VECTORS FOR EXPRESSION OF PROSTATE-ASSOCIATED ANTIGENS

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/898,966 filed November 1, 2013, which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

This application is being filed along with a sequence listing in electronic format. The sequence listing is provided as a file in .txt format entitled "PC72055A_FF_SEQ_LISTING_ST25.txt", created on October 6, 2014 and having a size of 429 KB. The sequence listing contained in the .txt file is part of the specification and is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates generally to immunotherapy and specifically to vaccines and methods for treating or preventing neoplastic disorders.

BACKGROUND OF THE INVENTION

Prostate cancer is the second most commonly diagnosed cancer and the fourth leading cause of cancer-related death in men in the developed countries worldwide. Various prostate-associated antigens (PAA), such as prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), and prostate stem cell antigen (PSCA) have been shown to be overexpressed by prostate cancer cells as compared to normal counterparts. These antigens, therefore, represent possible targets for inducing specific immune responses against cancers expressing the antigens via the use of vaccine-based immunotherapy. (See e.g. Marrari, A., M. Iero, et al. (2007). "Vaccination therapy in prostate cancer." Cancer Immunol Immunother 56(4): 429-45)

PSCA is a 123-amino acid membrane protein. The native full length human PSCA consists of amino acids 1 and 4-125 of SEQ ID NO:21 (wihtout the alanine and serine residues at the second and third position respectively). PSCA has high tissue specificity and is expressed on more than 85% of prostate cancer specimens, with expression levels increasing with higher Gleason scores and androgen independence. It is expressed in 80-100% of bone metastasis of prostate cancer patients.

PSA is a kallikrein-like serine protease that is produced exclusively by the columnar epithelial cells lining the acini and ducts of the prostate gland. PSA mRNA is translated as an inactive 261-amino acid preproPSA precursor. PreproPSA has 24 additional residues that constitute the pre-region (the signal polypeptide) and the

propolypeptide. Release of the propolypeptide results in the 237- amino acid, mature extracellular form, which is enzymatically active. The full length sequence of the native human PSA consists of amino acids 4-263 of SEQ ID NO: 15. PSA is organ-specific and, as a result, it is produced by the epithelial cells of benign prostatic hyperplastic (BPH) tissue, primary prostate cancer tissue, and metastatic prostate cancer tissue.

PSMA, also known as Folate hydrolase 1 (FOLH1), is composed of 750 amino acids. The amino acid sequence of the full length human PSMA is provided in SEQ ID NO:1. PSMA includes a cytoplasmic domain (amino acids 1-19), a transmembrane domain (amino acids 20-43), and an extracellular domain (amino acids 44-750). PSMA was found to be expressed in prostate cancer cells it at 1000-fold higher levels than normal tissues. It is abundantly expressed on neovasculature of a variety of other solid tumors such as colon, breast, liver, bladder, pancreas, lung, renal cancers as well as melanoma and sarcomas. Thus, PSMA is considered a target not only specific for prostate cancer cells but also a pan-carcinoma target for other cancers.

While a large number of tumor-associated antigens have been identified and many of these antigens have been explored as protein-based or DNA-based vaccines for the treatment or prevention of cancers, most clinical trials so far have failed to produce a therapeutic product. One of the challenges in developing cancer vaccines resides in the fact that the cancer antigens are usually self-derived and, therefore, poorly immunogenic because the immune system is self-regulated not to recognize self-proteins. Accordingly, a need exists for a method to enhance the immunogenicity or therapeutic effect of cancer vaccines.

Numerous approaches have been explored for enhancing the immunogenicity or enhancing anti-tumor efficacy of cancer vaccines. One of such approach involves the use of various immune modulators, such as TLR agonists, TNFR agonists, CTLA-4 inhibitors, and protein kinase inhibitors.

Toll-like receptors (TLRs) are type 1 membrane receptors that are expressed on hematopoietic and non-hematopoietic cells. At least 11 members have been identified in the TLR family. These receptors are characterized by their capacity to recognize pathogen-associated molecular patterns (PAMP) expressed by pathogenic organisms. These receptors in the innate immune systems exert control over the polarity of the ensuing acquired immune response. Among the TLRs, TLR9 has been extensively investigated for its functions in immune responses. Stimulation of the TLR9 receptor

directs antigen-presenting cells (APCs) towards priming potent, T_H1-dominated T-cell responses, by increasing the production of pro-inflammatory cytokines and the presentation of co-stimulatory molecules to T cells. CpG oligonucleotides, ligands for TLR9, were found to be a class of potent immunostimulatory factors. CpG therapy has been tested against a wide variety of tumor models in mice, and has consistently been shown to promote tumor inhibition or regression.

Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) is a member of the immunoglobulin superfamily and is expressed on the surface of Helper T cells. CTLA-4 is a negative regulator of CD28 dependent T cell activation, and acts as an inhibitory checkpoint for the adaptive immune response. Similar to the T-cell costimulatory protein CD28, CTLA-4 binds to CD80 and CD86 on antigen-presenting cells. CTLA-4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Human antibodies against human CTLA-4 have been described as immunostimulation modulators in a number of disease conditions, such as treating or preventing viral and bacterial infection and for treating cancer (WO 01/14424 and WO 00/37504). Various preclinical studies have shown that CTLA-4 blockade by monoclonal antibodies enhances the host immune response against immunogenic tumors, and can even reject established tumors. Two fully human anti-human CTLA-4 monoclonal antibodies (mAbs), ipilimumab (MDX-010) and Tremelimumab (also known as CP-675206), have been investigated in clinical trials in the treatment of various types of solid tumors.

The tumor necrosis factor (TNF) superfamily is a group of cytokines that engage specific cognate cell surface receptors, the TNF receptor (TNFR) superfamily. Members of the tumor necrosis factor superfamily act through ligand-mediated trimerization, causing recruitment of several intracellular adaptors to activate multiple signal transduction pathways, such as apoptosis, NF-kB pathway, JNK pathway, as well as immune and inflammatory responses. Examples of the TNF superfamily include CD40 ligands, OX40 ligands, 4-1BB ligands, CD27, CD30 ligand (CD153), TNF- alpha, TNF-beta, RANK ligands, LT- alpha, LT- beta, GITR ligands, and LIGHT. The TNFR superfamily includes, for example, CD40, OX40, 4-1BB, CD70 (CD27 ligand), CD30, TNFR2, RANK, LT- beta R, HVEM, GITR, TROY, and RELT. Among the TNF members, CD40 agonists, including various CD40 agonistic antibodies, such as the fully human agonist CD40 monoclonal antibody CP870893, have been extensively explored for usage in therapies.

Protein kinases are a family of enzymes that catalyze the phosphorylation of specific residues in proteins. A number of kinase inhibitors have been investigated in clinical investigation for use in anti-cancer therapies, which includes, for example, MK0457, VX-680, ZD6474, MLN8054, AZD2171, SNS-032, PTK787/ZK222584, Sorafenib (BAY43-9006), SU5416, SU6668 AMG706, Zactima (ZD6474), MP-412, Dasatinib, CEP-701, (Lestaurtinib), XL647, XL999, Tykerb, (Lapatinib), MLN518, (formerly known as CT53518), PKC412, ST1571, AMN107, AEE 788, OSI-930, OSI-817, Sunitinib malate (Sutent; SU11248), Vatalanib (PTK787/ZK 222584), SNS-032, SNS-314 and Axitinib (AG-013736). Gefitinib and Erlotinib are two orally available EGFR-TKIs.

SUMMARY OF THE INVENTION

The present disclosure relates to vectors constructed from chimpanzee adenovirus ChAd68 genomic sequences for expression of two or more immunogenic PAA polypeptides. The vector comprises (1) a C68 DNA sequence, (2) a multi-antigen construct for expression of two or more immunogenic PAA polypeptides, and (3) regulatory sequences that control the transcription and translation of the antigen products (i.e., the immunogenic PAA polypeptides). The C68 DNA sequence included in the vector is derived from C68 genomic sequence by functional deletion of one or more viral genes but is sufficient for production of an infectious viral particle. In a particular embodiment, the C68 DNA sequence used in the vector is the entire C68 genome with only functional deletions in the E1 and E3 regions.

The multi-antigen construct carried by the vector comprises nucleotide sequences encoding two or more immunogenic PAA polypeptides selected from immunogenic PSMA polypeptide, immunogenic PSA polypeptide, and immunogenic PSCA polypeptide. In some embodiments, the multi-antigen construct carried by the vector comprises (1) a nucleotide sequence encoding at least one immunogenic PSMA polypeptide, (2) a nucleotide sequence encoding at least one immunogenic PSA polypeptide, and (3) a nucleotide sequence encoding at least one immunogenic PSCA polypeptide. The multi-antigen constructs may also include separator sequences that enable expression of separate PAA polypeptides encoded by the construct. Examples of separator sequences include 2A peptide sequences and IRESs. In some embodiments, the vector comprises a multi-antigen construct having one of the following structures:

(1) PSA-F2A-PSMA-mIRES-PSCA;

(2) PSA-F2A-PSMA-T2A-PSCA;

(3) PSA-T2A-PSCA-F2A-PSMA; and

(4) PSCA-F2A-PSMA-mIRES-PSA.

In some embodiments, the nucleotide sequence encoding the immunogenic PSA polypeptide comprises nucleotides 1115-1825 of SEQ ID NO:58 or comprises nucleotides 1106-1825 of SEQ ID NO:58, the nucleotide sequence encoding the immunogenic PSCA polypeptide comprises nucleotides 1892-2257 of SEQ ID NO:58 or comprises nucleotides 1886-2257 of SEQ ID NO:58, and the nucleotide sequence encoding the immunogenic PSMA polypeptide comprises nucleotides 2333-4543 of SEQ ID NO:58 or comprises nucleotides 2324-4543 of SEQ ID NO:58. In some specific embodiments, the multi-antigen construct comprises nucleotide sequence selected from the group consisting of SEQ ID NOs:33, 34, 35, and 36. In a particular embodiment, the multi-antigen construct comprises a nucleotide sequence that encodes a polypeptide sequence of SEQ ID NO:60. In another particular embodiment, the multi-antigen construct comprises a nucleotide sequence of SEQ ID NO:61.

The present disclosure also provides compositions comprising the vectors. In some embodiments, the composition is an immunogenic composition useful for eliciting an immune response against a PAA in a mammal, such as a mouse, dog, monkey, or human. In some embodiments, the composition is a vaccine composition useful for immunization of a mammal, such as a human, for inhibiting abnormal cell proliferation, for providing protection against the development of cancer (used as a prophylactic), or for treatment of disorders (used as a therapeutic) associated with PAA over-expression, such as cancer, particularly prostate cancer.

The present disclosure further relates to methods of using the vectors or compositions for eliciting an immune response against a PAA, or for treating cancers, such as prostate cancers, in a mammal, particularly a human. In some embodiments, the vectors or compositions, including vaccine compositions, are administered to the mammal, particularly human, in combination with one or more immune modulators that enhance the immunogenicity or effect of the vectors or compositions. In some particular embodiments, the method involves co-administration of a vaccine provided by the present invention in combination with at least one immune-suppressive-cell inhibitor and at least one immune-effector-cell enhancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Schematic illustration of PJV7563 vector.

FIG. 2. Amino acid alignment of five viral 2A cassettes. The skipped glycine-proline bonds are indicated by asterisks.

FIG. 3. Sequence of the preferred EMCV IRES. The translation initiation site is indicated by the asterisk. The minimal IRES element excludes the underlined first 5 codons of the EMCV L protein.

FIG. 4. Graph showing the Kaplan-Meier survival curves of the groups of mice from a representative study evaluating the effect of sunitinib malate (Sutent) and an anti-murine CTLA-4 monoclonal antibody (clone 9D9) on the anti-tumor efficacy of a cancer vaccine (vaccine) in subcutaneous TUBO tumor bearing BALB/neuT mice.

FIG. 5. Graph depicting the IFN□ ELISPOT results from a representative study evaluating the effect of CpG7909 and an anti-CD40 antibody (Bioxcell #BE0016-2) on the antigen specific T cell responses induced by a cancer vaccine (rHER2).

FIG. 6. Graphs depicting results of a representative study that evaluates the immunomodulatory activity of CpG7909 on the quality of the immune responses induced by a cancer vaccine (PMED) using intracellular cytokine staining assay, in which cytokine positive CD8 T cells were measured. (* indicates $P<0.05$ by Student's T-test).

FIG. 7. Graphs depicting results of a representative study that evaluates the immunomodulatory activity of CpG7909 on the quality of the immune responses induced by a cancer vaccine (PMED) using intracellular cytokine staining assay, in which cytokine positive CD4 T cells (FIG. 7) were measured. (* indicates $P<0.05$ by Student's T-test).

FIG. 8. Graphs depicting results of a representative study that evaluates the immunomodulatory activity of an agonistic anti-murine CD40 monoclonal antibody on the quality of the immune responses induced by a cancer vaccine (PMED) using intracellular cytokine staining assay, in which cytokine positive CD8 T cells were measured. (*indicates $P<0.05$ by Student's T-test)

FIG. 9. Graphs depicting results of a representative study that evaluates the immunomodulatory activity of an agonistic anti-murine CD40 monoclonal antibody on the quality of the immune responses induced by a cancer vaccine (PMED) using intracellular cytokine staining assay, in which cytokine positive CD4 T cells were measured. (*indicates $P<0.05$ by Student's T-test)

FIG. 10. Graph showing the Kaplan-Meier survival curves of the groups of mice from a representative study that evaluates the effect of low dose sunitinib malate (Sutent) on the anti-tumor efficacy of a cancer vaccine in spontaneous mammary tumor bearing BALB/neuT mice.

FIG. 11. Graph showing the genomic organization of the AdC68-734 vector. CMV Enh/pro = human cytomegalovirus immediate early enhancer and promoter; tet op = tetracycline operator; T2A = Thosea asigna virus 2A; F2A = Foot and Mouth Disease Virus 2A; SV40 pA = Simian Virus 40 polyadenylation signal; LITR = left inverted terminal repeat; RITR = right inverted terminal repeat.

FIG. 12. Dot plots showing expression of PSMA and PSCA on the surface of A549 cells transduced with triple antigen expressing AdC68 vectors by flow cytometry.

FIG. 13. Western blot from lysates of A549 infected by AdC68 vectors.

DETAILED DESCRIPTION OF THE INVENTION

A. DEFINITIONS

The term "adjuvant" refers to a substance that is capable of enhancing, accelerating, or prolonging an immune response elicited by a vaccine immunogen.

The term "agonist" refers to a substance which promotes (induces, causes, enhances or increases) the activity of another molecule or a receptor. The term agonist encompasses substances which bind receptor (e.g., an antibody, a homolog of a natural ligand from another species) and substances which promote receptor function without binding thereto (e.g., by activating an associated protein).

The term "antagonist" or "inhibitor" refers to a substance that partially or fully blocks, inhibits, or neutralizes a biological activity of another molecule or receptor.

The term "co-administration" refers to administration of two or more agents to the same subject during a treatment period. The two or more agents may be encompassed in a single formulation and thus be administered simultaneously. Alternatively, the two or more agents may be in separate physical formulations and administered separately, either sequentially or simultaneously, to the subject. The term "administered simultaneously" or "simultaneous administration" means that the administration of the first agent and that of a second agent overlap in time with each other, while the term "administered sequentially" or "sequential administration" means that the administration of the first agent and that of a second agent does not overlap in time with each other.

The term "cytosolic" means that, after a nucleotide sequence encoding a particular polypeptide is expressed by a host cell, the expressed polypeptide is retained inside the host cell.

The terms "degenerate variant" refers to a nucleotide sequence that has substitutions of bases as compared to a reference nucleotide sequence but, due to degeneracy of the genetic code, encodes the same amino acid sequence as the reference nucleotide sequence.

The term "effective amount" refers to an amount administered to a mammal that is sufficient to cause a desired effect in the mammal.

The term "fragment" of a given polypeptide refers to a polypeptide that is shorter than the given polypeptide and shares 100% identity with the sequence of the given polypeptide.

The term "identical" or percent "identity," in the context of two or more nucleic acid or polypeptide sequences, refers to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence.

The term "immune-effector-cell enhancer" or "IEC enhancer" refers to a substance capable of increasing or enhancing the number, quality, or function of one or more types of immune effector cells of a mammal. Examples of immune effector cells include cytolytic CD8 T cells, CD40 T cells, NK cells, and B cells.

The term "immune modulator" refers to a substance capable of altering (e.g., inhibiting, decreasing, increasing, enhancing or stimulating) the working of any component of the innate, humoral or cellular immune system of a mammal. Thus, the term "immune modulator" encompasses the "immune-effector-cell enhancer" as defined herein and the "immune-suppressive-cell inhibitor" as defined herein, as well as substance that affects other components of the immune system of a mammal.

The term "immune response" refers to any detectable response to a particular substance (such as an antigen or immunogen) by the immune system of a host vertebrate animal, including, but not limited to, innate immune responses (e.g., activation of Toll receptor signaling cascade), cell-mediated immune responses (e.g., responses mediated by T cells, such as antigen-specific T cells, and non-specific cells of the immune system), and humoral immune responses (e.g., responses mediated by B cells, such as generation and secretion of antibodies into the plasma, lymph, and/or

tissue fluids). Examples of immune responses include an alteration (e.g., increase) in Toll-like receptor activation, lymphokine (e.g., cytokine (e.g., Th1, Th2 or Th17 type cytokines) or chemokine) expression or secretion, macrophage activation, dendritic cell activation, T cell (e.g., CD4+ or CD8+ T cell) activation, NK cell activation, B cell activation (e.g., antibody generation and/or secretion), binding of an immunogen (e.g., antigen (e.g., immunogenic polypolypeptide)) to an MHC molecule, induction of a cytotoxic T lymphocyte ("CTL") response, induction of a B cell response (e.g., antibody production), and, expansion (e.g., growth of a population of cells) of cells of the immune system (e.g., T cells and B cells), and increased processing and presentation of antigen by antigen presenting cells. The term "immune response" also encompasses any detectable response to a particular substance (such as an antigen or immunogen) by one or more components of the immune system of a vertebrate animal *in vitro*.

The term "immunogenic" refers to the ability of a substance to cause, elicit, stimulate, or induce an immune response, or to improve, enhance, increase or prolong a pre-existing immune response, against a particular antigen, whether alone or when linked to a carrier, in the presence or absence of an adjuvant.

The term "immunogenic PSA polypeptide" refers to a polypeptide that is immunogenic against human PSA protein or against cells expressing human PSA protein.

The term "immunogenic PSCA polypeptide" refers to a polypeptide that is immunogenic against human PSCA protein or against cells expressing human PSCA protein.

The term "immunogenic PSMA polypeptide" refers to a polypeptide that is immunogenic against human PSMA protein or against cells expressing human PSMA protein.

The term "immunogenic PAA polypeptide" refers to an "immunogenic PSA polypeptide," an "immunogenic PSCA polypeptide," or an "immunogenic PSMA polypeptide" as defined herein above.

The term "immune-suppressive-cell inhibitor" or "ISC inhibitor" refers to a substance capable of reducing or suppressing the number or function of immune suppressive cells of a mammal. Examples of immune suppressive cells include regulatory T cells ("T regs"), myeloid-derived suppressor cells, and tumor-associated macrophages.

The term "intradermal administration," or "administered intradermally," in the context of administering a substance, such as a therapeutic agent or an immune modulator, to a mammal including a human, refers to the delivery of the substance into the dermis layer of the skin of the mammal. The skin of a mammal is composed of three layers - the epidermis, dermis, and subcutaneous layer. The epidermis is the relatively thin, tough, outer layer of the skin. Most of the cells in the epidermis are keratinocytes. The dermis, the skin's next layer, is a thick layer of fibrous and elastic tissue (made mostly of collagen, elastin, and fibrillin) that gives the skin its flexibility and strength. The dermis contains nerve endings, sweat glands and oil (sebaceous) glands, hair follicles, and blood vessels. The dermis varies in thickness depending on the location of the skin. In humans it is about 0.3 mm on the eyelid and about 3.0 mm on the back. The subcutaneous layer is made up of fat and connective tissue that houses larger blood vessels and nerves. The thickness of this layer varies throughout the body and from person to person. The term "intradermal administration" refers to delivery of a substance to the inside of the dermis layer. In contrast, "subcutaneous administration" refers to the administration of a substance into the subcutaneous layer and "topical administration" refers to the administration of a substance onto the surface of the skin.

The term "local administration" or "administered locally" encompasses "topical administration," "intradermal administration," and "subcutaneous administration," each as defined herein above. This term also encompasses "intratumoral administration," which refers to administration of a substance to the inside of a tumor. Local administration is intended to allow for high local concentrations around the site of administration for a period of time until systemic biodistribution has been achieved with of the administered substance, while "systemic administration" is intended for the administered substance to be absorbed into the blood and attain systemic exposure rapidly by being distributed through the circulatory system to organs or tissues throughout the body.

The term "mammal" refers to any animal species of the Mammalia class. Examples of mammals include: humans; non-human primates such as monkeys; laboratory animals such as rats, mice, guinea pigs; domestic animals such as cats, dogs, rabbits, cattle, sheep, goats, horses, and pigs; and captive wild animals such as lions, tigers, elephants, and the like.

The term "membrane-bound" means that after a nucleotide sequence encoding a particular polypeptide is expressed by a host cell, the expressed polypeptide is bound to, attached to, or otherwise associated with, the membrane of the cell.

The term "neoplastic disorder" refers to a condition in which cells proliferate at an abnormally high and uncontrolled rate, the rate exceeding and uncoordinated with that of the surrounding normal tissues. It usually results in a solid lesion or lump known as "tumor." This term encompasses benign and malignant neoplastic disorders. The term "malignant neoplastic disorder", which is used interchangeably with the term "cancer" in the present disclosure, refers to a neoplastic disorder characterized by the ability of the tumor cells to spread to other locations in the body (known as "metastasis"). The term "benign neoplastic disorder" refers to a neoplastic disorder in which the tumor cells lack the ability to metastasize.

The term "operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a transgene is ligated in such a way that expression of the transgene is achieved under conditions compatible with the control sequences.

The term "pharmaceutically acceptable excipient" refers to a substance in an immunogenic or vaccine composition, other than the active ingredients (e.g., the antigen, antigen-coding nucleic acid, immune modulator, or adjuvant) that is compatible with the active ingredients and does not cause significant untoward effect in subjects to whom it is administered.

The terms "peptide," "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically, or biochemically modified or derivatized amino acids, and polypeptides having modified polypeptide backbones.

The term "preventing" or "prevent" refers to (a) keeping a disorder from occurring or (b) delaying the onset of a disorder or onset of symptoms of a disorder.

The term "prostate-associated-antigen" (or PAA) refers to the TAA (as defined herein) that is specifically expressed on prostate tumor cells or expressed at a higher frequency or density by tumor cells than by non-tumor cells of the same tissue type. Examples of PAA include PSA, PSCA, and PSMA.

The term "secreted" in the context of a polypeptide means that after a nucleotide sequence encoding the polypeptide is expressed by a host cell, the expressed polypeptide is secreted outside of the host cell.

The term "suboptimal dose" when used to describe the amount of an immune modulator, such as a protein kinase inhibitor, refers to a dose of the immune modulator that is below the minimum amount required to produce the desired therapeutic effect for the disease being treated when the immune modulator is administered alone to a patient.

The term "treating," "treatment," or "treat" refers to abrogating a disorder, reducing the severity of a disorder, or reducing the severity or occurrence frequency of a symptom of a disorder.

The term "tumor-associated antigen" or "TAA" refers to an antigen which is specifically expressed by tumor cells or expressed at a higher frequency or density by tumor cells than by non-tumor cells of the same tissue type. Tumor-associated antigens may be antigens not normally expressed by the host; they may be mutated, truncated, misfolded, or otherwise abnormal manifestations of molecules normally expressed by the host; they may be identical to molecules normally expressed but expressed at abnormally high levels; or they may be expressed in a context or milieu that is abnormal. Tumor-associated antigens may be, for example, proteins or protein fragments, complex carbohydrates, gangliosides, haptens, nucleic acids, or any combination of these or other biological molecules.

The term "vaccine" refers to an immunogenic composition for administration to a mammal for eliciting an immune response against a particular antigen.

The term "vector" refers to a nucleic acid molecule capable of transporting or transferring a foreign nucleic acid molecule. The foreign nucleic acid molecule is referred to as "insert" or "transgene." A vector generally consists of an insert and a larger sequence that serves as the backbone of the vector. The term "vector" encompasses both expression vectors and transcription vectors. The term "expression vector" refers to a vector capable of expressing the insert in the target cell. It generally contains control sequences, such as enhancer, promoter, and terminator sequences, that drive expression of the insert. The term "transcription vector" refers to a vector capable of being transcribed but not translated. Transcription vectors are used to amplify their insert. Based on the structure or origin of vectors, major types of vectors

include plasmid vectors, cosmid vectors, phage vectors such as lambda phage, viral vectors such as adenovirus (Ad) vectors, and artificial chromosomes.

B. VECTORS CONTAINING A MULTI-ANTIGEN CONSTRUCT

In one aspect, the present disclosure provides a voral vector constructed from the genome of chimpanzee adenovirus ChAd68 for expression of two or more immunogenic PAA polypeptides. Chimpanzee adenovirus ChAd68 is also referred in the literature as simian adenovirus 25, C68, Chad68, SAdV25, PanAd9, or Pan9. For convenience, the chimpanzee adenovirus ChAd68 may be referred to in this specification as "C68" and the viral vector constructed from the genome of chimpanzee adenovirus ChAd68 is refrred to as "C68 vector." The full length genomic sequence of C68 is available from Genbank (Accession Number AC_000011.1) and is provided in SEQ ID NO:57. In addition, the full length genomic sequence of C68 and location of the adenovirus genes E1a, E1b, E2a, E2b, E3, E4, I1, I2, L3, L4, and L5 are also provided in US Patent 6,083,716.

The C68 vector provided by the present disclosure comprises (1) a C68 DNA sequence, and (2) a multi-antigen construct for expression of two or more immunogenic PAA polypeptides. The vector may also contain non-native regulatory sequences that control the transcription and translation of the antigen products. The non-native regulatory sequences refer to sequences that are not part of the C68 genome. The C68 DNA sequence, multi-antigen construct, and regulatory sequences are operably linked to each other.

The C68 vector can be replication-competent, conditionally replication-competent, or replication-deficient. A replication-competent C68 vector can replicate in typical host cells, i.e., cells typically capable of being infected by an adenovirus. A replication-competent viral vector can have one or more mutations as compared to the wild-type adenovirus (e.g., one or more deletions, insertions, and/or substitutions) in the adenoviral genome that do not inhibit viral replication in host cells. A conditionally-replicating C68 vector is viral vector that has been engineered to replicate under pre-determined conditions. For example, replication-essential gene functions, e.g., gene functions encoded by the adenoviral early regions, can be operably linked to an inducible, repressible, or tissue-specific transcription control sequence, e.g., promoter. A replication-deficient C68 vector is a viral vector that requires complementation of one or more gene functions or regions of the viral genome that are required for replication, as a

result of, for example, a deficiency in one or more replication-essential gene function or regions, such that the vector does not replicate in typical host cells, especially those in a human to be infected by the vector.

The vectors are useful for cloning or expressing the immunogenic PAA polypeptides, or for delivering the multi-antigen construct in a composition, such as a vaccine, to a host cell or to a host animal, such as a human. In some particular embodiments, the present disclosure provides a vector selected from the group comsisting of (i) a vector comprsing or consisting of the nucleotide sequence of SEQ ID NO:58; (ii) a vector comprsing or consisting of nucleotides 9 – 34811 of SEQ ID NO:58; and (iii) a vector comprsing or consisting of the nucleotide sequence of SEQ ID NO:63.

The C68 vector provided by the present disclosure also encompasses functional variants of the vectors specifically described or exemplified in the present disclosure. A "functional variant" refers a vector that contains mutations (e.g., additions, deletions, or substitutions) relative to the sequence of a vector ("parent vector") specifically described or exemplified in the present disclosure but retains the function or property of the parent vector. For example, functional variant may comprise codon-optimized sequence corresponding to a parent vector examplified in the present disclosure.

B1. The C68 DNA Sequence

The term "C68 DNA sequence" refers to a DNA sequence that is part of the C68 genomic sequence. The C68 DNA sequence included in the vector is derived from C68 genomic sequence by functional deletion of one or more viral genes or genomic regions. The term "functional deletion" means that a sufficient amount of the gene region of the virus is removed or otherwise changed, e.g., by mutation or modification, so that the gene region is no longer capable of producing functional products of gene expression or is otherwise performing its normal function. Deletion of an entire gene region often is not required for disruption of a replication-essential gene function. However, for the purpose of providing sufficient space in the C68 genome for one or more transgenes, removal of a majority of one or more gene regions may be desirable. While deletion of genetic material is preferred, mutation of genetic material by addition or substitution also is appropriate for disrupting gene function.

In some embodiments, the C68 DNA sequence of the vector is derived from the C68 genomic sequence by functionally deleting the entire, or a sufficient portion of, the adenoviral immediate early gene E1a and delayed early gene E1b. In other

embodiments, in addition to the functional deletion of E1a and E1b, functional deletion may also be made to one or more other genes, such as the delayed early gene E2a, delayed early gene E3, E4, any of the late genes L1 through L5, the intermediate genes IX, and IVa2. Thus, the C68 DNA sequence for use in the construction of the vector of the invention may contain deletions in E1 only. Alternatively, deletions of entire genes or portions thereof effective to destroy their biological activity may be used in any combination. For example, in one exemplary vector, the C68 DNA sequence is derived from the C68 genomic sequence by functinal deletions of the E1 genes and the E4 gene, or of the E1, E2a and E3 genes, or of the E1 and E3 genes, or of E1, E2a and E4 genes, with or without deletion of E3, and so on. In addition, such deletions may be used in combination with other mutations, such as temperature-sensitive mutations, to achieve a desired result. In a particular embodiment, the C68 DNA sequence is the entire C68 genome with only functional deletions in the E1 and E3 regions.

In some particular embodiments, the functional deletion of E1 gene is accomplished by deletion of nucleotides 577-3403 of SEQ ID NO:57 or by deletion of nucleotides 456-3012 of SEQ ID NO:57, and the functional deletion of E3 gene is accomplished by deletion of nucleotides 27125–31831 of SEQ ID NO:57 or by deletion of nucleotides 27812-31330 of SEQ ID NO:57. In other particular embodiments, the C68 DNA sequence included in the vector comprises nucleodtides 3013 – 27811 of SEQ ID NO:57. In still other particular embodiments, the C68 DNA sequence included in the vector comprises nucleodtides 3013 – 27811 and 31331 – 36519 of SEQ ID NO:57.

The multi-antigen construct may be inserted into any deleted region of the adenovirus genome. The multi-antigen construct may also be inserted into an existing gene region to disrupt the function of that region. In some embodiments, the multi-antigen construct is inserted in the place of the deleted E1 gene.

B2. The Multi-antigen Constructs

The term "multi-antigen construct" refers to a nucleic acid molecule or sequence that encodes two or more PAA polypeptides. Such molecules or sequences may also be referred to as "multi-antigen vaccine" or "multi-antigen plasmid" in the present disclosure. A multi-antigen construct can carry two coding nucleotide sequences wherein each of the coding nucleotide sequences expresses an individual immunogenic PAA polypeptide. Such a construct is also referred to as "dual antigen construct," "dual

antigen vaccine," or "dual antigen plasmid" in this disclosure. A multi-antigen construct can also carry three coding nucleotide sequences wherein each of the coding nucleotide sequences expresses an individual immunogenic PAA polypeptide. Such a construct is also referred to as "triple antigen construct," "triple antigen vaccine," or "triple antigen plasmid" in this disclosure. The individual PAA polypeptides encoded by a multi-antigen construct may be immunogenic against the same antigen, such as PSMA, PSA, or PSCA. For example, a dual antigen construct may express two different PAA antigens that are both immunogenic against PSMA. The individual PAA polypeptides encoded by a multi-antigen construct may be immunogenic against different antigens, for example, a dual antigen construct may express two PAA polypeptides that are immunogenic against PSMA and PSA, respectively. It is preferred that a multi-antigen construct encodes two or more individual PAA polypeptides that are immunogenic against different antigens.

In some embodiments, the multi-antigen construct encodes at least two immunogenic PAA polypeptides in any one of the following combinations:

1) an immunogenic PSMA polypeptide and an immunogenic PSA polypeptide;

2) an immunogenic PSMA polypeptide and an immunogenic PSCA polypeptide; and

3) an immunogenic PSA polypeptide and an immunogenic PSCA polypeptide.

In some particular embodiments, the multi-antigen construct encodes at least one immunogenic PSMA polypeptide, at least one immunogenic PSA polypeptide, and at least one immunogenic PSCA polypeptide.

The immunogenic PSMA polypeptide expressed by a multi-antigen construct may be cytosolic, secreted, or membrane-bound, but preferably membrane-bound. In some embodiments, the immunogenic PSMA polypeptide comprises an amino acid sequence selected from the group consisting of:

1) the amino acid sequence of SEQ ID NO:1,

2) amino acids 15-750 of SEQ ID NO:1;

3) the amino acid sequence of SEQ ID NO:3;

4) the amino acid sequence of SEQ ID NO:5;

5) the amino acid sequence of SEQ ID NO:7;

6) amino acids 4 – 739 of SEQ ID NO:3;

7) amino acids 4 - 739 of SEQ ID NO:5;

8) amino acids 4 - 739 of SEQ ID NO:7;

9) the amino acid sequence of SEQ ID NO:9; and

10) amino acids 4 – 739 of SEQ ID NO:9.

The immunogenic PSA polypeptide expressed by a multi-antigen construct may be cytosolic, secreted, or membrane-bound, but preferably cytosolic. In some embodiments, the immunogenic PSA polypeptide comprises an amino acid sequence selected from the group consisting of:

1) amino acids 27-263 of SEQ ID NO: 15;

2) the amino acid sequence of SEQ ID NO:17; and

3) amino acids 4 - 240 of SEQ ID NO:17.

The immunogenic PSCA polypeptide expressed by a multi-antigen construct may be the full length human PSCA protein. In some embodiments, the immunogenic PSCA polypeptide comoprises an amino acid sequence selected from the group consisting of:

1) the amino acid sequence of SEQ ID NO:21;

2) amino acids 2-125 of SEQ ID NO;21; and

3) amino acids 4-125 ofSEQ ID NO:21.

In some other embodiments, the multi-antigen construct encodes at least one immunogenic PSA polypeptide, at least one immunogenic PSCA polypeptide, and at least one immunogenic PSMA polypeptide, wherein the immunogenic PSA polypeptide comprises the amino acid sequence of of SEQ ID NO:17 or amino acids 4 - 240 of SEQ ID NO:17, wherein the immunogenic PSCA polypeptide comprises the amino acid sequence of SEQ ID NO:21 or amino acids 2-125 of SEQ ID NO:21, and wherein the immunogenic PSMA polypeptide comprises an amino acid sequence selected from the group consisting of:

1) amino acids 15-750 of SEQ ID NO: 1;

2) amino acids 4 – 739 of SEQ ID NO:9; and

3) the amino acid sequence of SEQ ID NO: 9.

In some particular embodiments, the multi-antigen construct comprises a nucleotide sequence that encodes the amino acid sequence of SEQ ID NO:60 or 64.

In some particular embodiments, the multi-antigen construct comprises: (i) a nucleotide sequence encoding an immunogenic PSA polypeptide, (ii) a nucleotide sequence encoding an immunogenic PSCA polypeptide, and (iii) a nucleotide sequence encoding an immunogenic PSMA polypeptide, wherein:

(1) the nucleotide sequence encoding the immunogenic PSA polypeptide is selected from the group consisting of: (i) nucleotide sequence of SEQ ID NO:18; (ii) nucleotide sequence of SEQ ID NO:20; (iii) nucleotide sequence comprising nucleotides 10-720 of SEQ ID NO:18; (iv) nucleotide sequence comprising nucleotides 1115-1825 of SEQ ID NO:58 or SEQ ID NO:63; (v) nucleotide sequence comprising nucleotides 1106-1825 of SEQ ID NO:58 or SEQ ID NO:63; and (vi) a degerate variant of any of the nucleotide sequences provided in (i) – (v) above.

(2) the nucleotide sequence encoding the immunogenic PSCA polypeptide is selected from the group consisting of: (i) the nucleotide sequence of SEQ ID NO:22; (ii) a nucleotide sequence comprising nucleotides 10- 375 of SEQ ID NO:22; (iii) a nucleotide sequence comprising nucleotides 1892-2257 of SEQ ID NO:58 or SEQ ID NO:63; (iv) a nucleotide sequence comprising nucleotides 1886-2257 of SEQ ID NO:58 or SEQ ID NO:63; and (v) a degerate variant of any of the nucleotide sequences provided in (i) – (iv) above; and

(3) the nucleotide sequence encoding the immunogenic PSMA polypeptide is selected from the group consisting of: (i) the nucleotide sequence comprising nucleotides 43-2250 of SEQ ID NO:2; (ii) the nucleotide sequence of SEQ ID NO:4; (iii) the nucleotide sequence of SEQ ID NO:6; (iv) the nucleotide sequence of SEQ ID NO:8; (v) the nucleotide sequence of SEQ ID NO:10; (vi) a nucleotide sequence comprising nucleotides 10–2217 of SEQ ID NO:4; (vii) a nucleotide sequence comprising nucleotides 10–2217 of SEQ ID NO:6; (viii) a nucleotide sequence comprising nucleotides 10–2217 of SEQ ID NO:8; (ix) a nucleotide sequence comprising nucleotides 10–2217 of SEQ ID NO:10; (x) the nucleotide sequence comprising nucleotides 2333-4543 of SEQ ID NO:58 or SEQ ID NO:63; (xi) the nucleotide sequence comprising nucleotides 2324-4543 of SEQ ID NO:58 or SEQ ID NO:63; and (xii) a degerate variant of any of the nucleotide sequences provided in (i) – (xi) above.

In another specific embodiment, the multi-antigen construct comprises a nucleotide sequence encoding an immunogenic PSA polypeptide, a nucleotide sequence encoding an immunogenic PSCA polypeptide, and a nucleotide sequence encoding an immunogenic PSMA polypeptide, wherein: the nucleotide sequence encoding the immunogenic PSA polypeptide comprises nucleotides 1106-1825 of SEQ ID NO:58 or SEQ ID NO:63; the nucleotide sequence encoding the immunogenic PSCA polypeptide comprises nucleotides 1886-2257 of SEQ ID NO:58 or SEQ ID NO:62; and

the nucleotide sequence encoding the immunogenic PSMA polypeptide comprises nucleotides 2324-4543 of SEQ ID NO:58 or SEQ ID NO:63.

In order to enable expression of separate immunogenic PAA polypeptides from a single multi-antigen construct carried by the vector, intervening sequences are included between the sequences that encode the individual immunogenic PAA polypeptides (i.e., PSA, PSCA, and PSMA polypeptides). These intervening sequences enable the separate translation of the downstream immunogenic PAA polypeptide. Such an intervening sequence is referred to as "separator sequence" in the specification. Any sequences that can be used for the co-expression of multiple polypeptides from a single vector may be used as separator sequences in the vector provided by the present disclosure. Examples of useful separator sequences includes internal ribosomal entry sites (IRESs) and 2A peptide sequences.

2A peptide and 2A peptide-like sequences, also referred to as cleavage cassettes or CHYSELs (cis-acting hydrolase elements), are approximately 20 amino acids long with a highly conserved carboxy terminal D-V/I-EXNPGP motif (Figure 2). The sequences are rare in nature, most commonly found in viruses such as Foot-and-mouth disease virus (FMDV), Equine rhinitis A virus (ERAV), Encephalomyocarditis virus (EMCV), Porcine teschovirus (PTV), and Thosea asigna virus (TAV) (Luke, G. A., P. de Felipe, et al. (2008). "Occurrence, function and evolutionary origins of '2A-like' sequences in virus genomes." J Gen Virol 89(Pt 4): 1036-1042). With a 2A-based multi-antigen expression strategy, genes encoding multiple target antigens are linked together in a single open reading frame, separated by 2A sequences. The entire open reading frame is cloned into a vector with a single promoter and terminator. Upon delivery of the constructs to a host cell, mRNA encoding the multiple antigens is transcribed and translated as a single polyprotein. During translation of the 2A sequences, ribosomes skip the bond between the C-terminal glycine and proline. The ribosomal skipping acts like a cotranslational autocatalytic "cleavage" that releases upstream from downstream proteins. General information regarding use of various 2A peptide sequences in vectors co-expressing multiple polypeptides may be found in Andrea L. Szymczak & Darrio AA Vignali: Development of 2A peptide-based strategies in the design of multicistronic vectors. Expert Opinion Biol. Ther. (2005)5(5) 627-638, the disclosure of which is incorporated herein by reference. The incorporation of a 2A sequence between two protein antigens results in the addition of ~20 amino acids onto the C-terminus of the

upstream polypeptide and 1 amino acid (proline) to the N-terminus of downstream protein. In an adaptation of this methodology, protease cleavage sites can be incorporated at the N terminus of the 2A cassette such that ubiquitous proteases will cleave the cassette from the upstream protein (Fang, J., S. Yi, et al. (2007). "An antibody delivery system for regulated expression of therapeutic levels of monoclonal antibodies in vivo." Mol Ther 15(6): 1153-1159).

Examples of specific 2A-peptide sequences that may be used in the present invention are disclosed in Andrea L. Szymczak & Darrio AA Vignali: Development of 2A peptide-based strategies in the design of multicistronic vectors. Expert Opinion Biol. Ther. (2005)5(5) 627-638, and are provided in Table 1.

Table 1. 2A-peptide Sequences

Virus	**2A-peptide Sequence**
Foot and mouse disease virus (FMDV)	VKQTLNFDLLKLAGDVESNPG
Equine rhinitis A virus (ERAV)	QCTNYALLKLAGDVESNPG
Porcine teschovirus-1 (PTV1)	ATNF-SLLKQAGDVEENPG
Encephalomyocarditis virus (EMCV)	HYAGYFADLLIHDIETNPG
Encephalomyocarditis B variant (EMC-B)	GIFN-AHYAGYFADLLIHDIETNPG
Theiler murine encephalomyelitis GD7 (TME-GD7)	KAVRGYHADYYKQRLIHDVEMNPG
Equine rhinitis B virus (ERBV)	GATNF-SLLKLAGDVELNPG
Thosea asigna virus (TAV)	EGRGSLLTCGDVEENPG
Drosophilia C (DrosC)	AARQMLLLLSGDVETNPG
Cricket paralysis virus (CrPV)	FLRKRTQLLMSGDVESNPG
Acute bee paralysis virus (ABPV)	GSWTDILLLLSGDVETNPG
Infectious flacherie virus (IFV)	TRAEUEDELIRAGIESNPG
Porcine rotavirus	AKFQIDKILISGDVELNPG
Human rotavirus	SKFQIDKILISGDIELNPG
T. brucei TSR1	SSIIRTKMLVSGDVEENPG
T. cruzi AP endonuclease	CDAQRQKLLLSGDIEQNPG

Internal ribosomal entry sites (IRESs) are RNA elements (Figure 3) found in the 5' untranslated regions of certain RNA molecules (Bonnal, S., C. Boutonnet, et al.

(2003). "IRESdb: the Internal Ribosome Entry Site database." Nucleic Acids Res 31(1): 427-428). They attract eukaryotic ribosomes to the RNA to facilitate translation of downstream open reading frames. Unlike normal cellular 7-methylguanosine cap-dependent translation, IRES-mediated translation can initiate at AUG codons far within an RNA molecule. The highly efficient process can be exploited for use in multi-cistronic expression vectors (Bochkov, Y. A. and A. C. Palmenberg (2006). "Translational efficiency of EMCV IRES in bicistronic vectors is dependent upon IRES sequence and gene location." Biotechniques 41(3): 283-284, 286, 288). Sequence of a preferred EMCV IRES (pIRES) is provided in Figure 3 and SEQ ID NO:59. The minimal EMCV IRES (mIRES) excludes the underlined first five codons of the EMCV L protein as shown in Figure 3. Typically, two transgenes are inserted into a vector between a promoter and transcription terminator as two separate open reading frames separated by an IRES. Upon delivery of the constructs to a host cell, a single long transcript encoding both transgenes will be transcribed. The first ORF will be translated in the traditional cap-dependent manner, terminating at a stop codon upstream of the IRES. The second ORF will be translated in a cap-independent manner using the IRES. In this way, two independent proteins can be produced from a single mRNA transcribed from a vector with a single expression cassette. In some embodiments, the multi-antigen construct comprises a EMCV IRES comprising nucleotides 1-553 of SEQ ID NO:59.

Typically, only one separator sequence is needed between two immunogenic PAA polypeptide-coding sequences on a multi-antigen construct. The order of the separator sequences and the nucleotide sequences encoding the PAA polypeptides on a multi-antigen construct is shown in formula (I):

PAA1-SS1-PAA2-SS2-PAA3 (I)

Wherein: (i) PAA1, PAA2, and PAA3 each is a nucleotide sequence encoding an immunogenic PSA polypeptide, a nucleotide sequence encoding immunogenic PSCA polypeptide, or a nucleotide sequence encoding immunogenic PSMA polypeptide, provided that PAA1, PAA2, and PAA3 encode different PAA polypeptides, and (ii) SS1 and SS2 are separator sequences and can be same or different.

In some embodiments, the vector comprises a multi-antigen construct of formula (I) wherein:

(i) PAA1 is a nucleotide sequence encoding an immunogenic PSA polypeptide;

(ii) PAA2 is a nucleotide sequence encoding an immunogenic PSCA or PSMA polypeptide. (where PAA2 is nucleotide sequence encoding an immunogenic PSCA, then PAA3 is a nucleotide sequence encoding an immunogenic PSMA, or Vice Versa);

(iii) SS1 is a 2A-peptide sequence; and

(iv) SS2 is a 2A-peptide sequence or an IRES.

In some particular embodiments, the multi-antigen construct has a structure selected from the group consisting of:

(1) PSA-F2A-PSMA-mIRES-PSCA;

(2) PSA-F2A-PSMA-T2A-PSCA;

(3) PSA-T2A-PSCA-F2A-PSMA; and

(4) PSCA-F2A-PSMA-mIRES-PSA

In a specific embodiment, the vector comprises a multi-antigen construct having a structure of formula (I):

PAA1-SS1-PAA2-SS2-PAA3 (I)

wherein:

(i) PAA1 is a nucleotide sequence encoding an immunogenic PSA polypeptide and comprises nucleotides 1115-1825 SEQ ID NO: 58 or comprises 1106-1114 of SEQ ID NO: 58 or 63;

(ii) PAA2 is a nucleotide sequence encoding an immunogenic PSCA polypeptide and comprises nucleotides 1892-2257 of SEQ ID NO: 58 or comprises 1886-2257 of SEQ ID NO: 58 or 63;

(iii) PAA3 is a nucleotide sequence encoding an immunogenic PSMA polypeptide and comprises nucleotides 2333-4543 SEQ ID NO: 58 or comprises 2324-4543 of SEQ ID NO: 58 or 63;

(iv) SS1 is a nucleotide sequence encoding T2A; and

(v) SS2 is a nucleotide sequence encoding F2A.

The multi-antigen construct may also include a linker sequence positioned betyween a nucleotide sequence encoding an immunogenic PAA polypeptide (i.e, an immunogenic PSA, PSCA, or PSMA polypeptide) and a down-stream separator sequence. One example of such a linker sequence is a nucleotide sequence encoding glycine-serine.

In some particular embodiments, the multi-antigen construct comprises a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:60 or

encodes an amino acid sequence of SEQ ID NO:61. In a particular embodiment, the multi-antigen construct comprises a nucleotide sequence selected from the groups consisting of nucleotide sequence of SEQ ID NO:61, nucleotide sequence of SEQ ID NO:65, nucleotide sequence of SEQ ID NO:66, and degenerate variant of any of the nucleotide sequences.

B3. Regulatory Sequences

In addition to the separator sequences and linker sequences described herein above, the vector may comprise other non-native regulatory sequences to drive the efficient expression of the encoded PAA polypeptides. Examples of the regulatory sequences includes (1) transcription initiation, termination, promoter, and enhancer sequences; (2) efficient RNA processing signals such as splicing and polyadenylation signals; (3) sequences that stabilize cytoplasmic mRNA; (4) sequences that enhance translation efficiency (i.e., Kozak consensus sequence); (5) sequences that enhance protein stability; and (6) sequences that enhance protein secretion. Examples of promoter systems that can be used in the vectors provided by the present disclosure to drive efficient expression in mammalian cells include SV40 promoter, chicken B actin promoter, human elongation factor promoter, human cytomegalovirus (CMV) promoter, simian CMV promoter, murine CMV promoter, psudorabies promoter, Rous Sarcoma Virus promoter, phosphoglycerate kinase promoter, murine leukemia virus LTR promoter, avian leukosis virus LTR promoter, mouse mammary tumor virus LTR promoter, moloney murine leukemia virus LTR promoter, plasminogen activator inhibitor promoter, CYR61, adenovirus major late promoter, mouse metallothionein promoter, mouse phosphoenol-pyruvate carboxykinase promoter, bovine B-lactoglobulin promoter, bovine prolactin promoter, ubiquitin C promoter, and herpes simplex virus thymidine kinase promoter. Examples of transcription termination signals include SV40 polyadenylation (polyA); bovine growth hormone polyA; rabbit B globin polyA; HSV thymidine kinase , glycoprotein B , and glycoprotein D; HPV E and L, and synthetic terminators.

In some embodiments, the C68 vectors comprise a human cytomegalovirus (CMV) promoter, optionally with the CMV enhancer, and a SV40 polyA.

C. COMPOSITIONS COMPRISING A VECTOR CARRYING A MULTI-ANTIGEN CONSTRUCT (VECTOR COMPOSITIONS)

The present disclosure also provides a composition comprising a vector provided by the present disclosure (herein "vector composition'). The vector compositions are useful for eliciting an immune response against a PAA protein *in vitro* or *in vivo* in a mammal, including a human. The vector composition may comprise the vectors alone, or may further comprise an excipient.

In some embodiments, the vector composition is a pharmaceutical composition, which comprises a vector provided by the present disclosure and a pharmaceutically acceptable excipient. Suitable excipients for pharmaceutical compositions are known in the arts. The excipients may include aqueous solutions, non aqueous solutions, suspensions, and emulsions. Examples of non-aqueous excipients include propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Examples of aqueous excipient include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Suitable excipients also include agents that assist in cellular uptake of the vector.

In some embodiments, the pharmaceutical composition is a vaccine composition for administration to humans for inhibiting abnormal cell proliferation, providing protection against the development of cancer (used as a prophylactic), or for treatment of cancer (used as a therapeutic) associated with a PAA over–expression, or for eliciting an immune response to a particular human PAA, such as PSMA, PSA, and PSCA. The vaccine composition may further comprise one or more adjuvants. Examples of adjuvants that may be included in the vaccine compositions are provided herein below.

D. USES OF THE VECTORS AND VECTOR COMPOSITIONS

In other aspects, the present disclosure provides methods of using the vector or composition comprising the vectors described herein above.

In one aspect, the present disclosure provides a method of eliciting an immune response against a PAA in a mammal, particularly a human, comprising administering to the mammal an effective amount of (1) a vector containing a multi-antigen construct, or (2) a composition comprising such vectors.

In another aspect, the present disclosure provides a method of inhibiting abnormal cell proliferation in a human, wherein the abnormal cell proliferation is associated with over-expression of a PAA. The method comprises administering to the mammal an effective amount of (1) a vector containing a multi-antigen construct encoding two or more immunogenic PAA polypeptides, or (2) a composition comprising

such vectors. In some embodiments, the method is for inhibiting abnormal cell proliferation in prostate in a human. In a particular embodiment, the present disclosure provides a method of inhibiting abnormal cell proliferation in prostate over-expressing PSMA. In some embodiments, the disclosure provides a method of treating prostate cancer in a human, comprising administering to the human an effective amount of a (1) a vector containing a multi-antigen construct or (2) a composition comprising such vectors. In a preferred embodiment, the multi-antigen construct is a triple antigen construct that encodes an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, and an immunogenic PSCA polypeptide.

The vectors or vector compositions can be administered to an animal, including human, by a number of methods known in the art. Examples of suitable methods include: (1) intramuscular, intradermal, intraepidermal, intravenous, intraarterial, subcutaneous, or intraperitoneal administration, (2) oral administration, and (3) topical application (such as ocular, intranasal, and intravaginal application). One particular method of intradermal or intraepidermal administration of a nucleic acid vaccine composition involves the use of gene gun delivery technology, such the Particle Mediated Epidermal Delivery (PMED^{TM}) vaccine delivery device marketed by PowderMed. Another particular method for intramuscular administration of a nucleic acid vaccine is injection followed by electroporation.

The effective amount of the vector or vector composition to be administered in a given method can be readily determined by a person skilled in the art and will depend on a number of factors. In a method of treating cancer, such as prostate cancer, factors that may be considered in determining the effective amount include, but not limited: (1) the subject to be treated, including the subject's immune status and health, (2) the severity or stage of the cancer to be treated, (3) the specific immunogenic PAA polypeptides expressed, (4) the degree of protection or treatment desired, (5) the administration method and schedule, (6) formulations used, and (7) co-administration of other therapeutic agents (such as adjuvants or immune modulators). For example, the effective amounts of the vector may be in the range of 2 µg/dose – 10 mg/dose when the nucleic acid vaccine composition is formulated as an aqueous solution and administered by hypodermic needle injection or pneumatic injection, whereas only 16 ng/dose – 16 µg/dose may be required when the nucleic acid is prepared as coated gold beads and delivered using a gene gun technology.

The vectors or vector compositions, including vaccine compositions, provided by the present disclosure may be used together with one or more adjuvants. Examples of suitable adjuvants include: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl polypeptides or bacterial cell wall components), such as MF59™ (containing 5% Squalene, 0.5% Tween 80, and 0.5% sorbitan trioleate) and SAF (containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP); (2) saponin adjuvants, such as QS21, STIMULON™ (Cambridge Bioscience, Worcester, MA), Abisco® (Isconova, Sweden), or Iscomatrix® (Commonwealth Serum Laboratories, Australia); (3) complete Freund's Adjuvant (CFA) and incomplete Freund's Adjuvant (IFA); (4) oligonucleotides comprising CpG motifs, *i.e.* containing at least one CG dinucleotide, where the cytosine is unmethylated (e.g., Krieg, *Vaccine* (2000) 19:618-622; Krieg, *Curr Opin Mol Ther* (2001) 3:15-24; WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581); and (5) metal salt including aluminum salts (such as alum, aluminum phosphate, aluminum hydroxide); (12) a saponin and an oil-in-water emulsion (*e.g.* WO 99/11241).

The vectors or vector compositions provided by the present disclosure may be used together with one or more immune modulators. In a further aspect, the present disclosure provides a method of treating prostate cancer in a mammal, particularly a human, the method comprising administering to the mammal: (1) an effective amount of a vector, vector composition, or vaccine provided by the present invention; (2) an effective amount of at least one immune-suppressive-cell inhibitor (ISC inhibitor); and (3) an effective amount of at least one immune-effector-cell enhancer (IEC enhancer). This method is also referred to as "vaccine-based immunotherapy regimen" (or "VBIR") in the present disclosure.

The IEC enhancers and ISC inhibitors may be administered by any suitable methods and routes, including (1) systemic administration such as intravenous, intramuscular, or oral administration, and (2) local administration such intradermal and subcutaneous administration. Where appropriate or suitable, local administration is generally preferred over systemic administration. Local administration of any IEC enhancer and ISC inhibitor can be carried out at any location of the body of the mammal that is suitable for local administration of pharmaceuticals; however, it is more preferable that these immune modulators are administered locally at close proximity to the vaccine draining lymph node.

Two or more specific IEC enhancers from a single class of IEC enhancers (for examples, two CTLA-antagonists) may be administered in combination with the ISC inhibitors. In addition, two or more specific IEC enhancers from two or more different classes of IEC enhancers (for example, one CTLA-4 antagonist and one TLR agonist, or one CTLA-4 antagonist and one PD-1 antagonist) may be administered together. Similarly, two or more specific ISC inhibitors from a single class of ISC inhibitors (for examples, two or more protein kinase inhibitors) may be administered in combination with the IEC enhancers. In addition, two or more specific ISC inhibitors from two or more different classes of ISC inhibitors (for example, one protein kinase inhibitor and one COX-2 inhibitor) may be administered together.

The vectors or vector compositions may be administered simultaneously or sequentially with any or all of the immune modulators (i.e., ISC inhibitors and IEC enhancers) used. Similarly, when two or more immune modulators are used, they may be administered simultaneously or sequentially with respect to each other. In some embodiments, a vector or vector composition is administered simultaneously (e.g., in a mixture) with respect to one immune modulator, but sequentially with respect to one or more additional immune modulators. Co-administration of the vector or vector composition and the immune modulators can include cases in which the vaccine and at least one immune modulator are administered so that each is present at the administration site, such as vaccine draining lymph node, at the same time, even though the antigen and the immune modulators are not administered simultaneously. Co-administration of the vaccine and the immune modulators also can include cases in which the vaccine or the immune modulator is cleared from the administration site, but at least one cellular effect of the cleared vaccine or immune modulator persists at the administration site, such as vaccine draining lymph node, at least until one or more additional immune modulators are administered to the administration site. In cases where a nucleic acid vaccine is administered in combination with a CpG, the vaccine and CpG may be contained in a single formulation and administered together by any suitable method. In some embodiments, the nucleic acid vaccine and CpG in a co-formulation (mixture) is administered by intramuscular injection in combination with electroporation.

Any ISC inhibitors may be used in combination with the vectors or vector compositions provided by the present invention. Examples of classes of ISC inhibitors

include PD-1/PD-L1 antagonists, protein kinase inhibitors, cyclooxygenase-2 (COX-2) inhibitors, phosphodiesterase type 5 (PDE5) inhibitors, and DNA crosslinkers. Examples PD-1/PD-L1 antagonists include anti-PD-1 and PD-L1 monoclonal antibodies Examples of COX-2 inhibitors include celecoxib and rofecoxib. Examples of PDE5 inhibitors include avanafil, lodenafil, mirodenafil, sildenafil, tadalafil, vardenafil, udenafil, and zaprinast. An example of DNA crosslinkers is cyclophosphamide. Examples of specific protein kinase inhibitors are described in details below.

The term "protein kinase inhibitor" refers to any substance that acts as a selective or non-selective inhibitor of a protein kinase. The term "protein kinases" refers to the enzymes that catalyze the transfer of the terminal phosphate of adenosine triphosphate to tyrosine, serine or threonine residues in protein substrates. Protein kinases include receptor tyrosine kinases and non-receptor tyrosine kinases. Examples of receptor tyrosine kinases include EGFR (e.g., EGFR/HER1/ErbB1, HER2/Neu/ErbB2, HER3/ErbB3, HER4/ErbB4), INSR (insulin receptor), IGF-IR, IGF-II1R, IRR (insulin receptor-related receptor), PDGFR (e.g., PDGFRA, PDGFRB), c-KIT/SCFR, VEGFR-1/FLT-1, VEGFR-2/FLK-1/KDR, VEGFR-3/FLT-4, FLT-3/FLK-2, CSF-1R, FGFR 1-4, CCK4, TRK A-C, MET, RON, EPHA 1-8, EPHB 1-6, AXL, MER, TYRO3, TIE, TEK, RYK, DDR 1-2, RET, c-ROS, LTK (leukocyte tyrosine kinase), ALK (anaplastic lymphoma kinase), ROR 1-2, MUSK, AATYK 1-3, and RTK 106. Examples of non-receptor tyrosine kinases include BCR-ABL, Src, Frk, Btk, Csk, Abl, Zap70, Fes/Fps, Fak, Jak, Ack, and LIMK. In the vaccine-based immunotherapy regimen provided by the present disclosure, the protein kinase inhibitors are administered to the mammal at a suboptimal dose. The term "suboptimal dose" refers to the dose amount that is below the minimum effective dose when the tyrosine kinase inhibitor is administered in a monotherapy (i.e., where the protein kinase inhibitor is administered alone without any other therapeutic agents) for the target neoplastic disorder.

Examples of specific protein kinase inhibitors suitable for use in the vaccine-based immunotherapy regimen include lapatinib, AZD 2171, ET18OCH 3, indirubin-3'-oxime, NSC-154020, PD 169316, quercetin, roscovitine, triciribine, ZD1839, 5-Iodotubercidin, adaphostin, aloisine, alsterpaullone, aminogenistein, API-2, apigenin, arctigenin, ARRY-334543, axitinib, AY-22989, AZD 2171, Bisindolylmaleimide IX, CCI-779, chelerythrine, DMPQ, DRB, edelfosine, ENMD-981693, erbstatin analog, erlotinib, fasudil, gefitinib (ZD1839), H-7, H-8, H-89, HA-100, HA-1004, HA-1077, HA-1100,

hydroxyfasudil, kenpaullone, KN-62, KY12420, LFM-A13, luteolin, LY294002, LY-294002, mallotoxin, ML-9, MLN608, NSC-226080, NSC-231634, NSC-664704, NSC-680410, NU6102, olomoucine, oxindole I, PD 153035, PD 98059, phloridzin, piceatannol, picropodophyllin, PKI, PP1, PP2, PTK787/ZK222584, PTK787/ZK-222584, purvalanol A, rapamune, rapamycin, Ro 31-8220, rottlerin, SB202190, SB203580, sirolimus, SL327, SP600125, staurosporine, STI-571, SU1498, SU4312, SU5416, semaxanib, SU6656, SU6668, syk inhibitor, TBB, TCN, tyrphostin AG 1024, tyrphostin AG 490, tyrphostin AG 825, tyrphostin AG 957, U0126, W-7, wortmannin, Y-27632, zactima, ZM 252868, gefitinib, sunitinib malate, erlotinib, lapatinib, canertinib, semaxinib, vatalanib, sorafenib, imatinib, dasatinib, leflunomide, vandetanib, and nilotinib.

In some embodiments, the protein kinase inhibitor is a multi-kinase inhibitor, which is an inhibitor that acts on more than one specific kinase. Examples of multi-kinase inhibitors include imatinib, sorafenib, lapatinib, BIRB-796, and AZD-1152, AMG706, zactima, MP-412, sorafenib, dasatinib, lestaurtinib, XL647, XL999, lapatinib, MLN518, (also known as CT53518), PKC412, ST1571, AEE 788, OSI-930, OSI-817, sunitinib malate, erlotinib, gefitinib, axitinib, bosutinib, temsirolismus and nilotinib. In some particular embodiments, the tyrosine kinase inhibitor is sunitinib, sorafenib, or a pharmaceutically acceptable salt or derivative (such as a malate or a tosylate) of sunitinib or sorafenib.

Sunitinib malate, which is marketed by Pfizer Inc. under the trade name SUTENT, is described chemically as butanedioic acid, hydroxy-, (2S)-, compound with *N*-[2-(diethylamino)ethyl]-5-[(*Z*)-(5-fluoro-1,2-dihydro-2-oxo-*3H*-indol-3-ylidine)methyl]-2,4-dimethyl-*1H*-pyrrole-3-carboxamide (1:1). The compound, its synthesis, and particular polymorphs are described in U.S. Pat. No. 6,573,293, U.S. Patent Publication Nos. 2003-0229229, 2003-0069298 and 2005-0059824, and in J. M. Manley, M. J. Kalman, B. G. Conway, C. C. Ball, J. L Havens and R. Vaidyanathan, "Early Amidation Approach to 3-[(4-amido)pyrrol-2-yl]-2-indolinones," J. Org. Chew. 68, 6447-6450 (2003). Formulations of sunitinib and its L-malate salt are described in PCT Publication No. WO 2004/024127. Sunitinib malate has been approved in the U.S. for the treatment of gastrointestinal stromal tumor, advanced renal cell carcinoma, and progressive, well-differentiated pancreatic neuroendocrine tumors in patients with unresectable locally advanced or metastatic disease. The recommended dose of sunitinib malate for gastrointestinal stromal tumor (GIST) and advanced renal cell carcinoma (RCC) for

humans is 50 mg taken orally once daily, on a schedule of 4 weeks on treatment followed by 2 weeks off (Schedule 4/2). The recommended dose of sunitinib malate for pancreatic neuroendocrine tumors (pNET) is 37.5 mg taken orally once daily.

In the vaccine-based immunotherapy regimen, sunitinib malate may be administered orally in a single dose or multiple doses. Typically, sunitinib malate is delivered for two, three, four or more consecutive weekly doses followed by a "off" period of about 1 or 2 weeks, or more where no sunitinib malate is delivered. In one embodiment, the doses are delivered for about 4 weeks, with 2 weeks off. In another embodiment, the sunitinib malate is delivered for two weeks, with 1 week off. However, it may also be delivered without a "off" period for the entire treatment period. The effective amount of sunitinib malate administered orally to a human in the vaccine-based immunotherapy regimen is typically below 40 mg per person per dose. For example, it may be administered orally at 37.5, 31.25, 25, 18.75, 12.5, 6.25 mg per person per day. In some embodiments, sunitinib malate is administered orally in the range of 1 – 25 mg per person per dose. In some other embodiments, sunitinib malate is administered orally in the range of 6.25, 12.5, or 18.75 mg per person per dose. Other dosage regimens and variations are foreseeable, and will be determined through physician guidance.

Sorafenib tosylate, which is marketed under the trade name NEXAVAR, is also a multi-kinase inhibitor. Its chemical name is 4-(4-{3-[4-Chloro-3-(trifluoromethyl) phenyl]ureido}phenoxy)-N-methylpyrid-ine-2-carboxamide. It is approved in the U.S. for the treatment of primary kidney cancer (advanced renal cell carcinoma) and advanced primary liver cancer (hepatocellular carcinoma). The recommended daily dose is 400 mg taken orally twice daily. In the vaccine-based immunotherapy regimen provided by the present disclosure, the effective amount of sorafenib tosylate administered orally is typically below 400 mg per person per day. In some embodiments, the effective amount of sorafenib tosylate administered orally is in the range of 10 - 300 mg per person per day. In some other embodiments, the effective amount of sorafenib tosylate administered orally is between 10 - 200 mg per person per day, such as 10, 20, 60, 80, 100, 120, 140, 160, 180, or 200 mg per person per day.

Axitinib, which is marketed under the trade name INLYTA, is a selective inhibitor of VEGF receptors 1, 2, and 3. Its chemical name is (*N*-Methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1*H*-indazol-6-ylsulfanyl]-benzamide. It is approved for the treatment of advanced

renal cell carcinoma after failure of one prior systemic therapy. The starting dose is 5 mg orally twice daily. Dose adjustments can be made based on individual safety and tolerability. In the vaccine-based immunotherapy regimen provided by the present disclosure, the effective amount of axitinib administered orally is typically below 5 mg twice daily. In some other embodiments, the effective amount of axitinib administered orally is between 1-5 mg twice daily. In some other embodiments, the effective amount of axitinib administered orally is between 1, 2, 3, 4, or 5 mg twice daily.

In the vaccine-based immunotherapy regimens any IEC enhancers may be used. They may be small molecules or large molecules (such as protein, polypeptide, DNA, RNA, and antibody). Examples of IEC enhancers that may be used include TNFR agonists, CTLA-4 antagonists, TLR agonists, programmed cell death protein 1 (PD-1) antagonists (such as anti-PD-1 antibody CT-011), and programmed cell death protein 1 ligand 1 (PD-L1) antagonists (such as BMS-936559), lymphocyte-activation gene 3 (LAG3) antagonists, and T cell Immunoglobulin- and mucin-domain-containing molecule -3 (TIM-3) antagonists. Examples of specific TNFR agonists, CTLA-4 antagonists, and TLR agonists are provided in details herein below.

TNFR Agonists.

Examples of TNFR agonists include agonists of OX40, 4-1BB (such as BMS-663513), GITR (such as TRX518), and CD40. Examples of specific CD40 agonists are described in details herein below.

CD40 agonists are substances that bind to a CD40 receptor on a cell and are capable of increasing one or more CD40 or CD40L associated activities. Thus, CD40 "agonists" encompass CD40 "ligands".

Examples of CD40 agonists include CD40 agonistic antibodies, fragments CD40 agonistic antibodies, CD40 ligands (CD40L), and fragments and derivatives of CD40L such as oligomeric (e.g., bivalent, trimeric CD40L), fusion proteins containing and variants thereof.

CD40 ligands for use in the present invention include any peptide, polypeptide or protein, or a nucleic acid encoding a peptide, polypeptide or protein that can bind to and activate one or more CD40 receptors on a cell. Suitable CD40 ligands are described, for example, in U.S. Pat. No. 6,482,411; U.S. Pat. No.6,410,711; U.S. Pat. No. 6,391,637; and U.S. Pat. No. 5,981,724, all of which patents and application and the CD40L sequences disclosed therein are incorporated by reference in their entirety

herein. Although human CD40 ligands will be preferred for use in human therapy, CD40 ligands from any species may be used in the invention. For use in other animal species, such as in veterinary embodiments, a species of CD40 ligand matched to the animal being treated will be preferred. In certain embodiments, the CD40 ligand is a gp39 peptide or protein oligomer, including naturally forming gp39 peptide, polypeptide or protein oligomers, as well as gp39 peptides, polypeptides, proteins (and encoding nucleic acids) that comprise an oligomerization sequence. While oligomers such as dimers, trimers and tetramers are preferred in certain aspects of the invention, in other aspects of the invention larger oligomeric structures are contemplated for use, so long as the oligomeric structure retains the ability to bind to and activate one or more CD40 receptor(s).

In certain other embodiments, the CD40 agonist is an anti-CD40 antibody, or antigen-binding fragment thereof. The antibody can be a human, humanized or part-human chimeric anti-CD40 antibody. Examples of specific anti-CD40 monoclonal antibodies include the G28-5, mAb89, EA-5 or S2C6 monoclonal antibody, and CP870893. In a particular embodiment, the anti-CD40 agonist antibody is CP870893 or dacetuzumab (SGN-40).

CP-870,893 is a fully human agonistic CD40 monoclonal antibody (mAb) that has been investigated clinically as an anti-tumor therapy. The structure and preparation of CP870,893 is disclosed in WO2003041070 (where the antibody is identified by the internal identified "21.4.1"). The amino acid sequences of the heavy chain and light chain of CP-870,893 are set forth in SEQ ID NO: 40 and SEQ ID NO: 41, respectively. In clinical trials, CP870,893 was administered by intravenous infusion at doses generally in the ranges of 0.05 - 0.25 mg/kg per infusion. In a phase I clinical study, the maximum tolerated dose (MTD) of CP-870893 was estimated to be 0.2 mg/kg and the dose-limiting toxicities included grade 3 CRS and grade 3 urticaria. [Jens Ruter et al.: Immune modulation with weekly dosing of an agonist CD40 antibody in a phase I study of patients with advanced solid tumors. [Cancer Biology & Therapy 10:10, 983-993; November 15, 2010.]. In the vaccine-based immunotherapy regimen provided by the present disclosure, CP-870,893 can be administered intradermally, subcutaneously, or topically. It is preferred that it is administered intradermally. The effective amount of CP870893 to be administered in the regimen is generally below 0.2 mg/kg, typically in the range of 0.01 mg – 0.15 mg/kg, or 0.05 – 0.1 mg/kg.

Dacetuzumab (also known as SGN-40 or huS2C6; CAS number 88-486-59-9) is another anti-CD40 agonist antibody that has been investigated in clinical trials for indolent lymphomas, diffuse large B cell lymphomas and Multiple Myeloma. In the clinical trials, dacetuzumab was administered intravenously at weekly doses ranging from 2 mg/kg to 16 mg/kg. In the vaccine-based immunotherapy regimen provided by the present disclosure, dacetuzumab can be administered intradermally, subcutaneously, or topically. It is preferred that it is administered intradermally. The effective amount of dacetuzumab to be administered in the vaccine-based immunotherapy regimen is generally below 16 mg/kg, typically in the range of 0.2 mg – 14 mg/kg, or 0.5 –8 mg/kg, or 1 - 5 mg/kg.

CTLA-4 Inhibitors.

Suitable anti-CTLA-4 antagonist agents for use in the vaccine-based immunotherapy regimen provided by the disclosure include, without limitation, anti-CTLA-4 antibodies (such as human anti-CTLA-4 antibodies, mouse anti-CTLA-4 antibodies, mammalian anti-CTLA-4 antibodies, humanized anti-CTLA-4 antibodies, monoclonal anti-CTLA-4 antibodies, polyclonal anti-CTLA-4 antibodies, chimeric anti-CTLA-4 antibodies, anti-CTLA-4 domain antibodies), fragments of anti-CTLA-4 antibodies (such as (single chain anti-CTLA-4 fragments, heavy chain anti-CTLA-4 fragments, and light chain anti-CTLA-4 fragments), and inhibitors of CTLA-4 that agonize the co-stimulatory pathway. In some embodiments, the CTLA-4 inhibitor is Ipilimumab or Tremelimumab.

Ipilimumab (also known as MEX-010 or MDX-101), marketed as YERVOY, is a human anti-human CTLA-4 antibody. Ipilimumab can also be referred to by its CAS Registry No. 477202-00-9, and is disclosed as antibody 10DI in PCT Publication No. WO01/14424, which is incorporated herein by reference in its entirety. Examples of pharmaceutical composition comprising Ipilimumab are provided in PCT Publication No. WO2007/67959. Ipilimumab is approved in the U.S. for the treatment of unresectable or metastatic melanoma. The recommended dose of Ipilimumab as monotherapy is 3 mg/kg by intravenous administration every 3 weeks for a total of 4 doses. In the methods provided by the present invention, Ipilimumab is administered locally, particularly intradermally or subcutaneously. The effective amount of Ipilimumab administered locally is typically in the range of 5 – 200 mg/dose per person. In some embodiments, the effective amount of Ipilimumab is in the range of 10 – 150 mg/dose

per person per dose. In some particular embodiments, the effective amount of Ipilimumab is about 10, 25, 50, 75, 100, 125, 150, 175, or 200 mg/dose per person.

Tremelimumab (also known as CP-675,206) is a fully human IgG2 monoclonal antibody and has the CAS number 745013-59-6. Tremelimumab is disclosed as antibody 11.2.1 in U.S. Patent No: 6,682,736, incorporated herein by reference in its entirety and for all purposes. The amino acid sequences of the heavy chain and light chain of Tremelimumab are set forth in SEQ IND NOs:42 and 43, respectively. Tremelimumab has been investigated in clinical trials for the treatment of various tumors, including melanoma and breast cancer; in which Tremelimumab was administered intravenously either as single dose or multiple doses every 4 or 12 weeks at the dose range of 0.01 and 15 mg/kg. In the regimens provided by the present invention, Tremelimumab is administered locally, particularly intradermally or subcutaneously. The effective amount of Tremelimumab administered intradermally or subcutaneously is typically in the range of 5 – 200 mg/dose per person. In some embodiments, the effective amount of Tremelimumab is in the range of 10 –150 mg/dose per person per dose. In some particular embodiments, the effective amount of Tremelimumab is about 10, 25, 37.5, 40, 50, 75, 100, 125, 150, 175, or 200 mg/dose per person.

Toll-like Receptor (TLR) Agonists.

The term "toll-like receptor agonist" or "TLR agonist" refers to a compound that acts as an agonist of a toll-like receptor (TLR). This includes agonists of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, and TLR11 or a combination thereof. Unless otherwise indicated, reference to a TLR agonist compound can include the compound in any pharmaceutically acceptable form, including any isomer (e.g., diastereomer or enantiomer), salt, solvate, polymorph, and the like. In particular, if a compound is optically active, reference to the compound can include each of the compound's enantiomers as well as racemic mixtures of the enantiomers. Also, a compound may be identified as an agonist of one or more particular TLRs (e.g., a TLR7 agonist, a TLR8 agonist, or a TLR7/8 agonist).

In some embodiments, the TLR agonists are TLR9 agonists, particularly CpG oligonucleotides (or CpG.ODN). A CpG oligonucleotide is a short nucleic acid molecule containing a cytosine followed by a guanine linked by a phosphate bond in which the pyrimidine ring of the cytosine is unmethylated. A CpG motif is a pattern of bases that include an unmethylated central CpG surrounded by at least one base flanking (on the

3' and the 5' side of) the central CpG. CpG oligonucleotides include both D and K oligonucleotides. The entire CpG oligonucleotide can be unmethylated or portions may be unmethylated. Examples of CpG oligonucleotides useful in the methods provided by the present disclosure include those disclosed in U.S. Patent Nos. 6194388, 6207646, 6214806, 628371, 6239116, and 6339068.

Examples of particular CpG oligonucleotides useful in the methods provided by the present disclosure include:

5' TCGTCGTTTTGTCGTTTTGTCGTT3' (CpG 7909);

5' TCGTCGTTTTTCGGTGCTTTT3' (CpG 24555); and

5' TCGTCGTTTTTCGGTCGTTTT3' (CpG 10103).

CpG7909, a synthetic 24mer single stranded oligonucleotide, has been extensively investigated for the treatment of cancer as a monotherapy and in combination with chemotherapeutic agents, as well as an adjuvant for vaccines against cancer and infectious diseases. It was reported that a single intravenous dose of CpG 7909 was well tolerated with no clinical effects and no significant toxicity up to 1.05 mg/kg, while a single dose subcutaneous CpG 7909 had a maximum tolerated dose (MTD) of 0.45 mg/kg with dose limiting toxicity of myalgia and constitutional effects. [See Zent, Clive S, et al: Phase I clinical trial of CpG 7909 (PF-03512676) in patients with previously treated chronic lymphocytic leukemia. Leukemia and Lymphoma, 53(2):211-217(7)(2012)]. In the regimens provided by the present disclosure, CpG7909 may be administered by injection into the muscle or by any other suitable methods. It is preferred that it is administered locally in proximity to the vaccine draining lymph node, particularly by intradermal or subcutaneous administration. For use with a nucleic acid vaccine, such as a DNA vaccine, a CpG may be preferably co-formulated with the vaccine in a single formulation and administered by intramuscular injection coupled with electroporation. The effective amount of CpG7909 by intramuscular, intradermal, or subcutaneous administration is typically in the range of 10 µg/dose - 10 mg/dose. In some embodiments, the effective amount of CpG7909 is in the range of 0.05 mg – 14 mg/dose. In some particular embodiments, the effective amount of CpG7909 is about 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 05 1 mg/dose. Other CpG oligonucleotides, including CpG 24555 and CpG 10103, may be administered in similar manner and dose levels.

In some particular embodiments, the present disclosure provides a method of enhancing the immunogenicity or therapeutic effect of a vaccine for the treatment of a

neoplastic disorder in a human, comprising administering the human (1) an effective amount of at least one ISC inhibitor and (2) an effective amount of at least one IEC enhancer, wherein the at least one ISC inhibitor is protein kinase inhibitor selected from sorafenib tosylate, sunitinib malate, axitinib, erlotinib, gefitinib, axitinib, bosutinib, temsirolismus, or nilotinib and wherein the at least one IEC enhancer is selected from a CTLA-4 inhibitor, a TLR agonist, or a CD40 agonist. In some preferred embodiments, regimen comprises administering to the human (1) an effective amount of at least one ISC inhibitor and (2) effective amount of at least one IEC enhancer, wherein the at least one ISC inhibitor is a protein kinase inhibitor selected from axitinib, sorafenib tosylate, or sunitinib malate and wherein the at least one IEC enhancer is a CTLA-4 inhibitor selected from Ipilimumab or Tremelimumab. In some further preferred embodiments, the regimen comprises administering to the human (1) an effective amount of at least one ISC inhibitor and (2) an effective amount of at least two IEC enhancers, wherein the at least one ISC inhibitor is a protein kinase inhibitor selected from sunitinib or axitinib and wherein the at least two IEC enhancers are Tremelimumab and a TLR agonist selected from CpG7909, CpG2455, or CpG10103.

In some other embodiments, the present disclosure provides a method of treating prostate cancer in a human, comprising administering to the human (1) an effective amount of a vaccine capable of eliciting an immune response against a human PAA, (2) an effective amount of at least one ISC inhibitor, and (3) an effective amount of at least one IEC enhancer, wherein the at least one ISC inhibitor is a protein kinase inhibitor selected from sorafenib tosylate, sunitinib malate, axitinib, erlotinib, gefitinib, axitinib, bosutinib, temsirolismus, or nilotinib, and wherein the at least one IEC enhancer is selected from a CTLA-4 inhibitor, a TLR agonist, or a CD40 agonist. In some preferred embodiments, the method comprises administering to the human (1) an effective amount of a vaccine capable of eliciting an immune response against a human PAA, (2) an effective amount of at least one ISC inhibitor, and (3) an effective amount of at least one IEC enhancer, wherein the at least one ISC inhibitor is a protein kinase inhibitor selected from sorafenib tosylate, sunitinib malate, or axitinib and wherein the at least one IEC enhancer is a CTLA-4 inhibitor selected from Ipilimumab or Tremelimumab.

In some further specific embodiments, the method comprises administering to the human (1) an effective amount of at least one ISC inhibitor and (2) an effective amount of at least two IEC enhancers, wherein the at least one ISC inhibitor is a protein kinase

inhibitor selected from sunitinib or axitinib and wherein the at least two IEC enhancers are Tremelimumab and a TLR agonist selected from CpG7909, CpG2455, or CpG10103.

Additional therapeutic agents.

The vaccine-based immunotherapy regimen provided by the present disclosure may further comprise an additional therapeutic agent. A wide variety of cancer therapeutic agents may be used, including chemotherapeutic agents and hormone therapeutic agents. The term "chemotherapeutic agent" refers to a chemical or biological substance that can cause death of cancer cells, or interfere with growth, division, repair, and/or function of cancer cells. Examples of particular chemotherapeutic agents include: abiraterone acetate, cabazitaxel, degarelix, denosumab, docetaxel, enzalutamide, leuprolide acetate, prednisone, sipuleucel-T, and radium 223 dichloride. The term "hormone therapeutic agent" refers to a chemical or biological substance that inhibits or eliminates the production of a hormone, or inhibits or counteracts the effect of a hormone on the growth and/or survival of cancer cells. Examples of particular hormone therapeutic agents include leuprolide, goserelin, triptorelin, histrelin, bicalutamide, flutamide, and nilutamide. The VBIR provided by this disclosure may also be used in combination with other therapies, including (1) surgical methods that remove all or part of the organs or glands which participate in the production of the hormone, such as the ovaries, the testicles, the adrenal gland, and the pituitary gland, and (2) radiation treatment, in which the organs or glands of the patient are subjected to radiation in an amount sufficient to inhibit or eliminate the production of the targeted hormone.

E. EXAMPLES

The following examples are provided to illustrate certain embodiments of the invention. They should not be construed to limit the scope of the invention in any way. From the above discussion and these examples, one skilled in the art can ascertain the essential characteristics of the invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usage and conditions.

EXAMPLE 1. ANTIGENS IN CYTOSOLIC, SECRETED, AND MEMBRANE-BOUND FORMATS DERIVED FROM THE HUMAN PSMA PROTEIN

1A. Design of Immunogenic PSMA Polypeptides

DNA constructs encoding immunogenic PSMA polypeptides in cytosolic, secreted, and modified formats were constructed based on the native human PSMA protein sequence and tested for their ability to induce anti-tumor effector immune responses. The structure and preparation of each of the human PSMA antigen formats are provided as follows.

1A1. Human PSMA cytosolic antigen. An immunogenic PSMA polypeptide in cytosolic form was designed to retain the immunogenic polypeptide inside the cell once it is expressed. The cytoplasmic domain (amino acids 1-19) and the transmembrane domain (amino acids 20-43) of the human PSMA were removed, resulting in a cytosolic PSMA polypeptide that consists of amino acids 44-750 (extracellular domain or ECD) of the human PSMA of SEQ ID NO: 1. The optimal Kozak sequence "MAS" may be added to the N-terminus of the polypeptide for enhancing the expression or to facilitate cloning.

1A2. Human PSMA secreted antigen. An immunogenic PSMA polypeptide in secreted form was designed to secret the polypeptide outside of the cell once it is expressed. The secreted polypeptide is made with amino acids 44-750 (ECD) of the human PSMA of SEQ ID NO:1 and the Ig Kappa secretory element that has the amino acid sequence ETDTLLLWVLLLWVPGSTGD and a two-amino acid linker (AA) in the N-terminal in order to maximize the secretion of the PSMA antigen once it is expressed.

1A3. Human PSMA membrane-bound antigen. An immunogenic PSMA membrane-bound polypeptide was designed to stabilize the polypeptide on the cell surface. The first 14 amino acids of the human PSMA protein were removed and the resultant immunogenic polypeptide consists of amino acids 15 – 750 of the human PSMA protein of SEQ ID NO:1. The immunogenic polypeptide that consists of amino acids 15 – 750 of the native human PSMA protein of SES ID NO: 1 and share 100% sequence identity with the native human PSMA protein is also referred to as "human PSMA modified," "hPSMA modified," or "hPSMAmod" antigen in the present disclosure. The following three immunogenic PSMA polypeptides (referred to as "shuffled PSMA modified antigens") that are variants of the human PSMA modified antigen (SEQ ID NO:9) were also generated:

(1) shuffled PSMA modified antigen 1 having the amino acid sequence of SEQ ID NO:3;

(2) shuffled PSMA modified antigen 2 having the amino acid sequence of SEQ ID NO:5; and

(3) shuffled PSMA modified antigen 3 having the amino acid sequence of SEQ ID NO:7.

The nucleodie sequences encoding the shuffled PSMA modified antigens 1, 2, and 3 are set forth in SEQ ID NOs: 4, 6, and 8, respectively.

1B. Preparation of DNA Plasmids for Expressing the PSMA antigens

DNA constructs encoding the PSMA cytosolic, PSMA secreted, and PSMA modified antigens were cloned individually into PJV7563 vector that was suitable for in vivo testing in animals (Figure 1). Both strands of the DNA in the PJV7563 vectors were sequenced to confirm the design integrity.

A large scale plasmid DNA preparation (Qiagen/CsCl) was produced from a sequence confirmed clone. The quality of the plasmid DNA was confirmed by high 260/280 ratio, high super coiled/nicked DNA ratio, low endotoxin levels (< 10U/mg DNA) and negative bio burden.

1C. Expression of PSMA constructs in mammalian cells

The expression of the PSMA cytosolic, secreted, and modified antigens was determined by FACS. Mammalian 293 cells were transfected with the PJV7563 PMED vectors encoding the various immunogenic PSMA polypeptides. Three days later, the 293 cells were stained with mouse anti-PSMA antibody, followed with a fluorescent conjugated (FITC) rat anti-mouse secondary antibody. The results are presented tin Table 2. The data were reported as mean fluorescent intensity (MFI) over negative controls, confirmed that human PSMA modified antigen is expressed on the cell surface.

Table 2. Expression of Human PSMA Modified antigen on Cell Surface

Samples	Average mean fluorescent intensity
Untransfected 293 cells	231
293 cells transfected with full length human PSMA (SEQ ID NO:1)	6425
293 cells transfected with human PSMA modified antigen (SEQ ID NO: 9)	12270

EXAMPLE 2. DESIGN OF VARIOUS IMMUNOGENIC PSA POLYPEPTIDES

3A. Construction of Immunogenic PSA Polypeptides

Similar to what was described in Example 1 for the three different immunogenic PSMA polypeptide forms (e.g., the cytosolic, membrane-bound, and secreted forms),

immunogenic PSA polypeptides in the three forms were also designed based on the human PSA sequence. An immunogenic PSA polypeptide in cytosolic form, which consists of amino acids 25-261 of the native human PSA, is constructed by deleting the secretory signal and the pro domain (amino acids 1-24). The amino acid sequence of this cytosolic immunogenic PSA polypeptide is provided in SEQ ID NO: 17. The secreted form of the PSA polypeptide is the native full length human PSA (amino acids 1-261). An immunogenic PSA polypeptide in membrane-bound form is constructed by linking the immunogenic PSA polypeptide cytosolic form (amino acids 25-261 of the native human PSA) to the human PSMA transmembrane domain (amino acids 15-54 of the human PSMA).

3B. Immune responses in Pasteur and HLA A24 mice

Study design. Eight to 10 week old HLA A2 Pasteur mice or HLA A24 mice were immunized with DNA expressing the various PSA antigens using PMED provided in Example 3A in a prime/boost/boost regimen with two week intervals between each vaccination as described in Example 1. The antigen specific T and B cell responses were measured 7 days after the last immunization in an interferon-gamma (IFNγ) ELISPOT assay and sandwich ELISA.

ELISpot data shown in Table 3 indicates that immunogenic PSA polypeptides in both cytosolic and membrane-bound forms are capable of inducing T cells that recognize human tumor cells transduced with adenovirus to express the cytosolic PSA antigen (SKmel5-Ad-PSA) but not cells transduced with adenovirus to express eGFP (SKmel5-Ad-eGFP). These two antigens also elicited response to PSA protein. The PSA secreted antigen failed to induce T cells to both SKmel5-Ad-PSA or PSA protein. SFC>50 is considered positive.

Table 3. The induction of T cell responses by PSA antigens in Pasteur mice to PSA+ HLA A2.1+ SKmel5 human cancer cells

HLA A2.1+ human cancer cells or protein	IFN-γ SFC/1x10^6 splenocytes (SD)		
	PSA cytosolic	PSA membrane-bound	PSA secreted
SKmel5-Ad-eGFP	7.7 (9.6)	1.2 (1.4)	2.9 (2.7)
SKmel5-Ad-PSA	112.0 (169.3)	546.1 (379.6)	18.7 (18.5)
PSA protein	108.8 (161.0)	536.9 (380.9)	20.6 (21)

Table 4. The induction of anti-PSA antibody response as measured by a sandwich ELISA assay

Antigen Forms	ELISA (OD=1.0) Average (SD)	# of positive
PSA cytosolic	99 (0)	0/6
PSA membrane-bound	4838 (835)	6/6
PSA secreted	1151 2410)	2/6

Data in Table 4 demonstrates that immunogenic PSA polypeptides in both secreted and membrane-bound forms are capable of inducing anti-PSA antibody responses.

EXAMPLE 3. CONSTRUCTION OF MULTI-ANTIGEN VACCINE CONSTRUCTS

In this Example, constructions of plasmids comprising a multi-antigen construct using different strategies are described. These plasmids share the same general plasmid backbone as pPJV7563. Unless otherwise specified, the genes included in the multi-antigen constructs encode (1) an immunogenic PSMA polypeptide of SEQ ID NO:9, (2) an immunogenic PSCA polypeptide comprising amino acids 2-125 of SEQ ID NO:21, and (3) an immunogenic PSA polypeptide of SEQ ID NO:17.

EXAMPLE 3A. PLASMIDS COMPRISING A DUAL ANTIGEN CONSTRUCT

3A1. Construction of Plasmid utilizing multiple promoters

Plasmid 460 (PSMA/PSCA Dual promoter). Plasmid 460 was constructed using the techniques of site-directed mutagenesis, PCR, and restriction fragment insertion. First, a Kpn I restriction site was introduced upstream of the CMV promoter in plasmid 5259 using site-directed mutagenesis with MD5 and MD6 primers according to manufacturer's protocol (Quickchange kit, Agilent Technologies, Santa Clara, CA). Second, an expression cassette consisting of a minimal CMV promoter, human PSMA, and rabbit B globulin transcription terminator was amplified by PCR from plasmid 5166 using primers that carried Kpn I restriction sites (MD7 and MD8). The PCR amplicon was digested with Kpn I and inserted into the newly introduced Kpn I site of calf intestinal alkaline phosphatase (CIP)- treated plasmid 5259.

3A2. Construction of dual antigen constructs utilizing 2A peptides

Plasmid 451 (PSMA-T2A-PSCA). Plasmid 451 was constructed using the techniques of overlapping PCR and restriction fragment exchange. First, the gene encoding human PSMA amino acids 15-750 was amplified by PCR using plasmid 5166 as a template with primers 119 and 117. The gene encoding full-length human PSCA was amplified by PCR using plasmid 5259 as a template with primers 118 and 120. PCR resulted in the addition of overlapping TAV 2A (T2A) sequences at the 3' end of PSMA and 5' end of PSCA. The amplicons were mixed together and amplified by PCR with primers 119 and 120. The PSMA-T2A-PSCA amplicon was digested with Nhe I and Bgl II and inserted into similarly digested plasmid 5166. A glycine-serine linker was included between PSMA and the T2A cassette to promote high cleavage efficiency.

Plasmid 454 (PSCA-F2A-PSMA). Plasmid 454 was created using the techniques of PCR and restriction fragment exchange. First, the gene encoding full-length human PSCA was amplified by PCR using plasmid 5259 as a template with primers 42 and 132. The amplicon was digested with BamH I and inserted into similarly digested, CIP-treated plasmid 5300. A glycine-serine linker was included between PSCA and the FMDV 2A (F2A) cassette to promote high cleavage efficiency.

Plasmid 5300 (PSA-F2A-PSMA) Plasmid 5300 was constructed using the techniques of overlapping PCR and restriction fragment exchange. First, the gene encoding PSA amino acids 25-261 was amplified by PCR from plasmid 5297 with primers MD1 and MD2. The gene encoding human PSMA amino acids 15-750 was amplified by PCR from plasmid 5166 with primers MD3 and MD4. PCR resulted in the addition of overlapping F2A sequences at the 3' end of PSA and 5' end of PSMA. The amplicons were mixed together and extended by PCR. The PSA-F2A-PSMA amplicon was digested with Nhe I and Bgl II and inserted into similarly digested plasmid pPJV7563.

3A3. Dual antigen constructs utilizing internal ribosomal entry sites

Plasmid 449 (PSMA-mIRES-PSCA). Plasmid 449 was constructed using the techniques of overlapping PCR and restriction fragment exchange. First, the gene encoding full length human PSCA was amplified by PCR from plasmid 5259 with primers 124 and 123. The minimal EMCV IRES was amplified by PCR from pShuttle-IRES with primers 101 and 125. The overlapping amplicons were mixed together and amplified by PCR with primers 101 and 123. The IRES-PSCA amplicon was digested with Bgl II and BamH I and inserted into Bgl II-digested, CIP-treated plasmid 5166. In

order to fix a spontaneous mutation within the IRES, the IRES containing Avr II to Kpn I sequence was replaced with an equivalent fragment from pShuttle-IRES.

Plasmid 603 (PSCA-pIRES-PSMA). Plasmid 603 was constructed using the techniques of PCR and seamless cloning. The gene encoding full length human PSCA attached at its 3'end to a preferred EMCV IRES was amplified from plasmid 455 by PCR with primers SD546 and SD547. The gene encoding human PSMA amino acids 15-750 was amplified by PCR from plasmid 5166 using primers SD548 and SD550. The two overlapping PCR amplicons were inserted into Nhe I and Bgl II-digested pPJV7563 by seamless cloning according to manufacturer's instructions (Invitrogen, Carlsbad, CA).

Plasmid 455 (PSCA-mIRES-PSA). Plasmid 455 was constructed using the techniques of overlapping PCR and restriction fragment exchange. First, the gene encoding human PSA amino acids 25-261 was amplified by PCR from plasmid 5297 with primers 115 and 114. The minimal EMCV IRES was amplified by PCR from pShuttle-IRES with primers 101 and 116. The overlapping amplicons were mixed together and amplified by PCR with primers 101 and 114. The IRES-PSA amplicon was digested with Bgl II and BamH I and inserted into Bgl II-digested, CIP-treated plasmid 5259. In order to fix a spontaneous mutation within this clone, the Bgl II to BstE II sequence was replaced with an equivalent fragment from a fresh overlapping PCR reaction.

EXAMPLE 3B. PLASMIDS COMPRISING A TRIPLE ANTIGEN CONSTRUCT

General Strategy. A number of dual antigen plasmids, including PSA-F2A-PSMA, PSMA-mIRES-PSCA, PSMA-T2A-PSCA, PSA-T2A-PSCA, PSCA-F2A-PSMA, PSCA-pIRES-PSMA, and PSMA-mIRES-PSA, were selected for incorporation in various combinations into triple antigen plasmid vectors. In all cases, the plasmid vectors were based on the parental pPJV7563 plasmid backbone. Four plasmid vectors (plasmids 456, 457, 458, and 459) utilized a single full CMV promoter with a rabbit B globulin transcription terminator to drive expression of all three antigens. Two other plasmid vectors (plasmids 846 and 850) incorporated a dual promoter strategy in combination with either an IRES or 2A to drive expression of the three antigens. Plasmids with multiple 2A cassettes were engineered to carry different cassettes to minimize the likelihood of recombination between the first and second cassette during plasmid/vector amplification. Antigen expression was demonstrated by flow cytometry (FIGS. 7A and 7B) and western blotting (FIGS. 8A and 8B).

Plasmid 456 (PSA-F2A-PSMA-mIRES-PSCA). Plasmid 456 was constructed by restriction fragment exchange. Plasmid 5300 was digested with Nhe I and Hpa I and the ~1.8 kb insert was ligated into similarly digested plasmid 449.

Plasmid 457 (PSA-F2A-PSMA-T2A-PSCA). Plasmid 457 was constructed by restriction fragment exchange. Plasmid 5300 was digested with Nhe I and Hpa I and the ~1.8 kb insert was ligated into similarly digested plasmid 451.

Plasmid 458 (PSA-T2A-PSCA-F2A-PSMA). Plasmid 458 was constructed using the techniques of PCR and restriction fragment exchange. The gene encoding human PSA amino acids 25-261 was amplified by PCR from plasmid 5297 with primers 119 and 139, resulting in the addition of a T2A sequence and Nhe I restriction site at the 3' end. The amplicon was digested with Nhe I and inserted into similarly digested plasmid 454.

Plasmid 459 (PSCA-F2A-PSMA-mIRES-PSA). Plasmid 459 was constructed by restriction fragment exchange. Plasmid 454 was digested with Nhe I and Bgl II and the PSCA-F2A-PSMA containing insert was ligated into similarly digested plasmid 455.

Plasmid 846 (CBA-PSA, CMV-PSCA-pIRES-PSMA). Plasmid 846 was constructed using the techniques of PCR and seamless cloning. First, an expression cassette was synthesized that consisted of 1) the promoter and 5' untranslated region from the chicken beta actin (CBA) gene, 2) a hybrid chicken beta actin / rabbit beta globin intron, 3) the gene encoding human PSA amino acids 25-261, and 4) the bovine growth hormone terminator. This PSA expression cassette was amplified by PCR from plasmid 796 with primers 3SalICBA and 5SalIBGH. The amplicon was cloned into the *SalI* site of plasmid 603 using a GeneArt Seamless Cloning and Assembly Kit (Invitrogen, Carlsbad, CA). Upon delivery of this plasmid into a cell, PSA expression will be driven off the CBA promoter while PSCA and PSMA expression will be driven off the CMV promoter.

Plasmid 850 (CBA-PSA, CMV-PSCA-F2A-PSMA). Plasmid 850 was constructed using the techniques of PCR and seamless cloning. First, the CBA promoter-driven PSA expression cassette was amplified by PCR from plasmid 796 with primers 3SalICBA and 5SalIBGH. The amplicon was cloned into the *SalI* site of plasmid 454 using GeneArt Seamless Cloning. Upon delivery of this plasmid into a cell, PSA expression will be driven off the CBA promoter while PSCA and PSMA expression will be driven off the CMV promoter.

Plasmid 916 ((PSA-T2A-PSCA-F2A-PSMA). Plasmid 916 was constructed using the techniques of PCR and Gibson cloning. The genes encoding the three PAA polypeptides were amplified by PCR and ligated into the Nhe I / Bgl II sites of pPJV7563 by Gibson cloning techniques. The complete nucleotide sequence of Plasmid 916 is set forth in SEQ ID NO:62. Plasmid 458 and Plasmid 916 encode the same amino acid sequence that comprises the three immunogenic PAA polypeptides, which amino acid sequence is set forth in SEQ ID NO:60. The nucleotide sequence in Plasmid 916 that encodes the amino acid sequence comprising the three PAA polypeptides is codon-optimized and is also set forth in SEQ ID NO:61.

Table 21. List of Primers Used in the Construction of the Multi-antigen Plasmids

Primer	Sequence (5' to 3')	Strand
42	CGTTGACGCAAATGGGCGGTAGG	Sense
101	TCAGAGATCTGACCCCCTAACGTTACTGGC	Sense
114	TATAGGATCCTCAGGGGTTGGCCACGATG	Antisense
115	GAAAAACACGATGATAATATGGCCAGCATTGTGGGAG GCTGGGAGTG	Sense
116	CCACAATGCTGGCCATATTATCATCGTGTTTTTCAAAG GAAAACCACGTCC	Antisense
117	CATCTCCACAGGTCAATAATGAACCCCTACCTTCGGAT CCGGCTACTTCACTCAAAGTC	Antisense
118	GTTCATTATTGACCTGTGGAGATGTCGAAGAAAACCCA GGACCCGCAAGCAAGGCTGTGCTGCTTGCCCTG	Sense
119	TTGCCTCTCACATCTCGTCAATCTCCGCGAGGAC	Sense
120	GATCTTTTGTACAATATGATCTTGTGGCAATGTCCC	Antisense
123	TATAGGATCCCTATAGCTGGCCGGGTCC	Antisense
124	CACGATGATAATATGGCCAGCAAGGCTGTGCTGCTTG CC	Sense
125	CACAGCCTTGCTGGCCATATTATCATCGTGTTTTTCAAA GGAAAACCACGTCC	Antisense
132	TATAGGATCCTAGCTGGCCGGGTCCCCAGAG	Antisense
139	ATATGCTAGCGGGTCCTGGGTTTTCTTCGACATCTCCA CAGGTCAATAATGAACCCCTACCTTCGGATCCGGGG	Antisense

	TTGGCCACGATGGTGTCC	
SD546	CTGTGACGAACATGGCTAGCAAGG	Sense
SD547	ATTATCATCGTGTTTTTCAAAGGAAAACC	Antisense
SD548	AAACACGATGATAATATGGCCACAACCATGGCGCGCC GCCCGC	Sense
SD550	TTTTGTTAGGGCCCAGATCTTTAGGC	Antisense
MD1	GACGAACATGGCTAGCATTGTGGGAGGCTG	Sense
MD2	CCACATCGCCTGCCAGTTTCAGCAGATCAAAGTTCAGG GTCTGGGATCCGGGGTTGGCCACGATGGTGTC	Antisense
MD3	GATCTGCTGAAACTGGCAGGCGATGTGGAAAGCAACC CAGGCCCAATGGCAAGCGCGCGCCGCCCGCGCTG	Sense
MD4	GTTAGGGCCCAGATCTTTAGGCTACTTCACTCAAAGTC	Antisense
MD5	CTTGTATTACTGTTTATGTAAGCAGACAGGGTACCAAT ATTGGCTATTGGCCATTGCATAC	Sense
MD6	GTATGCAATGGCCAATAGCCAATATTGGTACCCTGTCT GCTTACATAAACAGTAATACAAG	Antisense
MD7	CATGCATGGGTACCAATCTTCCGAGTGAGAGACACAAA AAATTCC	Sense
MD8	GATCGATCGGTACCCTGCAGGTCGAGCACCAAAATCA ACGGG	Antisense
5SalIB GH	GTTTATGTAAGCAGACAGGTCGACCCATAGAGCCCAC CGCATCCCCAGC	Antisense
3SalIC BA	TGGCCAATAGCCAATATTGTCGACTGGGTCGAGGTGA GCCCCACGTTCTG	Sense

EXAMPLE 3C. TRIPLE ANTIGEN ADENOVIRUS VECTORS

General Strategy. As with DNA plasmids, viral vectors can be engineered to deliver multiple prostate cancer antigens. The three multi-antigen expression strategies described above for multi-antigen constructs - dual promoters, 2A peptides, and internal ribosome entry sites – were incorporated in various combinations to create triple antigen adenovirus vectors. Briefly, the multi-antigen expression cassettes were cloned into a pShuttle-CMV plasmid modified to carry two copies of the tetracycline operator

sequence (TetO2). Recombinant adenovirus serotype 5 vectors were created using the AdEasy Vector System according to manufacturer's protocols (Agilent Technologies, Inc., Santa Clara, CA). Viruses were amplified in HEK293 cells and purified by double cesium chloride banding according to standard protocols. Prior to in vivo studies, viral stocks were thoroughly characterized for viral particle concentration, infectivity titer, sterility, endotoxin, genomic and transgene integrity, transgene identity and expression.

Adenovirus-733 (PSA-F2A-PSMA-T2A-PSCA). Ad-733 is the viral equivalent of plasmid 457. Expression of the three antigens is driven off a single CMV promoter with a tetracycline operator for repressing transgene expression during large scale production in Tet repressor expressing HEK293 lines. Multi-antigen expression strategies include two different 2A sequences.

Adenovirus-734 (PSA-T2A-PSCA-F2A-PSMA). Ad-734 is the viral equivalent of plasmid 458. Expression of the three antigens is driven off a single CMV promoter with a tetracycline operator for repressing transgene expression during large scale production in Tet repressor expressing HEK293 lines. Multi-antigen expression strategies include two different 2A sequences.

Adenovirus-735 (PSCA-F2A-PSMA-mIRES-PSA). Ad-735 is the viral equivalent of plasmid 459. Expression of the three antigens is driven off a single CMV promoter with a tetracycline operator for repressing transgene expression during large scale production in Tet repressor expressing HEK293 lines. Multi-antigen expression strategies include a 2A sequence and an IRES.

Adenovirus-796 (CBA-PSA, CMV-PSCA-pIRES-PSMA). Ad-796 is the viral equivalent of plasmid 846. Expression of PSA is driven off the chicken beta actin promoter while PSCA and PSMA expression is driven off the CMV-TetO2 promoter. Multi-antigen expression strategies include two promoters and an IRES.

Adenovirus-809 (CBA-PSA, CMV-PSCA-F2A-PSMA). Ad-809 is the viral equivalent of plasmid 850. Expression of PSA is driven off the chicken beta actin promoter while PSCA and PSMA expression is driven off the CMV-TetO2 promoter. Multi-antigen expression strategies include two promoters and a 2A sequence.

EXAMPLE 4. ANTI-CANCER EFFICACY OF VACCINE IN COMBINATION WITH SUNITINIB AND ANTI-CTLA-4 ANTIBODY

The anti-tumor efficacy of a cancer vaccine in combination with sunitinib and anti-CTLA-4 monoclonal antibody (clone 9D9) was investigated in subcutaneous TUBO tumor bearing BALB/neuT mice.

Study Procedure. Briefly, ten mice per each group were daily orally dosed with either vehicle or sunitinib malate at 20 mg/kg starting at day 10 post tumor implant until day 64. Vaccination with DNA constructs that either encode no antigen (control vaccine) or a rat Her-2 antigen of SEQ Id NO: 54 (cancer vaccine) as adenovirus vectors initiated on day 13 subsequently followed by two weekly immunizations, two biweekly immunizations, and seven weekly immunizations of respective antigens (HBV antigens or rHer-2) by DNA. The groups of mice (closed circle and open triangle) that were treated with anti-murine CTLA-4 monoclonal antibody were intraperitoneally injected with 250 μg of the antibody on day 20, 27, 41, 55, 62, 69, 76, 83, 90, and 97 right after the PMED injections.

Results. Figure 4 shows the Kaplan-Meier survival curve of the groups of mice from a representative study evaluating the anti-tumor efficacy of sunitinib and anti-murine CTLA-4 monoclonal antibody (clone 9D9) in combination with a cancer vaccine. Increased survival time was observed in mice treated with Sutent with control vaccine (open circle), anti-murine CTLA-4 monoclonal antibody (open triangle) or cancer vaccine (closed triangle). A further increase of survival was observed in mice treated with Sutent and cancer vaccine in combination with anti-murine CTLA-4 (closed circle). P values were calculated by log-rank test.

EXAMPLE 5. EFFECT OF CPG OR CD40 AGONIST ON THE IMMUNE RESPONSES INDUCED BY CANCER VACCINE

Immunogenicity Studies in BALB/c Mice

The effect of local administration of immune modulators on the magnitude and quality of antigen specific immune responses induced by a cancer was investigated in BALB/c mice, in which the immune response was assessed by measuring rHER2 specific T cell responses using the IFNγ ELISPOT assay or intracellular cytokine staining assay. Briefly, 4 to 6 female BALB/c mice per group as indicated were immunized with DNA plasmid expression constructs encoding rHER2 antigen sequences (SEQ ID NO:54) by PMED delivery system. The immune modulators, CpG7909 (PF-03512676) and anti-CD40 monoclonal agonistic antibody, were administered locally by intradermal injections in proximity to the vaccine draining

inguinal lymph node subsequently after the PMED actuations. Antigen specific T cell responses were measured by IFNγ ELISPOT or intracellular cytokine staining assay according to the procedure described below.

Intracellular Cytokine Staining (ICS) Assay

The rHer-2 specific polyfunctional (multi-cytokine positive) T cell immune responses were measured from splenocytes or PBMCs isolated from individual animals by ICS assay. Typically 1e6 splenocytes were incubated with Brefeldin A at 1μg/ml and peptide stimulant (rHer-2 specific CD8 p66, rHer-2 specific CD4 p169 or irrelevant HBV p87) at 10 μg/ml for 5hr at 37^{0}C in a 5% CO_2 incubator. After the stimulation, the splenocytes were washed and blocked with Fc□ block (anti-mouse CD16/CD32) for 10 min. at 4^{0}C followed by a 20min staining with Live/dead aqua stain, anti-mouse CD3ePE-Cy7, anti-mouse CD8a Pacific blue, and anti-mouse CD45R/B220 PerCP-Cy5.5. The cells were washed, fixed with 4% paraformaldehyde overnight at 4^{0}C, permeabilized with BD fix/perm solution for 30 min at RT and incubated with anti-mouse IFNγ APC, anti-mouse TNF□ Alexa488 and anti-mouse IL-2 PE for 30 min at RT. The cells were washed and 20,000 CD4 or CD8 T cells were acquired for analysis by flow cytometry. The total number of antigen specific single, double or triple cytokine positive T cells per total spleen of each animal is calculated by subtracting the rHer-2 specific responses to the irrelevant peptide HBV from the vaccine specific responses and normalized to the total number of splenocytes isolated from the spleen.

IFNγ ELISPOT Assay Results

Figure 5 shows the IFNγ ELISPOT results from groups of mice from a representative study evaluating the magnitude of antigen specific T cell responses induced by the rHER2 vaccine when given with the immune modulators as indicated. Briefly, each mouse per treatment group (n=4) was immunized with DNA plasmid expression constructs encoding rHER2 antigen sequences (SEQ ID NO:54) by PMED immediately followed by either 100ug of control rat IgG monoclonal antibody (Bioxcell #BE0089: control mAb) or 50□g CpG7909 or 100ug of anti-CD40 monoclonal antibody (Bioxcell #BE0016-2: a-CD40 mAb) as indicated. The antigen specific immune responses were measured by IFNγ ELISPOT assay from 5e5 splenocytes mixed with control or rHer-2 specific p66 peptides at 10μg/ml concentration, 7 days after the PMED actuation. The number of total IFNγ secreting cells from splenocytes containing1e5 CD8 T cells was calculated from the ELISPOT results from individual animals and the % of

CD8 T cells in splenocytes and mean and standard error of mean of each group are plotted. As shown, both CpG7909 and the anti-CD40 monoclonal antibody significantly enhanced the magnitude of antigen specific immune responses induced by rHer-2 DNA compared to mice that received control antibodies.

Intracellular Cytokine Staining (ICS) Assay Results. Figures 6 and 7 show the results of a representative study that evaluates the immunomodulatory activity of CpG 7909 on the quality of the vaccine induced immune responses by intracellular cytokine staining assay. Briefly, each animal was immunized twice with the DNA plasmid expression constructs encoding rHER2 antigen sequences (SEQ ID NO:54) delivered by PMED with a 4-week interval. The mice in each group (n=5) were given intradermal injections of either PBS (PMED group) or 50□g of CpG 7909 (PMED+CpG group) in proximity to the right side vaccine draining inguinal node immediately following both DNA immunizations by PMED. Seven days after the last immunization by PMED, an ICS assay was performed on the splenocytes isolated from each individual mice to detect antigen specific polyfunctional CD8 or CD4 T cells that secrete IFNy, TNF□ and/ or IL-2. A significant increase in rHer-2 specific multi-cytokine positive CD8 and CD4 T cell responses were detected from mice treated with the local delivery of CpG 7909 compared to PBS. An increase in the single cytokine positive CD8 population was observed in the animals that received local delivery of CpG7909 administration compared to PBS.

Figures 8 and 9 show the results of a representative study that evaluates the immunomodulatory activity of an agonistic anti-CD40 monoclonal antibody on the quality of the vaccine induced immune responses by intracellular cytokine staining assay. Briefly, each animal was immunized twice by DNA plasmid expression constructs encoding rHER2 antigen sequences (SEQ ID NO:54) delivered by PMED with a 4week interval. The mice in each group (n=6) were given 100 □g of intradermal injections of either isotype IgG control (PMED with IgG) or anti-CD40 monoclonal antibody (PMED with aCD40) in proximity to the right side vaccine draining inguinal node, one day after the first immunization was administered by PMED. Seven days after the last PMED, an ICS assay was performed on the splenocytes isolated from each individual mice to detect rHer-2 specific polyfunctional CD8 or CD4 T cells that secrete IFN□, TNF□ and/ or IL-2.. A significant increase in the rHer-2 specific triple-cytokine positive CD8 and CD4 T cell responses were detected from mice treated with the local delivery of anti-

CD40 monoclonal antibody compared to isotype IgG control. There were also significant increases in rHer-2 specific single and double cytokine positive CD4 T cells by anti-CD40 monoclonal antibody given locally.

EXAMPLE 6. ANTI-CANCER EFFICACY OF CANCER VACCINE IN COMBINATIOIN WITH LOW DOSE SUNITINIB

Anti-tumor efficacy of anti-cancer vaccine in combination with low dose sunitinib was investigated in BALB/neuT mice with spontaneous mammary pad tumors.

Animal treatment. Briefly, 13-14 weeks old female mice were orally given sunitinib malate (Sutent) at 5mg/kg for 112 days twice a day. The control vaccine, which delivers no antigen, and cancer vaccine which delivers a rat Her-2 antigen of SEQ ID NO: 54 (rHer-2), were given by adenovirus injections on day 3 as a prime followed by 7 biweekly administrations by PMED of DNA delivering HBV antigens (control vaccine) or rHer-2 (cancer vaccine) respectively. The survival end point was determined when all ten mammary pads became tumor positive or when the volume of any of the mammary tumors reached 2000mm^3.

Results. The results are presented in Figure 10. Compared to previously published pharmacokinetic profile of Sutent (Mendel, D., Laird, D., et al.: "In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship". Clinical Cancer Research, 203, 9:327-337), the C_{Max} of Sutent in mice dosed twice a day at 5 mg/kg is expected to be significantly lower than the minimum blood levels necessary to achieve efficient anti-tumor efficacy in mice and man. The data shows a quick and temporary improvement in the survival of the mice treated with low dose Sutent monotherapy. However when given with the cancer vaccine, a more persistent and significant improvement of survival was observed ($P< 0.0001$ by Log rank test).

EXAMPLE 7. ENHANCEMENT OF VACCINE-INDUCED IMMUNE RESPONSES BY LOCAL ADMINISTRATION OF CPG

The immune enhancement of local administration of CpG (PF-03512676) on the immune responses induced by a human PSMA nucleic acid provided by the invention was investigated in a monkey study, in which the immune response was assessed by measuring PSMA specific T cell responses using an IFNγ ELISPOT assay.

Animal Treatment and Sample Collection. Six groups of Chinese cynomolgus macaques, six (#1 to 6) per each test group, were immunized with a plasmid DNA encoding the human PSMA modified antigen (the polypeptide of SEQ ID NO:9) delivered by electroporation. Briefly, all animals received bilateral intramuscular injections of 5 mg of plasmid DNA followed by electroporation (DNA EP) on day 0. Subsequently right after the electroporation, group 2 received bilateral intramuscular injections of 2 mg of CpG mixed with 1 mg Alum in proximity to the DNA injection sites. Groups 3 and 4 received bilateral intramuscular injections of 2 mg of CpG delivered without alum in proximity to the DNA injection sites either on day 0 or day 3, respectively. Group 5 received 2 mg of bilateral intradermal injections of CpG delivered in proximity to the vaccine draining inguinal nodes on day 3. Group 6 received bilateral injections of 200 □g of CpG mixed with the DNA solution which was co-electroporated into the muscle on day 0.

IFNγ ELISPOT Assay Procedure. Peripheral blood samples were collected from each animal fifteen days after the DNA immunization. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples and were subjected to an IFNγ ELISPOT assay to measure the PSMA specific T cell responses. Briefly, 4e5 PBMCs from individual animals were plated per well with pools of PSMA specific peptides or nonspecific control peptides (human HER2 peptide pool) each at 2ug/ml in IFNγ ELISPOT plates. The composition of each of the PSMA specific peptide pool is provided in Table 24A. The plates were incubated for 16 hrs at 37°C and 5% CO2 and washed and developed after incubation as per manufacturer's instruction. The number of IFNγ spot forming cells (SFC) was counted by CTL reader. Each condition was performed in duplicates.

Results. Table 6 shows the result of a representative IFNγ ELISPOT assay that evaluates and compares the IFNγ T cell responses induced by the vaccine without (group 1) or with CpG (PF-03512676) given locally by intramuscular (groups 2, 3, 4, and 5) or intradermal injections (group 6). The reported PSMA specific response was calculated by subtracting the average number of the SFC to the nonspecific control peptides (human HER2 peptide pool) from the average number of SFC to the PSMA peptide pools and normalized to the SFC observed with 1e6 PBMCs. ^ indicates that the count is not accurate because the numbers of spots were too numerous to count. ND indicates not determined.

The PSMA specific IFNγ T cell responses were detected to multiple PSMA specific peptide pools in the absence of CpG (PF-03512676) in all six animals (group 1). The total responses to the PSMA peptides measured were modestly higher in a few animals that additionally received CpG (PF-03512676) either by intramuscular (group 4: 3/6) or intradermal (group 5: 2/6) injections 3 days after DNA electroporation. However, when CpG was delivered subsequently right after electroporation on the same day (groups 2 and 3), there were several animals that failed to produce high responses (group 2: 4/6 and group3: 3/6) whether mixed or not mixed with Alum. However, higher net responses were detected in 4/6 animals when a ten-fold lower dose of CpG was co-electroporated with the DNA solution into the muscle (group 6) with a statistically higher response ($P<0.05$) to peptide pools H1 and R1 compared to animals that did not receive CpG (group 1). The data shows that low dose of CpG can effectively enhance IFNγ T cell responses induced by a DNA vaccine when co-electroporated into the muscle.

Table 6. PSMA specific IFNγ T cell responses induced by the DNA vaccine without (Group 1) or with CpG (Groups 2, 3, 4, 5 and 6) is measured by IFNγ ELISPOT assay from PBMCs, 15 days after DNA electroporation

Group	Animal ID	Recall Antigen						
		P1	P2	P3	H1	H2	R1	R2
1	#1	36	31	1	126	183	5	14
	#2	6	3	13	61	524	6	141
	#3	11	4	8	108	1049	3	56
	#4	10	0	13	20	151	13	10
	#5	8	6	11	39	469	14	18
	#6	26	5	0	145	356	8	30
2	#1	3	10	0	15	35	0	0
	#2	0	0	8	4	6	13	0
	#3	3	0	0	0	10	11	0
	#4	6	209	4	111	414	23	9
	#5	15	5	30	171	104	68	6
	#6	0	0	0	9	9	6	8
3	#1	14	19	8	123	1066	10	60
	#2	14	16	20	384	393	104	8
	#3	0	0	15	0	6	0	0
	#4	0	0	0	33	21	0	4
	#5	4	91	1	875	^1235	233	109
	#6	0	0	0	0	3	0	0
4	#1	0	33	15	1025	^1209	280	90
	#2	0	313	3	23	656	6	31
	#3	61	120	61	428	1190	143	53
	#4	0	0	8	599	870	34	111
	#5	0	1	8	19	226	10	36
	#6	111	55	39	231	613	121	99
5	#1	21	9	0	355	1131	73	5
	#2	0	0	0	118	233	0	0
	#3	0	0	0	18	129	0	0
	#4	0	28	78	68	294	58	8
	#5	25	0	28	329	1125	134	5
	#6	0	0	0	23	39	4	0
6	#1	0	0	13	650	1096	270	5
	#2	34	1	74	124	474	29	15
	#3	0	3	14	684	1074	126	64
	#4	8	9	0	136	321	49	1
	#5	13	23	35	ND	^1235	333	195
	#6	0	0	0	421	^1201	138	29

EXAMPLE 8. ENHANCEMENT OF VACCINE-INDUCED IMMUNE RESPONSES BY LOCAL ADMINISTRATION OF ANTI-CTLA-4 ANTIBODY

The effect of low dose subcutaneous administration of anti-CTLA-4 monoclonal antibody (CP-675, 206) on the immune responses induced by a rhesus PSMA nucleic acid was investigated in a monkey study, in which the immune response was assessed by measuring PSMA specific T cell responses using an IFNγ ELISPOT assay. The rhesus PSMA nucleic acid used in the study has the sequence as set forth in SEQ ID NO: 56) and encodes an immunogenic PSMA polypeptide of SEQ ID NO: 55.

Animal Treatment and Sample Collection. Five groups of male Indian rhesus macaques, seven (#1 to 7) per each test group, were immunized with an adenovirus encoding a rhesus PSMA modified polypeptide delivered by bilateral intramuscular injections (2x 5e10 V.P.). Immediately following the adenovirus injections, group 1 received vehicle, and groups 2 to 4 received bilateral subcutaneous injections of anti-CTLA-4 antibody (CP-675, 206) at doses 2x 25mg, 2x 16.7mg and 2x 8.4mg respectively in proximity to the vaccine draining lymph node.

Nine days after the immunization, peripheral blood mononuclear cells (PBMCs) were isolated from each animal and were subjected to an IFNγ ELISPOT assay to measure the rhesus PSMA specific T cell responses. Briefly, 4e5 PBMCs from individual animals were plated per well with pools of rhesus PSMA specific peptides (P1, P2, P3 or R1+R2 defined in Table 24A) or nonspecific control peptides (human HER2 peptide pool) each at 2ug/ml in IFN□ ELISPOT plates. The plates were incubated for 16 hrs at 37 °C and 5% CO2 and washed and developed after incubation as per manufacturer's instruction. The number of IFNγ spot forming cells (SFC) was counted by CTL reader. Each condition was performed in duplicates. The average of the duplicates from the background adjusted SFC of the rhesus PSMA specific peptide pools was normalized to the response in 1e6 PBMCs. The individual and sum responses to the peptide pools from each individual animal are presented in Table 29.

IFNγ ELISPOT Assay Procedure. A capture antibody specific to IFNγ (□BD Bioscience, #51-2525kc) is coated onto a polyvinylidene fluoride (PVDF) membrane in a microplate overnight at 4^0C. The plate is blocked with serum/protein to prevent nonspecific binding to the antibody. After blocking, effector cells (such as splenocytes isolated from immunized mice or PBMCs isolated from rhesus macaques) and targets (such as PSMA peptides from peptide library, target cells pulsed with antigen specific

peptides or tumor cells expressing the relevant antigens) are added to the wells and incubated overnight at 37^0C in a 5% CO_2 incubator. Cytokine secreted by effector cells are captured by the coating antibody on the surface of the PVDF membrane. After removing the cells and culture media, 100 µl of a biotinylated polyclonal anti-humanIFNγ antibody was added to each of the wells for detection. The spots are visualized by adding streptavidin-horseradish peroxidase and the precipitate substrate, 3-amino-9-ethylcarbazole (AEC), to yield a red color spot as per manufacturer's (Mabtech) protocol. Each spot represents a single cytokine producing T cell.

Results. Table 7 shows the results of a representative IFNγ ELISPOT assay that compares the T cell responses induced by the vaccine without (group 1) or with (groups 2-4) anti-CTLA-4 monoclonal antibody (CP-675,206) given locally by subcutaneous injections in proximity to the vaccine draining lymph node. The vaccine generated an immune response (group1) that was significantly enhanced by the local administration of the anti-CTLA-4 antibody (CP-675, 206) at a dose of 50mg (group 2, P=0.001 by Student's T-test using underestimated values). The response was also significantly enhanced by low doses of anti-CTLA-4 antibody at 33.4 mg (group3: P=0.004 by Student T-test using underestimated values) and 16.7mg (group4: P=0.05 by Student T-test) respectively. The data suggests that low doses of anti-CTLA-4 delivered by subcutaneous injection can significantly enhance the vaccine induced immune responses.

Table 7. IFNγ T cell responses induced by the vaccine without (Group 1) or with subcutaneous injections of anti-CTLA-4 antibody (CP-675,206). ^ indicates that the count is underestimated due to the high spot numbers. TNTC means too numerous to count.

Group	aCTLA4 dose, mg	animal ID	peptide pool				Sum
			P1	P2	P3	R1+R2	
1	NA	1	21	0	0	108	129
		2	59	480	28	353	920
		3	133	29	359	305	826
		4	0	28	1	35	64
		5	41	6	30	99	176
		6	1	0	849	169	1019
		7	0	0	0	23	23
2	50.0	1	^1105	704	^1116	^1116	^4041
		2	371	26	661	779	1837
		3	393	559	216	198	1366
		4	^1100	^1100	406	1078	^3684
		5	778	325	554	419	2076
		6	^1079	^1079	844	^1079	^4081
		7	423	103	535	398	1459
3	33.4	1	^425	^425	^425	^425	^1700
		2	^580	^580	^580	^580	^2320
		3	TNTC	TNTC	TNTC	TNTC	TNTC
		4	321	778	370	409	1878
		5	331	466	311	446	1554
		6	545	121	^631	^1194	^2491
		7	446	299	^1078	^1060	^2883
4	16.7	1	^964	296	^964	^964	^3188
		2	76	76	76	76	304
		3	^984	^984	^984	^984	^3936
		4	260	489	648	^1109	^2506
		5	119	45	28	140	332
		6	55	76	43	198	372
		7	146	726	141	400	1413

EXAMPLE 9. IMMUNOMODULATION OF MYELOID DERIVED SUPPRESSOR CELLS BY LOW DOSE SUNITINIB

The following example is provided to illustrate the immunomodulatory effects of low dose sunitinib on Myeloid Derived Suppressor Cells (MDSC) *in vivo*, in a non-tumor mouse model.

Study Procedures.

To generate MDSC enriched splenocytes, TUBO cells ($1x10^6$) were implanted into the flanks of 5 BALB/neuT mice, and left for approx. 20-30 days until tumor volume reached between 1000-1500mm^3. Mice were then sacrificed, spleens removed and the MDSC enriched splenocytes recovered. Splenocytes were labeled for 10 minutes with 5 µM CFSE, washed with PBS and counted. Labeled cells were subsequently resuspended at $5x10^7$ splenocytes/ml in PBS solution and adoptively transferred via an i.v. tail vein injection into naïve BALB/c recipient mice. Three days prior to adoptive transfer, the recipient mice began bi-daily dosing with vehicle or sunitinib malate (Sutent) at 5 mg/kg, 10 mg/kg and 20 mg/kg. Following adoptive transfer, recipient mice continued to receive bi-daily dosing of Vehicle or sunitinib for two further days, after which point the mice were sacrificed, spleens removed, splenocytes recovered and processed for phenotypic analysis.

Splenocytes were counted and resuspended at $5x10^6$ cells/ml in FACS staining buffer (PBS, 0.2% (w/v) bovine serum albumin, and 0.02% (w/v) Sodium Azide). For flow cytometry staining of splenocytes, $2.5x10^6$ cells were first incubated with anti-bodies to CD16/CD32, 10 minutes at 4ºC, to block Fc receptors and minimize non-specific binding. Splenocytes were then stained for 20 minutes at 4ºC with appropriate fluorophore conjugated antibodies (Biolegend) to murine cell surface markers. For T cells (anti-CD3 (Pacific Blue), clone 17A2) and for MDSC (anti-GR-1 (APC), clone RB6-8C5 and anti-CD11b (PerCp Cy5.5), clone M1/70). A live/dead stain was also included. Following antibody incubation, stained splenocytes were washed with 2mls of FACS buffer, pelleted by centrifugation and resuspended in 0.2ml of FACS buffer prior to data acquisition on a BD CANTO II flow cytometer. To monitor the effect of Sunitinib or Vehicle on the adoptively transferred MDSC survival, we calculated the percentage of CFSE+,CD3-,GR1+,CD11b+ in the live, singlet gate. We then determined the number of adoptively transferred MDSC per spleen by calculating what actual cell number the percentage represented of total splenocytes count. Data was analyzed by FloJo and Graph pad software.

Results. The data presented in Table 27 represents the mean number of adoptively transferred CSFE+,CD3-,GR1+,CD11b+ cells recovered per spleen (n=7/group), 2 days post adoptive transfer, from mice bi-daily dosed with either Vehicle or 5mg/kg, 10mg/kg and 20 mg/kg Sunitinib. Statistical significance was determined by

one-way ANOVA using the Dunnett's multiple comparison test, comparing the Sunitinib dosed groups against the 0mg/kg (vehicle) group.The data demonstrates that Sunitinib, dosed bi-daily, *in vivo,* has an immunomodulatory effect on MDSCs, even when dosed as low as 5mg/kg, resulting in a statistically significant reduction in the numbers recovered when compared to the vehicle treated control group.

Table 8. Mean number of CFSE+,CD3-,GR1+,CD11b+ MDSCs recovered from spleen

	Sunitinib Dose (mg/kg)			
	0 (Vehicle)	5	10	20
MDSC # /spleen Mean +/- SEM	17470 +/- 2017	10980 +/- 1082	4207 +/- 338	4440 +/- 440
Statistical significance, p<0.05	NA	Yes	Yes	Yes

EXAMPLE 10. IMMUNOGENICITY OF TRIPLE ANTIGEN ADENOVIRUS AND DNA CONSTRUCTS

The following example is provided to illustrate the capability of triple antigen vaccine constructs (either in the form of adenovirus vector or DNA plasmid) expressing three antigens PSMA, PSCA and PSA provided by the invention to elicit specific T cell responses to all three encoded antigens in nonhuman primates.

In Vivo Study Procedures. The T cell immunogenicity of five adenovirus vectors each expressing three antigens (PSMA, PSCA and PSA; Ad-733, Ad-734, Ad-735, Ad-796 and Ad-809) provided by the invention were compared to the mix of three adenovirus vectors each only expressing a single antigen (PSMA, PSA or PSCA), 9 days post prime. The response to single adenovirus expressing a single antigen (groups 1-3) was evaluated to demonstrate the specificity. Briefly, Indian rhesus macaques (n=6 for groups 1 and 3, n=7 for group 2 and n=8 for groups 4-9) were intramuscularly injected with a total of 1e11 V.P. followed by intradermal injections of anti-CTLA-4 at 10 mg/kg on the same day. Nine days after the injections, peripheral blood mononuclear cells (PBMCs) were isolated from each animal and were subjected to an IFN□ ELISPOT assay to measure the PSMA, PSA and PSCA specific T cell responses.

Thirteen weeks after the adenovirus and anti-CTLA-4 injections when the T cell responses have contracted, the monkeys received DNA (Group 1: PSMA, plasmid 5166; Group 2: PSA, plasmid 5297; Group 3: PSCA, plasmid 5259; Group 4: mix of PSMA,

PSA and PSCA, plasmids 5166, 5259 and 5297; Group 4: plasmid 457; Group 6: plasmid 458; Group 7: plasmid 459; Group 8: plasmid 796 and Group 9: plasmid 809) boost vaccinations delivered by electroporation. In summary, each animal received a total 5mg of plasmid DNA provided by the invention which delivers the same expression cassette encoded in the adenovirus used in the prime. Nine days after the boost vaccination, peripheral blood mononuclear cells (PBMCs) were isolated from each animal and were subjected to an IFNγ ELISPOT assay.

IFNγ ELISPOT assay. Briefly, 4e5 PBMCs from individual animals were plated per well with PSMA specific peptide pools P1, P2, P3 or H1 and H2 (Table 9A), PSA specific pool 1 or 2 (Table 9B), PSCA specific pool (Table 10) or nonspecific control peptides (human HER2 peptide pool) each at 2ug/ml in IFNγ ELISPOT plates. The plates were incubated for 16 hrs at 37 °C and 5% CO2 and washed and developed after incubation as per manufacturer's instruction. The number of IFNγ spot forming cells (SFC) was counted by CTL reader. Each condition was performed in duplicates. The average of the duplicates from the background adjusted SFC of the antigen specific peptide pools was normalized to the response in 1e6 PBMCs. The antigen specific responses in the tables present the sum of the responses to the corresponding antigen specific peptides or peptide pools.

Results: Table 11 represents a study that evaluates the T cell immunogenicity of five different adenoviruses each expressing all three antigens in comparison to the mixture of three adenoviruses each expressing a single antigen in Indian rhesus macaques by IFNγ ELISPOT. The majority of animals that only received Ad-PSMA (group 1) injections induced specific responses to PSMA but not to PSA or PSCA (Student's T-test, $P<0.03$. One animal (#4) that induced responses to PSCA preferentially was removed from the statistical analysis). The animals that only received injections of Ad-PSA (group 2) induced specific responses to PSA but not to PSMA or PSCA (Student's T-test, $P<0.02$). The animals that only received injections of Ad-PSCA (group 3) induced specific responses to PSCA but not to PSMA or PSA (Student's T-test, $P<0.03$). All five triple-antigen expressing adenovirus vectors (groups 5-9) induced IFN□ T cell responses to all three antigens which the magnitude varied by animal. The magnitude of the responses to PSCA induced by the triple antigen expressing adenoviruses was similar to the mix of individual vectors (group 4). However the magnitude of responses to PSMA induced by Ad-809 (group 9) and responses to PSA

induced by Ad-796 (group 8) were each significantly superior to the mix (Student's T-test, P=0.04 and P=0.02) respectively. These results indicate that vaccinating with an adenovirus expressing triple antigens can elicit equivalent or superior T cell immune responses to vaccinating with the mix of individual adenoviruses in nonhuman primates.

Table 12 shows the IFNγ ELISPOT results represents a study that evaluates the immunogenicity of the five different triple antigen expression cassettes provided in the invention delivered by an adenovirus prime in combination with anti-CTLA-4 followed by an electroporation boost of the corresponding plasmid DNA. The immune responses are compared to the mix of three constructs expressing a single antigen delivered similarly by an adenovirus prime with anti-CTLA-4 and DNA electroporation boost immunizations.

All of the animals that only received Ad-PSMA with anti-CTLA-4 followed by plasmid-PSMA (group 1) immunizations induced specific responses to PSMA but not to PSA or PSCA. Similarly all of the animals that only received Ad-PSA with anti-CTLA-4 followed by plasmid-PSA immunizations (group 2) induced specific responses to PSA but not to PSMA or PSCA and finally all of the animals that only received Ad-PSCA with anti-CTLA-4 followed by plasmid-PSCA (group 3) immunizations induced specific responses to PSCA but not to PSMA or PSA (Student's T-test, $P<0.01$).

All animals that have been immunized with either the triple-antigen expressing vectors (groups 5-9) or the mix (group 4) induced IFNγ T cell responses to all three antigens. The frequency of PSCA or PSA specific IFγ T cells detected were similar in all of these groups (groups 4-9) respectively. However construct groups 7 and 9 that received triple antigen expression vector vaccinations produced significantly higher frequency of responses to PSMA than the mix of three single antigen expressing constructs (group 4). These results indicate that adenovirus and DNA vaccines expressing triple antigens in one cassette can elicit equivalent or superior IFNγ T cell responses to the mix of adenoviruses and DNAs expressing the single antigens in nonhuman primates.

Table 9A. PSMA peptide pools*

P1	P2	P3	H1	H2	R1	R2
h 1-15	h 249-263	h 449-463	h 33-47	h 465-479	r 33-47	r 465-479
h 5-19	h 253-267	h 453-467	h 37-51	h 469-483	r 37-51	r 469-483
h 9-23	h 257-271	h 457-471	h 41-55	h 473-487	r 41-55	r 473-487
h 13-27	h 261-275	h 485-499	h 45-59	h 477-491	r 45-59	r 477-491
h 17-31	h 265-279	h 489-503	h 61-75	h 481-495	r 61-75	r 481-495
h 21-35	h 269-283	h 493-507	h 65-79	h 537-551	r 65-79	r 537-551
h 25-39	h 273-287	h 497-511	h 69-83	h 541-555	r 69-83	r 541-555
h 29-43	h 277-291	h 501-515	h 73-87	h 545-559	r 73-87	r 545-559
h 49-63	h 281-295	h 505-519	h 97-111	h 577-591	r 97-111	r 577-591
h 53-67	h 285-299	h 509-523	h 101-115	h 581-595	r 101-115	r 581-595
h 57-71	h 289-303	h 513-527	h 105-119	h 585-599	r 105-119	r 585-599
h 77-91	h 293-307	h 517-531	h 109-123	h 589-603	r 109-123	r 589-603
h 81-95	h 297-311	h 521-535	h 137-151	h 601-615	r 137-151	r 601-615
h 85-99	h 317-331	h 525-539	h 141-155	h 605-619	r 141-155	r 605-619
h 89-103	h 321-335	h 529-543	h 145-159	h 609-623	r 145-159	r 609-623
h 93-107	h 325-339	h 533-547	h 149-163	h 613-627	r 149-163	r 613-627
h 113-127	h 329-343	h 549-563	h 209-223	h 637-651	r 209-223	r 637-651
h 117-131	h 333-347	h 553-567	h 213-227	h 641-655	r 213-227	r 641-655
h 121-135	h 353-367	h 557-571	h 217-231	h 645-659	r 217-231	r 645-659
h 125-139	h 357-371	h 561-575	h 221-235	h 649-663	r 221-235	r 649-663
h 129-143	h 361-375	h 565-579	h 301-315	h 653-667	r 301-315	r 653-667
h 133-147	h 365-379	h 569-583	h 305-319	h 657-671	r 305-319	r 657-671
h 153-167	h 369-383	h 573-587	h 309-323	h 709-723	r 309-323	r 709-723
h 157-171	h 373-387	h 593-607	h 313-327	h 713-727	r 313-327	r 713-727
h 161-175	h 377-391	h 597-611	h 337-351	h 717-731	r 337-351	r 717-731
h 165-179	h 381-395	h 617-631	h 341-355	h 721-735	r 341-355	r 721-735

h 169-183	h 385-399	h 621-635	h 345-359	h 725-739	r 345-359	r 725-739
h 173-187	h 389-403	h 625-639	h 349-363	h 729-743	r 349-363	r 729-743
h 177-191	h 393-407	h 629-643	h 461-475	h 733-747	r 461-475	r 733-747
h 181-195	h 397-411	h 633-647				
h 185-199	h 401-415	h 661-675				
h 189-203	h 405-419	h 665-679				
h 193-207	h 409-423	h 669-683				
h 197-211	h 413-427	h 673-687				
h 201-215	h 417-431	h 677-691				
h 205-219	h 421-435	h 681-695				
h 225-239	h 425-439	h 685-699				
h 229-243	h 429-443	h 689-703				
h 233-247	h 433-447	h 693-707				
h 237-251	h 437-451	h 697-711				
h 241-255	h 441-455	h 701-715				
h 245-259	h 445-459	h 705-719				
		h737-750				

Table 9B. PSA peptide pools: the amino acid position and sequence of fifteen amino acid peptides overlapping by thirteen amino acids from PSA peptide library is shown.

PSA peptide pool 1		PSA peptide pool 2	
amino acid no.	PSA peptide sequence	amino acid no.	PSA peptide sequence
5-19	VVFLTLSVTWIGAAP	129-143	PAELTDAVKVMDLPT
9-23	TLSVTWIGAAPLILS	131-145	ELTDAVKVMDLPTQE
11-25	SVTWIGAAPLILSRI	133-147	TDAVKVMDLPTQEPA
13-27	TWIGAAPLILSRIVG	135-149	AVKVMDLPTQEPALG
15-29	IGAAPLILSRIVGGW	137-151	KVMDLPTQEPALGTT
17-31	AAPLILSRIVGGWEC	139-153	MDLPTQEPALGTTCY
19-33	PLILSRIVGGWECEK	141-155	LPTQEPALGTTCYAS

21-35	ILSRIVGGWECEKHS	143-157	TQEPALGTTCYASGW
23-37	SRIVGGWECEKHSQP	145-159	EPALGTTCYASGWGS
25-39	IVGGWECEKHSQPWQ	147-161	ALGTTCYASGWGSIE
27-41	GGWECEKHSQPWQVL	149-163	GTTCYASGWGSIEPE
29-43	WECEKHSQPWQVLVA	151-165	TCYASGWGSIEPEEF
31-45	CEKHSQPWQVLVASR	153-167	YASGWGSIEPEEFLT
33-47	KHSQPWQVLVASRGR	155-169	SGWGSIEPEEFLTPK
35-49	SQPWQVLVASRGRAV	157-171	WGSIEPEEFLTPKKL
37-51	PWQVLVASRGRAVCG	159-173	SIEPEEFLTPKKLQC
39-53	QVLVASRGRAVCGGV	161-175	EPEEFLTPKKLQCVD
41-55	LVASRGRAVCGGVLV	163-177	EEFLTPKKLQCVDLH
43-57	ASRGRAVCGGVLVHP	165-179	FLTPKKLQCVDLHVI
45-59	RGRAVCGGVLVHPQW	167-181	TPKKLQCVDLHVISN
47-61	RAVCGGVLVHPQWVL	169-183	KKLQCVDLHVISNDV
49-63	VCGGVLVHPQWVLTA	171-185	LQCVDLHVISNDVCA
51-65	GGVLVHPQWVLTAAH	173-187	CVDLHVISNDVCAQV
53-67	VLVHPQWVLTAAHCI	175-189	DLHVISNDVCAQVHP
55-69	VHPQWVLTAAHCIRN	177-191	HVISNDVCAQVHPQK
57-71	PQWVLTAAHCIRNKS	179-193	ISNDVCAQVHPQKVT
59-73	WVLTAAHCIRNKSVI	181-195	NDVCAQVHPQKVTKF
61-75	LTAAHCIRNKSVILL	183-197	VCAQVHPQKVTKFML
63-77	AAHCIRNKSVILLGR	185-199	AQVHPQKVTKFMLCA
65-79	HCIRNKSVILLGRHS	187-201	VHPQKVTKFMLCAGR
67-81	IRNKSVILLGRHSLF	189-203	PQKVTKFMLCAGRWT
69-83	NKSVILLGRHSLFHP	191-205	KVTKFMLCAGRWTGG
71-85	SVILLGRHSLFHPED	193-207	TKFMLCAGRWTGGKS
73-87	ILLGRHSLFHPEDTG	195-209	FMLCAGRWTGGKSTC
75-89	LGRHSLFHPEDTGQV	197-211	LCAGRWTGGKSTCSG
77-91	RHSLFHPEDTGQVFQ	199-213	AGRWTGGKSTCSGDS
79-93	SLFHPEDTGQVFQVS	201-215	RWTGGKSTCSGDSGG
81-95	FHPEDTGQVFQVSHS	203-217	TGGKSTCSGDSGGPL
83-97	PEDTGQVFQVSHSFP	205-219	GKSTCSGDSGGPLVC

85-99	DTGQVFQVSHSFPHP	207-221	STCSGDSGGPLVCNG
87-101	GQVFQVSHSFPHPLY	209-223	CSGDSGGPLVCNGVL
89-103	VFQVSHSFPHPLYDM	211-225	GDSGGPLVCNGVLQG
91-105	QVSHSFPHPLYDMSL	213-227	SGGPLVCNGVLQGIT
93-107	SHSFPHPLYDMSLLK	215-229	GPLVCNGVLQGITSW
95-109	SFPHPLYDMSLLKNR	217-231	LVCNGVLQGITSWGS
97-111	PHPLYDMSLLKNRFL	219-233	CNGVLQGITSWGSEP
99-113	PLYDMSLLKNRFLRP	221-235	GVLQGITSWGSEPCA
101-115	YDMSLLKNRFLRPGD	223-237	LQGITSWGSEPCALP
103-117	MSLLKNRFLRPGDDS	225-239	GITSWGSEPCALPER
105-119	LLKNRFLRPGDDSSH	227-241	TSWGSEPCALPERPS
107-121	KNRFLRPGDDSSHDL	229-243	WGSEPCALPERPSLY
109-123	RFLRPGDDSSHDLML	231-245	SEPCALPERPSLYTK
111-125	LRPGDDSSHDLMLLR	233-247	PCALPERPSLYTKVV
113-127	PGDDSSHDLMLLRLS	235-249	ALPERPSLYTKVVHY
115-129	DDSSHDLMLLRLSEP	237-251	PERPSLYTKVVHYRK
117-131	SSHDLMLLRLSEPAE	239-253	RPSLYTKVVHYRKWI
119-133	HDLMLLRLSEPAELT	241-255	SLYTKVVHYRKWIKD
121-135	LMLLRLSEPAELTDA	243-257	YTKVVHYRKWIKDTI
123-137	LLRLSEPAELTDAVK	245-259	KVVHYRKWIKDTIVA
125-139	RLSEPAELTDAVKVM	247-261	VHYRKWIKDTIVANP
127-141	SEPAELTDAVKVMDL	249-261	YRKWIKDTIVANP
		251-261	KWIKDTIVANP

Table 10. PSCA peptide pool: The amino acid position and sequence of fifteen amino acid peptides overlapping by thirteen amino acids from PSCA peptide library is shown.

amino acid no.	PSCA peptide sequence
1-15	MKAVLLALLMAGLAL
3-17	AVLLALLMAGLALQP
5-19	LLALLMAGLALQPGT
7-21	ALLMAGLALQPGTAL
9-23	LMAGLALQPGTALLC
11-25	AGLALQPGTALLCYS
13-27	LALQPGTALLCYSCK
15-29	LQPGTALLCYSCKAQ
17-31	PGTALLCYSCKAQVS
19-33	TALLCYSCKAQVSNE
21-35	LLCYSCKAQVSNEDC
23-37	CYSCKAQVSNEDCLQ
25-39	SCKAQVSNEDCLQVE
27-41	KAQVSNEDCLQVENC
29-43	QVSNEDCLQVENCTQ
31-45	SNEDCLQVENCTQLG
33-47	EDCLQVENCTQLGEQ
35-49	CLQVENCTQLGEQCW
37-51	QVENCTQLGEQCWTA
39-53	ENCTQLGEQCWTARI
41-55	CTQLGEQCWTARIRA
43-57	QLGEQCWTARIRAVG
45-59	GEQCWTARIRAVGLL
47-61	QCWTARIRAVGLLTV
49-63	WTARIRAVGLLTVIS
51-65	ARIRAVGLLTVISKG
53-67	IRAVGLLTVISKGCS
55-69	AVGLLTVISKGCSLN

57-71	GLLTVISKGCSLNCV
59-73	LTVISKGCSLNCVDD
61-75	VISKGCSLNCVDDSQ
63-77	SKGCSLNCVDDSQDY
65-79	GCSLNCVDDSQDYYV
67-81	SLNCVDDSQDYYVGK
69-83	NCVDDSQDYYVGKKN
71-85	VDDSQDYYVGKKNIT
73-87	DSQDYYVGKKNITCC
75-89	QDYYVGKKNITCCDT
77-91	YYVGKKNITCCDTDL
79-93	VGKKNITCCDTDLCN
81-95	KKNITCCDTDLCNAS
83-97	NITCCDTDLCNASGA
85-99	TCCDTDLCNASGAHA
87-101	CDTDLCNASGAHALQ
89-103	TDLCNASGAHALQPA
91-105	LCNASGAHALQPAAA
93-107	NASGAHALQPAAAIL
95-109	SGAHALQPAAAILAL
97-111	AHALQPAAAILALLP
99-113	ALQPAAAILALLPAL
101-115	QPAAAILALLPALGL
103-117	AAAILALLPALGLLL
105-119	AILALLPALGLLLWG
107-121	LALLPALGLLLWGPG
109-123	LLPALGLLLWGPGQL
111-125	PALGLLLWGPGQL

Table 11. IFNγ T cell responses induced by the single antigen (Group 1: Ad-PSMA; Group 2: Ad-PSA; Group 3: Ad-PSCA; Group 4: mix of Ad-PSMA, Ad-PSA and Ad-PSCA) or triple antigen expressing adenovirus vectors (Group 4: Ad-733; Group 6: Ad-

734; Group 7: Ad-735; Group 8: Ad-796 and Group 9: Ad-809) after adenovirus prime with anti-CTLA-4 analyzed by ELISPOT assay.

Response to PSMA peptides		animal ID							
		1	2	3	4	5	6	7	8
Group No.	1	2356	988	1505	335	501	2145	NA	NA
	2	342	1776	154	329	158	438	321	NA
	3	0	1276	40	126	20	0	NA	NA
	4	304	1198	774	2007	1277	1310	1159	2774
	5	943	2670	2757	780	1082	2251	1566	544
	6	472	2092	4248	1369	1760	2964	1447	263
	7	2161	2202	939	869	3513	1654	3424	900
	8	1166	799	2566	663	1043	497	1334	560
	9	1621	3247	2031	980	2942	1882	1918	3805

Response to PSA peptides		animal ID							
		1	2	3	4	5	6	7	8
Group No.	1	0	0	0	48	0	42	NA	NA
	2	1419	1426	298	1223	1346	1120	1694	NA
	3	6	462	91	0	77	0	NA	NA
	4	790	1093	1611	790	186	783	2016	1964
	5	101	510	955	665	336	1512	1052	119
	6	236	673	2155	724	504	1600	930	83
	7	0	1086	494	663	2265	117	1712	84
	8	1893	2060	1490	1759	2352	1700	2232	1326
	9	1193	1432	207	1738	1886	949	492	1940

Response to PSCA peptides		animal ID							
		1	2	3	4	5	6	7	8
Group No.	1	795	425	874	1069	219	203	NA	NA
	2	669	713	391	199	164	560	461	NA

3	510	1234	1099	1115	1194	339	NA	NA
4	778	528	680	1101	165	531	1175	1009
5	378	1061	1161	143	71	756	766	204
6	118	380	1190	403	829	1225	148	261
7	615	1141	794	564	1175	490	856	204
8	968	1136	745	290	550	976	955	841
9	929	434	1150	745	1120	246	1195	970

Table 12. IFNγ T cell responses induced by the single antigen (Group 1: PSMA; Group 2: PSA; Group 3: PSCA; Group 4: mix of PSMA, PSA and PSCA) or triple antigen expressing vectors (Groups 5 - 9) after adenovirus prime with anti-CTLA-4 and DNA electroporation boost immunizations analyzed by ELISPOT assay.

Response to PSMA peptides		animal ID							
		1	2	3	4	5	6	7	8
Group No.	1	1327	1535	1643	535	1506	1267	NA	NA
	2	15	266	26	191	10	46	1305	NA
	3	0	445	5	75	4	6	NA	NA
	4	365	675	731	1134	244	714	999	1683
	5	270	1623	2254	626	860	2245	1453	1046
	6	541	1151	2923	1094	1061	1746	691	489
	7	1183	1183	1453	1649	2844	1470	2321	991
	8	486	69	399	216	351	758	416	1389
	9	1430	2631	2015	475	1368	1826	1851	3141

Response to PSA peptides		animal ID							
		1	2	3	4	5	6	7	8
Group No.	1	0	0	0	1	0	26	NA	NA
	2	1883	1236	1574	393	461	941	1565	NA
	3	33	30	9	13	8	11	NA	NA

	4	571	1129	1180	210	88	274	924	360
	5	50	1255	1344	628	210	638	948	1161
	6	88	228	1390	489	1006	908	683	51
	7	0	211	321	156	1509	56	199	85
	8	414	611	85	105	544	1080	331	1883
	9	434	821	556	343	1160	510	144	1115

Response to PSCA peptides		animal ID							
		1	2	3	4	5	6	7	8
Group No.	1	615	799	533	74	258	61	NA	NA
	2	194	170	133	133	8	66	405	NA
	3	819	1071	873	839	1045	724	NA	NA
	4	543	506	664	470	70	673	761	1235
	5	154	455	1218	109	218	1094	285	569
	6	56	293	603	506	745	911	63	165
	7	429	298	939	589	1226	263	803	451
	8	279	214	871	61	144	511	193	963
	9	379	191	1196	73	699	198	616	836

EXAMPLE 11. CONSTRUCTION OF C68 VECTORS

11A. Vector AdC68-734 Construciton

AdC68-734 is a replication incompetent adenovirus vector based upon the chimpanzee adenovirus C68 that encodes three immunogenic PAA polypeptides – an immunogenic PSA polypeptide, immunogenic PSCA polypeptide, and immunogenic PSMA polypeptide. The vector sequence was designed *in silico*. First, the baseline full length C68 sequence was obtained from Genbank (Definition: Simian adenovirus 25, complete genome; accession number AC_000011.1). Five point mutations described in the literature were introduced into the sequence. (Roshorm, Y., M. G. Cottingham, et al. (2012). "T cells induced by recombinant chimpanzee adenovirus alone and in prime-boost regimens decrease chimeric EcoHIV/NDK challenge virus load." *Eur J Immunol* 42(12): 3243-3255) Next, 2.6 kilobases of the viral early transcription region 1 (E1) were

deleted to render the vector replication incompetent, and 3.5 kilobases of the early transcription region 3 (E3) were removed to create space in the vector for the transgene expression cassette. (Tatsis, N., L. Tesema, et al. (2006). Chimpanzee-origin adenovirus vectors as vaccine carriers. *Gene Ther.* 13: 421-429) A highly efficient eukaryotic expression cassette was then introduced into the E1 region. The expression cassette included the following components: (A) Cytomegalovirus (CMV) immediate early enhancer/promoter, (B) Tet operator (binding site for the tetracycline repressor), (C) the multi-antigen construct comprising (1) nucleotide sequence encoding amino acids 25 through 261 of the human PSA, (2) Cis acting hydrolase element encoding a glycine-serine linker and Thosea asigna virus 2A peptide (T2A), (3) nucleotide sequence encoding amino acids 2 through 123 of the human PSCA, (4) Cis acting hydrolase element encoding a glycine-serine linker and Foot and Mouth Disease Virus 2A peptide (F2A), and (5) nucleotide sequence encoding amino acids 15 through 750 the human PSMA, and (D) SV40 polyA transcription termination signal. Finally, PacI restriction sites were inserted at each end of the viral genome to facilitate the release of the genome from the parent Bacmid. Nucleotides from the PacI restriction sites are removed during viral propagation and, therefore, are not incorporated into the genome of the vector product itself. A nucleotid sequence of the entire vector AdC68-734, including the PacI restriction sites, is set forth in SEQ ID NO:58. The multi-antigen construct (PSA-T2A-PSCA-F2A-PSMA) incorporated in vector AdC68-734 (as well as in Plasmid 458) is also set forth in SEQ ID NO:61. The amino acid sequence encoded by the multi-agtigen construct of SEQ ID NO:61 is set forth in SEq ID NO:60. The components of vector AdC68-734 are provided in Table 13.

Table 13. Components of Vector AdC68-734

Base Numbers	Feature
1-8	PacI restriction site
9-463	Bases 1-455 of AC000011.1 (SEQ ID NO:57)
464-1096	CMV enhancer/promoter
1031-1070	Tetracycline operator / repressor binding site
1106-1825	Sequence encoding amino acids 25 through 261 of the human PSA and the preceding methionine-alanine-serine linker
1826-1831	Linker encoding glycine - serine

1832-1885	Cis acting hydrolase element encoding a Thosea asigna virus 2A peptide
1886-2257	Sequence encoding amino acids 2 through 123 of the human PSCA and the preceding alanine-serine linker
2258-2263	Linker encoding glycine - serine
2264-2323	Cis acting hydrolase element encoding a Foot and Mouth Disease Virus 2A peptide
2324-4543	Sequence encoding amino acids 15 through 750 of the human PSMA and the preceding methionine-alanine-serine linker
4541-4543	Stop codon
4596-4823	SV40 polyA transcription termination signal
4824-29622	Bases 3013-27811 of AC000011.1 (SEQ ID NO:57)
29623-34811	Bases 31331-36519 of AC000011.1 (SEQ ID NO:57)
10730	C to G substitution at base 8919 of AC000011.1 (SEQ ID NO:57)
17569	G to C substitution at base 15758 of AC000011.1 (SEQ ID NO:57)
18967	A to T substitution at base 17156 of AC000011.1 (SEQ ID NO:57)
19245	C to A substitution at base 17434 of AC000011.1 (SEQ ID NO:57)
33520	G to C substitution at base 35228 of AC000011.1 (SEQ ID NO:57)
34812-34819	PacI restriction site

Following in silico design, the 34,819 base-pair sequence was biochemically synthesized in a multi-stage process utilizing in vitro oligo synthesis and subsequent recombination-mediated intermediate assembly in E. coli and yeast. The viral genome was ultimately inserted into a bacterial artificial chromosome (pCC1BAC-LCyeast-TRP Trunc) for propagation. Next generation sequencing (MiSeq technology) was performed at multiple steps in the production process, including the final Bacmid 17.3.3.22 lot that was used to create the viral seed stock. Viral seed stock was generated by digesting

Bacmid 17.3.3.22 with PacI to release the AdC68-734 genome from the BAC backbone. The linearized nucleic acid was transfected into an E1 complimenting adherent HEK293 cell line and upon visible cytopathic effects and adenovirus foci formation, cultures were harvested by multiple rounds of freezing/thawing to release virus from the cells. Viruses were amplified and purified by standard techniques. The genetic organization of Bacmid 17.3.3.22 is provided in Figure 11.

11B. Constructions of Additonal C68 Vectors

Additional triple antigen C68 vectors were constructed in a similar fashion to AdC68-734. Some of the additional vectors involve functional deletions in the C68 genome that are slightly different from those in Vector AdC68-734, while others incorporate different multi-antigen constructs. Based on these examples and other description of the presentdisclosure, a person skilled in the art would be able construct additional vectors from C68 for expressing vrious multi-antigen constructs, all of which are within the scope of the present invention.

(1) AdC68X-734 and AdC68W-734

Vector AdC68X-734 was constructed from C68 by functional deletion of the E1 and E3 regions of the C68 genome through deletions of nucleotides 577-3403 (E1 region) and 27125– 31831 (E2 region) of the C68 genome of SEQ ID NO:57 and by insertion of the triple antigen construct (PSA-T2A-PSCA-F2A-PSMA) of SEQ ID NO:61 in the deleted E1 region. Vector AdC68W-734 is identical to Vector vector AdC68-734 except that AdC68W-734 contains one or more mutitions in the C68 NDA sequence.

(2) AdC68X-733 and AdC68X-735

Vectors AdC68X-733 and AdC68X-735 were created by replacing the triple antige-construct incorporated in the AdC68X-734 vector with the triple antigen construct of SEQ ID NOs:65 and 66, respectively. The multi-antigen construct incorporated in vector AdC68X-733 (i.e, PSA-F2A-PSMA-T2A-PSCA) is the same as that incorporated in Plasmid 457 and the multi-antigen construct incorporated in vector AdC68X-735 (i.e., PSCA-F2A-PSMA-mIRES-PSA) is the same as that in Plasmid 459.

11C. Research Productivity Characterization

Various research grade lots of AdC68-734 were produced and tested for productivity. Bacmid was digested with PacI to release the vector genome from the BAC backbone and the linearized nucleic acid was transfected into E1 complimenting

adherent HEK293 cell lines. When extensive cytopathic effects and adenovirus foci were visible, cultures were harvested by multiple rounds of freezing/thawing to release virus from the cells. Viruses from these Passage 0 (P0) cultures were amplified at least one additional passage in tissue culture flasks and then used as seed stocks for research scale production runs (~0.5 to 3e13 total viral particles per lot). In total, 11 production runs were executed (five in HEK293 suspension cells and six in HEK293 adherent cells). The average specific productivity was 15,000 +/- 6,000 viral particles purified per initial infected cell, with a viral particle:infectious unit ratio of 55. Research scale productivities are summarized in Table 14.

Table 14. Specific productivities and infectivities of research scale production lots

Lot	Specific productivity (purified viral particles / cell)	Viral particle : infectious unit ratio
20039	17000	33
20424	19000	49
20542	12000	76
20609	25000	54
20626	16000	58
20671	19000	ND
130502	17000	51
130718*	3500	52
130820	7400	55
130821	9300	70
130822	19000	54

* Late passage HEK293 suspension cells used in production

11D. Antigen Expression

The surface expression of PSMA and PSCA was measured by flow cytometry (Figure 12) and total cellular expression of PSMA, PSCA and PSA was measured by western blot analysis (Figure 13) from AdC68-vector infected A549 cells at an MOI=10,000. Mock and AdC68 infected cells were stained with anti-PSCA (fluorescein isothiocyanate-conjugated monoclonal antibody 1G8 [1:200]) and PSMA antibodies (allophycocyanin- conjugated monoclonal antibody J591 [1:200]) for flow cytometric

analysis, 2 days post infection. Surface expression of PSCA and PSMA were detected from majority of the cells infected with the different triple antigen-expressing AdC68 vectors with varying levels. Relatively higher levels of expression of PSCA and PSMA were detected from AdC68X-809 infected cells and lower levels were detected from AdC68X-733 infected cell.Two days after infection, total cellular lysates from approximately $1x10^5$ infected cells were loaded onto each lane of a sodium dodecyl sulfate polyacrylamide gel. The gel was subsequently transferred to a membrane for the detection of PSA, PSMA, and PSCA proteins using primary antibodies specific to PSA, PSMA, and PSCA by western blot analysis. The expressions of all three antigens were detected in the infected cells to varying degrees. While relatively similar levels of PSMA and PSCA were detected from AdC68-734 and AdC68X-735 infected lysates, higher levels of PSA were detected from AdC68-734 lysates compared to those from AdC68X-735

11E. Immunogenicity

A head-to head comparison of the CD8 IFNγ responses induced by various triple antigen AdC68 vectors was performed. Each group of mice (n=5 per group) was immunized with AdC68-734, AdC68X-735, AdC68X-809, or Ad5-734 at 1e9 or 1e10 VP in the quadriceps. IFNγ CD8+ T cell responses in the mice were measured by collecting the spleens from each animal on day 13 post immunization. Splenocytes were isolated and subjected to an IFNγ ELISPOT assay to measure the PSMA, PSCA, and PSA-specific T cell responses. Briefly, 2.5 to $5x10^5$ splenocytes from immunized animals were cultured in the presence of individual human PSMA, PSCA, or PSA-specific peptides at 10μg/ml. The 15-mer peptides were previously defined to contain CD8+ T cell epitopes to each prostate antigen. Splenocytes cultured with medium alone served as a control. Each condition was performed in triplicate. The plates were incubated for 20h at 37°C and 5% CO_2, washed, and developed after incubation as per the manufacturer's instructions. The number of IFNγ SFC was counted by a CTL reader. The results show the average number of PSMA, PSCA, and PSA-specific SFCs with the medium alone background values subtracted, and normalized to $1x10^6$ splenocytes.

In summary, all triple antigen expressing AdC68 vectors induced immune responses to all three antigens but to different magnitude. At 1e9 VP, the response to PSMA by the AdC68 vectors was similar to Ad5. The response to PSCA by the three AdC68 vectors was similar or lower than the response induced by Ad5 while the

response to PSA was lower with Ad68-735 compared to all of the vectors tested. However at 1e10VP, AdC68-809 induced similar or better responses to all three antigens compared to AdC68-734, AdC68-735 or Ad5. Resutls are presented in Table 15.

Table 15. IFNγ T cellular Immunogenicity by AdC68 vectors co-expressing PSMA, PSA and PSCA in C57BL6 mice by IFNγ ELISPOT assay

Construct	Ad5-734		AdC68-734		AdC68-809		AdC68-735	
Titer, vp	1e9	1e10	1e9	1e10	1e9	1e10	1e9	1e10
PSMA	473	1221	699	296	489	684	288	503
	491	831	143	513	221	687	203	261
	435	740	149	607	315	809	256	745
	248	596	224	116	347	317	317	1197
	709	711	269	681	296	536	320	368
PSA	1299	1472	1180	1741	1973	1979	533	695
	939	1025	1327	1985	841	1532	313	1615
	1096	797	672	780	1869	1979	277	1420
	989	933	904	635	1009	1669	535	616
	1971	1047	1309	1901	907	1920	824	403
PSCA	104	64	228	61	115	197	148	92
	160	80	11	41	59	92	80	897
	163	52	15	116	25	235	47	39
	119	223	32	57	24	96	107	33
	207	100	8	53	17	35	32	16

SELECT RAW SEQUENCES

SEQ ID NO: 1. AMINO ACID SEQUENCE OF THE FULL LENGTH HUMAN PSMA

MWNLLHETDSAVATARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNITPKHNM
KAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKEFGLDSVELAHYDVLLS
YPNKTHPNYISIINEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSPQGMPEGDLVYVN
YARTEDFFKLERDMKINCSGKIVIARYGKVFRGNKVKNAQLAGAKGVILYSDPADYFAP
GVKSYPDGWNLPGGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPV
HPIGYYDAQKLLEKMGGSAPPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTNE
VTRIYNVIGTLRGAVEPDRYVILGGHRDSWVFGGIDPQSGAAVVHEIVRSFGTLKKEGW
RPRRTILFASWDAEEFGLLGSTEWAEENSRLLQERGVAYINADSSIEGNYTLRVDCTPL
MYSLVHNLTKELKSPDEGFEGKSLYESWTKKSPSPEFSGMPRISKLGSGNDFEVFFQR
LGIASGRARYTKNWETNKFSGYPLYHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVF
ELANSIVLPFDCRDYAVVLRKYADKIYSISMKHPQEMKTYSVSFDSLFSAVKNFTEIASK
FSERLQDFDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGES
FPGIYDALFDIESKVDPSKAWGEVKRQIYVAAFTVQAAAETLSEVA

SEQ ID NO: 2. NUCLEOTIDE SEQUENCE ENCODING THE FULL LENGTH HUMAN PSMA OF SEQ ID NO: 1

atgtggaatctccttcacgaaaccgactcggctgtggccaccgcgcgccgcccgcgctggctgtgcgctggggcgctggt
gctggcgggtggcttctttctcctcggcttcctcttcgggtggtttataaaatcctccaatgaagctactaacattactccaaagc
ataatatgaaagcatttttggatgaattgaaagctgagaacatcaagaagttcttatataatttttacacagataccacatttag
caggaacagaacaaaactttcagcttgcaaagcaaattcaatcccagtggaaagaatttggcctggattctgttgagctag
cacattatgatgtcctgttgtcctacccaaataagactcatcccaactacatctcaataattaatgaagatggaaatgagatttt
caacacatcattatttgaaccacctcctccaggatatgaaaatgtttcggatattgtaccacctttcagtgctttctctcctcaag
gaatgccagagggcgatctagtgtatgttaactatgcacgaactgaagacttctttaaattggaacgggacatgaaaatca
attgctctgggaaaattgtaattgccagatatgggaaagttttcagaggaaataaggttaaaaatgcccagctggcagggg
ccaaaggagtcattctctactccgaccctgctgactactttgctcctggggtgaagtcctatccagatggttggaatcttcctgg
aggtggtgtccagcgtggaaatatcctaaatctgaatggtgcaggagaccctctcacaccaggttacccagcaaatgaat
atgcttataggcgtggaattgcagaggctgttggtcttccaagtattcctgttcatccaattggatactatgatgcacagaagct
cctagaaaaaatgggtggctcagcaccaccagatagcagctggagaggaagtctcaaagtgccctacaatgttggacct
ggctttactggaaacttttctacacaaaaagtcaagatgcacatccactctaccaatgaagtgacaagaatttacaatgtgat
aggtactctcagaggagcagtggaaccagacagatatgtcattctgggaggtcaccgggactcatgggtgtttggtggtatt
gaccctcagagtggagcagctgttgttcatgaaattgtgaggagctttggaacactgaaaaaggaagggtggagacctag

aagaacaattttgtttgcaagctgggatgcagaagaatttggtcttcttggttctactgagtgggcagaggagaattcaagac
tccttcaagagcgtggcgtggcttatattaatgctgactcatctatagaaggaaactacactctgagagttgattgtacaccgc
tgatgtacagcttggtacacaacctaacaaaagagctgaaaagccctgatgaaggctttgaaggcaaatctctttatgaaa
gttggactaaaaaaagtccttccccagagttcagtggcatgcccaggataagcaaattgggatctggaaatgattttgaggt
gttcttccaacgacttggaattgcttcaggcagagcacggtatactaaaaattgggaaacaaacaaattcagcggctatcc
actgtatcacagtgtctatgaaacatatgagttggtggaaaagttttatgatccaatgtttaaatatcacctcactgtggcccag
gttcgaggagggatggtgtttgagctagccaattccatagtgctccctttgattgtcgagattatgctgtagttttaagaaagtat
gctgacaaaatctacagtatttctatgaaacatccacaggaaatgaagacatacagtgtatcatttgattcacttttttctgcag
taaagaattttacagaaattgcttccaagttcagtgagagactccaggactttgacaaaagcaacccaatagtattaagaat
gatgaatgatcaactcatgtttctggaaagagcatttattgatccattagggttaccagacaggccttttataggcatgtcatct
atgctccaagcagccacaacaagtatgcaggggagtcattcccaggaatttatgatgctctgtttgatattgaaagcaaagt
ggacccttccaaggcctggggagaagtgaagagacagatttatgttgcagccttcacagtgcaggcagctgcagagactt
tgagtgaagtagcc

SEQ ID NO: 3. AMINO ACID SEQUENCE OF PSMA SHUFFLED ANTIGEN 1

SEQ ID NO: 4. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE OF PSMA SHUFFLED ANTIGEN 1 OF SEQ ID NO: 3

SEQ ID NO: 5. AMINO ACID SEQUENCE OF PSMA SHUFFLED ANTIGEN 2

SEQ ID NO: 6. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE OF PSMA SHUFFLED ANTIGEN 2 OF SEQ ID NO:5

SEQ ID NO: 7. AMINO ACID SEQUENCE OF PSMA SHUFFLED ANTIGEN 3

SEQ ID NO: 8. NUCLEOTIDE SEQEUNCE ENCODING AMINO ACID SEQUENCE OF PSMA SHUFFLED ANTIGEN 3 OF SEQ ID NO:7

SEQ ID NO: 9. AMINO ACID SEQUENCE OF A MEMBRANE-BOUND PSMA ANTIGEN

MASARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNITPKHNMKAFLDELKAENI
KKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKEFGLDSVELAHYDVLLSYPNKTHPNYISI
INEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSPQGMPEGDLVYVNYARTEDFFKLE
RDMKINCSGKIVIARYGKVFRGNKVKNAQLAGAKGVILYSDPADYFAPGVKSYPDGWN
LPGGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPIGYYDAQKLL
EKMGGSAPPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTNEVTRIYNVIGTLRG
AVEPDRYVILGGHRDSWVFGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWD
AEEFGLLGSTEWAEENSRLLQERGVAYINADSSIEGNYTLRVDCTPLMYSLVHNLTKEL
KSPDEGFEGKSLYESWTKKSPSPEFSGMPRISKLGSGNDFEVFFQRLGIASGRARYTK

NWETNKFSGYPLYHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELANSIVLPFDC
RDYAVVLRKYADKIYSISMKHPQEMKTYSVSFDSLFSAVKNFTEIASKFSERLQDFDKS
NPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIYDALFDIE
SKVDPSKAWGEVKRQIYVAAFTVQAAAETLSEVA

SEQ ID NO: 10. NUCLEOTIDE SEQEUNCE ENCODING AMINO ACID SEQUENCE OF THE MEMBRANE-BOUND PSMA ANTIGEN OF SEQ ID NO: 9

atggctagcgcgcgccgcccgcgctggctgtgcgctggggcgctggtgctggcgggtggcttctttctcctcggcttcctcttc
gggtggtttataaaatcctccaatgaagctactaacattactccaaagcataatatgaaagcatttttggatgaattgaaagct
gagaacatcaagaagttcttatataatttacacagataccacatttagcaggaacagaacaaaactttcagcttgcaaag
caaattcaatcccagtggaaagaatttggcctggattctgttgagctggcacattatgatgtcctgttgtcctacccaaataag
actcatcccaactacatctcaataattaatgaagatggaaatgagattttcaacacatcattatttgaaccacctcctccagg
atatgaaaatgtttcggatattgtaccacctttcagtgctttctctcctcaaggaatgccagagggcgatctagtgtatgttaact
atgcacgaactgaagacttctttaaattggaacgggacatgaaaatcaattgctctgggaaaattgtaattgccagatatgg
gaaagttttcagaggaaataaggttaaaaatgcccagctggcaggggccaaaggagtcattctctactccgaccctgctg
actactttgctcctggggtgaagtcctatccagatggttggaatcttcctggaggtggtgtccagcgtggaaatatcctaaatct
gaatggtgcaggagaccctctcacaccaggttacccagcaaatgaatatgcttataggcgtggaattgcagaggctgttgg
tcttccaagtattcctgttcatccaattggatactatgatgcacagaagctcctagaaaaaatgggtggctcagcaccacca
gatagcagctggagaggaagtctcaaagtgccctacaatgttggacctggctttactggaaacttttctacacaaaaagtca
agatgcacatccactctaccaatgaagtgacaagaatttacaatgtgataggtactctcagaggagcagtggaaccagac
agatatgtcattctgggaggtcaccgggactcatgggtgtttggtggtattgaccctcagagtggagcagctgttgttcatgaa
attgtgaggagctttggaacactgaaaaaggaagggtggagacctagaagaacaattttgtttgcaagctgggatgcaga
agaatttggtcttcttggttctactgagtgggcagaggagaattcaagactccttcaagagcgtggcgtggcttatattaatgct
gactcatctatagaaggaaactacactctgagagttgattgtacaccgctgatgtacagcttggtacacaacctaacaaaa
gagctgaaaagccctgatgaaggctttgaaggcaaatctctttatgaaagttggactaaaaaaagtccttccccagagttc
agtggcatgcccaggataagcaaattgggatctggaaatgattttgaggtgttcttccaacgacttggaattgcttcaggcag
agcacggtatactaaaaattgggaaacaaacaaattcagcggctatccactgtatcacagtgtctatgaaacatatgagtt
ggtggaaaagttttatgatccaatgtttaaatatcacctcactgtggcccaggttcgaggagggatggtgtttgagctggcca
attccatagtgctccctttgattgtcgagattatgctgtagttttaagaaagtatgctgacaaaatctacagtatttctatgaaac
atccacaggaaatgaagacatacagtgtatcatttgattcacttttttctgcagtaaagaattttacagaaattgcttccaagttc
agtgagagactccaggactttgacaaaagcaacccaatagtattaagaatgatgaatgatcaactcatgtttctggaaaga
gcatttattgatccattagggttaccagacaggccttttataggcatgtcatctatgctccaagcagccacaacaagtatgca
ggggagtcattcccaggaatttatgatgctctgtttgatattgaaagcaaagtggacccttccaaggcctggggagaagtga
agagacagatttatgttgcagccttcacagtgcaggcagctgcagagactttgagtgaagtagcc

SEQ ID NO: 11. AMINO ACID SEQUENCE OF A CYTOSOLIC PSMA ANTIGEN

SEQ ID NO: 12. NUCLEOTIDE SEQEUNCE ENCODING AMINO ACID SEQUENCE OF THE CYTOSOLIC PSMA ANTIGEN OF SEQ ID NO: 11

SEQ ID NO: 13. AMINO ACID SEQUENCE OF A SECRETED PSMA ANTIGEN

SEQ ID NO: 14. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE OF THE SECRETED PSMA ANTIGEN OF SEQ ID NO:13

SEQ ID NO: 15. AMINO ACID SEQUENCE OF THE FULL LENGTH HUMAN PSA

MASWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLV
HPQWVLTAAHCIRNKSVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRP
GDDSSHDLMLLRLSEPAELTDAVKVMDLPTQEPALGTTCYASGWGSIEPEEFLTPKKL
QCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCSGDSGGPLVCNGVLQGITS
WGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

SEQ ID NO: 16. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE OF THE FULL LENGTH HUMAN PSA OF SEQ ID NO: 15

atggctagctgggtcccggttgtcttcctcaccctgtccgtgacgtggattggcgctgcgcccctcatcctgtctcggattgtgg
gaggctgggagtgcgagaagcattcccaaccctggcaggtgcttgtggcctctcgtggcagggcagtctgcggcggtgtt
ctggtgcacccccagtgggtcctcacagctgcccactgcatcaggaacaaaagcgtgatcttgctgggtcggcacagctt
gtttcatcctgaagacacaggccaggtatttcaggtcagccacagcttcccacacccgctctacgatatgagcctcctgaag
aatcgattcctcaggccaggtgatgactccagccacgacctcatgctgctccgcctgtcagagcctgccgagctcacggat
gctgtgaaggtcatggacctgcccacccaggagccagcactggggaccacctgctacgcctcaggctggggcagcatt
gaaccagaggagttcttgaccccaaagaaacttcagtgtgtggacctccatgttatttccaatgacgtgtgtgcgcaagttca
ccctcagaaggtgaccaagttcatgctgtgtgctggacgctggacaggggcaaaagcacctgctcgggtgattctgggg
gcccacttgtctgtaatggtgtgcttcaaggtatcacgtcatggggcagtgaaccatgtgccctgcccgaaaggccttccctg
tacaccaaggtggtgcattaccggaagtggatcaaggacaccatcgtggccaacccc

SEQ ID NO: 17. AMINO ACID SEQUENCE OF A CYTOSOLIC PSA ANTIGEN

MASIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGRH
SLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAV
KVMDLPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTK
FMLCAGRWTGGKSTCSGDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYR
KWIKDTIVANP

SEQ ID NO: 18. NUCLEOTIDE SEQEUNCE ENCODING AMINO ACID SEQUENCE OF THE CYTOSOLIC PSA ANTIGEN OF SEQ ID NO: 17

atggctagcattgtgggaggctgggagtgcgagaagcattcccaaccctggcaggtgcttgtggcctctcgtggcagggc
agtctgcggcggtgttctggtgcacccccagtgggtcctcacagctgcccactgcatcaggaacaaaagcgtgatcttgct
gggtcggcacagcttgtttcatcctgaagacacaggccaggtatttcaggtcagccacagcttcccacacccgctctacgat
atgagcctcctgaagaatcgattcctcaggccaggtgatgactccagccacgacctcatgctgctccgcctgtcagagcct
gccgagctcacggatgctgtgaaggtcatggacctgcccacccaggagccagcactggggaccacctgctacgcctca
ggctggggcagcattgaaccagaggagttcttgaccccaaagaaacttcagtgtgtggacctccatgttatttccaatgacg
tgtgtgcgcaagttcaccctcagaaggtgaccaagttcatgctgtgtgctggacgctggacaggggcaaaagcacctgc
tcgggtgattctgggggcccacttgtctgtaatggtgtgcttcaaggtatcacgtcatggggcagtgaaccatgtgccctgcc
cgaaaggccttccctgtacaccaaggtggtgcattaccggaagtggatcaaggacaccatcgtggccaacccc

SEQ ID NO: 19. AMINO ACID SEQUENCE OF A MEMBRANE-BOUND PSA ANTIGEN

MASARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNITPGIVGGWECEKHSQP
WQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGRHSLFHPEDTGQVFQVSH
SFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMDLPTQEPALGTTC
YASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTC
SGDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

SEQ ID NO:20. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE OF THE MEMBRANE-BOUND PSA ANTIGEN OF SEQ ID NO:19

atggctagcgcgccgcccgcgctggctgtgcgctggggcgctggtgctggcgggtggcttctttctcctcggcttcctcttc
gggtggtttataaaatcctccaatgaagctactaacattactccaggaattgtgggaggctgggagtgcgagaagcattcc
caaccctggcaggtgcttgtggcctctcgtggcagggcagtctgcggcggtgttctggtgcacccccagtgggtcctcaca
gctgcccactgcatcaggaacaaaagcgtgatcttgctgggtcggcacagcttgtttcatcctgaagacacaggccaggta
tttcaggtcagccacagcttcccacacccgctctacgatatgagcctcctgaagaatcgattcctcaggccaggtgatgact
ccagccacgacctcatgctgctccgcctgtcagagcctgccgagctcacggatgctgtgaaggtcatggacctgcccacc
caggagccagcactggggaccacctgctacgcctcaggctggggcagcattgaaccagaggagttcttgaccccaaag
aaacttcagtgtgtggacctccatgttatttccaatgacgtgtgtgcgcaagttcaccctcagaaggtgaccaagttcatgctg
tgtgctggacgctggacaggggcaaaagcacctgctcgggtgattctgggggcccacttgtctgtaatggtgtgcttcaag
gtatcacgtcatggggcagtgaaccatgtgccctgcccgaaaggccttccctgtacaccaaggtggtgcattaccggaagt
ggatcaaggacaccatcgtggccaacccctga

SEQ ID NO: 21. AMINO ACID SEQUENCE OF THE FULL LENGTH HUMAN PSCA

MASKAVLLALLMAGLALQPGTALLCYSCKAQVSNEDCLQVENCTQLGEQCWTARIRAV
GLLTVISKGCSLNCVDDSQDYYVGKKNITCCDTDLCNASGAHALQPAAAILALLPALGLL
LWGPGQL

SEQ ID NO: 22. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE OF THE FULL LENGTH HUMAN PSCA OF SEQ ID NO: 21

atggctagcaaggctgtgctgcttgccctgttgatggcaggcttggccctgcagccaggcactgccctgctgtgctactcctg
caaagcccaggtgagcaacgaggactgcctgcaggtggagaactgcacccagctgggggagcagtgctggaccgcg
cgcatccgcgcagttggcctcctgaccgtcatcagcaaaggctgcagcttgaactgcgtggatgactcacaggactacta
cgtgggcaagaagaacatcacgtgctgtgacaccgacttgtgcaacgccagcggggcccatgccctgcagccggctgc
cgccatccttgcgctgctccctgcactcggcctgctgctctggggacccggccagcta

SEQ ID NO:23. NUCLEOTIDE SEQUENCE OF PLASMID 5166

SEQ ID NO:24. NUCLEOTIDE SEQUENCE OF PLASMID 5259

SEQ ID NO:25. NUCLEOTIDE SEQUENCE OF PLASMID 5297

SEQ ID NO:26. NUCLEOTIDE SEQUENCE OF PLASMID 460

SEQ ID NO:27. NUCLEOTIDE SEQUENCE OF PLASMID 451

SEQ ID NO:28. NUCLEOTIDE SEQUENCE OF PLASMID 454

SEQ ID NO:29. NUCLEOTIDE SEQUENCE OF PLASMID 5300

SEQ ID NO:30. NUCLEOTIDE SEQUENCE OF PLASMID 449

SEQ ID NO:31. NUCLEOTIDE SEQUENCE OF PLASMID 603

SEQ ID NO:32. NUCLEOTIDE SEQUENCE OF PLASMID 455

SEQ ID NO:33. NUCLEOTIDE SEQUENCE OF PLASMID 456

SEQ ID NO:34. NUCLEOTIDE SEQUENCE OF PLASMID 457

SEQ ID NO:35. NUCLEOTIDE SEQUENCE OF PLASMID 458

GGCGTAATGCTCTGCCAGTGTTACAACCAATTAACCAATTCTGATTAGAAAAACTCA
TCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTT
GAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATG
GCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTAT
TAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGAC
TGAATCCGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGACTTGTTCAACAGG
CCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCG
TGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAA
CAGGAATCAAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCA
CCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGT

GGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAG
GCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAA
CGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAT
CGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATA
TAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGAGCAAGACGTTTCCCGTT
GAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGGTCGACAA
TATTGGCTATTGGCCATTGCATACGTTGTATCTATATCATAATATGTACATTTATATT
GGCTCATGTCCAATATGACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAG
TAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAA
CTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGT
CAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAAT
GGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATG
CCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATG
CCCAGTACATGACCTTACGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCA
TCGCTATTACCATGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGGATAGCG
GTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGT
TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCCGCCCCGTTG
ACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTT
TAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGA
AGACACCGGGACCGATCCAGCCTCCGCGGCCGGGAACGGTGCATTGGAACGCGG
ATTCCCCGTGCCAAGAGTGACTCACCGTCCGGATCTCAGCAAGCAGGTATGTACTC
TCCAGGGTGGGCCTGGCTTCCCCAGTCAAGACTCCAGGGATTTGAGGGACGCTGT
GGGCTCTTCTCTTACATGTACCTTTTGCTTGCCTCAACCCTGACTATCTTCCAGGTC
AGGATCCCAGAGTCAGGGGTCTGTATTTTCCTGCTGGTGGCTCCAGTTCAGGAACA
GTAAACCCTGCTCCGAATATTGCCTCTCACATCTCGTCAATCTCCGCGAGGACTGG
GGACCCTGTGACGAACATGGCTAGCATTGTGGGAGGCTGGGAGTGCGAGAAGCAT
TCCCAACCCTGGCAGGTGCTTGTGGCCTCTCGTGGCAGGGCAGTCTGCGGCGGT
GTTCTGGTGCACCCCCAGTGGGTCCTCACAGCTGCCCACTGCATCAGGAACAAAA
GCGTGATCTTGCTGGGTCGGCACAGCTTGTTTCATCCTGAAGACACAGGCCAGGT
ATTTCAGGTCAGCCACAGCTTCCCACACCCGCTCTACGATATGAGCCTCCTGAAGA
ATCGATTCCTCAGGCCAGGTGATGACTCCAGCCACGACCTCATGCTGCTCCGCCT
GTCAGAGCCTGCCGAGCTCACGGATGCTGTGAAGGTCATGGACCTGCCCACCCAG
GAGCCAGCACTGGGGACCACCTGCTACGCCTCAGGCTGGGGCAGCATTGAACCA

GAGGAGTTCTTGACCCCAAAGAAACTTCAGTGTGTGGACCTCCATGTTATTTCCAAT
GACGTGTGTGCGCAAGTTCACCCTCAGAAGGTGACCAAGTTCATGCTGTGTGCTG
GACGCTGGACAGGGGGCAAAAGCACCTGCTCGGGTGATTCTGGGGGCCCACTTG
TCTGTAATGGTGTGCTTCAAGGTATCACGTCATGGGGCAGTGAACCATGTGCCCTG
CCCGAAAGGCCTTCCCTGTACACCAAGGTGGTGCATTACCGGAAGTGGATCAAGG
ACACCATCGTGGCCAACCCCGGATCCGAAGGTAGGGGTTCATTATTGACCTGTGG
AGATGTCGAAGAAAACCCAGGACCCGCTAGCAAGGCTGTGCTGCTTGCCCTGTTG
ATGGCAGGCTTGGCCCTGCAGCCAGGCACTGCCCTGCTGTGCTACTCCTGCAAAG
CCCAGGTGAGCAACGAGGACTGCCTGCAGGTGGAGAACTGCACCCAGCTGGGGG
AGCAGTGCTGGACCGCGCGCATCCGCGCAGTTGGCCTCCTGACCGTCATCAGCAA
AGGCTGCAGCTTGAACTGCGTGGATGACTCACAGGACTACTACGTGGGCAAGAAG
AACATCACGTGCTGTGACACCGACTTGTGCAACGCCAGCGGGGCCCATGCCCTGC
AGCCGGCTGCCGCCATCCTTGCGCTGCTCCCTGCACTCGGCCTGCTGCTCTGGG
GACCCGGCCAGCTAGGATCCCAGACCCTGAACTTTGATCTGCTGAAACTGGCAGG
CGATGTGGAAAGCAACCCAGGCCCAATGGCAAGCGCGCGCCGCCCGCGCTGGCT
GTGCGCTGGGGCGCTGGTGCTGGCGGGTGGCTTCTTTCTCCTCGGCTTCCTCTTC
GGGTGGTTTATAAAATCCTCCAATGAAGCTACTAACATTACTCCAAAGCATAATATG
AAAGCATTTTTGGATGAATTGAAAGCTGAGAACATCAAGAAGTTCTTATATAATTTTA
CACAGATACCACATTTAGCAGGAACAGAACAAAACTTTCAGCTTGCAAAGCAAATTC
AATCCCAGTGGAAAGAATTTGGCCTGGATTCTGTTGAGCTGGCACATTATGATGTC
CTGTTGTCCTACCCAAATAAGACTCATCCCAACTACATCTCAATAATTAATGAAGAT
GGAAATGAGATTTTCAACACATCATTATTTGAACCACCTCCTCCAGGATATGAAAAT
GTTTCGGATATTGTACCACCTTTCAGTGCTTTCTCTCCTCAAGGAATGCCAGAGGG
CGATCTAGTGTATGTTAACTATGCACGAACTGAAGACTTCTTTAAATTGGAACGGGA
CATGAAAATCAATTGCTCTGGGAAAATTGTAATTGCCAGATATGGGAAAGTTTTCAG
AGGAAATAAGGTTAAAAATGCCCAGCTGGCAGGGGCCAAAGGAGTCATTCTCTACT
CCGACCCTGCTGACTACTTTGCTCCTGGGGTGAAGTCCTATCCAGATGGTTGGAAT
CTTCCTGGAGGTGGTGTCCAGCGTGGAAATATCCTAAATCTGAATGGTGCAGGAGA
CCCTCTCACACCAGGTTACCCAGCAAATGAATATGCTTATAGGCGTGGAATTGCAG
AGGCTGTTGGTCTTCCAAGTATTCCTGTTCATCCAATTGGATACTATGATGCACAGA
AGCTCCTAGAAAAAATGGGTGGCTCAGCACCACCAGATAGCAGCTGGAGAGGAAG
TCTCAAAGTGCCCTACAATGTTGGACCTGGCTTTACTGGAAACTTTTCTACACAAAA
AGTCAAGATGCACATCCACTCTACCAATGAAGTGACAAGAATTTACAATGTGATAGG

TACTCTCAGAGGAGCAGTGGAACCAGACAGATATGTCATTCTGGGAGGTCACCGG
GACTCATGGGTGTTTGGTGGTATTGACCCTCAGAGTGGAGCAGCTGTTGTTCATGA
AATTGTGAGGAGCTTTGGAACACTGAAAAAGGAAGGGTGGAGACCTAGAAGAACA
ATTTTGTTTGCAAGCTGGGATGCAGAAGAATTTGGTCTTCTTGGTTCTACTGAGTGG
GCAGAGGAGAATTCAAGACTCCTTCAAGAGCGTGGCGTGGCTTATATTAATGCTGA
CTCATCTATAGAAGGAAACTACACTCTGAGAGTTGATTGTACACCGCTGATGTACA
GCTTGGTACACAACCTAACAAAAGAGCTGAAAAGCCCTGATGAAGGCTTTGAAGGC
AAATCTCTTTATGAAAGTTGGACTAAAAAAAGTCCTTCCCCAGAGTTCAGTGGCATG
CCCAGGATAAGCAAATTGGGATCTGGAAATGATTTTGAGGTGTTCTTCCAACGACT
TGGAATTGCTTCAGGCAGAGCACGGTATACTAAAAATTGGGAAACAAACAAATTCA
GCGGCTATCCACTGTATCACAGTGTCTATGAAACATATGAGTTGGTGGAAAAGTTTT
ATGATCCAATGTTTAAATATCACCTCACTGTGGCCCAGGTTCGAGGAGGGATGGTG
TTTGAGCTGGCCAATTCCATAGTGCTCCCTTTTGATTGTCGAGATTATGCTGTAGTT
TTAAGAAAGTATGCTGACAAAATCTACAGTATTTCTATGAAACATCCACAGGAAATG
AAGACATACAGTGTATCATTTGATTCACTTTTTTCTGCAGTAAAGAATTTTACAGAAA
TTGCTTCCAAGTTCAGTGAGAGACTCCAGGACTTTGACAAAAGCAACCCAATAGTA
TTAAGAATGATGAATGATCAACTCATGTTTCTGGAAAGAGCATTTATTGATCCATTA
GGGTTACCAGACAGGCCTTTTTATAGGCATGTCATCTATGCTCCAAGCAGCCACAA
CAAGTATGCAGGGGAGTCATTCCCAGGAATTTATGATGCTCTGTTTGATATTGAAAG
CAAAGTGGACCCTTCCAAGGCCTGGGGAGAAGTGAAGAGACAGATTTATGTTGCA
GCCTTCACAGTGCAGGCAGCTGCAGAGACTTTGAGTGAAGTAGCCTAAAGATCTG
GGCCCTAACAAAACAAAAAGATGGGGTTATTCCCTAAACTTCATGGGTTACGTAATT
GGAAGTTGGGGGACATTGCCACAAGATCATATTGTACAAAAGATCAAACACTGTTTT
AGAAAACTTCCTGTAAACAGGCCTATTGATTGGAAAGTATGTCAAAGGATTGTGGG
TCTTTTGGGCTTTGCTGCTCCATTTACACAATGTGGATATCCTGCCTTAATGCCTTT
GTATGCATGTATACAAGCTAAACAGGCTTTCACTTTCTCGCCAACTTACAAGGCCTT
TCTAAGTAAACAGTACATGAACCTTTACCCCGTTGCTCGGCAACGGCCTGGTCTGT
GCCAAGTGTTTGCTGACGCAACCCCCACTGGCTGGGGCTTGGCCATAGGCCATCA
GCGCATGCGTGGAACCTTTGTGGCTCCTCTGCCGATCCATACTGCGGAACTCCTA
GCCGCTTGTTTTGCTCGCAGCCGGTCTGGAGCAAAGCTCATAGGAACTGACAATTC
TGTCGTCCTCTCGCGGAAATATACATCGTTTCGATCTACGTATGATCTTTTTCCCTC
TGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAAT
AAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGG

AAGGAATTCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTAT
TGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTG
CGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAG
GGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCG
TAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCAT
CACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATA
CCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCG
CTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAG
CTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGT
GTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTC
TTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAA
CAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGG
CCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCC
AGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTG
GTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCT
CAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTC
ACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGAC
AGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCA
TCCATAGTTGCCTGACTC

SEQ ID NO:36. NUCLEOTIDE SEQUENCE OF PLASMID 459

SEQ ID NO:37. NUCLEOTIDE SEQUENCE OF PSHUTTLE IRES

SEQ ID NO:38. Amino acid sequence of Her-2 antigen:

SEQ ID NO:39. Nucleic acid sequence encoding the Her-2 antigen amino acid sequence of SEQ ID NO: 38

SEQ ID NO:40. Amino acid sequence of heavy chain of the anti-CD40 antibody CP870,893:

MDWTWRILFLVAAATGAHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWV
RQAPGQGLEWMGWINPDSGGTNYAQKFQGRVTMTRDTSISTAYMELNRLRSDDTAV
YYCARDQPLGYCTNGVCSYFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYT
CNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGK

EYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPGK.

SEQ ID NO:41. Acid sequence of the light chain of the anti-CD40 antibody CP870,893:

MRLPAQLLGLLLLWFPGSRCDIQMTQSPSSVSASVGDRVTITCRASQGIYSWLAWYQQ
KPGKAPNLLIYTASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANIFPLTF
GGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS
GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC.

SEQ ID NO:42. Acid sequence of the heavy chain of the anti-CTLA-4 antibody Tremelimumab

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDGS
NKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDPRGATLYYYYYGMD
VWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC
VECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGV
EVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPM
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:43. Acid sequence of the light chain of the anti-CTLA-4 antibody Tremelimumab

DIQMTQSPSSLSASVGDRVTITCRASQSINSYLDWYQQKPGKAPKLLIYAASSLQSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPFTFGPGTKVEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:44. Nucleotide sequence of CpG 7909

5' TCGTCGTTTTGTCGTTTTGTCGTT3'

SEQ ID NO:45. Nucleotide sequence of CpG 24555

5' TCGTCGTTTTTCGGTGCTTTT3'

SEQ ID NO:46. Nucleotide sequence of CpG 10103

5' TCGTCGTTTTTCGGTCGTTTT3'

SEQ ID NO:47. Amino acid sequence of eGFP

SEQ ID NO:48. Amino acid sequence of HBV core antigen

SEQ ID NO:49. Amino acid sequence of HBV surface antigen

SEQ ID NO:50. Amino acid sequence of Rhesus PSMA ECD protein:

SEQ ID NO:51. Amino acid sequence of rat Her-2 p66 peptide (H-2d T cell epitope)

SEQ ID NO:52. Amino acid sequence of rat Her-2 p169 peptide (H-2d T cell epitope)

SEQ ID NO:53. Amino acid sequence of HBV core antigen p87 peptide

SEQ ID NO:54. Amino acid sequence of a Rat Her-2 Antigen (rHer-2):

SEQ ID NO:55. Amino Acid Sequence of Rhesus PSMA antigen:

SEQ ID NO:56. Nucleotide sequence encoding the rhesus PSMA antigen of SEQ ID NO: 55"

SEQ ID NO:57. Complete Genome of Simian Adenovirus 25 (C68)

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SEQ ID NO:59: Nucleotide Seqeunce of Preferred EMCV IRES (pIRES)

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CACAACCATG

(The minimal EMCV IRES (mIRES) lacks the underlined 15 nucleotides)

SEQ ID NO: 60. Amino Acid Sequence Comprising an Immunogenic PSA, PSCA, and PSMA Polypeptide (Encoded by by Plasmid 916 and Vectors AdC68-734 and AdC68W-734)

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SEQ ID NO:61. Nucleotide Sequence Encoding the Amino Acid Sequence of SEQ ID NO:60.

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SEQ ID NO:64. Amino Acid Sequence Comprising an Immunogenic PSA, PSMA, and PSCA Polypeptide (Encoded by Plasmid 457 and Vector AdC68X-733)

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SEQ ID NO:65. Nucleotide Sequence Encoding the Amino Acid Sequence of SEQ ID NO:64

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GCACCCAGCTCGGAGAACAATGCTGGACTGCACGGATCCGCGCTGTCGGCCTGCT
GACCGTGATCTCCAAAGGGTGCTCATTGAACTGCGTGGACGATAGCCAGGACTAC
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AGCGCACGCCCTGCAGCCAGCAGCCGCCATTCTGGCCCTGCTTCCGGCCCTGGG
GTTGCTGCTCTGGGGTCCGGGCCAGCTC

SEQ ID NO:66. Nucleotide Sequence of the Multi-antigen Construct (PSCA-F2A-PSMA-mIRES-PSA) Incorporated in Plasmid 459 and Vector AdC68X-735

ATGGCTAGCAAAGCAGTGCTGCTGGCGCTCCTGATGGCTGGACTCGCGCTGCAGC
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TGCACTACCGCAAATGGATTAAAGATACCATCGTCGCAAACCCT

CLAIMS

1. A C68 vector comprising:
(a) a C68 nucleotide sequence; and
(b) a multi-antigen construct of formula (I):
PAA1-SS1-PAA2-SS2-PAA3 (I)
Wherein the C68 nucleotide sequence is the sequence of SEQ ID NO: 57 lacking at least one gene selected from the group consisting of E1A, E1B, E2A, E2B, E3, E4, L1, L2, L3, L4 and L5 genes;
and wherein in formula (I) (1) PAA1, PAA2, and PAA3 each is a nucleotide sequence encoding an immunogenic prostate-specific antigen (PSA) polypeptide comprising amino acids 4-240 of SEQ ID NO:17,
a nucleotide sequence encoding an immunogenic prostate stem cell antigen (PSCA) polypeptide, or a nucleotide sequence encoding an immunogenic prostate-specific membrane antigen (PSMA polypeptide),provided the PAA1, PAA2, and PAA3 encode different polypeptides, and (2) SS1
and SS2 are separator sequences and can be the same or different.

2. The C68 vector according to claim 1, wherein the immunogenic PSCA polypeptide comprises the amino acid sequence of SEQ ID NO:21 or amino acids 4-125 0f SEQ ID NO:21, and wherein the immunogenic PSMA polypeptide is selected from the group consisting of:

1) a polypeptide comprising amino acids 15-750 of SEQ ID NO: 1;
2) a polypeptide comprising the amino acid sequence of SEQ ID NO:3;
3) a polypeptide comprising the amino acid sequence of SEQ ID NO:5;
4) a polypeptide comprising the amino acid sequence of SEQ ID NO:7;
5) a polypeptide comprising the amino acids 4 – 739 of SEQ ID NO:9;
6) a polypeptide comprising the amino acids 4 – 739 of SEQ ID NO:3;
7) a polypeptide comprising the amino acids 4 - 739 of SEQ ID NO:5;
8) a polypeptide comprising the amino acids 4 - 739 of SEQ ID NO:7; and
9) a polypeptide comprising the amino acid sequence of SEQ ID NO: 9.

3. The C68 vector according to claim 2, wherein the C68 nucleotide sequence is the sequence of SEQ ID NO: 57 lacking the genes of E1A, E1B, and E3..

4. The C68 vector according to claim 3, wherein the separator sequences are selected from 2A peptide sequences and internal ribosomal entry sites (IRESs).

5. The C68 vector according to claim 4, wherein the 2A peptide sequence is selected from the group consisting of the 2A-peptide sequence of foot and mouth disease virus (FMDV), equine rhinitis A virus (ERAV), porcine teschovirus-1 (PTV1), encephalomycarditis B variant (EMC-B), encephalomycarditis virus (EMCV), Theiler murine encephalomyelitis GD7 (TME-GD7), equime equine rhinitis B virus (ERBV), thosea asigna virus (TaV), drosophilia C (DrosC), criket paralysis virus (CrPV), acute bee paralysis virus (ABPV), infectious flacherie virus (IFV), Porcine rotavirus, human rotavirus, T brucei TSR1, and T cruzi AP endonuclease; and wherein the IRES is a EMCV IRES.

6. The vector according to claim 5, wherein PAA1 in formula (I) is a nucleotide sequence encoding the immunogenic PSA polypeptide or a nucleotide sequence encoding the immunogenic PSCA polypeptide.

7. The vector according to claim 6, wherein:

(i) PAA1 is a nucleotide sequence encoding the immunogenic PSA polypeptide;

(ii) PAA2 is a nucleotide sequence encoding an immunogenic PSCA or PSMA polypeptide;

(iii) SS1 is a 2A-peptide sequence; and

(iv) SS2 is a 2A-peptide sequence or an EMCV IRES.

8. The C68 vector according to claim 7, wherein the 2A-peptide sequence is the FMDV 2A-peptide sequence or the TAV 2A-peptide sequence.

9. The C68 vector according to claim 8, wherein:

(1) the nucleotide sequence encoding the immunogenic PSA polypeptide is selected from the group consisting of: (i) the nucleotide sequence of SEQ ID NO: 18; (ii) the nucleotide sequence of SEQ ID NO: 20; (iii) the nucleotide sequence comprising nucleotides 10-720 of SEQ ID NO:18; (iv) the nucleotide sequence comprising

nucleotides 1115-1825 of SEQ ID NO:58; and (v) the nucleotide sequence comprising nucleotides 1106-1825 of SEQ ID NO:58;

(2) the nucleotide sequence encoding the immunogenic PSCA polypeptide is selected from the group consisting of: (i) the nucleotide sequence of SEQ ID NO:22; (ii) a nucleotide sequence comprising nucleotides 10- 372 of SEQ ID NO:22; (iii) a nucleotide sequence comprising nucleotides 1892-2257 of SEQ ID NO:58; and (iv) a nucleotide sequence comprising nucleotides 1886-2257 of SEQ ID NO:58; and

(3) the nucleotide sequence encoding the immunogenic PSMA polypeptide is selected from the group consisting of: (i) the nucleotide sequence comprising nucleotides 43-2250 of SEQ ID NO:2; (ii) the nucleotide sequence of SEQ ID NO:4; (iii) the nucleotide sequence of SEQ ID NO:6; (iv) the nucleotide sequence of SEQ ID NO:8; (v) the nucleotide sequence of SEQ ID NO:10; (vi) a nucleotide sequence comprising nucleotides 10 – 2217 of SEQ ID NO:4; (vii) a nucleotide sequence comprising nucleotides 10 – 2217 of SEQ ID NO:6; (viii) a nucleotide sequence comprising nucleotides 10 – 2217 of SEQ ID NO:8; (ix) a nucleotide sequence comprising nucleotides 10 – 2217 of SEQ ID NO:10; (x) a nucleotide sequence comprising nucleotides 2333-4543 of SEQ ID NO:58; and (xi) a nucleotide sequence comprising nucleotides 2324-4543 of SEQ ID NO:58.

10. The C68 vector according to claim 3, wherein in formula (I):

(1) PAA1 is a nucleotide sequence encoding an immunogenic PSA polypeptide and comprises nucleotides 1115-1825 of SEQ ID NO: 58 or comprises 1106-1114 of SEQ ID NO: 58;

(2) PAA2 is a nucleotide sequence encoding an immunogenic PSCA polypeptide and comprises nucleotides 1892-2257 of SEQ ID NO: 58 or comprises 1886-2257 of SEQ ID NO: 58;

(3) PAA3 is a nucleotide sequence encoding an immunogenic PSMA polypeptide and comprises nucleotides 2333-4543 of SEQ ID NO: 58 or comprises 2324-4543 of SEQ ID NO: 58;

(4) SS1 is a nucleotide sequence encoding the TAV 2A peptide ; and

(5) SS2 is a nucleotide sequence encoding the FMDV 2A peptide .

11. The C68 vector according to claim 1, wherein the multi-antigen construct comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:60 or SEQ ID NO:64.

12. The C68 vector according to claim 1, wherein the multi-antigen construct comprises a nucleotide sequence of SEQ ID NO:61, SEQ ID NO:65, SEQ ID NO:66, or a degenerate variant of the nucleotide sequence of SEQ ID NO:61, SEQ ID NO:65, SEQ ID NO:66.

13. The C68 vector according to claim 11 or claim 12, further comprising a CMV promoter.

14. The C68 vector according to claim 1, which comprises the nucleotide sequence of SEQ ID NO:58, nucleotides 9-34811 of SEQ ID NO:58, or the nucleotide sequence of SEQ ID NO:63.

15. A composition comprising a C68 vector according to any one of claims 1-14.

16. A cell comprising a C68 vector according to any one of claims 1-14.

17. A pharmaceutical composition comprising a vector according to any one of claims 1-14 and a pharmaceutically acceptable excipient.

18. A pharmaceutical composition according to claim 17 for use in the treatment of prostate cancer.

19. The composition according to claim 18, wherein an effective amount of an immune modulator is also to be administered during the treatment.

20. The composition according to claim 18, wherein (a) an effective amount of at least one immune- suppressive-cell inhibitor and (b) an effective amount of at least one immune-effector-cell enhancer is also to be administered during the treatment.

21. The composition according to claim 20, wherein the immune-suppressive-cell inhibitor is selected from the group consisting of a protein kinase inhibitor, a COX-2 inhibitor, and a PDE5 inhibitor, and wherein the immune-effector-cell enhancer is selected from the group consisting of a CTLA-4 inhibitor, a CD40 agonist, a TLR agonist, a 4-1BB agonist, a OX40 agonist, a GITR agonist, a PD-1 antagonist, and a PD-L1 antagonist.

22. The composition according to claim 21, wherein:

(1) the protein kinase inhibitor is selected from the group consisting of imatinib, sorafenib, lapatinib, zactima MP-412, dasatinib, lestaurtinib, sunitinib malate, axitinib, erlotinib, gefitinib, bosutinib, temsirolismus, and nilotinib;

(2) the CTLA-4 inhibitor is selected from the group consisting of ipilimumab and tremelimumab;

(3) the CD40 agonist is an anti-CD40 antibody selected from the group consisting of G28-5, mAb89, EA-5 , S2C6, CP870893, and dacetuzumab; and

(4) the TLR agonist is a CpG oligonucleotide selected from the group consisting of CpG 24555, CpG 10103, CpG7909, and CpG1018.

23. The composition according to claim 22, wherein the immune-suppressive-cell inhibitor is a protein kinase inhibitor selected from the group consisting of sorafenib, dasatinib, imatinib, axitinib, and sunitinib malate, and wherein the immune-effector-cell enhancer is tremelimumab.

24. The composition according to claim 22, wherein the immune-suppressive-cell inhibitor is a protein kinase inhibitor selected from the group consisting of sorafenib, dasatinib, imatinib, axitinib, and sunitinib malate, and wherein the immune-effector-cell enhancer is a CpG oligonucleotide selected from the group consisting of CpG24555, CpG10103, CpG7909, and CpG1018.

25. The use of a pharmaceutical composition according to claim 17, in the manufacture of a medicament for the treatment of prostate cancer.

26. The use according to claim 25, wherein an effective amount of an immune

modulator is to be administered as well as the medicament.

27. The use according to claim 25, wherein (a) an effective amount of at least one immune-suppressive-cell inhibitor and (b) an effective amount of at least one immune-effector-cell enhancer is to be administered as well as the medicament.

28. The use according to claim 27, wherein the immune-suppressive-cell inhibitor is selected from the group consisting of a protein kinase inhibitor, a COX-2 inhibitor, and a PDE5 inhibitor, and wherein the immune-effector-cell enhancer is selected from the group consisting of a CTLA-4 inhibitor, a CD40 agonist, a TLR agonist, a 4-1BB agonist, a OX40 agonist, a GITR agonist, a PD-1 antagonist, and a PD-L1 antagonist.

29. The use according to claim 28, wherein:

(1) the protein kinase inhibitor is selected from the group consisting of imatinib, sorafenib, lapatinib, zactima MP-412, dasatinib, lestaurtinib, sunitinib malate, axitinib, erlotinib, gefitinib, bosutinib, temsirolismus, and nilotinib;

(2) the CTLA-4 inhibitor is selected from the group consisting of ipilimumab and tremelimumab;

(3) the CD40 agonist is an anti-CD40 antibody selected from the group consisting of G28-5, mAb89, EA-5 , S2C6, CP870893, and dacetuzumab; and

(4) the TLR agonist is a CpG oligonucleotide selected from the group consisting of CpG 24555, CpG 10103, CpG7909, and CpG1018.

30. The use according to claim 29, wherein the immune-suppressive-cell inhibitor is a protein kinase inhibitor selected from the group consisting of sorafenib, dasatinib, imatinib, axitinib, and sunitinib malate, and wherein the immune-effector-cell enhancer is tremelimumab.

31. The use according to claim 29, wherein the immune-suppressive-cell inhibitor is a protein kinase inhibitor selected from the group consisting of sorafenib, dasatinib, imatinib, axitinib, and sunitinib malate, and wherein the immune-effector-cell enhancer is a CpG oligonucleotide selected from the group consisting of CpG24555, CpG10103, CpG7909, and CpG1018.