally processed MHC class I and II epitopes (Iwasaki, et al. 1997, *J Immunol*, 159:11-14). This direct expression by host cells, including MHC class I expressing bystander cells, has been demonstrated to lead to vigorous CD8+CTL responses specific for the targeted antigen (Iwasaki et al., 1997, *J. Immunol.* 159:11-14; Chen et al., 1998, *J. Immunol.*, 160: 2425-2432; Thomson et al., 1998, *J. Immunol.*, 160:1717-1723; and Cho et al., 2000, *Nat. Biotechnol.* 18:509-514).

Clinical trials have suggested that plasmid DNA vaccines are safe and immunologically effective in humans. Boyer and colleagues reported that doses of 300 µg of plasmid DNA encoding HIV rev and env proteins administered intramuscularly were capable of eliciting antigen-specific, IFNγ-secreting T cell responses in HIV-seronegative patients (Boyer et al., 2000, *J. Infect. Dis.* 181:476-83). In addition, results of a clinical trial targeting prostate-specific membrane antigen (PSMA) in patients with prostate cancer by means of plasmid DNA and adenovirus have been

reported (Mincheff et al., 2000, Eur. Urol., 38:208-217). In this study, 26 patients were immunized either in a prime/boost strategy with an adenoviral vector expressing PSMA followed by immunization with plasmid DNA expressing PSMA, or with plasmid DNA alone. The authors report no significant toxicity with doses of 100-800 µg of plasmid DNA administered intradermally, and suggest that patients receiving plasmid DNA expressing PSMA and CD86 with soluble GM-CSF as an adjuvant were all successfully immunized.

A DNA vaccine for the treatment of prostate cancer based on prostatic acid phosphatase (PAP) has also been described (US 2004/0142890).

BRIEF SUMMARY OF THE INVENTION

The present invention is based, in part, on the inventors' discovery that patients with prostate cancer have antibodies specific for the androgen receptor, that androgen receptor ligand-binding domain as well as four fragments thereof (SEQ ID NO:9-12) can elicit immune responses in vivo, and that animals vaccinated with a DNA vaccine encoding the androgen receptor (AR) ligand-binding domain (LBD) inhibited prostate tumor growth in vivo.

In one aspect, the invention relates a method for inducing an immune reaction to androgen receptor in a mammal in need thereof, the method comprising administering to the mammal an effective amount of a recombinant DNA construct comprising a polynucleotide operatively linked to a transcriptional regulatory element (e.g., a promoter such as a heterologous promoter) wherein the polynucleotide encodes a member selected from (i) a mammalian androgen receptor (e.g., a human androgen receptor), (ii) a fragment of the androgen receptor that comprises the ligand-binding domain, (iii) a fragment of the ligand-binding domain defined by SEQ ID NO:9, (iv) a fragment of the ligand-binding domain defined by SEQ ID NO:10, (v) a fragment of the ligand-binding domain defined by SEQ ID NO:11, and (vi) a fragment of the ligand-binding domain defined by SEQ ID NO:12, whereby the mammal develops immune reaction against the androgen receptor. In one form, the polynucleotide employed in the method encodes the ligand-binding domain of a mammalian androgen receptor. In another form, multiple DNA constructs with each comprising a polynucleotide that encodes a different fragment selected from (iii)-(vi) are administered. For example, two DNA constructs covering fragments (iii) and (iv) can be administered together. As another example, four DNA constructs covering all four fragments (iii)-(vi) can be administered together. The method disclosed can be practiced with a mammal, preferably a human, who either currently has or previously had prostate cancer.

In one embodiment, the polynucleotide encodes a human androgen receptor or a fragment of the human androgen receptor that comprises the ligand-binding domain. The polynucleotide is preferably a nucleotide sequence of the human androgen receptor gene. In one form of this embodiment, the polynucleotide encodes the ligand-binding domain of a human androgen receptor.

The above method employing the DNA construct induces cytotoxic immune reaction against cells expressing androgen receptor. Preferably, both humoral and cellular immune reactions against androgen receptor are induced.

In another aspect, the present invention relates to a method for inducing an immune reaction to androgen receptor in a mammal in need thereof, the method comprising administering to the mammal an effective amount of a

polypeptide selected from (i) a mammalian androgen receptor (e.g., a human androgen receptor), (ii) a fragment of the androgen receptor that comprises the ligand-binding domain, (iii) a fragment of the ligand-binding domain defined by SEQ ID NO:9, (iv) a fragment of the ligand-binding domain defined by SEQ ID NO:10, (v) a fragment of the ligand-binding domain defined by SEQ ID NO:11, and (vi) a fragment of the ligand-binding domain defined by SEQ ID NO:12, whereby the mammal develops immune reaction against the androgen receptor. In one form, the polypeptide employed is the ligand-binding domain of a mammalian androgen receptor. In another form, multiple fragments of the ligand-binding domain (e.g. SEQ ID NO:9 and SEQ ID NO:10, and optionally SEQ ID NO:11 and SEQ ID NO:12) are administered. The method disclosed can be practiced with a mammal, preferably a human, who either currently has or previously had prostate cancer.

In one embodiment, the human androgen receptor or a fragment of the human androgen receptor that comprises the ligand-binding domain is administered. In one form of this embodiment, the ligand-binding domain of the human androgen receptor is administered.

The above method employing the polypeptide induces cellular or humoral immune reaction against cells expressing androgen receptor. Preferably, both humoral and cellular immune reactions against androgen receptor are induced.

According to one embodiment of the invention, the recombinant DNA construct or the polypeptide is administered to the mammal intradermally, intramuscularly, subcutaneously, or intravascularly, including intravenously and intraarterially. Preferably, the recombinant DNA construct is administered intradermally, intramuscularly, or intravascularly and the polypeptide is administered subcutaneously.

In another aspect, the present invention relates to an isolated polypeptide selected from SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12. In another aspect, the present invention relates to a composition that comprises one or more of the above polypeptides and a pharmaceutically acceptable carrier.

According to another aspect of the present invention, a DNA vaccine is contemplated which comprises a plasmid vector comprising a polynucleotide operatively linked to a transcriptional regulatory element (e.g., a promoter such as a heterologous promoter) wherein the polynucleotide encodes a member selected from (i) a mammalian androgen receptor (e.g., a human androgen receptor), (ii) a fragment of the androgen receptor that comprises the ligand-binding domain, (iii) a fragment of the ligand-binding domain defined by SEQ ID NO:9, (iv) a fragment of the ligand-binding domain defined by SEQ ID NO:10, (v) a fragment of the ligand-binding domain defined by SEQ ID NO:11, and (vi) a fragment of the ligand-binding domain defined by SEQ ID NO:12, wherein upon administration of said vaccine to a mammal a cytotoxic immune reaction against cells expressing androgen receptor is induced. The vaccine of the present invention preferably is suitable for intradermal, intramuscular, subcutaneous, or intravascular (including intravenous and intraarterial) administration to a mammal such as a human. According to a preferred embodiment, the plasmid vector comprises (a) a backbone of pNGVL3, (b) a polynucleotide operably inserted therein wherein the polynucleotide encodes a member selected from (i) a mammalian androgen receptor (e.g., a human androgen receptor), (ii) a fragment of the androgen receptor that comprises the ligand-binding domain, (iii) a fragment of the ligand-binding domain defined by SEQ ID NO:9, (iv) a fragment of the ligand-binding domain defined by SEQ ID NO:10, (v) a

fragment of the ligand-binding domain defined by SEQ ID NO:11, and (vi) a fragment of the ligand-binding domain defined by SEQ ID NO:12, and, optionally, (c) one or a plurality of an immunostimulatory sequence (ISS) motif.

Preferably, the DNA vaccine according to the invention comprises a plasmid vector that comprises (a) a polynucleotide operatively linked to a CMV promoter wherein the polynucleotide encodes a member selected from (i) a mammalian androgen receptor (e.g., a human androgen receptor), (ii) a fragment of the androgen receptor that comprises the ligand-binding domain, (iii) a fragment of the ligand-binding domain defined by SEQ ID NO:9, (iv) a fragment of the ligand-binding domain defined by SEQ ID NO:10, (v) a fragment of the ligand-binding domain defined by SEQ ID NO:11, and (vi) a fragment of the ligand-binding domain defined by SEQ ID NO:12, (b) a CMV intron A operatively linked to the polynucleotide for enhancing expression of the polynucleotide, and, optionally, (c) at least one copy of an immunostimulatory fragment comprising 5'-GTCGTT-3'. In one embodiment, the plasmid construct does not express in eukaryotic cells any gene other than a member selected from (i) a mammalian androgen receptor, (ii) a fragment of the androgen receptor that comprises the ligand-binding domain, (iii) a fragment of the ligand-binding domain defined by SEQ ID NO:9, (iv) a fragment of the ligand-binding domain defined by SEQ ID NO:10, (v) a fragment of the ligand-binding domain defined by SEQ ID NO:11, and (vi) a fragment of the ligand-binding domain defined by SEQ ID NO:12. The plasmid vector pTVG4 is particularly preferred.

According to another aspect of the present invention, a peptide vaccine is contemplated which comprises a member selected from (i) a mammalian androgen receptor (e.g., a human androgen receptor), (ii) a fragment of the androgen receptor that comprises the ligand-binding domain, (iii) a fragment of the ligand-binding domain defined by SEQ ID NO:9, (iv) a fragment of the ligand-binding domain defined by SEQ ID NO:10, (v) a fragment of the ligand-binding domain defined by SEQ ID NO:11, and (vi) a fragment of the ligand-binding domain defined by SEQ ID NO:12. The peptide vaccine also comprises a pharmaceutically acceptable carrier. The peptide vaccine preferably is suitable for intradermal, intramuscular, subcutaneous, or intravascular (including intravenous and intraarterial) administration to a mammal such as a human.

Also disclosed are pharmaceutical compositions comprising a DNA or peptide vaccine of the invention (the polypeptides or recombinant plasmid vectors described above), and a pharmaceutically acceptable carrier. Preferably, the pharmaceutical composition further comprises a suitable amount of immuno-stimulant such as GM-CSF.

A kit containing the DNA or peptide vaccine of the invention and an instruction manual directing administering the vaccine to a mammal that has or previously had prostate cancer (e.g., a human prostate cancer patient) is also within the scope of the invention.

In another aspect, the present invention relates to a method for determining the effectiveness of a treatment for prostate cancer. The method includes the steps of (a) measuring the frequency or amount of cytotoxic T lymphocytes (CTLs) specific for a peptide selected from SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12 prior to providing at least a portion of the treatment to a mammal (e.g., a human) having prostate cancer, (b) measuring the frequency or amount of CTLs specific for the peptide after said portion of the treatment is provided to the mammal, and (c) comparing the frequency or amount of CTLs of (a) and

that of (b) wherein the frequency or amount of CTLs of (b) being higher than that of (a) indicates that the treatment is effective.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1 shows that patients with prostate cancer have antibodies specific for the androgen receptor. Panel A: Sera from patients with various stages of prostate cancer were analyzed for the presence of AR-specific antibodies by screening titrated sera samples using ELISA. Panel B: The presence or absence of AR-specific antibodies was confirmed using Western blotting against thioredoxin-tagged AR LBD or thioredoxin (trx) alone, followed by incubation with patient's sera. Panel C: ELISA was used to evaluate sera samples for the presence of AR-specific antibodies. Samples were analyzed from healthy male blood donors (n=41), patients with prostatitis (n=38), or patients with prostate cancer (n=105), and relative antibody concentrations were calculated by referencing delta absorbance values to titrated Ig protein standards. Positive antibody responses were defined by values higher than three standard deviations above the mean of the healthy donor group (greater than 0.22 µg/mL, indicated by the line). Statistically significant differences were calculated using the Chi square test.

FIG. 2 shows that patients with AR LBD-specific IgG antibodies have concurrent AR-specific cellular immune responses. PBMC were analyzed for the presence of AR-specific T cells and IFN γ -secreting cells among patients with antibody responses specific for the AR (n=6) or patients with no detectable antibody responses (n=9). PBMC were stimulated with thioredoxin-tagged AR LBD, thioredoxin alone, media only, or PHA. After a 96-hour stimulation, these cells were analyzed for the presence of CD4+ and CD8+ T cell proliferation in response to antigen stimulation. Example data is shown in panel A from a subject with a strong IgG2 response. The numbers in the upper-right corner of each panel indicate the percentage of CD4+ or CD8+ T cells that co-stained with BrdU. Proliferation indexes (PI) were calculated by normalizing experimental values to values obtained from PBMCs stimulated with media alone, and compiled PI values are shown for CD4+(panel B, left) and CD8+(panel B, right) T-cells. Supernatants from cultured PBMC were also analyzed for the presence of IFN γ secretion by capture ELISA (panel C). A comparison of results among different antigen-stimulation conditions was performed using the Student's T test.

FIG. 3 shows that patients with AR LBD-specific IgG antibodies have a mixed Th1/Th2-type immune response. PBMC from prostate cancer patients with (n=5) or without (n=5) antibody responses specific for the AR were analyzed for the presence of antigen-specific IFN γ or IL-10-secretion. PBMC were stimulated with thioredoxin-tagged AR LBD, thioredoxin alone, media only, or PHA. After a 96-hour stimulation, supernatants were analyzed for the presence of IFN γ (panel A) and IL-10 (panel B) secretion by quantitative ELISA. A comparison of results among different antigen-stimulation conditions was performed using a Student's T test. The results shown are representative of duplicate experiments with the same peripheral blood samples.

FIG. 4 shows the plasmid map of pTVG4.

FIG. 5 shows the plasmid map of pTVG-ARLBD (pTVG-androgen receptor ligand-binding domain).

FIG. 6 shows that AR LBD peptide-specific T-cells can lyse peptide-pulsed and prostate cancer cell line target cells. Peptide-specific T-cell lines were cultured from the periph-

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eral blood of HLA-A2-expressing patients with prostate cancer using peptides AR811 (LLFSIIPV, SEQ ID NO:9, panel A) or AR859 (QLTKLLDSV, SEQ ID NO:12, panel B). After several *in vitro* restimulations, cultures were tested for cytolytic activity to T2 cells loaded with AR811 peptide (diamond), AR859 (square), or the LNCaP HLA-A2 expressing prostate cancer cell line (triangle). Shown is a representative graph results obtained from the cytotoxicity assay of T-cell cultures performed in triplicate at three different effector-to-target ratios as indicated.

FIG. 7 shows that a T-cell clone specific for the AR811 peptide secretes IFN γ and TNF α in response to peptide stimulation. A clonal T-cell line specific for the AR811 peptide was derived by limited dilution following multiple restimulations *in vitro*. This line was restimulated for 5 hours *in vitro* with T2 cells and media only (panel A), an irrelevant peptide (panel B), AR811 peptide (panel C), or PMA/Ionomycin (panel D). IFN γ and TNF α cytokine accumulation were assessed by intracellular flow cytometric analysis (Cytofix/cytoperm kit, BD Pharmingen). Cells were first stained for CD3 and CD8 expression and CD3+/CD8+ cells were analyzed for IFN γ and TNF α expression. Shown are the plots gated on CD3+/CD8+ cells.

FIG. 8 shows that the AR811 epitope is a naturally processed HLA-A2-restricted T-cell epitope. The AR811 T-cell clone was tested for its ability to lyse prostate cancer cell lines (expressing the AR) that express HLA-A2 (LNCaP, diamond and MDA-Pca-2B, triangle), or do not express HLA-A2 (DU145, X). In addition, lysis was evaluated following pre-treatment of LNCaP with an HLA-A2 blocking monoclonal antibody (starburst). Shown is a cytotoxicity assay of an AR811 CD8+ cell clone. Cells were restimulated for seven days using peptide-pulsed antigen presenting cells, followed on day seven by a rest period of three days before they were analyzed for their cytotoxic activity.

FIG. 9 shows that HHD-II mice immunized with AR811 peptide develop peptide-specific CTL (contain splenocytes that can specifically lyse AR811 target cells). Male HHD-II mice (n=3 per group) were immunized once with 100 μ g AR811 peptide in CFA or with CFA alone. One week after immunization, splenocytes were collected and stimulated *in vitro* with 10 μ g/mL peptide for two hours, and on the second day, recombinant murine IL-2 and IL-7 (Fitzgerald Industries) were added to 10 U/mL and 30 U/mL, respectively. The cultures were then allowed to incubate an additional six days before analysis. Cultured cells from from AR811-immunized animals (panel A) or control immunized animals (panel B) were then tested for cytolytic activity to T2 cells pulsed with the AR811 peptide (square) or T2 cells pulsed with an influenza matrix peptide (diamond). Shown are the mean and standard deviation of triplicate wells at three effector-to-target ratios as indicated, each from a single animal per group, and representative of the other animals per group.

FIG. 10 shows that rats immunized with a plasmid vector encoding AR LBD develop AR LBD-specific antibody responses. Copenhagen rats were immunized 4 times at 14-day intervals with pTVG4 control vector or pTVG-ARLBD. Two weeks after the fourth immunization, blood was collected and assessed for antibody responses to the AR-LBD protein by ELISA. Shown are the mean and standard error for 10 animals per experimental group.

FIG. 11 shows tumor growth in pTVG4 control vector or pTVG-ARLBD immunized rats (Kaplan-Meier analysis of animal endpoint survival following treatment with the pTVG4 control vector or pTVG-ARLBD). Male Copenhagen rats were immunized four times every other week with

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pTVG4 control vector or pTVG-ARLBD. Two weeks after immunization, the rats were challenged with 1×10^4 syngeneic Mat-LyLu prostate cancer cells given with Matrigel. Tumors were measured every other day, and rats were sacrificed when tumors grew larger than 10 cm³ (Kaplan-Meier survival end point being tumor volume over 10 cm³).

DETAILED DESCRIPTION OF THE INVENTION

This invention provides pharmaceutical compositions and methods that relate to the use of plasmid DNA and peptide vaccines for the treatment of prostate cancer. Specifically, this invention provides polypeptides such as the ligand-binding domain of an androgen receptor or certain fragments thereof and recombinant plasmid vectors comprising genes or polynucleotide molecules encoding the polypeptides for preventing or treating prostate cancer, including metastatic tumors thereof. In a preferred embodiment, the polypeptides or recombinant plasmid vectors are administered to prostate cancer patients to treat prostate cancer. In another preferred embodiment, the polypeptides or recombinant plasmid vectors are administered to stage D0 or D1 prostate cancer patients to prevent recurrence or metastasis of prostate cancer.

A polypeptide vaccine of the present invention, which comprises a pharmaceutically acceptable carrier and an effective amount of a mammalian androgen receptor, a fragment of the mammalian androgen receptor that comprises the ligand-binding domain, or certain fragments of the ligand-binding domain, can be administered into a mammal such as a human being to elicit an immune response against androgen receptor in the mammal. An "effective amount" or an "immunologically effective amount" means that the administration of that amount to a subject, either in a single dose or as part of a series, is effective for inducing an immune reaction and preferably for treating or preventing prostate cancer. Pharmaceutically acceptable carriers are well known to those of ordinary skill in the art (Arnon, R. (Ed.) *Synthetic Vaccines* 1:83-92, CRC Press, Inc., Boca Raton, Fla., 1987). They include liquid media suitable for use as vehicles to introduce the peptide into a patient but should not in themselves induce the production of antibodies harmful to the individual receiving the composition. An example of such liquid media is saline solution. Moreover, the vaccine formulation may also contain an adjuvant for stimulating the immune response and thereby enhancing the effect of the vaccine.

The plasmid DNA vaccines of the present invention, when directly introduced into mammals such as humans *in vivo*, induce the expression of encoded polypeptides within the mammals, and cause the mammals' immune system to become reactive against the polypeptides. The vaccines may be any polynucleotides that are capable of eliciting immune responses to an encoded polypeptide.

The instant invention also provides a method of using a polynucleotide which, upon introduction into a mammal, induces the expression, *in vivo*, of the polynucleotide thereby producing the encoded polypeptide, and causes the mammal to become immune reactive against the polypeptide so produced.

DNA vaccines, like peptide-based vaccines, are relatively easy and inexpensive to manufacture, and are not individualized for patients, as are dendritic cell-based vaccines. With recombinant protein vaccines, the antigen is taken up by antigen presenting cells and expressed predominantly in the context of MHC class II. DNA in nucleic acid vaccines is

taken up and expressed by antigen-presenting cells directly, leading to antigen presentation through naturally processed MHC class I and II epitopes (Iwasaki, et al. 1997, J Immunol, 159:11-14).

Given their potential ability to elicit antigen-specific cytotoxic T lymphocytes (CTL) immunity in an MHC class I diverse population, DNA-based vaccines for various diseases have recently entered human clinical trials (Mincheff et al., 2000, Eur. Urol., 38:208-217). This method of immunization is similar to the use of viral immunization vectors, but without the additional foreign antigens introduced with a viral vector and therefore with less risk of an overwhelming immune response to the vector itself (Irvine and Restifo, 1995, Seminars in Canc. Biol. 6:337-347). Direct expression by host cells, including MHC class I-expressing bystander cells, has been demonstrated to lead to vigorous CD8+CTL responses specific for the targeted antigen (Iwasaki et al., 1997, J. Immunol. 159:11-4; Chen et al., 1998, J. Immunol. 160:2425-2432; Thomson et al., 1998, J. Immunol. 160:1717-1723; and Cho et al., 2000, Nat. Biotechnol. 18:509-14). In addition, plasmid DNA used for immunization may remain within cells at the site of immunization, providing a constant source of antigenic stimulation. Persistent antigen expression may lead to long-lived immunity (Raz et al., 1994, Proc. Natl. Acad. Sci. USA 91:9519-23).

The present invention provides DNA-based vaccines that express a polypeptide antigen, the ligand-binding domain of a mammalian androgen receptor or certain fragments thereof, and methods for treating prostate cancers in a human or non-human animal using the vaccines. In addition to the reasons explained above, plasmid vaccines are advantageous over viral vaccines. For example, viral vaccines are not amenable to repeated immunizations. With viral vectors, one is trying to elicit an immune response against a "self" protein encoded by a foreign virus. The immune system preferentially recognizes the foreign proteins, sometimes hundreds of proteins, encoded by the virus. For example, the inventors have found in rats that repeated immunizations with a vaccinia virus encoding human prostatic acid phosphatase (hPAP) elicits a strong vaccinia response but no hPAP-specific response (Johnson et al., 2007, Canc. Immunol. Immunoth. 56:885). That same finding was also shown in humans, in a trial in which repeated immunization with the vaccinia virus encoding human prostate-specific antigen (PSA) elicited weak PSA-specific immunity, but potent vaccinia immunity (Sanda et al., 1999, Urology 53:260). The direction in the field of viral-based vaccines is to "prime" with a virus encoding the antigen, and then "boost" with a different virus (like adenovirus or fowl pox) encoding the same antigen. The advantage of plasmid DNA vaccines is that they encode a defined, often small, number of proteins. Therefore, one can repetitively immunize the animal or patient. Furthermore, a virus may kill cells, incorporate into the genome, or potentially induce other unwanted immune responses. All these are disadvantages that are likely avoided by DNA plasmid vaccines.

It is readily recognizable that the ligand-binding domain of an androgen receptor of any origin, or any of the ligand-binding domain's derivatives, equivalents, variants, mutants etc., is suitable for the instant invention, as long as the ligand-binding domain or derivatives, equivalents, variants, or mutants thereof is able to induce an immune reaction in the host human or non-human animal substantially similar to that induced by an autoantigenic or xenoantigenic ligand-binding domain of the androgen receptor in the animal. Similarly, a polynucleotide sequence of an androgen receptor gene of any origin that encodes the ligand-binding

domain of the receptor, or any of the polynucleotide's derivatives, equivalents, variants, mutants etc., is suitable for the instant invention, as long as the polynucleotide sequence and the polypeptide or protein encoded by the polynucleotide sequence, or derivatives, equivalents, variants, or mutants thereof is able to induce an immune reaction in the host human or non-human animal substantially similar to that induced by an autoantigenic or xenoantigenic ligand-binding domain of the androgen receptor in the animal.

Androgen receptor genes are known and have been cloned from many species. For example, the human, mouse, rat, dog, chimpanzee, macaque, and lemur androgen receptor cDNA along with amino acid sequences can be found at GenBank Accession Nos. NM_000044 (cDNA-SEQ ID NO:1 and amino acid sequence-SEQ ID NO:2), NM_013476 (cDNA-SEQ ID NO:3 and amino acid sequence-SEQ ID NO:4), NM_012502 (cDNA-SEQ ID NO:5 and amino acid sequence-SEQ ID NO:6), NM_001003053, NM_001009012, U94179, and U94178, respectively. Androgen receptor genes from other species are also known. These species include but are not limited to *Sus scrofa*, *Astatotilapia burtoni*, *Gallus gallus*, *Kryptolebias marmoratus*, *Alligator mississippiensis*, *Leucoraja erinacea*, *Haplochromis burtoni*, *Pimephales promelas*, *Dicentrarchus labrax*, *Gambusia affinis*, *Micropogonias undulatus*, *Oryzias latipes*, *Acanthopagrus schlegelii*, *Rana catesbeiana*, *Crocota crocata*, *Eulemur fulvus collaris*, and *Anguilla japonica* (see GenBank Accession Nos. NM_214314 (or AF161717), AY082342, NM_001040090, DQ339105, AB186356, DQ382340, AF121257, AY727529, AY647256, AB099303, AY701761, AB076399, AY219702, AY324231, AY128705, U94178, and AB023960, respectively). The ligand-binding domains of androgen receptors are well known in the art. For the purpose of the present invention, the ligand-binding domain of the human androgen receptor refers to a polypeptide that starts at any amino acid from amino acid positions 651 to 681 and ends at any amino acid from amino acid positions 900 to 920. For example, human androgen receptor or a fragment of the human androgen receptor that comprises amino acids 681-900 as well as DNA vaccines containing a polynucleotide encoding the above are suitable vaccines. The corresponding ligand-binding domains of androgen receptors from other species can be readily determined by sequence alignment (to the human sequence) (e.g., by the methods described below in connection with sequence identity or homology). In a preferred embodiment, a polypeptide from the human androgen receptor that starts at any amino acid from amino acid positions 661 to 671 and ends at any amino acid from amino acid positions 910 to 920 is used in the present invention. In a more preferred embodiment, a polypeptide containing amino acids 661 to 920 or 664 to 920 of the human androgen receptor is used in the present invention. To help determine the corresponding fragments of the androgen receptors from other species, it is noted here that the amino acid positions on rat, dog, chimpanzee, macaque, and lemur androgen receptors that correspond to amino acid positions 661 to 920 of the human androgen receptor are 640 to 899, 643 to 902, 648 to 907, 652 to 910, 636 to 895, and 625 to 884, respectively. It is noted that the above fragments of the human, mouse, rat, dog, chimpanzee, macaque, and lemur androgen receptors have the same amino acid sequence. The ligand-binding domains of the androgen receptors of other species are also known or can be readily identified through sequence alignment. As will be readily recognized by one of ordinary skill in the art, any DNA sequence that encodes a ligand-binding domain or a larger fragment of an androgen

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receptor including the full-length receptor from one of the above species as well as other animals is suitable for the present invention.

As is well-known to those skilled in the art, polypeptides having substantial sequence similarities cause identical or very similar immune reaction in a host animal. As discussed below, this phenomenon is the basis for using a xenoantigen for inducing autoreactive reaction to an otherwise tolerated autoantigen. Accordingly, a derivative, equivalent, variant, fragment, or mutant of the ligand-binding domain of any of the known or to-be-identified androgen receptors or any DNA sequence encoding the above is also suitable for the present invention. The polypeptides encoded by these DNA sequences are useful as long as the polypeptides encoded by the DNA sequences are structurally similar to the ligand-binding domain of the autologous androgen receptor, and are sufficiently immunogenic.

It is readily apparent to those ordinarily skilled in the art that variations or derivatives of the nucleotide sequence encoding the polypeptide or protein antigen can be produced which alter the amino acid sequence of the encoded polypeptide or protein. The altered polypeptide or protein may have an altered amino acid sequence, for example by conservative substitution, yet still elicits immune responses which react with the unaltered protein antigen, and are considered functional equivalents. According to a preferred embodiment, the derivative, equivalents, variants, or mutants of the ligand-binding domain of an androgen receptor are polypeptides that are at least 85% homologous to the ligand-binding domain of a human androgen receptor. More preferably, the homology is at least 88%, at least 90%, at least 95%, or at least 98%.

As used in this application, "percent identity" between amino acid or nucleotide sequences is synonymous with "percent homology," which can be determined using the algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 87, 2264-2268, 1990), modified by Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90, 5873-5877, 1993). The noted algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (*J. Mol. Biol.* 215, 403-410, 1990). BLAST nucleotide searches are performed with the NBLAST program, score=100, wordlength=12, to obtain nucleotide sequences homologous to a polynucleotide of the invention. BLAST protein searches are performed with the XBLAST program, score=50, wordlength=3, to obtain amino acid sequences homologous to a reference polypeptide. To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul et al. (*Nucleic Acids Res.* 25, 3389-3402, 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used.

As used herein, the term "conservative substitution" denotes the replacement of an amino acid residue by another, biologically similar residue. It is well known in the art that the amino acids within the same conservative group can typically substitute for one another without substantially affecting the function of a protein. For the purpose of the present invention, such conservative groups are set forth in Table 1 based on shared properties.

TABLE 1

Conservative substitution.	
Original Residue	Conservative Substitution
Ala (A)	Val, Leu, Ile
Arg (R)	Lys, Gln, Asn
Asn (N)	Gln, His, Lys, Arg

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TABLE 1-continued

Conservative substitution.	
Original Residue	Conservative Substitution
Asp (D)	Glu
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
His (H)	Asn, Gln, Lys, Arg
Ile (I)	Leu, Val, Met, Ala, Phe
Leu (L)	Ile, Val, Met, Ala, Phe
Lys (K)	Arg, Gln, Asn
Met (M)	Leu, Phe, Ile
Phe (F)	Leu, Val, Ile, Ala
Pro (P)	Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr, Phe
Tyr (Y)	Trp, Phe, Thr, Ser
Val (V)	Ile, Leu, Met, Phe, Ala

In addition, fragments of a ligand binding domain of an androgen receptor such as those that can bind to HLA-A2 are also useful antigens which elicit cytotoxic responses against cells expressing the androgen receptor or its ligand binding domain. Polynucleotides that encode these fragments are considered functional equivalents. Examples of these fragments are provided in the examples below. In particular, the use of the following four fragments are contemplated: SEQ ID NO:9 (amino acids 811-819 of SEQ ID NO:2), SEQ ID NO:10 (amino acids 761-770 of SEQ ID NO:2), SEQ ID NO:11 (amino acids 805-813 of SEQ ID NO:2), and SEQ ID NO:12 (amino acids 859-867 of SEQ ID NO:2).

A polynucleotide useful in the present invention is preferably ligated into an expression vector which has been specifically optimized for polynucleotide vaccinations. Elements include a transcriptional promoter, immunogenic epitopes, and additional cistrons encoding immunoenhancing or immunomodulatory genes, with their own promoters, transcriptional terminator, bacterial origin of replication and antibiotic resistance gene, as well known to those skilled in the art. Optionally, the vector may contain internal ribosome entry sites (IRES) for the expression of polycistronic mRNA.

In one embodiment of this invention, a polynucleotide useful in the present invention is directly linked to a transcriptional promoter. The use of tissue-specific promoters or enhancers, for example the muscle creatine kinase (MCK) enhancer element, may be desirable to limit expression of the polynucleotide to a particular tissue type. For example, myocytes are terminally differentiated cells which do not divide. Integration of foreign DNA into chromosomes appears to require both cell division and protein synthesis. Thus, limiting protein expression to non-dividing cells such as myocytes may be preferable. In addition, a PSA promoter may be used to limit expression of the protein to prostate tissue. In one embodiment, tissue- or cell-specific promoters may be used to target the expression of the protein to antigen-presenting cells. For example, an α -fetoprotein (AFP) promoter (see e.g., Peyton et al. 2000, Proc. Natl. Acad. Sci., USA. 97:10890-10894) may be used to limit expression to liver tissues. However, use of the CMV promoter is adequate for achieving expression in many tissues into which the plasmid DNA vaccine is introduced.

Suitable vectors include any plasmid DNA construct encoding an androgen receptor, a fragment of the androgen receptor that comprises the ligand-binding domain, a suit-

able fragment of the ligand-binding domain, or a functional equivalent or derivative thereof, operatively linked to a suitable promoter. Examples of such vectors include the pCMV series of expression vectors, commercially available from Stratagene (La Jolla, Calif.); or the pCDNA or pREP series of expression vectors by Invitrogen Corporation (Carlsbad, Calif.).

A preferred vector is pNGVL3 available from the National Gene Vector Laboratory at the University of Michigan. This vector, similar to the pCDNA3.1 eukaryotic expression vector of Invitrogen Corp. (Carlsbad, Calif.), drives transcription from the CMV promoter, but also includes the CMV intron A sequence to enhance protein expression (Lee et al., 1997, *Mol. Cells* 7:495-501). The vector also contains a multi-cloning site, and does not express a eukaryotic antibiotic resistance gene, such that the only protein expression expected in a eukaryotic system is the one driven from the CMV promoter, unlike the pCDNA vector. Another preferred vector is the pTVG4 vector described in US 2004/0142890, which is herein incorporated by reference in its entirety. The pTVG4 vector can be made by incorporating 2 copies of a 36-bp immunostimulatory (ISS) fragment containing the 5'-GTCGTT-3' motif previously identified (Hartmann et al., 2000, *J. Immunol.* 164: 1617-24) into pNGVL3.

There are many embodiments of the instant invention which those skilled in the art can appreciate from the specification. Thus, different transcriptional promoters, terminators, and other transcriptional regulatory elements may be used successfully. Examples of other eukaryotic transcription promoters include the Rous sarcoma virus (RSV) promoter, the simian virus 40 (SV40) promoter, the human elongation factor-1 α (EF-1 α) promoter, and the human ubiquitin C (UbC) promoter.

A Kozak sequence can be provided upstream of the polynucleotide useful in the present invention to enhance the translation of the corresponding mRNA from the polynucleotide. For vertebrates, the Kozak sequence is (GCC)NC-CATGG (SEQ ID NO:7) wherein N is A or G and GCC is less conserved. For example, ACCATGG can be used. See Kozak, M. *Nucleic Acids Res.* 1987, 15:8125-48.

The vectors of the present invention may be delivered intradermally, intramuscularly, subcutaneously, or intravascularly (including intravenously and intraarterially). In preferred embodiments, delivery may be a combination of two or more of the various delivery methods.

"Naked" plasmid DNA expressing a transgene could be directly injected intradermally or intramuscularly, taken up, and expressed (see e.g., Wolff et al., 1990, *Science* 247: 1465-8). The efficiency of this approach may be low, with only a small percentage of myocytes being directly transformed in vivo, and within only a limited area of muscle tissue targeted by this directed delivery. Various alternative approaches yielding a higher gene delivery efficiency are known (see e.g., Acsadi et al., 1991, *New Biol.* 3:71-81). Subsequent work on strategies that increase uptake of plasmid DNA by muscle tissue focused on various carrier solutions and molecules (Wolff et al., 1991, *Biotechniques* 11:474-85; and Budker et al., 1996, *Nat. Biotechnol.* 14:760-4), the use of myotoxic agents to enhance DNA uptake (Davis et al., 1993, *Hum. Gene Ther.* 4:151-9; and Danko et al., 1994, *Gene Ther.* 1:114-21), and the use of various transcriptional promoters and plasmid DNA backbones (Manthorpe et al., 1993, *Hum. Gene Ther.* 4:419-31).

In a preferred embodiment, plasmid vectors of the present invention may be delivered to the patient in need thereof intravascularly. Plasmid DNA delivered intravascularly

resulted in 100-fold higher uptake in downstream tissues in rodent studies (Budker et al., 1996, *Gene Ther.* 3:593-8). Intravascular delivery may be intravenous, e.g. by direct injection of plasmid DNA into the portal vein of rodents with uptake and expression demonstrated in hepatocytes (Budker et al., 1996, *Gene Ther.* 3:593-8; and Zhang et al., 1997, *Hum. Gene Ther.* 8:1763-72). Intravascular delivery may also be performed more directly by intraarterial delivery. For example, initial studies in rodents demonstrated that high levels of gene expression in hind limb muscle could be obtained by rapid injection of plasmid DNA into the femoral artery (Budker et al., 1998, *Gene Ther.* 5:272-276). This approach is efficient and safe in non-human primates as well, with an average of 7% of downstream myofibers expressing a β -galactosidase reporter construct two weeks after intraarterial DNA administration (Zhang et al., 2001, *Hum. Gene Ther.* 12:427-438). Parallel studies in T cell immuno-suppressed rats showed that gene expression was stable for at least 10 weeks (Zhang et al., 2001, *Hum. Gene Ther.* 12:427-438).

Accordingly, delivery of plasmid DNA vaccines of the present invention can be done by direct intraarterial administration. This method provides more effective delivery to MHC class I expressing cells. Administrations of plasmid DNA vaccines intravascularly may result in increased antigen expression and subsequently lead to enhanced immune responses, and increased antigen expression in MHC class I expressing cells by means of intraarterial delivery of DNA plasmid may lead to a more robust immune response with androgen receptor-specific CTL. An intraarterial method of DNA delivery has been shown to be at least as effective as or more effective than traditional intradermal administration of DNA in eliciting prostatic acid phosphatase-specific immunity.

In another embodiment, intravenous delivery may also be used, employing methods well known to those skilled in the art (See e.g., Budker et al., 1998, *Gene Ther.* 5:272-276; and Budker et al., 1996, *Gene Ther.* 3:593-598). This delivery method may lead to a high level of antigen expression in hepatocytes. Expression of the antigen in liver, a tissue more rich with antigen-presenting cells, may lead to a more pronounced Th1/CTL response than expression in muscle tissue.

The DNA or peptide vaccines of the present invention can be used in a prime-boost strategy to induce robust and long-lasting immune response to androgen receptor. Priming and boosting vaccination protocols based on repeated injections of the same antigenic construct are well known and result in strong CTL responses. In general, the first dose may not produce protective immunity, but only "primes" the immune system. A protective immune response develops after the second or third dose.

In one embodiment, the DNA or peptide vaccines of the present invention may be used in a conventional prime-boost strategy, in which the same antigen is administered to the animal in multiple doses. In a preferred embodiment, the DNA or peptide vaccine is used in one or more inoculations. These boosts are performed according to conventional techniques, and can be further optimized empirically in terms of schedule of administration, route of administration, choice of adjuvant, dose, and potential sequence when administered with another vaccine, therapy or homologous vaccine.

The peptide or DNA vaccines of the present invention may be used in a prime-boost strategy using an alternative administration of xenoantigen and autoantigen or xenoantigen- and autoantigen-encoding vectors. Specifically, according to the present invention, the animal is first treated, or

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“primed,” with a peptide antigen of foreign origin (a “xeno-antigen”) or DNA vaccine encoding the antigen of foreign origin. The animal is then treated with another peptide antigen which corresponds to the xenoantigen but is of self origin (“autoantigen”) or another DNA vaccine encoding the autoantigen. This way, the immune reaction to the antigen is boosted. The boosting step may be repeated one or more times.

A xenoantigen, as compared to a self-antigen or an autoantigen, is an antigen originated in or derived from a species different from the species that generates an immune reaction against the antigen. Xenoantigens usually are highly homologous molecules to a corresponding autoantigen. Xenoantigens have been shown to be able to elicit auto-reactive immunity. For example, molecular mimicry by highly homologous viral antigens has been one theory to explain the occurrence of several autoimmune diseases (von Herrat and Oldstone, 1996, *Curr. Opin. Immunol.* 8:878-885; and Oldstone, 1998, *Faseb J.* 12:1255-1265). That is, the induction of immune responses following infection by viral antigens that closely resemble normal autologous proteins may then lead to an autoimmune reaction to the autologous protein.

The use of highly homologous foreign antigens or xenoantigens as vaccine antigens to elicit autoreactive immunity has been explored in animal models. For example, xenoantigens derived from zona pellucida of foreign species can elicit autoreactive T cell responses and disrupt ovarian function in a variety of animal species studied (Mahi-Brown et al., 1992, *J. Reprod. Immunol.* 21:29-46; and Mahi-Brown, 1996, *J. Reprod. Fertil. Suppl.* 50:165-74). While not wishing to be bound by any theory on mechanism, it is believed that because T cells involved in autoimmune processes recognize peptide epitopes presented in the context of MHC molecules, the uptake and MHC presentation of a homologous foreign antigen presumably exposes T cell epitopes with enhanced MHC binding or unmasks cryptic epitopes of the native antigen not normally recognized.

While the prime-boost strategy is known to work with antigens of different origins, it is readily apparent to those ordinarily skilled in the art that variants, derivatives or equivalents, as discussed above, of the nucleotide sequence encoding a self-antigen can also be used to achieve the same results as xenoantigens.

The peptide or DNA vaccines of the present invention may be used together with prostate cancer vaccines based on other antigens such as prostatic acid phosphatase-based antigens. The androgen receptor-based vaccines and vaccines based on other antigens can be used simultaneously or at different times. Each may be used in a prime-boost strategy.

The present invention also provides a method for determining the effectiveness of a treatment for prostate cancer. The method includes the steps of (a) measuring the frequency or amount of cytotoxic T lymphocytes (CTLs) specific for a peptide selected from SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12 prior to providing at least a portion of the treatment to a mammal having prostate cancer, (b) measuring the frequency or amount of CTLs specific for the peptide after said portion of the treatment is provided to the mammal, and (c) comparing the frequency or amount of CTLs of (a) and that of (b) wherein the frequency or amount of CTLs of (b) being higher than that of (a) indicates that the treatment is effective. For example, a biological sample containing CTLs such as a blood sample or a sample of peripheral blood mononuclear cells (PBMC) can be taken from the mammal and

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the frequency or amount of CTLs in the blood sample can be measured. In one embodiment, the method is used to determine the effectiveness of a treatment provided to a human prostate cancer patient.

One of ordinary skill in the art is familiar with the techniques for functional and quantitative measurements of antigen-specific T cells. Examples include but are not limited to limited dilution assays (LDA), enzyme linked immunosorbent assay on a single cell level (ELISPOT), intracellular staining, and MHC/HLA multimer (e.g., dimer, tetramer, and pentamer) staining. Description on the MHC/HLA multimer staining technique can be found, for example, in Arnold H Bakker and Ton N M Schumacher (*Current Opinion in Immunology*, 2005, 17:428-433), Meidenbauer N et al. (*Methods*, 2003, 31:160-171), and U.S. patent application publication 20072036812.

In one embodiment, a biological sample (e.g., a blood sample or PBMC sample) containing CTLs from a patient is obtained and the sample is brought into contact with an HLA multimer (e.g., an HLA tetramer). The frequency or amount of CTLs specific for a peptide antigen bound to the HLA tetramer can then be measured by known techniques such as flow cytometry.

The invention will be more fully understood upon consideration of the following non-limiting examples.

Example 1

Immune Responses to Androgen Receptor Ligand-Binding Domain can Exist in Patients with Prostate Cancer

We evaluated whether or not patients with prostate cancer have existing immune responses to the androgen receptor (AR). We focused these studies on responses specific for the AR ligand-binding domain (LBD). As shown in FIG. 1, we found that prostate cancer patients, but not healthy male blood donors, have antibodies that are specific for the AR LBD. These antibodies were predominantly of the IgG isotype and IgG2 sub-isotype (data not shown). Moreover, we identified that patients with antibody responses to the AR LBD have CD4+ and CD8+ T cells that proliferate, as well as cells that secrete interferon-gamma (IFN γ), in response to stimulation with the AR LBD (FIG. 2). Antigen-specific IL-10 secretion was observed in many patients who did not have evidence of antibody responses to AR LBD (FIG. 3). Taken together, these results demonstrate that some patients with prostate cancer can have pre-existing cellular immune responses specific for the AR. These findings further suggest that tolerance against the AR, which may be prevalent, is not absolute and can be overcome in some patients with prostate cancer.

Example 2

Materials and Methods

Subject Population:

Peripheral blood mononuclear cells (PBMC) were obtained from eleven patients with prostate cancer at the University of Wisconsin Hospital and Clinics between 2001 and 2007. All subjects gave Institutional Review Board-approved written informed consent for their blood products to be used for immunological research. PBMC were prepared from heparinized blood by gradient centrifugation.

T2 Binding Assay:

After passing T2 cells into fresh media the day before the assay, these cells were pulsed with 50 μ g/mL peptide overnight at 37° C./5% CO $_2$. The next day, the levels of HLA-A2 expression on the surface of these cells were measured using

a fluorescently-labeled HLA-A2 antibody (Clone BB7.2, BD Biosciences, San Jose, Calif.) followed by flow cytometry using FACSCaliber system (BD Biosciences). The reported "fold change" in fluorescence intensity was calculated by averaging the mean fluorescent intensity results from triplicate samples and dividing these values by the average mean fluorescent intensity of the negative control (media only) samples.

T-Cell Culturing:

Human prostate cancer patients underwent leukapheresis, and PBMCs were isolated using a Ficoll-Paque gradient (Pharmacia, Kirkland, Quebec). Immature dendritic cells (iDCs) were generated by incubating flask-adherent PBMCs with 20 ng/mL granulocyte-macrophage colony stimulating factor and 10 ng/mL interleukin four (IL-4) for six days at 37° C./5% CO₂ (cytokines from: Fitzgerald Industries, Concord, Mass.). These iDCs were then treated with 150 ng/mL IL-6, 10 ng/mL IL-1 β , 10 ng/mL tumor necrosis factor alpha (TNF- α), and 1 mg/mL prostaglandin E2 (Fitzgerald Industries) to generate mature dendritic cells (mDCs). These mDCs were then pulsed with 20 μ g/mL AR LBD-derived peptide, and after being irradiated were co-incubated with CD4+ and CD8+ T-cells negatively isolated from autologous PBMCs (Invitrogen, Carlsbad, Calif.). After incubating for 24 hours, these cultures were then treated with 10 U/mL IL-2 and 30 U/mL IL-7 (Fitzgerald Industries), and incubated for another 6 days. These cultured T-cells were re-stimulated weekly using peptide-pulsed antigen presenting cells (either mDCs or the TK6 lymphoma cell line).

Lactate Dehydrogenase Cytotoxicity Assay:

T-cell cultures that underwent at least two stimulations (or splenocyte cultures that had undergone one stimulation) were collected and incubated for four hours with target cells (either T2 cells pulsed for two hours with a specific or non-specific peptide, the LNCaP prostate cancer cell line, or media alone) at various effector-to-target ratios. After incubation, supernatants were collected and levels of lactate dehydrogenase were measured using the CytoTox 96 Non-Radioactive Assay (Promega). The relative percentage of peptide-specific lysis was quantitated using the following equation:

% Cytotoxicity =

$$\frac{\text{Experimental} - \text{Effector Spontaneous} - \text{Target Spontaneous}}{\text{Target Maximum} - \text{Target Spontaneous}}$$

To conduct a mini-cytotoxicity assay of limited-dilution clones, 50 μ l (1/4) of the cultured clones were incubated for four hours with either T2 cells pulsed with the specific peptide, a non-specific peptide, or media alone. For the subsequent characterization of CTL clone cytotoxicity against prostate cancer cells, cryopreserved CTL clones were thawed, washed 3 times, and restimulated by incubating the clones with peptide-pulsed irradiated TK6 cells for 7 days. These clones were then resuspended in 10 U/mL IL-2, and allowed to incubate for three days at 37° C./5% CO₂. They were then analyzed for cytotoxicity (as described above) against four prostate cancer cell lines: LNCaP, DU145, LAPC4, and MDAPCa-2b cell lines. To characterize HLA-A2-restriction of observed cytotoxicity, a HLA-A2 antibody was added to the reaction (Clone BB7.2, BD Biosciences, 1 μ g/mL).

Limited-Dilution T-Cell Cloning and T-Cell Expansion:

After T-cell cultures containing AR LBD peptide-specific T-cells were identified, peptide-specific T-cell clones were isolated using limited-dilution cloning. Briefly, cultured T-cells were diluted to 400 cells/mL, and were diluted at a 1:1 ratio down the rows of a 96-well plate. These T-cells were mixed with 2 \times 10⁵ autologous PBMCs, as well as an anti-CD3 antibody (Clone UCHT1, BD Biosciences, 120 ng/mL) and IL-2 (Fitzgerald Industries, 200 U/mL), and were incubated at 37° C./5% CO₂ for 12-14 days. Cultures generated from a single cell were identified, and their peptide-specificity was analyzed using a mini-cytotoxicity assay (as above). Peptide-specific T-cell clones were then expanded in the presence of autologous PBMCs, TK6 cells, and an anti-CD3 antibody (30 ng/mL). The next day, 50 U/mL IL-2 was added to the cultures. Six days later, CTL clones were resuspended in media with 80 U/mL IL2, and three days later were again resuspended in 20 U/mL IL-2. After three additional days, expanded T-cell clones were analyzed for cytotoxicity against peptide-specific or non-specific T2-pulsed cells (as described above).

Surface Molecule Staining:

T-cell clones were thawed, washed 3 times, and resuspended in media supplemented with 10 U/mL IL-2 for 18 hours at 37° C./5% CO₂. These recovered clones were then resuspended in staining buffer (phosphate-buffered saline+5% fetal bovine serum) and incubated with fluorescently-labeled antibodies specific for CD3, CD4, CD8, and CD56 (clones SK7, RPA-T4, RPA-T8, or NCAM16.2, respectively; BD Biosciences) or the appropriate controls, for 30 minutes on ice. Cells were subsequently analyzed using an LSR II flow cytometer (BD Biosciences), counting 100,000 events. Cells were gated based on CD3+/CD56+ expression and CD4+/CD8+ expression.

Intracellular Cytokine Staining:

Recovered CTL clones were restimulated for one hour with media alone, the specific peptide, a non-specific peptide (peptides both at 2 μ g/mL), or Phorbol Myristate Acetate (Sigma-Aldrich, St. Louis, Mo.; 10 μ g/mL) and Ionomycin (MP Biomedicals, Solon, Ohio; 1 μ g/mL). Cells were then treated with monensin (BD Biosciences; 1 μ l per 1.5 mL cell culture) for four hours at 37° C./5% CO₂, followed by a brief blocking treatment with mouse IgG. Cells were then resuspended in staining buffer (phosphate-buffered saline+5% fetal bovine serum) and incubated with fluorescently-labeled CD3- and CD8-specific antibodies (BD Biosciences), or the appropriate controls, for 30 minutes on ice. After fixation and permeabilization, intracellular staining was conducted using fluorescently-labeled IFN γ and TNF α antibodies (Clones 4S.B3 and Mab11, respectively; BD Biosciences), or the appropriate isotype controls. Cells were subsequently analyzed using an LSR II flow cytometer, counting 100,000 events. IFN γ and TNF α -positive events were determined by gating CD3+/CD8+ cells and analyzing this population for co-expression of IFN γ and TNF α .

Immunization of HLA-A2/HLA-DR1 Mice:

Groups of four 6-10 week old HLA-A2/DR1 transgenic male mice (Charles River Laboratory—France with the permission of Dr. Francois Lemmonier) were immunized subcutaneously with 100 μ m AR811 peptide with Complete Freund's Adjuvant (CFA) or with CFA alone (Sigma-Aldrich), and seven days later, the mice were euthanized. Spleens were collected, and splenocytes were isolated by gradient centrifugation (Histopaque 3130, Sigma-Aldrich). Splenocytes were stimulated with 10 μ g/mL peptide for two hours, and on the second day, recombinant murine IL-2 and IL-7 (Fitzgerald Industries) were added to 10 U/mL and 30

U/mL, respectively. The cultures were then allowed to incubate an additional six days before analysis.

Construction of pTVG4 and pTVG-ARLBD:

Plasmid DNA expression vectors have been developed for use in human vaccines. Shown in FIG. 4 is a plasmid map of the pTVG4 vector as constructed for animal (e.g., rat and mouse) and human immunization. The coding sequence for the ligand-binding domain of the human androgen receptor gene has been inserted into the pTVG4 vector to create the immunization vector pTVG-ARLBD (see below).

The plasmid vector pNGVL3 was obtained from the National Gene Vector Laboratory at the University of Michigan (courtesy, Dr. Robert Gerard). This vector, similar to the pCDNA3.1 expression vector from Invitrogen Corp. (Carlsbad, Calif.), drives transcription from the CMV promoter, but also includes the CMV intron A sequence to enhance transcription (Lee et al., 1997, Mol. Cells 7:495-501). The vector also contains a multi-cloning site, and does not express a eukaryotic antibiotic resistance gene, such that the only protein expression expected in a eukaryotic system is the one driven from the CMV promoter, unlike the pCDNA vector. To this vector has been added 2 copies of a 36-bp immunostimulatory (ISS) fragment containing the 5'-GTCGTT-3' motif previously identified (Hartmann et al., 2000, J. Immunol. 164:1617-24), to create the vector pTVG4 (FIG. 4). ColE1, the DNA sequence for Colicin E1 which can be used for cloning purpose, is provided in the vector. Kan, the DNA sequence encoding a kanamycin resistance gene which can also be used for cloning purpose, is also provided in the vector. The coding sequence for the ligand-binding domain of the human androgen receptor gene has been cloned into this vector and a Kozak sequence has been provided directly upstream of the coding sequence to enhance the translation of the corresponding mRNA (FIG. 5). Expression of the ligand-binding domain has been confirmed by in vitro expression studies (not shown). This construct, named pTVG-ARLBD, is used for the immunization of animals and humans.

Immunization and Tumor Protection of Copenhagen Rats:

Groups of ten 9-11 week old Copenhagen rats (Harlan) were immunized intradermally with 100 µg pTVG-ARLBD or 100 µg pTVG4 alone with 1 µg rat GM-CSF. Rats received three booster immunizations (100 µg) every 14 days, and 14 days after the last immunization, rats were challenged with 10,000 syngeneic Mat-LyLu prostate tumor cells, given along with Matrigel Matrix (BD Biosciences). Tumors (long and short diameters) were measured every two days, and volumes were calculated using the following equation:

$$\text{Tumor volume} = (\pi/6) \times (d_{\text{short}})^2 \times (d_{\text{long}})$$

Results

AR LBD-Specific CD8+ T-Cells from Prostate Cancer Patients can Lyse Prostate Cancer Cells:

To characterize CD8+ T-cell responses to the AR LBD, the amino acid sequence of the AR LBD was evaluated for potential HLA-A2-binding epitopes that fit the consensus peptide binding sequence of X-L/M-X-X-X-V-X-X-V/L (SEQ ID NO:8), using the algorithm of Parker and colleagues (Parker K C et al., 1994, J. Immunol. 152:163-175). As demonstrated in Table 1, ten unique peptides were identified. These peptides were synthesized and then characterized for their affinity for HLA-A2 in vitro using T2 binding assays. The results from these binding studies are also shown in Table 1. As demonstrated, several potential epitopes were predicted and found experimentally to bind strongly to HLA-A2. Peptide-specific T-cell lines were then cultured from the peripheral blood of 11 HLA-A2+ patients with prostate cancer, using each of these peptides, and then tested for their cytolytic activity against both peptide-pulsed HLA-A2-expressing target cells and the HLA-A2+ LNCaP prostate cancer cell line. Specifically, naive T cells were isolated by magnetic negative selection (Dyna) from the peripheral blood mononuclear cells (PBMCs) of HLA-A2-expressing prostate cancer patients, and cultured in the presence of autologous cytokine-matured irradiated dendritic cells (mDC) that had been loaded with individual peptides. These cultures received interleukin 2 (IL-2) and IL-7 the day after the culture, and were re-stimulated weekly with peptide-pulsed mDCs. Beginning after two weeks of stimulation, the T-cell cultures were tested weekly for their cytolytic activity using peptide-loaded T2 cells as target cells. Peptide-specific T-cells could be cultured from the majority of patients to at least one of these peptides, and peptide-specific T-cell lines could be cultured from the majority (7/11) of HLA-A2-expressing patients against the AR811 peptide in particular (Table 2).

Table 1. Prediction of AR LBD-derived HLA-A2-specific peptide epitopes. AR LBD peptide epitopes were identified by scanning the protein sequence of the AR LBD for 9-mer or 10-mer peptides that fit the HLA-A2 consensus binding sequence X-L/M-X-X-X-V-X-X-V/L (SEQ ID NO:8) and by their predicted binding affinity to HLA-A2 (Bioinformatics and Molecular Analysis Section). These peptides were synthesized and then analyzed for their affinity for HLA-A2 using a T2 binding assay. Shown is the ratio of the mean fluorescent intensity, calculated from triplicate samples, of peptide-loaded T cells normalized against unloaded T cells. Influenza: positive control influenza matrix protein peptide; negative control: vehicle.

Peptide	Sequence	Predicted Binding Affinity ($t_{1/2}$ (min) of Dissociation)	In Vitro HLA-A2 Expression (Relative Mean Fluorescent Intensity)
AR677	VLEAIEPGV (SEQ ID NO: 13)	7.6	1.37 ± 0.14
AR700	ALLSSLNEL (SEQ ID NO: 14)	182	2.66 ± 0.28
AR708	LGERQLVHV (SEQ ID NO: 15)	0.114	1.16 ± 0.06
AR742	WMGLMVFAM (SEQ ID NO: 16)	220	1.32 ± 0.06
AR761	RMLYFAPDLV (SEQ ID NO: 10)	217	2.15 ± 0.13
AR805	FLCMKALLL (SEQ ID NO: 11)	98	2.19 ± 0.14

-continued

Peptide	Sequence	Predicted Binding Affinity ($t_{1/2}$ (min) of Dissociation)	In Vitro HLA-A2 Expression (Relative Mean Fluorescent Intensity)
AR811	LLLSIIPV (SEQ ID NO: 9)	1006	2.54 \pm 0.25
AR814	FSIIPVDGL (SEQ ID NO: 17)	111	1.01 \pm 0.12
AR859	QLTKLLDSV (SEQ ID NO: 12)	78	1.33 \pm 0.15
AR862	KLLDSVQPI (SEQ ID NO: 18)	1274	1.65 \pm 0.19
Influenza	GILGFVFTL (SEQ ID NO: 19)	30	1.88 \pm 0.24
Negative Control		0	1.00 \pm 0.07

Table 2. Compiled results of T cell culturing and cytotoxicity assays. Shown in this table are the results obtained from cytotoxicity assays of T cell cultures from eleven HLA-A2-expressing patients with prostate cancer. In the second column is the number of patients for which peptide-specific T cells could be identified following 2-4 in vitro stimulations. The third column shows results as to whether those peptide-specific T cells were able to lyse the HLA-A2+ LNCaP prostate cancer cell line (+: positive lysis, -: no lysis, +/-: inconclusive).

Peptide	Peptide-specific lysis (Number of patients)	Prostate cancer cell lysis
AR677	2/11	-
AR700	1/11	-
AR708	1/11	-
AR742	2/11	-
AR761	6/11	+/-
AR805	3/11	+/-
AR811	7/11	+
AR814	3/11	-
AR859	2/11	+/-
AR862	5/11	-

As shown in FIG. 6, AR811 peptide-specific T-cells were found to lyse T2 cells in a peptide-specific fashion, and could lyse the LNCaP cell line. Several other peptides demonstrated peptide-specific lysis and variable amounts of lysis against the LNCaP cell line (Table 2). In contrast, T-cells specific for the AR859 peptide could be cultured, and while these showed peptide-specific lysis they did not lyse the LNCaP cell line (FIG. 6B). AR811-specific T-cells were cloned by limited dilution and found to be CD8+, and to secrete both IFN γ and TNF α in response to peptide stimulation (FIG. 7). Moreover, these cells lysed prostate cancer cells in an MHC class I-restricted fashion (FIG. 8). These findings confirm that the AR811 peptide is a naturally processed and presented HLA-A2 epitope from the AR.

HLA A2 Transgenic Mice Immunized with the AR811 Peptide Developed Peptide-Specific CTL:

Human HLA transgenic mice have become a valuable tool for the identification and study of human MHC class I-specific epitopes and CTL responses. In work published by others, transgenic mice expressing human HLA-A201 have been immunized directly with peptides, or with DNA encoding antigens, or protein antigens, to identify HLA-A2-specific epitopes (Carralot J P et al., 2005, Int Immunol 17:591-7; Gallez-Hawkins G et al., 2003, J Virol 77:4457-62; and Loirat D et al., 2000, J Immunol 165:4748-55).

Unfortunately, many of the early studies with these transgenic strains were complicated by the preference to develop H-2-restricted murine responses rather than HLA-A2-restricted CTL responses, thus limiting the usefulness of these strains. This led to the development of the HHD strains by Dr. Francois Lemonnier and colleagues at the Institut Pasteur, in which the mouse MHC class I H-2Db was knocked out, and mice express human β 2-microglobulin and HLA-A201 monochains fused to the α 3, transmembrane and cytoplasmic domains of the mouse MHC class I molecule (Pascolo S et al., 1997, J Exp Med 185:2043-51). These strains and derivatives have been particularly useful as these mice are forced to use a diverse repertoire of CD8+ T-cells specific for HLA-A2 (Pascolo S et al., 1997, J Exp Med 185:2043-51), and have been demonstrated to be superior in eliciting HLA-A2-restricted CTL (Ramage J M et al., 2004, Vaccine 22:1728-31). The HHD-II transgenic mouse strain developed by Dr. Lemonnier expresses both human HLA-A201 and HLA-DR1, and has both the murine H-2 class I and MHC class II knocked out (Pajot A et al., 2004, Eur J Immunol 34:3060-9). This particular strain has been used for the identification of HLA-DR1-restricted CD4+ T-cell epitopes as well as HLA-A2-restricted epitopes (Pajot A et al., 2006, Microbes Infect 8:2783-90). HHD-II mice were immunized once with 100 μ g of the AR811 peptide in complete Freund's adjuvant (CFA) or with CFA alone. As shown in FIG. 9, AR811 peptide-specific CTL could be identified after immunization.

DNA Vaccine Encoding AR LBD can Elicit Antigen-Specific Responses and Retard Prostate Cancer Cell Growth In Vivo:

cDNA was prepared from a prostate cancer cell line, and AR LBD (amino acids 664-920) was cloned into the pTVG4 vector as described above (similar to cloning prostatic acid phosphatase into the pTVG4 vector described in Johnson L E, et al., 2007, Canc Immunol Immunoth 56:885-895, which is herein incorporated by reference in its entirety). CHO cells transiently transfected with this pTVG-ARLBD construct produced AR LBD mRNA and protein that could be detected by RT-PCR and by Western blot analysis (data not shown). Male Copenhagen rats, 2-3 months of age, were then immunized with 100 μ g of pTVG-ARLBD four times at 14-day intervals, intradermally with 5 μ g rat GM-CSF given as a vaccine adjuvant. Two weeks after the final immunization, blood was collected for immunological analysis. As shown in FIG. 10, animals immunized with pTVG-ARLBD, but not the pTVG4 vector, developed AR LBD-specific IgG antibody responses. To assess anti-tumor efficacy, Copenhagen rats that had been immunized four

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times at 14-day intervals were then challenged with 1×10^4 syngeneic Mat-LyLu prostate tumor cells implanted subcutaneously. As shown in FIG. 11, immunization with pTVG-ARLBD, but not the pTVG4 vector, retarded the growth of these Mat-LyLu prostate tumors.

Example 3 (Prophetic)

Prostate Cancer Therapy with pTVG-ARLBD DNA Vaccine

Groups of ten suitable rats or mice such as Copenhagen rats are challenged with a suitable number of prostate cancer cells (e.g., 1×10^4 Mat-LyLu prostate cancer cells). These rats or mice are then immunized with either the pTVG4 (negative control) or pTVG-ARLBD constructs. One example is to immunize the rats or mice at days 2, 9, and 16 after the tumor challenge with 100 μ g injected intradermally along with 5 μ g

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rat or mouse GM-CSF as an adjuvant. Suitable schemes with fewer or additional immunizations may be used as alternatives. Optionally, boosts (e.g., on weekly basis) can be provided. Other suitable amounts of DNA or adjuvant can be used, as can different adjuvants (such as Freund's adjuvant) or additional vaccines (such as those targeting prostatic acid phosphatase or the synovial sarcoma X chromosome family of proteins). In addition, other suitable routes of administration may be used (such as intravenously). Tumor growth is monitored daily using bi-dimensional measurements. Sera from these rats or mice may be obtained and used to evaluate the presence of AR LBD antibodies. It is expected that immunization with pTVG-ARLBD DNA vaccine will elicit therapeutic anti-tumor response.

The present invention is not intended to be limited to the foregoing examples, but encompasses all such modifications and variations as come within the scope of the appended claims.

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				Met	Glu	Val	Gln	Leu	Gly							
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ctg	gga	agg	gtc	tac	cct	cgg	ccg	ccg	tcc	aag	acc	tac	cga	gga	gct	1181
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Leu	Leu	Leu	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	
	55				60					65					70	
cag	cag	cag	cag	cag	cag	cag	cag	cag	caa	gag	act	agc	ccc	agg	cag	1373
Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Glu	Thr	Ser	Pro	Arg	Gln	
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cag	cag	cag	cag	cag	ggt	gag	gat	ggt	tct	ccc	caa	gcc	cat	cgt	aga	1421
Gln	Gln	Gln	Gln	Gln	Gly	Glu	Asp	Gly	Ser	Pro	Gln	Ala	His	Arg	Arg	
			90					95					100			
ggc	ccc	aca	ggc	tac	ctg	gtc	ctg	gat	gag	gaa	cag	caa	cct	tca	cag	1469
Gly	Pro	Thr	Gly	Tyr	Leu	Val	Leu	Asp	Glu	Glu	Gln	Gln	Pro	Ser	Gln	
	105					110						115				
ccg	cag	tcg	gcc	ctg	gag	tgc	cac	ccc	gag	aga	ggt	tgc	gtc	cca	gag	1517
Pro	Gln	Ser	Ala	Leu	Glu	Cys	His	Pro	Glu	Arg	Gly	Cys	Val	Pro	Glu	
	120					125					130					
cct	gga	gcc	gcc	gtg	gcc	gcc	agc	aag	ggg	ctg	ccg	cag	cag	ctg	cca	1565
Pro	Gly	Ala	Ala	Val	Ala	Ala	Ser	Lys	Gly	Leu	Pro	Gln	Gln	Leu	Pro	
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gca	cct	ccg	gac	gag	gat	gac	tca	gct	gcc	cca	tcc	acg	ttg	tcc	ctg	1613
Ala	Pro	Pro	Asp	Glu	Asp	Asp	Ser	Ala	Ala	Pro	Ser	Thr	Leu	Ser	Leu	
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ctg	ggc	ccc	act	ttc	ccc	ggc	tta	agc	agc	tgc	tcc	gct	gac	ctt	aaa	1661
Leu	Gly	Pro	Thr	Phe	Pro	Gly	Leu	Ser	Ser	Cys	Ser	Ala	Asp	Leu	Lys	
		170					175						180			
gac	atc	ctg	agc	gag	gcc	agc	acc	atg	caa	ctc	ctt	cag	caa	cag	cag	1709
Asp	Ile	Leu	Ser	Glu	Ala	Ser	Thr	Met	Gln	Leu	Leu	Gln	Gln	Gln	Gln	
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Gln	Glu	Ala	Val	Ser	Glu	Gly	Ser	Ser	Ser	Gly	Arg	Ala	Arg	Glu	Ala	
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tcg	ggg	gct	ccc	act	tcc	tcc	aag	gac	aat	tac	tta	ggg	ggc	act	tcg	1805
Ser	Gly	Ala	Pro	Thr	Ser	Ser	Lys	Asp	Asn	Tyr	Leu	Gly	Gly	Thr	Ser	
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acc	att	tct	gac	aac	gcc	aag	gag	ttg	tgt	aag	gca	gtg	tcg	gtg	tcc	1853
Thr	Ile	Ser	Asp	Asn	Ala	Lys	Glu	Leu	Cys	Lys	Ala	Val	Ser	Val	Ser	
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atg	ggc	ctg	ggt	gtg	gag	gcg	ttg	gag	cat	ctg	agt	cca	ggg	gaa	cag	1901
Met	Gly	Leu	Gly	Val	Glu	Ala	Leu	Glu	His	Leu	Ser	Pro	Gly	Glu	Gln	
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Leu	Arg	Gly	Asp	Cys	Met	Tyr	Ala	Pro	Leu	Leu	Gly	Val	Pro	Pro	Ala	
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gtg	cgt	ccc	act	cct	tgt	gcc	cca	ttg	gcc	gaa	tgc	aaa	ggt	tct	ctg	1997
Val	Arg	Pro	Thr	Pro	Cys	Ala	Pro	Leu	Ala	Glu	Cys	Lys	Gly	Ser	Leu	
	280					285				290						
cta	gac	gac	agc	gca	ggc	aag	agc	act	gaa	gat	act	gct	gag	tat	tcc	2045
Leu	Asp	Asp	Ser	Ala	Gly	Lys	Ser	Thr	Glu	Asp	Thr	Ala	Glu	Tyr	Ser	
	295				300					305					310	
cct	ttc	aag	gga	ggt	tac	acc	aaa	ggg	cta	gaa	ggc	gag	agc	cta	ggc	2093
Pro	Phe	Lys	Gly	Gly	Tyr	Thr	Lys	Gly	Leu	Glu	Gly	Glu	Ser	Leu	Gly	
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tgc	tct	ggc	agc	gct	gca	gca	ggg	agc	tcc	ggg	aca	ctt	gaa	ctg	ccg	2141
Cys	Ser	Gly	Ser	Ala	Ala	Ala	Gly	Ser	Ser	Gly	Thr	Leu	Glu	Leu	Pro	
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Ser Thr Leu Ser Leu Tyr Lys Ser Gly Ala Leu Asp Glu Ala Ala Ala	
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Tyr Gln Ser Arg Asp Tyr Tyr Asn Phe Pro Leu Ala Leu Ala Gly Pro	
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Pro Pro Pro Pro Pro Pro Pro His Pro His Ala Arg Ile Lys Leu Glu	
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aac ccg ctg gac tac ggc agc gcc tgg gcg gct gcg gcg gcg cag tgc	2333
Asn Pro Leu Asp Tyr Gly Ser Ala Trp Ala Ala Ala Ala Ala Gln Cys	
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Arg Tyr Gly Asp Leu Ala Ser Leu His Gly Ala Gly Ala Ala Gly Pro	
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Gly Ser Gly Ser Pro Ser Ala Ala Ala Ser Ser Ser Trp His Thr Leu	
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Phe Thr Ala Glu Glu Gly Gln Leu Tyr Gly Pro Cys Gly Gly Gly Gly	
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ggt ggt ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc	2525
Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly	
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Gly Gly Gly Glu Ala Gly Ala Val Ala Pro Tyr Gly Tyr Thr Arg Pro	
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Pro Gln Gly Leu Ala Gly Gln Glu Ser Asp Phe Thr Ala Pro Asp Val	
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Trp Tyr Pro Gly Gly Met Val Ser Arg Val Pro Tyr Pro Ser Pro Thr	
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Cys Val Lys Ser Glu Met Gly Pro Trp Met Asp Ser Tyr Ser Gly Pro	
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Tyr Gly Asp Met Arg Leu Glu Thr Ala Arg Asp His Val Leu Pro Ile	
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Asp Tyr Tyr Phe Pro Pro Gln Lys Thr Cys Leu Ile Cys Gly Asp Glu	
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Ala Ser Gly Cys His Tyr Gly Ala Leu Thr Cys Gly Ser Cys Lys Val	
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Phe Phe Lys Arg Ala Ala Glu Gly Lys Gln Lys Tyr Leu Cys Ala Ser	
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Arg Asn Asp Cys Thr Ile Asp Lys Phe Arg Arg Lys Asn Cys Pro Ser	
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Cys Arg Leu Arg Lys Cys Tyr Glu Ala Gly Met Thr Leu Gly Ala Arg	
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Lys Leu Lys Lys Leu Gly Asn Leu Lys Leu Gln Glu Glu Gly Glu Ala	
635 640 645	
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Ser Ser Thr Thr Ser Pro Thr Glu Glu Thr Thr Gln Lys Leu Thr Val	
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gaa gcc att gag cca ggt gta gtg tgt gct gga cac gac aac aac cag	3197
Glu Ala Ile Glu Pro Gly Val Val Cys Ala Gly His Asp Asn Asn Gln	
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ccc gac tcc ttt gca gcc ttg ctc tct agc ctc aat gaa ctg gga gag	3245
Pro Asp Ser Phe Ala Leu Leu Ser Ser Leu Asn Glu Leu Gly Glu	
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Arg Gln Leu Val His Val Val Lys Trp Ala Lys Ala Leu Pro Gly Phe	
715 720 725	
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Arg Asn Leu His Val Asp Asp Gln Met Ala Val Ile Gln Tyr Ser Trp	
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Met Gly Leu Met Val Phe Ala Met Gly Trp Arg Ser Phe Thr Asn Val	
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Asn Ser Arg Met Leu Tyr Phe Ala Pro Asp Leu Val Phe Asn Glu Tyr	
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cgc atg cac aag tcc cgg atg tac agc cag tgt gtc cga atg agg cac	3485
Arg Met His Lys Ser Arg Met Tyr Ser Gln Cys Val Arg Met Arg His	
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Leu Ser Gln Glu Phe Gly Trp Leu Gln Ile Thr Pro Gln Glu Phe Leu	
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tgc atg aaa gca ctg cta ctc ttc agc att att cca gtg gat ggg ctg	3581
Cys Met Lys Ala Leu Leu Phe Ser Ile Ile Pro Val Asp Gly Leu	
810 815 820	
aaa aat caa aaa ttc ttt gat gaa ctt cga atg aac tac atc aag gaa	3629
Lys Asn Gln Lys Phe Phe Asp Glu Leu Arg Met Asn Tyr Ile Lys Glu	
825 830 835	
ctc gat cgt atc att gca tgc aaa aga aaa aat ccc aca tcc tgc tca	3677
Leu Asp Arg Ile Ile Ala Cys Lys Arg Lys Asn Pro Thr Ser Cys Ser	
840 845 850	
aga cgc ttc tac cag ctc acc aag ctc ctg gac tcc gtg cag cct att	3725
Arg Arg Phe Tyr Gln Leu Thr Lys Leu Leu Asp Ser Val Gln Pro Ile	
855 860 865 870	
gcg aga gag ctg cat cag ttc act ttt gac ctg cta atc aag tca cac	3773
Ala Arg Glu Leu His Gln Phe Thr Phe Asp Leu Leu Ile Lys Ser His	
875 880 885	
atg gtg agc gtg gac ttt ccg gaa atg atg gca gag atc atc tct gtg	3821
Met Val Ser Val Asp Phe Pro Glu Met Met Ala Glu Ile Ile Ser Val	
890 895 900	
caa gtg ccc aag atc ctt tct ggg aaa gtc aag ccc atc tat ttc cac	3869
Gln Val Pro Lys Ile Leu Ser Gly Lys Val Lys Pro Ile Tyr Phe His	
905 910 915	
acc cag tga agcattggaa accctatttc cccaccccag ctcatgcccc	3918
Thr Gln	
920	
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Val Ile Gln Asn Pro Gly Pro Arg His Pro Glu Ala Ala Ser Ala Ala
 35          40          45

Pro Pro Gly Ala Ser Leu Leu Leu Leu Gln Gln Gln Gln Gln Gln
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Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
 65          70          75          80

Glu Thr Ser Pro Arg Gln Gln Gln Gln Gln Gln Gly Glu Asp Gly Ser
 85          90          95

Pro Gln Ala His Arg Arg Gly Pro Thr Gly Tyr Leu Val Leu Asp Glu
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Glu Gln Gln Pro Ser Gln Pro Gln Ser Ala Leu Glu Cys His Pro Glu
115          120          125

Arg Gly Cys Val Pro Glu Pro Gly Ala Ala Val Ala Ala Ser Lys Gly
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Leu Pro Gln Gln Leu Pro Ala Pro Pro Asp Glu Asp Asp Ser Ala Ala
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Pro Ser Thr Leu Ser Leu Leu Gly Pro Thr Phe Pro Gly Leu Ser Ser
165          170          175

Cys Ser Ala Asp Leu Lys Asp Ile Leu Ser Glu Ala Ser Thr Met Gln
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Leu Leu Gln Gln Gln Gln Gln Glu Ala Val Ser Glu Gly Ser Ser Ser
195          200          205

Gly Arg Ala Arg Glu Ala Ser Gly Ala Pro Thr Ser Ser Lys Asp Asn
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Tyr Leu Gly Gly Thr Ser Thr Ile Ser Asp Asn Ala Lys Glu Leu Cys
225          230          235          240

Lys Ala Val Ser Val Ser Met Gly Leu Gly Val Glu Ala Leu Glu His
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Leu Ser Pro Gly Glu Gln Leu Arg Gly Asp Cys Met Tyr Ala Pro Leu
260          265          270

Leu Gly Val Pro Pro Ala Val Arg Pro Thr Pro Cys Ala Pro Leu Ala
275          280          285

Glu Cys Lys Gly Ser Leu Leu Asp Asp Ser Ala Gly Lys Ser Thr Glu
290          295          300

Asp Thr Ala Glu Tyr Ser Pro Phe Lys Gly Gly Tyr Thr Lys Gly Leu
305          310          315          320

Glu Gly Glu Ser Leu Gly Cys Ser Gly Ser Ala Ala Ala Gly Ser Ser
325          330          335

Gly Thr Leu Glu Leu Pro Ser Thr Leu Ser Leu Tyr Lys Ser Gly Ala
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	370					375						380			
Ala	Arg	Ile	Lys	Leu	Glu	Asn	Pro	Leu	Asp	Tyr	Gly	Ser	Ala	Trp	Ala
	385				390					395					400
Ala	Ala	Ala	Ala	Gln	Cys	Arg	Tyr	Gly	Asp	Leu	Ala	Ser	Leu	His	Gly
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Ala	Gly	Ala	Ala	Gly	Pro	Gly	Ser	Gly	Ser	Pro	Ser	Ala	Ala	Ala	Ser
				420				425					430		
Ser	Ser	Trp	His	Thr	Leu	Phe	Thr	Ala	Glu	Glu	Gly	Gln	Leu	Tyr	Gly
		435					440					445			
Pro	Cys	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly
	450					455					460				
Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Glu	Ala	Gly	Ala	Val	Ala	Pro
	465				470					475					480
Tyr	Gly	Tyr	Thr	Arg	Pro	Pro	Gln	Gly	Leu	Ala	Gly	Gln	Glu	Ser	Asp
				485					490						495
Phe	Thr	Ala	Pro	Asp	Val	Trp	Tyr	Pro	Gly	Gly	Met	Val	Ser	Arg	Val
			500					505					510		
Pro	Tyr	Pro	Ser	Pro	Thr	Cys	Val	Lys	Ser	Glu	Met	Gly	Pro	Trp	Met
		515					520					525			
Asp	Ser	Tyr	Ser	Gly	Pro	Tyr	Gly	Asp	Met	Arg	Leu	Glu	Thr	Ala	Arg
	530					535					540				
Asp	His	Val	Leu	Pro	Ile	Asp	Tyr	Tyr	Phe	Pro	Pro	Gln	Lys	Thr	Cys
	545				550					555					560
Leu	Ile	Cys	Gly	Asp	Glu	Ala	Ser	Gly	Cys	His	Tyr	Gly	Ala	Leu	Thr
				565					570						575
Cys	Gly	Ser	Cys	Lys	Val	Phe	Phe	Lys	Arg	Ala	Ala	Glu	Gly	Lys	Gln
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Lys	Tyr	Leu	Cys	Ala	Ser	Arg	Asn	Asp	Cys	Thr	Ile	Asp	Lys	Phe	Arg
		595					600					605			
Arg	Lys	Asn	Cys	Pro	Ser	Cys	Arg	Leu	Arg	Lys	Cys	Tyr	Glu	Ala	Gly
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Met	Thr	Leu	Gly	Ala	Arg	Lys	Leu	Lys	Lys	Leu	Gly	Asn	Leu	Lys	Leu
	625				630					635					640
Gln	Glu	Glu	Gly	Glu	Ala	Ser	Ser	Thr	Thr	Ser	Pro	Thr	Glu	Glu	Thr
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Thr	Gln	Lys	Leu	Thr	Val	Ser	His	Ile	Glu	Gly	Tyr	Glu	Cys	Gln	Pro
			660					665					670		
Ile	Phe	Leu	Asn	Val	Leu	Glu	Ala	Ile	Glu	Pro	Gly	Val	Val	Cys	Ala
		675					680					685			
Gly	His	Asp	Asn	Asn	Gln	Pro	Asp	Ser	Phe	Ala	Ala	Leu	Leu	Ser	Ser
	690				695						700				
Leu	Asn	Glu	Leu	Gly	Glu	Arg	Gln	Leu	Val	His	Val	Val	Lys	Trp	Ala
	705				710					715					720
Lys	Ala	Leu	Pro	Gly	Phe	Arg	Asn	Leu	His	Val	Asp	Asp	Gln	Met	Ala
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Val	Ile	Gln	Tyr	Ser	Trp	Met	Gly	Leu	Met	Val	Phe	Ala	Met	Gly	Trp
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Arg	Ser	Phe	Thr	Asn	Val	Asn	Ser	Arg	Met	Leu	Tyr	Phe	Ala	Pro	Asp
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Leu Val Phe Asn Glu Tyr Arg Met His Lys Ser Arg Met Tyr Ser Gln
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 Cys Val Arg Met Arg His Leu Ser Gln Glu Phe Gly Trp Leu Gln Ile
 785 790 795 800
 Thr Pro Gln Glu Phe Leu Cys Met Lys Ala Leu Leu Leu Phe Ser Ile
 805 810 815
 Ile Pro Val Asp Gly Leu Lys Asn Gln Lys Phe Phe Asp Glu Leu Arg
 820 825 830
 Met Asn Tyr Ile Lys Glu Leu Asp Arg Ile Ile Ala Cys Lys Arg Lys
 835 840 845
 Asn Pro Thr Ser Cys Ser Arg Arg Phe Tyr Gln Leu Thr Lys Leu Leu
 850 855 860
 Asp Ser Val Gln Pro Ile Ala Arg Glu Leu His Gln Phe Thr Phe Asp
 865 870 875 880
 Leu Leu Ile Lys Ser His Met Val Ser Val Asp Phe Pro Glu Met Met
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Gly Arg Val Tyr Pro Arg Pro Ser Lys Thr Tyr Arg Gly Ala Phe	
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Gln Asn Leu Phe Gln Ser Val Arg Glu Ala Ile Gln Asn Pro Gly Pro	
25 30 35	
agg cac cct gag gcc gct aac ata gca cct ccc ggc gcc tgt tta cag	197
Arg His Pro Glu Ala Ala Asn Ile Ala Pro Pro Gly Ala Cys Leu Gln	
40 45 50 55	
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Gln Arg Gln Glu Thr Ser Pro Arg Arg Arg Arg Arg Gln Gln His Thr	
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Glu Asp Gly Ser Pro Gln Ala His Ile Arg Gly Pro Thr Gly Tyr Leu	
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gcc ctg gag gag gaa cag cag cct tca cag cag cag gca gcc tcc gag	341
Ala Leu Glu Glu Glu Gln Gln Pro Ser Gln Gln Gln Ala Ala Ser Glu	
90 95 100	
ggc cac cct gag agc agc tgc ctc ccc gag cct ggg gcg gcc acc gct	389
Gly His Pro Glu Ser Ser Cys Leu Pro Glu Pro Gly Ala Ala Thr Ala	
105 110 115	
cct ggc aag ggg ctg ccg cag cag cca cca gct cct cca gat cag gat	437
Pro Gly Lys Gly Leu Pro Gln Gln Pro Pro Ala Pro Pro Asp Gln Asp	
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Gly Leu Ser Ser Cys Ser Ala Asp Ile Lys Asp Ile Leu Asn Glu Ala	
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ggc acc atg caa ctt ctt cag cag cag caa caa cag cag cag cac caa	581
Gly Thr Met Gln Leu Leu Gln Gln Gln Gln Gln Gln Gln His Gln	
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Gln Gln His Gln Gln His Gln Gln Gln Gln Glu Val Ile Ser Glu Gly	
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Ser Ser Ala Arg Ala Arg Glu Ala Thr Gly Ala Pro Ser Ser Ser Lys	
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Asp Ser Tyr Leu Gly Gly Asn Ser Thr Ile Ser Asp Ser Ala Lys Glu	
220 225 230	
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Leu Cys Lys Ala Val Ser Val Ser Met Gly Leu Gly Val Glu Ala Leu	
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Glu His Leu Ser Pro Gly Glu Gln Leu Arg Gly Asp Cys Met Tyr Ala	
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Ser Leu Leu Gly Gly Pro Pro Ala Val Arg Pro Thr Cys Ala Pro	
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280 285 290 295	
act gaa gag act gct gag tat tcc tct ttc aag gga ggt tac gcc aaa	965
Thr Glu Glu Thr Ala Glu Tyr Ser Ser Phe Lys Gly Gly Tyr Ala Lys	
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gga ttg gaa ggt gag agc ttg ggg tgc tct ggc agc agt gaa gca ggt	1013
Gly Leu Glu Gly Glu Ser Leu Gly Cys Ser Gly Ser Ser Glu Ala Gly	
315 320 325	
agc tct ggg aca ctt gag atc ccg tcc tct ctg tct ctg tat aaa tct	1061
Ser Ser Gly Thr Leu Glu Ile Pro Ser Ser Leu Ser Leu Tyr Lys Ser	
330 335 340	
gga gca cta gac gag gca gca gca tac cag aat cgc gac tac tac aac	1109
Gly Ala Leu Asp Glu Ala Ala Ala Tyr Gln Asn Arg Asp Tyr Tyr Asn	
345 350 355	
ttt ccg ctg gct ctg tcc ggg ccg ccg cac ccc ccg ccc cct acc cat	1157
Phe Pro Leu Ala Leu Ser Gly Pro Pro His Pro Pro Pro Thr His	
360 365 370 375	
cca cac gcc cgt atc aag ctg gag aac cca ttg gac tac ggc agc gcc	1205
Pro His Ala Arg Ile Lys Leu Glu Asn Pro Leu Asp Tyr Gly Ser Ala	
380 385 390	
tgg gct gcg gcg gca gcg caa tgc cgc tat ggg gac ttg ggt agt cta	1253
Trp Ala Ala Ala Ala Ala Gln Cys Arg Tyr Gly Asp Leu Gly Ser Leu	
395 400 405	
cat gga ggg agt gta gcc ggg ccc agc act gga tcg ccc cca gcc acc	1301
His Gly Gly Ser Val Ala Gly Pro Ser Thr Gly Ser Pro Pro Ala Thr	
410 415 420	
acc tct tct tcc tgg cat act ctc ttc aca gct gaa gaa ggc caa tta	1349
Thr Ser Ser Ser Trp His Thr Leu Phe Thr Ala Glu Glu Gly Gln Leu	
425 430 435	
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Tyr Gly Pro Gly Gly Gly Gly Gly Ser Ser Ser Pro Ser Asp Ala Gly	
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Pro Val Ala Pro Tyr Gly Tyr Thr Arg Pro Pro Gln Gly Leu Thr Ser	
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Gln Glu Ser Asp Tyr Ser Ala Ser Glu Val Trp Tyr Pro Gly Gly Val	
475 480 485	
gtg aac aga gta ccc tat ccc agt ccc aat tgt gtc aaa agt gaa atg	1541
Val Asn Arg Val Pro Tyr Pro Ser Pro Asn Cys Val Lys Ser Glu Met	
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Gly Pro Trp Met Glu Asn Tyr Ser Gly Pro Tyr Gly Asp Met Arg Leu	
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Asp Ser Thr Arg Asp His Val Leu Pro Ile Asp Tyr Tyr Phe Pro Pro	
520 525 530 535	
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Gln Lys Thr Cys Leu Ile Cys Gly Asp Glu Ala Ser Gly Cys His Tyr	
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Gly Ala Leu Thr Cys Gly Ser Cys Lys Val Phe Phe Lys Arg Ala Ala	
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Glu Gly Lys Gln Lys Tyr Leu Cys Ala Ser Arg Asn Asp Cys Thr Ile	
570 575 580	
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Asp Lys Phe Arg Arg Lys Asn Cys Pro Ser Cys Arg Leu Arg Lys Cys	
585 590 595	
tat gaa gca ggg atg act ctg gga gct cgt aag ctg aag aaa ctt gga	1877
Tyr Glu Ala Gly Met Thr Leu Gly Ala Arg Lys Leu Lys Lys Leu Gly	
600 605 610 615	
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Asn Leu Lys Leu Gln Glu Glu Gly Glu Asn Ser Asn Ala Gly Ser Pro	
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Thr Glu Asp Pro Ser Gln Lys Met Thr Val Ser His Ile Glu Gly Tyr	
635 640 645	
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Glu Cys Gln Pro Ile Phe Leu Asn Val Leu Glu Ala Ile Glu Pro Gly	
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Val Val Cys Ala Gly His Asp Asn Asn Gln Pro Asp Ser Phe Ala Ala	
665 670 675	
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Leu Leu Ser Ser Leu Asn Glu Leu Gly Glu Arg Gln Leu Val His Val	
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gtc aag tgg gcc aag gcc ttg cct ggc ttc cgc aac ttg cat gtg gat	2165
Val Lys Trp Ala Lys Ala Leu Pro Gly Phe Arg Asn Leu His Val Asp	
700 705 710	
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Asp Gln Met Ala Val Ile Gln Tyr Ser Trp Met Gly Leu Met Val Phe	
715 720 725	
gcc atg ggt tgg cgg tcc ttc act aat gtc aac tcc agg atg ctc tac	2261
Ala Met Gly Trp Arg Ser Phe Thr Asn Val Asn Ser Arg Met Leu Tyr	
730 735 740	
ttt gca cct gac ttg gtt ttc aat gag tac cgc atg cac aag tct cgg	2309
Phe Ala Pro Asp Leu Val Phe Asn Glu Tyr Arg Met His Lys Ser Arg	
745 750 755	
atg tac agc cag tgt gtg agg atg agg cac ctg tct caa gag ttt gga	2357
Met Tyr Ser Gln Cys Val Arg Met Arg His Leu Ser Gln Glu Phe Gly	
760 765 770 775	

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Trp Leu Gln Ile Thr Pro Gln Glu Phe Leu Cys Met Lys Ala Leu Leu	
780 785 790	
ctc ttc agc att att cca gtg gat ggg ctg aaa aat caa aaa ttc ttt	2453
Leu Phe Ser Ile Ile Pro Val Asp Gly Leu Lys Asn Gln Lys Phe Phe	
795 800 805	
gat gaa ctt cga atg aac tac atc aag gaa ctc gat cgc atc att gca	2501
Asp Glu Leu Arg Met Asn Tyr Ile Lys Glu Leu Asp Arg Ile Ile Ala	
810 815 820	
tgc aaa aga aag aat ccc aca tcc tgc tca agg cgc ttc tac cag ctc	2549
Cys Lys Arg Lys Asn Pro Thr Ser Cys Ser Arg Arg Phe Tyr Gln Leu	
825 830 835	
acc aag ctc ctg gat tct gtg cag cct att gca aga gag ctg cat cag	2597
Thr Lys Leu Leu Asp Ser Val Gln Pro Ile Ala Arg Glu Leu His Gln	
840 845 850 855	
ttc act ttt gac ctg cta atc aag tcc cat atg gtg agc gtg gac ttt	2645
Phe Thr Phe Asp Leu Leu Ile Lys Ser His Met Val Ser Val Asp Phe	
860 865 870	
cct gaa atg atg gca gag atc atc tct gtg caa gtg ccc aag atc ctt	2693
Pro Glu Met Met Ala Glu Ile Ile Ser Val Gln Val Pro Lys Ile Leu	
875 880 885	
tct ggg aaa gtc aag ccc atc tat ttc cac aca cag tga agatttggaa	2742
Ser Gly Lys Val Lys Pro Ile Tyr Phe His Thr Gln	
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tctgcactac ttctctgcag tgccttgggg gaaattcctc tactgatgta cagtctgtcg	2862
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<210> SEQ ID NO 4

<211> LENGTH: 899

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 4

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Ala Ile Gln Asn Pro Gly Pro Arg His Pro Glu Ala Ala Asn Ile Ala	
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Pro Pro Gly Ala Cys Leu Gln Gln Arg Gln Glu Thr Ser Pro Arg Arg	
50 55 60	
Arg Arg Arg Gln Gln His Thr Glu Asp Gly Ser Pro Gln Ala His Ile	
65 70 75 80	
Arg Gly Pro Thr Gly Tyr Leu Ala Leu Glu Glu Glu Gln Gln Pro Ser	
85 90 95	
Gln Gln Gln Ala Ala Ser Glu Gly His Pro Glu Ser Ser Cys Leu Pro	
100 105 110	
Glu Pro Gly Ala Ala Thr Ala Pro Gly Lys Gly Leu Pro Gln Gln Pro	
115 120 125	
Pro Ala Pro Pro Asp Gln Asp Asp Ser Ala Ala Pro Ser Thr Leu Ser	
130 135 140	
Leu Leu Gly Pro Thr Phe Pro Gly Leu Ser Ser Cys Ser Ala Asp Ile	
145 150 155 160	

Lys	Asp	Ile	Leu	Asn	Glu	Ala	Gly	Thr	Met	Gln	Leu	Leu	Gln	Gln	Gln
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Gln	Gln	Gln	Gln	Gln	His	Gln	Gln	Gln	His	Gln	Gln	His	Gln	Gln	Gln
				180				185					190		
Gln	Glu	Val	Ile	Ser	Glu	Gly	Ser	Ser	Ala	Arg	Ala	Arg	Glu	Ala	Thr
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Gly	Ala	Pro	Ser	Ser	Ser	Lys	Asp	Ser	Tyr	Leu	Gly	Gly	Asn	Ser	Thr
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Ile	Ser	Asp	Ser	Ala	Lys	Glu	Leu	Cys	Lys	Ala	Val	Ser	Val	Ser	Met
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Gly	Leu	Gly	Val	Glu	Ala	Leu	Glu	His	Leu	Ser	Pro	Gly	Glu	Gln	Leu
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Arg	Gly	Asp	Cys	Met	Tyr	Ala	Ser	Leu	Leu	Gly	Gly	Pro	Pro	Ala	Val
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Arg	Pro	Thr	Pro	Cys	Ala	Pro	Leu	Pro	Glu	Cys	Lys	Gly	Leu	Pro	Leu
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Asp	Glu	Gly	Pro	Gly	Lys	Ser	Thr	Glu	Glu	Thr	Ala	Glu	Tyr	Ser	Ser
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Phe	Lys	Gly	Gly	Tyr	Ala	Lys	Gly	Leu	Glu	Gly	Glu	Ser	Leu	Gly	Cys
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Ser	Gly	Ser	Ser	Glu	Ala	Gly	Ser	Ser	Gly	Thr	Leu	Glu	Ile	Pro	Ser
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Ser	Leu	Ser	Leu	Tyr	Lys	Ser	Gly	Ala	Leu	Asp	Glu	Ala	Ala	Ala	Tyr
				340				345					350		
Gln	Asn	Arg	Asp	Tyr	Tyr	Asn	Phe	Pro	Leu	Ala	Leu	Ser	Gly	Pro	Pro
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His	Pro	Pro	Pro	Pro	Thr	His	Pro	His	Ala	Arg	Ile	Lys	Leu	Glu	Asn
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Pro	Leu	Asp	Tyr	Gly	Ser	Ala	Trp	Ala	Ala	Ala	Ala	Ala	Gln	Cys	Arg
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Tyr	Gly	Asp	Leu	Gly	Ser	Leu	His	Gly	Gly	Ser	Val	Ala	Gly	Pro	Ser
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Thr	Gly	Ser	Pro	Pro	Ala	Thr	Thr	Ser	Ser	Ser	Trp	His	Thr	Leu	Phe
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Thr	Ala	Glu	Glu	Gly	Gln	Leu	Tyr	Gly	Pro	Gly	Gly	Gly	Gly	Gly	Ser
				435			440					445			
Ser	Ser	Pro	Ser	Asp	Ala	Gly	Pro	Val	Ala	Pro	Tyr	Gly	Tyr	Thr	Arg
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Pro	Pro	Gln	Gly	Leu	Thr	Ser	Gln	Glu	Ser	Asp	Tyr	Ser	Ala	Ser	Glu
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Val	Trp	Tyr	Pro	Gly	Gly	Val	Val	Asn	Arg	Val	Pro	Tyr	Pro	Ser	Pro
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Asn	Cys	Val	Lys	Ser	Glu	Met	Gly	Pro	Trp	Met	Glu	Asn	Tyr	Ser	Gly
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Pro	Tyr	Gly	Asp	Met	Arg	Leu	Asp	Ser	Thr	Arg	Asp	His	Val	Leu	Pro
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Ile	Asp	Tyr	Tyr	Phe	Pro	Pro	Gln	Lys							

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Ser Arg Asn Asp Cys Thr Ile Asp Lys Phe Arg Arg Lys Asn Cys Pro
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 Ser Cys Arg Leu Arg Lys Cys Tyr Glu Ala Gly Met Thr Leu Gly Ala
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 Arg Lys Leu Lys Lys Leu Gly Asn Leu Lys Leu Gln Glu Glu Gly Glu
 610 615 620
 Asn Ser Asn Ala Gly Ser Pro Thr Glu Asp Pro Ser Gln Lys Met Thr
 625 630 635 640
 Val Ser His Ile Glu Gly Tyr Glu Cys Gln Pro Ile Phe Leu Asn Val
 645 650 655
 Leu Glu Ala Ile Glu Pro Gly Val Val Cys Ala Gly His Asp Asn Asn
 660 665 670
 Gln Pro Asp Ser Phe Ala Ala Leu Leu Ser Ser Leu Asn Glu Leu Gly
 675 680 685
 Glu Arg Gln Leu Val His Val Val Lys Trp Ala Lys Ala Leu Pro Gly
 690 695 700
 Phe Arg Asn Leu His Val Asp Asp Gln Met Ala Val Ile Gln Tyr Ser
 705 710 715 720
 Trp Met Gly Leu Met Val Phe Ala Met Gly Trp Arg Ser Phe Thr Asn
 725 730 735
 Val Asn Ser Arg Met Leu Tyr Phe Ala Pro Asp Leu Val Phe Asn Glu
 740 745 750
 Tyr Arg Met His Lys Ser Arg Met Tyr Ser Gln Cys Val Arg Met Arg
 755 760 765
 His Leu Ser Gln Glu Phe Gly Trp Leu Gln Ile Thr Pro Gln Glu Phe
 770 775 780
 Leu Cys Met Lys Ala Leu Leu Leu Phe Ser Ile Ile Pro Val Asp Gly
 785 790 795 800
 Leu Lys Asn Gln Lys Phe Phe Asp Glu Leu Arg Met Asn Tyr Ile Lys
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 Glu Leu Asp Arg Ile Ile Ala Cys Lys Arg Lys Asn Pro Thr Ser Cys
 820 825 830
 Ser Arg Arg Phe Tyr Gln Leu Thr Lys Leu Leu Asp Ser Val Gln Pro
 835 840 845
 Ile Ala Arg Glu Leu His Gln Phe Thr Phe Asp Leu Leu Ile Lys Ser
 850 855 860
 His Met Val Ser Val Asp Phe Pro Glu Met Met Ala Glu Ile Ile Ser
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 His Thr Gln

<210> SEQ ID NO 5
 <211> LENGTH: 4137
 <212> TYPE: DNA
 <213> ORGANISM: Rattus norvegicus
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (994)..(3702)

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acataccacg ccagccccag cccagcgaca gccaacgcct gttgcagagc ggcggttcg	180

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gtagtcgaaa gcaaaacaaa agcaaaaaga aaacaaaag aaaatagccc agttcttatt	660
tgcacctgct tcagtggaca ttgactttgg aaggcagaga attttccttc ccccagtca	720
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gga agg gtc tac cca cgg ccc ccg tcc aag acc tat cga gga gcg ttc	1062
Gly Arg Val Tyr Pro Arg Pro Pro Ser Lys Thr Tyr Arg Gly Ala Phe	
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Gln Asn Leu Phe Gln Ser Val Arg Glu Ala Ile Gln Asn Pro Gly Pro	
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Arg His Pro Glu Ala Ala Ser Ile Ala Pro Pro Gly Ala Cys Leu Gln	
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Gln Arg Gln Glu Thr Ser Pro Arg Arg Arg Arg Gln Gln His Pro	
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gag gat ggc tct cct caa gcc cac atc aga ggc acc aca ggc tac ctg	1254
Glu Asp Gly Ser Pro Gln Ala His Ile Arg Gly Thr Thr Gly Tyr Leu	
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gcc ctg gag gag gaa cag cag cct tca cag cag cag tca gcc tcc gag	1302
Ala Leu Glu Glu Glu Gln Gln Pro Ser Gln Gln Gln Ser Ala Ser Glu	
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Gly His Pro Glu Ser Gly Cys Leu Pro Glu Pro Gly Ala Ala Thr Ala	
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cct ggc aag ggg ctg ccg cag cag cca cca gct cct cca gat cag gat	1398
Pro Gly Lys Gly Leu Pro Gln Gln Pro Pro Ala Pro Pro Asp Gln Asp	
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Asp Ser Ala Ala Pro Ser Thr Leu Ser Leu Leu Gly Pro Thr Phe Pro	
140 145 150	
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Gly Leu Ser Ser Cys Ser Ala Asp Ile Lys Asp Ile Leu Ser Glu Ala	
155 160 165	
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Gly Thr Met Gln Leu Leu Gln Gln Gln Gln Gln Gln Gln Gln Gln	
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185 190 195	

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Ser Ser Lys Asp Ser Tyr Leu Gly Gly Asn Ser Thr Ile Ser Asp Ser	
220 225 230	
gcc aag gag ttg tgt aaa gca gtg tct gtg tcc atg ggg ttg ggt gtg	1734
Ala Lys Glu Leu Cys Lys Ala Val Ser Met Gly Leu Gly Val	
235 240 245	
gaa gca ctg gaa cat ctg agt cca ggg gag cag ctt cgg ggc gac tgc	1782
Glu Ala Leu Glu His Leu Ser Pro Gly Glu Gln Leu Arg Gly Asp Cys	
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Met Tyr Ala Ser Leu Leu Gly Gly Pro Pro Ala Val Arg Pro Thr Pro	
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Cys Ala Pro Leu Ala Glu Cys Lys Gly Leu Ser Leu Asp Glu Gly Pro	
280 285 290 295	
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Gly Lys Gly Thr Glu Glu Thr Ala Glu Tyr Ser Ser Phe Lys Gly Gly	
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Tyr Ala Lys Gly Leu Glu Gly Glu Ser Leu Gly Cys Ser Gly Ser Ser	
315 320 325	
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Glu Ala Gly Ser Ser Gly Thr Leu Glu Ile Pro Ser Ser Leu Ser Leu	
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Tyr Lys Ser Gly Ala Val Asp Glu Ala Ala Ala Tyr Gln Asn Arg Asp	
345 350 355	
tac tac aac ttt ccg ctc gct ctg tcc ggg ccg ccg cac ccc ccg ccc	2118
Tyr Tyr Asn Phe Pro Leu Ala Leu Ser Gly Pro Pro His Pro Pro Pro	
360 365 370 375	
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Pro Thr His Pro His Ala Arg Ile Lys Leu Glu Asn Pro Ser Asp Tyr	
380 385 390	
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Gly Ser Ala Trp Ala Ala Ala Ala Ala Gln Cys Arg Tyr Gly Asp Leu	
395 400 405	
gct agc cta cat gga ggg agt gta gcc gga ccc agc act gga tcg ccc	2262
Ala Ser Leu His Gly Gly Ser Val Ala Gly Pro Ser Thr Gly Ser Pro	
410 415 420	
cca gcc acc gcc tct tct tcc tgg cat act ctc ttc aca gct gaa gaa	2310
Pro Ala Thr Ala Ser Ser Ser Trp His Thr Leu Phe Thr Ala Glu Glu	
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Gly Gln Leu Tyr Gly Pro Gly Gly Gly Gly Gly Ser Ser Ser Pro Ser	
440 445 450 455	
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Asp Ala Gly Pro Val Ala Pro Tyr Gly Tyr Thr Arg Pro Pro Gln Gly	
460 465 470	
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Leu Ala Ser Gln Glu Gly Asp Phe Ser Ala Ser Glu Val Trp Tyr Pro	
475 480 485	
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Gly Gly Val Val Asn Arg Val Pro Tyr Pro Ser Pro Ser Cys Val Lys	
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Phe Pro Pro Gln Lys Thr Cys Leu Ile Cys Gly Asp Glu Ala Ser Gly	
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Cys His Tyr Gln Ala Leu Thr Cys Gly Ser Cys Lys Val Phe Phe Lys	
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Arg Ala Ala Glu Gly Lys Gln Lys Tyr Leu Cys Ala Ser Arg Asn Asp	
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tgc acc att gat aaa ttt cgg agg aaa aat tgt cca tcg tgt cgt ctc	2790
Cys Thr Ile Asp Lys Phe Arg Arg Lys Asn Cys Pro Ser Cys Arg Leu	
585 590 595	
cgg aaa tgt tat gaa gca ggg atg act ctg gga gct cgt aag ctg aag	2838
Arg Lys Cys Tyr Glu Ala Gly Met Thr Leu Gly Ala Arg Lys Leu Lys	
600 605 610 615	
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Lys Leu Gly Asn Leu Lys Leu Gln Glu Glu Gly Glu Asn Ser Ser Ala	
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Gly Ser Pro Thr Glu Asp Pro Ser Gln Lys Met Thr Val Ser His Ile	
635 640 645	
gaa ggc tat gaa tgt caa cct atc ttt ctt aat gtc ctg gaa gcc att	2982
Glu Gly Tyr Glu Cys Gln Pro Ile Phe Leu Asn Val Leu Glu Ala Ile	
650 655 660	
gag cca gga gtg gtg tgt gcc gga cat gac aac aac cag cct gat tcc	3030
Glu Pro Gly Val Val Cys Ala Gly His Asp Asn Asn Gln Pro Asp Ser	
665 670 675	
ttt gct gcc ttg tta tct agt ctc aac gag ctt ggc gag aga cag ctt	3078
Phe Ala Ala Leu Leu Ser Ser Leu Asn Glu Leu Gly Glu Arg Gln Leu	
680 685 690 695	
gta cat gtg gtc aag tgg gcc aag gcc ttg cct ggc ttc cgc aac ttg	3126
Val His Val Val Lys Trp Ala Lys Ala Leu Pro Gly Phe Arg Asn Leu	
700 705 710	
cat gtg gat gac cag atg gca gtc att cag tat tcc tgg atg gga ctg	3174
His Val Asp Asp Gln Met Ala Val Ile Gln Tyr Ser Trp Met Gly Leu	
715 720 725	
atg gta ttt gcc atg ggt tgg cgg tcc ttc act aat gtc aac tct agg	3222
Met Val Phe Ala Met Gly Trp Arg Ser Phe Thr Asn Val Asn Ser Arg	
730 735 740	
atg ctc tac ttt gca cct gac ctg gtt ttc aat gag tat cgc atg cac	3270
Met Leu Tyr Phe Ala Pro Asp Leu Val Phe Asn Glu Tyr Arg Met His	
745 750 755	
aag tct cga atg tac agc cag tgc gtg agg atg agg cac ctt tct caa	3318
Lys Ser Arg Met Tyr Ser Gln Cys Val Arg Met Arg His Leu Ser Gln	
760 765 770 775	
gag ttt gga tgg ctc cag ata acc ccc cag gaa ttc ctg tgc atg aaa	3366
Glu Phe Gly Trp Leu Gln Ile Thr Pro Gln Glu Phe Leu Cys Met Lys	
780 785 790	
gca ctg cta ctc ttc agc att att cca gtg gat ggg ctg aaa aat caa	3414
Ala Leu Leu Leu Phe Ser Ile Ile Pro Val Asp Gly Leu Lys Asn Gln	
795 800 805	
aaa ttc ttt gat gaa ctt cga atg aac tac atc aag gaa ctt gat cgc	3462
Lys Phe Phe Asp Glu Leu Arg Met Asn Tyr Ile Lys Glu Leu Asp Arg	
810 815 820	
atc att gca tgc aaa aga aaa aat ccc aca tcc tgc tca agg cgc ttc	3510
Ile Ile Ala Cys Lys Arg Lys Asn Pro Thr Ser Cys Ser Arg Arg Phe	
825 830 835	

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tac cag ctc acc aag ctc ctg gat tct gtg cag cct att gca aga gag	3558
Tyr Gln Leu Thr Lys Leu Leu Asp Ser Val Gln Pro Ile Ala Arg Glu	
840 845 850 855	
ctg cat caa ttc act ttt gac ctg cta atc aag tcc cat atg gtg agc	3606
Leu His Gln Phe Thr Phe Asp Leu Leu Ile Lys Ser His Met Val Ser	
860 865 870	
gtg gac ttt cct gaa atg atg gca gag atc atc tct gtg caa gtg ccc	3654
Val Asp Phe Pro Glu Met Met Ala Glu Ile Ile Ser Val Gln Val Pro	
875 880 885	
aag atc ctt tct ggg aaa gtc aag ccc atc tat ttc cac aca cag tga	3702
Lys Ile Leu Ser Gly Lys Val Lys Pro Ile Tyr Phe His Thr Gln	
890 895 900	
agatttgga accctaatac ccaaacccac cttgttccct tttcagatgt cttctgctg	3762
ttatataact ctgcactact tctctggcat gggccttggg ggaaattcct ctactgatgt	3822
acagtctgtc atgaacatgt tccccaaagt ctatttcctg ggcttttctc tctttctttt	3882
tcttctcttc tgctcttttt accctcccat ggcacatttt gaatccgctg cgtgtgtgtg	3942
ctcctgctg tgttttgagt tttgttgat ttcttcaagt ctgtgatgat cttctgtgg	4002
cccagtgtea actgtgcttg tttatagcac tgtgctgtgt gccaaccaag caaatgttta	4062
ctcaccttat gccatggcaa gtttagagag ctataagtat cttgggaaga aacaaacaga	4122
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<210> SEQ ID NO 6

<211> LENGTH: 902

<212> TYPE: PRT

<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 6

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

Glu 210	Ala	Thr	Gly	Ala	Pro	Ser 215	Ser	Ser	Lys	Asp	Ser 220	Tyr	Leu	Gly	Gly
Asn 225	Ser	Thr	Ile	Ser	Asp 230	Ser	Ala	Lys	Glu	Leu 235	Cys	Lys	Ala	Val	Ser 240
Val	Ser	Met	Gly	Leu 245	Gly	Val	Glu	Ala	Leu 250	Glu	His	Leu	Ser	Pro 255	Gly
Glu	Gln	Leu	Arg	Gly 260	Asp	Cys	Met	Tyr 265	Ala	Ser	Leu	Leu	Gly 270	Gly	Pro
Pro	Ala	Val	Arg	Pro	Thr	Pro	Cys 280	Ala	Pro	Leu	Ala	Glu 285	Cys	Lys	Gly
Leu	Ser 290	Leu	Asp	Glu	Gly 295	Pro	Gly	Lys	Gly	Thr 300	Glu	Glu	Thr	Ala	Glu
Tyr 305	Ser	Ser	Phe	Lys	Gly 310	Gly	Tyr	Ala	Lys	Gly 315	Leu	Glu	Gly	Glu	Ser 320
Leu	Gly	Cys	Ser	Gly 325	Ser	Ser	Glu	Ala	Gly 330	Ser	Ser	Gly	Thr	Leu 335	Glu
Ile	Pro	Ser	Ser	Leu 340	Ser	Leu	Tyr	Lys 345	Ser	Gly	Ala	Val 350	Asp	Glu	Ala
Ala	Ala	Tyr 355	Gln	Asn	Arg	Asp	Tyr 360	Tyr	Asn	Phe	Pro	Leu 365	Ala	Leu	Ser
Gly 370	Pro	Pro	His	Pro	Pro	Pro 375	Pro	Thr	His	Pro	His 380	Ala	Arg	Ile	Lys
Leu 385	Glu	Asn	Pro	Ser	Asp 390	Tyr	Gly	Ser	Ala	Trp 395	Ala	Ala	Ala	Ala	Ala 400
Gln	Cys	Arg	Tyr	Gly 405	Asp	Leu	Ala	Ser	Leu 410	His	Gly	Gly	Ser	Val 415	Ala
Gly	Pro	Ser	Thr	Gly 420	Ser	Pro	Pro	Ala 425	Thr	Ala	Ser	Ser	Ser	Trp	His
Thr	Leu	Phe 435	Thr	Ala	Glu	Glu	Gly 440	Gln	Leu	Tyr	Gly 445	Pro	Gly	Gly	Gly
Gly 450	Gly	Ser	Ser	Ser	Pro	Ser 455	Asp	Ala	Gly	Pro	Val 460	Ala	Pro	Tyr	Gly
Tyr 465	Thr	Arg	Pro	Pro	Gln 470	Gly	Leu	Ala	Ser	Gln 475	Glu	Gly	Asp	Phe	Ser 480
Ala	Ser	Glu	Val	Trp 485	Tyr	Pro	Gly	Gly	Val 490	Val	Asn	Arg	Val	Pro 495	Tyr
Pro	Ser	Pro	Ser	Cys 500	Val	Lys	Ser	Glu 505	Met	Gly	Pro	Trp 510	Met	Glu	Asn
Tyr	Ser	Gly 515	Pro	Tyr	Gly	Asp	Met 520	Arg	Leu	Asp	Ser	Thr 525	Arg	Asp	His
Val 530	Leu	Pro	Ile	Asp	Tyr	Tyr 535	Phe	Pro	Pro	Gln 540	Lys	Thr	Cys	Leu	Ile
Cys 545	Gly	Asp	Glu	Ala	Ser 550	Gly	Cys	His	Tyr	Gly 555	Ala	Leu	Thr	Cys	Gly 560
Ser	Cys	Lys	Val	Phe 565	Phe	Lys	Arg	Ala	Ala 570	Glu	Gly	Lys	Gln	Lys	Tyr 575
Leu	Cys	Ala	Ser	Arg 580	Asn	Asp	Cys	Thr 585	Ile	Asp	Lys	Phe 590	Arg	Arg	Lys
Asn	Cys 595	Pro	Ser	Cys	Arg	Leu	Arg 600	Lys	Cys	Tyr	Glu 605	Ala	Gly	Met	Thr
Leu 610	Gly	Ala	Arg	Lys	Leu	Lys 615	Lys	Leu	Gly	Asn 620	Leu	Lys	Leu	Gln	Glu

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Glu Gly Glu Asn Ser Ser Ala Gly Ser Pro Thr Glu Asp Pro Ser Gln
 625 630 635 640
 Lys Met Thr Val Ser His Ile Glu Gly Tyr Glu Cys Gln Pro Ile Phe
 645 650 655
 Leu Asn Val Leu Glu Ala Ile Glu Pro Gly Val Val Cys Ala Gly His
 660 665 670
 Asp Asn Asn Gln Pro Asp Ser Phe Ala Ala Leu Leu Ser Ser Leu Asn
 675 680 685
 Glu Leu Gly Glu Arg Gln Leu Val His Val Val Lys Trp Ala Lys Ala
 690 695 700
 Leu Pro Gly Phe Arg Asn Leu His Val Asp Asp Gln Met Ala Val Ile
 705 710 715 720
 Gln Tyr Ser Trp Met Gly Leu Met Val Phe Ala Met Gly Trp Arg Ser
 725 730 735
 Phe Thr Asn Val Asn Ser Arg Met Leu Tyr Phe Ala Pro Asp Leu Val
 740 745 750
 Phe Asn Glu Tyr Arg Met His Lys Ser Arg Met Tyr Ser Gln Cys Val
 755 760 765
 Arg Met Arg His Leu Ser Gln Glu Phe Gly Trp Leu Gln Ile Thr Pro
 770 775 780
 Gln Glu Phe Leu Cys Met Lys Ala Leu Leu Leu Phe Ser Ile Ile Pro
 785 790 795 800
 Val Asp Gly Leu Lys Asn Gln Lys Phe Phe Asp Glu Leu Arg Met Asn
 805 810 815
 Tyr Ile Lys Glu Leu Asp Arg Ile Ile Ala Cys Lys Arg Lys Asn Pro
 820 825 830
 Thr Ser Cys Ser Arg Arg Phe Tyr Gln Leu Thr Lys Leu Leu Asp Ser
 835 840 845
 Val Gln Pro Ile Ala Arg Glu Leu His Gln Phe Thr Phe Asp Leu Leu
 850 855 860
 Ile Lys Ser His Met Val Ser Val Asp Phe Pro Glu Met Met Ala Glu
 865 870 875 880
 Ile Ile Ser Val Gln Val Pro Lys Ile Leu Ser Gly Lys Val Lys Pro
 885 890 895
 Ile Tyr Phe His Thr Gln
 900

<210> SEQ ID NO 7
 <211> LENGTH: 10
 <212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic oligonucleotide
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (4)..(4)
 <223> OTHER INFORMATION: n is a or g

<400> SEQUENCE: 7

gccnccatgg

10

<210> SEQ ID NO 8
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic peptide

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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: X can be any amino acid
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X is Leu or Met
<220> FEATURE:
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<223> OTHER INFORMATION: X can be any amino acid
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<223> OTHER INFORMATION: X can be any amino acid
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<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X is Val or Leu

<400> SEQUENCE: 8

Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Xaa
1 5

<210> SEQ ID NO 9
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 9

Leu Leu Leu Phe Ser Ile Ile Pro Val
1 5

<210> SEQ ID NO 10
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 10

Arg Met Leu Tyr Phe Ala Pro Asp Leu Val
1 5 10

<210> SEQ ID NO 11
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 11

Phe Leu Cys Met Lys Ala Leu Leu Leu
1 5

<210> SEQ ID NO 12
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 12

Gln Leu Thr Lys Leu Leu Asp Ser Val
1 5

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<210> SEQ ID NO 13
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 13

Val Leu Glu Ala Ile Glu Pro Gly Val
1 5

<210> SEQ ID NO 14
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 14

Ala Leu Leu Ser Ser Leu Asn Glu Leu
1 5

<210> SEQ ID NO 15
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 15

Leu Gly Glu Arg Gln Leu Val His Val Val
1 5 10

<210> SEQ ID NO 16
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 16

Trp Met Gly Leu Met Val Phe Ala Met
1 5

<210> SEQ ID NO 17
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 17

Phe Ser Ile Ile Pro Val Asp Gly Leu
1 5

<210> SEQ ID NO 18
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 18

Lys Leu Leu Asp Ser Val Gln Pro Ile
1 5

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<210> SEQ ID NO 19
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
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<400> SEQUENCE: 19

Gly Ile Leu Gly Phe Val Phe Thr Leu
 1 5

We claim:

1. A DNA vaccine comprising a plasmid vector comprising (a) a polynucleotide operatively linked to a transcriptional regulatory element, the polynucleotide encoding a polypeptide that consists of one of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, and (b) at least one immunostimulatory sequence (ISS) motif (5'-GTCGTT-3'), wherein upon administration of said vaccine to a mammal a cytotoxic immune reaction against cells expressing androgen receptor is induced.

2. The DNA vaccine of claim 1, wherein the plasmid vector comprises

- (a) a backbone of pNGVL3;
- (b) a polynucleotide operably inserted therein wherein the polynucleotide encodes a polypeptide that consists of one of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12; and
- (c) an immunostimulatory sequence (ISS) motif.

3. The DNA vaccine of claim 1, wherein the plasmid vector comprises

- (a) a polynucleotide operatively linked to a CMV promoter wherein the polynucleotide encodes a polypeptide that consists of one of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12;
- (b) a CMV intron A operatively linked to the polynucleotide for enhancing expression of the polynucleotide; and
- (c) at least one copy of an immunostimulatory fragment comprising 5'-GTCGTT-3'.

4. A pharmaceutical composition comprising the DNA vaccine of claim 1, and a pharmaceutically acceptable carrier.

5. A pharmaceutical composition comprising the DNA vaccine of claim 4, further comprising an adjuvant.

6. The pharmaceutical composition of claim 5, wherein the adjuvant is GM-CSF.

* * * * *