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(54) Title: METHODS AND COMPOSITIONS FOR PROSTATE CANCER IMMUNOTHERAPY

(57) Abstract: The present invention features methods and compositions (e.g., immune response stimulating peptides (e.g., ERG or SIM2 peptides), activated immune cells, antigen-presenting cells, and antibodies or antigen-binding fragments thereof) for generating an immune response for the treatment of cancer (e.g., prostate cancer).

METHODS AND COMPOSITIONS FOR PROSTATE CANCER IMMUNOTHERAPY

Field of the Invention

In general, the invention relates to methods and compositions for the treatment of cancer (e.g., prostate cancer).

Background of the Invention

The failure of immunotherapy for the treatment of prostate cancer in clinical trials is partly due to the lack of a causative oncogene antigen target for such therapy. However, the identification of cancer-specific antigens, which are specifically expressed in targeted cancer cells, provides rational targets for cancer immunotherapy. For example, existing vaccines for prostate cancer utilize cell lines without discrete or identified antigens. In some instances, these vaccines include antigenic proteins that are not specific to targeted cancer cells, antigenic proteins that are not immunogenic, or antigenic proteins that are not expressed in sufficient amounts on the surface of the tumor cells, thus reducing the efficacy of these vaccines and potentially resulting in undesirable side effects. Therefore, there is a need in the art for effective, more specific immunotherapies for the treatment of cancer, such as prostate cancer.

Summary of the Invention

The present invention features methods and compositions (e.g., immune response stimulating peptides (e.g., ERG or SIM2 peptides), activated immune cells, antigen-presenting cells, and antibodies or antigen-binding fragments thereof) for generating an immune response for the treatment of cancer (e.g., prostate cancer).

In a first aspect, the invention features an immune response stimulating peptide having at least 90% sequence identity (e.g., 95, 96, 97, 98, or 99% sequence identity) to a contiguous amino acid sequence set forth in SEQ ID NO:1 (ERG2), SEQ ID NO:5 (SIM2), SEQ ID NO:11 (AMACR), SEQ ID NO:12 (BICD1), SEQ ID NO:13 (C10orf137), SEQ ID NO:14 (CDCL6), SEQ ID NO:15 (ICA1), SEQ ID NO:16 (KIAA1661), SEQ ID NO:17 (MAP7), SEQ ID NO:18 (MYO6), SEQ ID NO:19 (OR51E2), SEQ ID NO:20 (PAICS), SEQ ID NO:21 (PCSK6), SEQ ID NO:22 (PVT1), SEQ ID NO:23 (RGS10), SEQ ID NO:24 (SGEF), SEQ ID NO:25 (SMARCA4/// MRPL43), or SEQ ID NO:26 (SS18), wherein the peptide has at least 7 but fewer than 50 amino acid residues (e.g., between 7 and 30, 25, 20, 15, 14, 13, 12, 11, or 10 amino acid residues, preferably 8, 9, or 10 amino acid residues, most preferably 9 amino acid residues) and is capable of activating immune cells in a mammalian host when bound to an antigen-presenting molecule. In another embodiment, the immune response stimulating peptide has the amino acid sequence set forth in SEQ ID NOs: 2, 3, 4, 6, 7, 8, 9, or 10.

In a second aspect, the invention features an activated immune cell (e.g., a cytotoxic T lymphocyte) capable of specifically binding to an immune response stimulating peptide of the first aspect of the invention when the peptide is bound to an antigen-presenting molecule.

In a third aspect, the invention features an antigen-presenting cell having on its surface a peptide of the first aspect of the invention that is bound to an antigen-presenting molecule of an antigen-presenting cell.

In a fourth aspect, the invention features an antibody or antigen-binding fragment thereof that specifically binds to a peptide of the first aspect of the invention when the peptide is bound to an antigen-presenting molecule.

In a fifth aspect, the invention features a method of treating cancer (e.g., prostate cancer) in a subject (e.g., a mammal, such as a human) by administering a composition containing a peptide, activated immune cell, antigen-presenting cell, or antibody or antigen-binding fragment thereof of the first, second, third, and fourth aspects of the invention, respectively, in an

amount sufficient to treat the cancer. The method may include administering an adjuvant, cytokine, or hormone therapy (e.g., androgen ablation). The composition administered to the subject (e.g., a human) may include a pharmaceutically acceptable diluent, excipient, or carrier and may be administered by any means known in the art (e.g., injection).

In a sixth aspect, the invention features a method of diagnosing a subject (e.g., a mammal, such as a human) as having cancer (e.g., prostate cancer) by measuring the level of ERG (e.g., SEQ ID NOs: 1, 2, 3, or 4) or SIM2 polypeptide (e.g., SEQ ID NOs: 5, 6, 7, 8, 9, or 10), or fragment thereof, in a sample from the subject and comparing it to a reference, wherein an alteration (e.g., an increase) in the level of ERG or SIM2 polypeptide compared to a reference is a diagnostic indicator of cancer (e.g., prostate cancer). The sample may be a bodily fluid (e.g., urine, blood, serum, plasma, and cerebrospinal fluid), cell, or tissue sample from a subject in which ERG or SIM2 polypeptide is normally detectable.

In other embodiments of all aspects of the invention, the immune cells of the invention include, for example, T cells (e.g., cytotoxic T lymphocytes); the antigen-presenting molecules are, e.g., histocompatibility molecules (e.g., HLA molecules (e.g., HLA class I molecules)) that may be present on the surface of a cell, e.g., a prostate cancer cell. Antigen-presenting cells contemplated by the invention include, e.g., dendritic cells, macrophages, B cells, monocytes, fibroblasts, thymic epithelial cells, thyroid epithelial cells, glial cells, pancreatic beta cells, and vascular endothelial cells. Any of the agents described by the invention (e.g., immune response stimulating peptides, activated immune cells, antigen-presenting cells, and antibodies or antigen-binding fragments thereof) may be conjugated to a heterologous compound, e.g., a therapeutic agent or cytotoxic agent. In yet other embodiments, the compositions of the invention may be administered as a vaccine and may include, for example, an additional therapeutic agent (e.g., a chemotherapeutic agent) or an adjuvant.

As used herein, "activation of immune cells" is meant an increase in immune cell (e.g., T cells (e.g., cytotoxic T lymphocytes), B cells, macrophages, and NK cells) function, for example, the release of cytokines, antibodies, and/or the induction of apoptosis following stimulation with one or more stimulatory molecules.

By "adjuvant" is meant any substance that is used to specifically or non-specifically potentiate an antigen-specific immune response through, e.g., activation of antigen-presenting cells. Exemplary adjuvants include an oil emulsion (e.g., complete or incomplete Freund's adjuvant), a chemokine, a cytokine, or an ADP-ribosylating exotoxin (bΛRE)). An adjuvant may be administered with an antigen or may be administered by itself. A single molecule may have both adjuvant and antigen properties.

As used herein, by "administering" is meant a method of giving a dosage of a composition of the invention to a subject in need thereof. The compositions described herein can be administered by any acceptable route known in the art and including, e.g., parenteral, dermal, transdermal, ocular, inhalation, buccal, sublingual, perilingual, nasal, rectal, topical, and oral administration. Parenteral administration includes intra-arterial, intravenous, intraperitoneal, subcutaneous, and intramuscular administration. The preferred method of administration can vary depending on various factors (e.g., the components of the composition being administered, the condition being treated and its severity, and the age, weight, and health of the patient).

By "an amount sufficient to treat" is meant the amount of a composition of the invention administered to improve, inhibit, or ameliorate a condition of a subject, or a symptom thereof, in a clinically relevant manner (e.g., improve, inhibit, prevent, or ameliorate prostate cancer or symptoms thereof). Any improvement in the subject is considered sufficient to achieve treatment. Preferably, an amount sufficient to treat is an amount that reduces, inhibits, or prevents the occurrence of one or more symptoms of, e.g., cancer (e.g., prostate cancer) or is an amount that reduces the severity of, or the length of time during which a subject suffers from, one or more symptoms of the cancer (e.g., by at

least 10%, 20%, or 30%, more preferably by at least 50%, 60%, or 70%, and most preferably by at least 80%, 90%, 95%, 99%, or more, relative to a control subject that is not treated with a composition of the invention). A sufficient amount of a composition used to practice the methods described herein varies depending upon the manner of administration and the age, body weight, and general health of the subject being treated. A physician or researcher can decide the appropriate amount and dosage regimen.

The term "antibody," as used herein, includes whole antibodies or immunoglobulins and any antigen-binding fragment or single chains thereof. Antibodies, as used herein, can be mammalian (e.g., human or mouse), humanized, chimeric, recombinant, synthetically produced, or naturally isolated. Antibodies of the present invention include all known forms of antibodies and other protein scaffolds with antibody-like properties. For example, the antibody can be a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, or a protein scaffold with antibody-like properties, such as fibronectin or ankyrin repeats. The antibody also can be a Fab, Fab'2, scFv, SMIP, diabody, nanobody, aptamers, or a domain antibody. The antibody can have any of the following isotypes: IgG (e.g., IgG1, IgG2, IgG3, and IgG4), IgM, IgA (e.g., IgA1, IgA2, and IgAsec), IgD, or IgE.

The term "antigen-binding fragment," as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to a cancer-specific antigen (e.g., a prostate cancer-specific antigen (e.g., ERG or SIM2)). The antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding fragment" of an antibody include, but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L, and C_H1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an

antibody, (v) a dAb including V_H and V_L domains; (vi) a dAb fragment (Ward et al., *Nature* 341:544-546 (1989)), which consists of a V_H domain; (vii) a dAb which consists of a V_H or a V_L domain; (viii) an isolated complementarity determining region (CDR); and (ix) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see, e.g., Bird et al., *Science* 242: 423-426 (1988) and Huston et al., *Proc. Natl. Acad. Sci. USA* 85: 5879-5883 (1988)). These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as intact antibodies. Antigen-binding fragments can be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact immunoglobulins.

By "antigen-presenting cell" is meant a cell that displays an antigen (e.g., a cancer-specific antigen, such as a prostate cancer-specific antigen) complexed with a major histocompatibility complex (MHC) molecule on its surface. In some embodiments, antigen-presenting cells are capable of activating an immune cell (e.g., a T cell) that has not been exposed to an antigen (e.g., a naive T cell). These antigen-presenting cells internalize the antigen (e.g., either by phagocytosis or by receptor-mediated endocytosis) and display a fragment of the antigen bound to an MHC molecule on the cell surface. The immune cell (e.g., T cell) recognizes and interacts with the MHC molecule complex on the surface of the antigen-presenting cell. An additional co-stimulatory signal is then produced by the antigen-presenting cell, leading to activation of the T cell. Antigen-presenting cells include, e.g., dendritic cells, macrophages, B cells, monocytes, fibroblasts, thymic epithelial cells, thyroid epithelial cells, glial cells, pancreatic beta cells, and vascular endothelial cells. Antigen-presenting cells may be isolated from any of a variety of biological fluids, tissues, and organs (e.g., peripheral blood, bone marrow, tumor-

infiltrating cells, peritumoral tissues, infiltrating cells, lymph nodes, spleen, skin, and umbilical cord blood).

As used herein, an "antigen-presenting molecule" refers to a class I or class II major histocompatibility (MHC) molecule (e.g., a human leukocyte antigen (HLA) molecule) or any other molecule capable of binding to an antigen and presenting the antigen on the surface of a cell such that it can be recognized by an immune cell (e.g., a naïve T cell) as a complex of antigen and antigen-presenting molecule, thereby leading to, e.g., activation of the immune cell.

The term "cytotoxic agent," as used herein, refers to a substance that inhibits or prevents one or more functions of cells (e.g., cellular replication, division, or secretion of proteins) or causes apoptosis or necrosis of cells. The term is intended to include radioactive isotopes (e.g., I^{131}, I^{125}, Y^{90}, and Re^{186}), chemotherapeutic agents, and toxins, such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof. Additional cytotoxic agents include, but are not limited to, alkylating agents, antibiotics, antimetabolites, tubulin inhibitors, topoisomerase I and II inhibitors, hormonal agonists or antagonists, or immunomodulators. Cytotoxic agents may be cytotoxic when activated by light or infrared radiation (Photofrin, IR dyes; Nat. Biotechnol. 19(4):327-331, 2001), may operate through other mechanistic pathways, or be supplementary potentiating agents.

The term "immune cells," as used herein, refers to any cell that is involved in the generation, regulation, or effect of the acquired or innate immune system. Immune cells include, e.g., T cells (e.g., $CD4^+$ cells or $CD8^+$ cells), B cells, natural killer (NK) cells, macrophages, monocytes and dendritic cells, and neutrophils.

By "immune response stimulating peptide" is meant a peptide that is a tumor-specific antigen, such as a prostate cancer-specific antigen (e.g., a peptide of ERG or SIM2) that is presented by an antigen-presenting cell histocompatibility molecule (e.g., a major histocompatibility complex molecule (MHC), such as an HLA class I molecule) expressed in, e.g., dendritic cells,

macrophages, monocytes, and B cells; the binding of a naïve immune cell (e.g., a T cell) to the peptide/histocompatibility molecule complex activates an immune cell against the tumor-specific antigen. Such immune response stimulating peptides generally comprise at least 7 amino acid residues, but may comprise up to 50 amino acid residues. Immune response stimulating peptides can generally be identified using well-known techniques including, e.g., screening peptides for the ability to react with antigen-specific antibodies, antisera, or T cell lines or clones. T cell responses to the immune response stimulating peptide may include, e.g., the release of cytokines, increased T cell proliferation, or changes in intracellular calcium concentrations, as known in the art.

By "pharmaceutically acceptable carrier" is meant a diluent, excipient, or adjuvant which is physiologically acceptable to the subject while retaining the therapeutic properties of the composition with which it is administered. One exemplary pharmaceutically acceptable carrier is physiological saline. Other physiologically acceptable diluents, excipients, carriers, or adjuvants and their formulations are known to one skilled in the art.

By "proliferative disease" or "cancer" is meant any condition characterized by abnormal or unregulated cell growth. An example of a proliferative disease is, e.g., prostate cancer. Other exemplary cancers include solid tumors such as: sarcomas (e.g., clear cell sarcoma), carcinomas (e.g., renal cell carcinoma), and lymphomas; tumors of the breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, bilecyst, bile duct, small intestine, urinary system (including the kidney, bladder, and epithelium of the urinary tract), female genital system (including the uterine neck, uterus, ovary, chorioma, and gestational trophoblast), male genital system (including the seminal vesicle and testicles), endocrine glands (including the thyroid gland, adrenal gland, and pituitary body), skin (including angioma, melanoma, sarcoma originating from bone or soft tissue, and Kaposi's sarcoma), brain and meninges (including astrocytoma, neuroastrocytoma, spongioblastoma, retinoblastoma, neuroma, neuroblastoma, neurinoma and

neuroblastoma), nerves, eyes, hemopoietic system (including chloroleukemia, plasmacytoma and dermal T lymphoma/leukemia), and immune system (including lymphoma, e.g., Hodgkin's lymphoma and non-Hodgkin's lymphoma). An example of a non-solid tumor proliferative disease is leukemia (e.g., acute lymphoblastic leukemia).

By "protein," "polypeptide," or "peptide" is meant any chain of more than two amino acids, regardless of post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally occurring polypeptide or peptide, or constituting a non-naturally occurring polypeptide or peptide. A polypeptide or peptide may be said to be "isolated" or "substantially pure" when physical, mechanical, or chemical methods have been employed to remove the polypeptide from cellular constituents. An "isolated polypeptide or peptide," "substantially pure polypeptide or peptide," or "substantially pure and isolated polypeptide or peptide" is typically considered removed from cellular constituents and substantially pure when it is at least 60% by weight free from the proteins and naturally occurring organic molecules with which it is naturally associated. Preferably, the polypeptide or peptide is at least 75%, more preferably at least 90%, and most preferably at least 99% by weight pure. A substantially pure polypeptide or peptide may be obtained by standard techniques, for example, by extraction from a natural source (e.g., cell lines), by expression of a recombinant nucleic acid encoding the polypeptide, or by chemically synthesizing the polypeptide or peptide. Purity can be measured by any appropriate method, e.g., by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. Alternatively, a polypeptide or peptide is considered isolated if it has been altered by human intervention, placed in a location that is not its natural site, or if it is introduced into one or more cells.

By "specifically bind" is meant the preferential association of a binding moiety (e.g., an antibody, histocompatibility molecule, antigen-binding fragment, receptor, ligand, small molecule, or a cell (e.g., an immune cell)) to a target molecule (e.g., an antigen (or fragment thereof), cytokine, chemokine,

hormone, receptor, antigen/MHC complex, or ligand) or to a cell or tissue bearing the target molecule (e.g., a cell surface antigen, a receptor, and a ligand) and not to non-target cells or tissues lacking the target molecule. In the context of an antigen/MHC complex, the term "specifically bind" applies, e.g., to the preferential association between the antigen/MHC complex and a receptor (e.g., a T cell receptor) on a naïve immune cell, such as a T cell. It is recognized that a certain degree of non-specific interaction may occur between a binding moiety and a non-target molecule (present alone or in combination with a cell or tissue). Nevertheless, specific binding may be distinguished as mediated through specific recognition of the target molecule or complex. Specific binding results in a much stronger association between the binding moiety (e.g., an antibody or antigen-binding fragment) and, e.g., cells bearing the target molecule (e.g., an antigen or antigen/MHC complex) than between the binding moiety (e.g., an antibody or immune cell) and, e.g., cells lacking the target molecule. Specific binding typically results in greater than 2-fold, preferably greater than 5-fold, more preferably greater than 10-fold and most preferably greater than 100-fold increase in the amount of bound binding moiety (per unit time) to, e.g., a cell or tissue bearing the target molecule as compared to a cell or tissue lacking that target molecule. Binding moieties bind to the target molecule with a dissociation constant of, e.g., less than 10^{-6} M, more preferably less than 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M, and most preferably less than 10^{-13} M, 10^{-14} M, or 10^{-15} M. Specific binding to a target molecule, e.g., a protein or peptide (e.g., an antigen) under such conditions requires a binding moiety that is selected for its specificity for that particular target molecule. A variety of assay formats are appropriate for selecting binding moieties (e.g., antibodies or immune cells) capable of specifically binding to a particular target molecule (e.g., an antigen or antigen/MHC complex), and vice versa. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow & Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, 1988, for a description

of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

By "subject" is meant any animal, e.g., a mammal (e.g., a human). A subject to be treated according to the methods described herein (e.g., a subject diagnosed with cancer (e.g., prostate cancer)) may be one who has been diagnosed by a medical practitioner as having such a condition or one at risk for developing the condition (e.g., cancer (e.g., prostate cancer)). Diagnosis may be performed by any suitable means. One skilled in the art will understand that a subject to be treated according to the present invention may have been subjected to standard tests or may have been identified, without examination, as one at high risk due to the presence of one or more risk factors (e.g., elevated prostate specific antigen (PSA) or a history of cancer).

The term "substantial identity" or "substantially identical," when used in the context of comparing a polynucleotide or polypeptide sequence to a reference sequence, means that the polynucleotide or polypeptide sequence is the same as the reference sequence or has a specified percentage of nucleotides or amino acid residues that are the same at the corresponding locations within the reference sequence when the two sequences are optimally aligned. For instance, an amino acid sequence that is "substantially identical" to a reference sequence has at least about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher percentage identity (up to 100%) to the reference sequence (e.g., the full-length amino acid sequence of ERG or SIM2 as set forth in SEQ ID NOs:11 or 12, respectively, or a fragment thereof), when compared and aligned for maximum correspondence over the full length of the reference sequence as measured using BLAST or BLAST 2.0 sequence comparison algorithms with default parameters, or by manual alignment and visual inspection (see, e.g., NCBI website).

The term "vaccine," as used herein, is defined as a composition used to provoke an immune response and confer immunity, at least briefly, after administration of the composition to a subject.

Other features and advantages of the invention will be apparent from the detailed description and from the claims.

Brief Description of the Drawings

Figure 1 is a Venn diagram highlighting the genes overexpressed in prostate cancer in our data set and in the Stanford data set and those underexpressed in extraprostatic human adult male tissues as deduced from the Novartis Gene Expression Atlas.

Figure 2 is a series of graphs showing qRT-PCR validation of mRNA expression levels of individual genes (AMACR, BICD1, C10orf137, CDC2L6, ICA1, KIAA1661, MAP7, MYO6, OR51E2, PAICS, PCSK6, PVT1, RGS10, SGEF, and SIM2). qRT-PCR validation was performed using the TaqMan® gene expression assay. Only the 15 genes that were significantly overexpressed ($P < .05$) in seven prostate cancer specimens compared to eight normal prostate specimens are shown here.

Figures 3A-3B are graphs showing that ERG epitopes bind human HLA-A2.1 and induce cytotoxic T lymphocytes (CTLs). Binding of predicted peptides to HLA-A2.1 was assessed using the assembly assay on T2 cells (Figure 3A). Out of the 12 peptides tested, eight showed high-binding ability, as compared to a non-binding peptide (Ctrl). The rate of dissociation of peptides from HLA-A2.1 was determined by monitoring the decrease in HLA-A2.1 expression over time after incubation with binder peptides. Immunization of HHD mice with the eight peptides revealed three immunogenic ERG peptides, two of which are shown in Figure 3B.

Figures 4A-4B show the methods and results of microarray experiments using nucleic acid programmable protein arrays (NAPPA). Figure 4A is a schematic representation of NAPPA. Figure 4B shows microarray data of autoantibodies to ACPP, AMACR, BRD2, ERG, and ETV1 by screening one prostate cancer serum against 800 tumor-associated antigens (TAAs).

Figure 5 is a bar graph showing that SIM2 elicits spontaneous humoral responses in prostate cancer patients. Sera from nine prostate cancer patients

and five healthy donors were subjected to an *in vitro*, cell-free protein expression-based ELISA to detect autoantibodies to SIM2. SIM2 was expressed as a GST-tagged protein, and an anti-GST-coated plate was used in the assay. Serum antibodies that bound to immobilized SIM2 were detected using a labeled anti-human antibody. In each assay, wells containing a GST-expressing vector were used as a negative control. Signals obtained from GST wells were subtracted from those obtained from wells that contained GST-SIM2. Three experiments were performed with triplicate wells for each serum sample per experiment. Statistics were performed on the triplicates' mean and SD, and antibody amounts were plotted as the difference of OD signals produced by GST-SIM2 and GST alone.

Figures 6A-6B are graphs showing that SIM2 harbors HLA-A2.1-restricted immunogenic epitopes. The binding to and rate of dissociation of peptides from HLA-A2.1 was determined by monitoring the decrease in HLA-A2.1 expression over time after incubation with binder peptides (Figure 6A). Immunization of A2.1 transgenic HHD mice with the nine binding peptides revealed five immunogenic SIM2 peptides, as demonstrated by an IFN-γ ELISPOT assay (Figure 6B).

Figures 7A-7B are bar graphs showing that androgen suppression attenuates prostate-specific tolerance. Hybrid A2.1/PSA (males, castrated males, or females) transgenic and A2.1 mice were immunized with vac-prostate specific antigen (PSA), and splenocytes were restimulated with PSA protein-loaded dendritic cells and tested by ELISPOT (Figure 7A) or PSA-tetramers (Figure 7B).

Figures 8A-8B are bar graphs showing qRT-PCR quantitation of ERG and SIM2 in Vcap (Figure 8A) and LNCap cell lines (Figure 8B), respectively, with increasing doses of androgen.

Figures 9A-9D are bar graphs showing the increased generation of regulatory T lymphocytes (Tregs) in mice with prostate cancer and the effect of anti-Tim-1 agonist antibody. Figures 9A and 9B describe sorted naïve CD4 and CD8 T cells from splenocytes of TRAMP and control mice cultured with

anti-CD3 and anti-CD28 Abs in the presence of TGF-β. The percentage of newly differentiated Tregs was determined by flow cytometry after three days of culture. Figures 9C and 9D show that treatment with an agonist anti-Tim-1 antibody at the time of immunization enhances the CTL response to Tag antigen in B6 mice, as evidenced by an increased number of Tag-specific CTLs.

Figure 10 is a bar graph showing that anti-Tim-1Ab treatment elicits enhanced CTL responses to Tag in TRAMP mice ($P < .01$ versus an isotype control).

Figure 11 is the AMACR amino acid sequence (SEQ ID NO:11).

Figure 12 is the BICD1 amino acid sequence (SEQ ID NO:12).

Figure 13 is the C10orf137 amino acid sequence (SEQ ID NO:13).

Figure 14 is the CDC2L6 amino acid sequence (SEQ ID NO:14).

Figure 15 is the ICA1 amino acid sequence (SEQ ID NO:15).

Figure 16 is the KIAA1661 amino acid sequence (SEQ ID NO:16).

Figure 17 is the MAP7 amino acid sequence (SEQ ID NO:17).

Figure 18 is the MYO6 amino acid sequence (SEQ ID NO:18).

Figure 19 is the OR51E2 amino acid sequence (SEQ ID NO:19).

Figure 20 is the PAICS amino acid sequence (SEQ ID NO:20).

Figure 21 is the PCSK6 amino acid sequence (SEQ ID NO:21).

Figure 22 is the PVT1 nucleic acid sequence (SEQ ID NO:22).

Figure 23 is the RGS10 amino acid sequence (SEQ ID NO:23).

Figure 24 is the SGEF amino acid sequence (SEQ ID NO:24).

Figure 25 is the SMARCA4///MRPL43 nucleic acid sequence (SEQ ID NO:25).

Figure 26 is the SS18 amino acid sequence (SEQ ID NO:26).

Figure 27 is the ERG amino acid sequence (SEQ ID NO:1).

Figure 28 is the SIM2 amino acid sequence (SEQ ID NO:5).

Detailed Description

It was recently discovered that the ERG transcription factor is overexpressed in prostate cancer cells and not expressed in non-cancerous prostate cells. We hypothesized that, because normal tissues do not express ERG, immune tolerance to ERG could be overcome and that immune responses could be generated against ERG. We identified ERG-derived, immune response stimulating peptides that can be presented to human immune cells (e.g., naïve T cells) via, e.g., the human HLA-A2.1 antigen-presenting molecule that is found on the surface of all cells in the majority of humans. In addition, we identified immune response stimulating peptides from the protein SIM2, another protein overexpressed in prostate cancer cells and not expressed in non-cancerous prostate cells that may serve as an immunotherapy against prostate cancer. Our studies have also identified 16 additional proteins overexpressed in prostate cancer cells, peptides of which can also be used in immunotherapy.

Accordingly, the present invention features methods and compositions (e.g., immune response stimulating peptides (e.g., ERG or SIM2 peptides), activated immune cells, antigen-presenting cells, and antibodies or antigen-binding fragments thereof) for generating an immune response for the treatment of cancer (e.g., prostate cancer).

ERG, SIM2, and Other Prostate Tumor-Associated Antigens

An immune response stimulating peptide that is a tumor-specific antigen, such as a prostate cancer-specific antigen (e.g., a peptide of ERG or SIM2) that is presented by an antigen-presenting cell histocompatibility molecule (e.g., a major histocompatibility complex molecule (MHC), such as HLA class I) expressed in, e.g., dendritic cells, macrophages, monocytes, and B cells; the binding of a naïve immune cell (e.g., a T cell) to the peptide/histocompatibility molecule complex activates an immune cell against the tumor-specific antigen. Such immune response stimulating peptides generally contain at least 7 amino acid residues but fewer than 50 amino acid

residues. For example, the immune response stimulating peptide may include, e.g., 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 49 amino acid residues. Non-limiting examples of immune response stimulating peptides for use in the treatment of prostate cancer are described in Table 1.

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Table 1

Peptide Name	Peptide Sequence
ERG(157) (SEQ ID NO:2)	GLPDVNILL
ERG(295) (SEQ ID NO:3)	QLWQFLLEL
ERG(412) (SEQ ID NO:4)	FVAPHPPAL
SIM2(87) (SEQ ID NO:6)	TLDGFVFVV
SIM2(205) (SEQ ID NO:7)	YQIVGLVAV
SIM2(237) (SEQ ID NO:8)	SLDLKLIFL
SIM2(241) (SEQ ID NO:9)	KLIFLDSRV
SIM2(244) (SEQ ID NO:10)	FLDSRVTEV
SEQ ID NO:27	YGLPDVNILL
SEQ ID NO:28	GLPDVNILLF
SEQ ID NO:29	YGLPDVNILLF
SEQ ID NO:30	EYGLPDVNILL
SEQ ID NO:31	GLPDVNILLFG
SEQ ID NO:32	EYGLPDVNILLFG
SEQ ID NO:33	IQLWQFLLEL
SEQ ID NO:34	QLWQFLLELS
SEQ ID NO:35	IQLWQFLLELS
SEQ ID NO:36	QIQLWQFLLEL
SEQ ID NO:37	QLWQFLLELSD
SEQ ID NO:38	QIQLWQFLLELSD

SEQ ID NO:39	NFVAPHPPAL
SEQ ID NO:40	FVAPHPPALP
SEQ ID NO:41	NFVAPHPPALP
SEQ ID NO:42	MNFVAPHPPAL
SEQ ID NO:43	FVAPHPPALPV
SEQ ID NO:44	MNFVAPHPPALPV
SEQ ID NO:45	QTLDGFVFVV
SEQ ID NO:46	TLDGFVFVVA
SEQ ID NO:47	QTLDGFVFVVA
SEQ ID NO:48	LQTLDGFVFVV
SEQ ID NO:49	TLDGFVFVVAS
SEQ ID NO:50	LQTLDGFVFVVAS
SEQ ID NO:51	CYQIVGLVAV
SEQ ID NO:52	YQIVGLVAVG
SEQ ID NO:53	CYQIVGLVAVG
SEQ ID NO:54	SCYQIVGLVAV
SEQ ID NO:55	YQIVGLVAVGQ
SEQ ID NO:56	SCYQIVGLVAVGQ
SEQ ID NO:57	ASLDLKLIFL
SEQ ID NO:58	SLDLKLIFLD
SEQ ID NO:59	ASLDLKLIFLD
SEQ ID NO:60	RASLDLKLIFL
SEQ ID NO:61	SLDLKLIFLDS
SEQ ID NO:62	RASLDLKLIFLDS
SEQ ID NO:63	LKLIFLDSRV
SEQ ID NO:64	KLIFLDSRVT

SEQ ID NO:65	LKLIFLDSRVT
SEQ ID NO:66	DLKLIFLDSRV
SEQ ID NO:67	KLIFLDSRVTE
SEQ ID NO:68	DLKLIFLDSRVTE
SEQ ID NO:69	IFLDSRVTEV
SEQ ID NO:70	FLDSRVTEVT
SEQ ID NO:71	IFLDSRVTEVT
SEQ ID NO:72	LIFLDSRVTEV
SEQ ID NO:73	FLDSRVTEVTG
SEQ ID NO:74	LIFLDSRVTEVTG
SEQ ID NO:75	ALPDVNILL
SEQ ID NO:76	QLWQFVLEL
SEQ ID NO:77	FVAPHPPGL
SEQ ID NO:78	TLDGFLFVV
SEQ ID NO:79	YQIVALVAV
SEQ ID NO:80	SLDVKLIFL
SEQ ID NO:81	KLIYLDSRV
SEQ ID NO:82	FLDTRVTEV

Immune response stimulating peptides may be identified using well-known techniques, such as those described in Paul, W. E. (ed.), Fundamental Immunology, 3rd ed., pages 243-247 (Raven Press, 1993), hereby incorporated by reference. Such techniques include, e.g., screening polypeptides for the ability to react with antigen-specific antibodies, antisera, MHC molecules (e.g., HLA class I molecules), and/or T cell lines or clones. T cell responses to the immune response stimulating peptide may include, e.g., the release of cytokines, increased T cell proliferation, or changes in intracellular calcium concentrations, as known in the art. Activated T cells can now target cells

expressing the peptide and can cause apoptosis or necrosis due to, e.g., changes in cytokine expression.

Immune response stimulating peptides, as described herein, can be produced by chemical synthesis using, for example, Merrifield solid phase synthesis, solution phase synthesis, or a combination of both (see, for example, the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., 1984, The Pierce Chemical Co., Rockford, IL, hereby incorporated by reference). Immune response stimulating peptides may then be condensed by standard peptide assembly chemistry. The peptides of the present invention may also be obtained by biological or genetic engineering processes (e.g., recombinant production in bacteria, mammalian cells, such as CHO cells, or in transgenic animals). For example, an expression vector, known to one of skill in the art, may be used that includes a polynucleotide sequence encoding the peptide of interest; the expression vector can be incorporated into a cell and the peptide encoded thereby can be expressed in the cell.

Peptides having the desired activity may be modified as necessary to provide certain desired attributes, e.g., improved pharmacological characteristics, while increasing or at least retaining substantially all of the biological activity of the unmodified peptide to bind the desired antigen-presenting molecule and activate the appropriate immune cell. For example, the immune response stimulating peptides may be subject to various changes, such as, e.g., substitutions (either conservative or non-conservative), deletions, or insertions, wherein such changes might provide for certain advantages in their use (e.g., improved binding to antigen-presenting molecules). Conservative substitutions may include, e.g., replacing an amino acid residue with another residue that is biologically and/or chemically similar (e.g., one hydrophobic residue for another or one polar residue for another). The substitutions include combinations such as Gly, Ala; Val, Ile, Leu, Met; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. The effect of single amino acid substitutions may also be probed using D-amino acids. Such modifications may be made using well-known peptide synthesis procedures, as

described in, e.g., Merrifield, *Science* 232: 341-347 (1986), Barany and Merrifield, The Peptides, Gross and Meienhofer, eds., 1979, pages 1-284, Academic Press, New York.

The immune response stimulating peptides may also be modified by lengthening or shortening the amino acid sequence of the peptide, e.g., by the addition or deletion of amino acids. The peptides may also be modified by altering the order or composition of certain residues, though certain amino acid residues essential for biological activity, e.g., those at critical contact sites or conserved residues, may generally not be altered without an adverse effect on biological activity. The non-critical amino acids need not be limited to those naturally occurring in proteins, such as L-α-amino acids (or their D-isomers), but may include non-natural amino acids as well, such as β-γ-δ-amino acids, as well as many derivatives of L-α-amino acids.

Modifications of the immune response stimulating peptides with various amino acid residue mimetics or unnatural amino acids are particularly useful in increasing the stability of the peptide *in vivo*.

The immune response stimulating peptides of the present invention may be modified to provide desired attributes other than improved half-life *in vivo*. For example, the ability of the peptides to induce immune cell (e.g., cytotoxic T lymphocyte) activity may be enhanced by linkage to a sequence that contains at least one epitope that is capable of inducing, e.g., a T helper cell response. Exemplary epitopes include the non-structural protein from influenzae virus, NS1 (hemaglutinin) and the tetanus toxoid. Alternatively, the epitope can be selected so as to increase the solubility of the peptide or to enable the peptide to be targeted to desired intracellular compartments. The peptide may also be conjugated to any known cytotoxic or therapeutic moiety known to treat, inhibit, reduce, or ameliorate disease (e.g., prostate cancer)

In one embodiment, the immune response stimulating peptides of the invention activate immune cells in a subject when bound to an antigen-presenting molecule. An antigen-presenting molecule is a class I or class II major histocompatibility (MHC) molecule (e.g., a human leukocyte antigen

(HLA) molecule) or any other molecule capable of binding to an antigen that presents the antigen on the surface of a cell and is recognized by cell(s) of the immune system as a complex of antigen and antigen-presenting molecule. Exemplary HLA molecules include, without limitation, HLA-A1 (A*0101); HLA-A2 (A*0206); HLA-A2 (A*0201); HLA-A2 (A*0207); HLA-A2 (A*02011); HLA-A3 (A*0301); HLA-A11 (A*11011); HLA-A24 (A*24021); HLA-A24 (A*2420); HLA-A26 (A*2601); HLA-A26 (A*2603); HLA-A31 (A*31012); HLA-A33 (A*3303); HLA-B7 (B*07021); HLA-B8 (B*0801); HLA-B15 (B*15011); HLA-B35 (B*35011); HLA-B38 (B*3801); HLA-B39 (B*39011); HLA-B40 (B*40012); HLA-B40 (B*4002); HLA-B44 (B*4401); HLA-B44 (B*44031); HLA-B46 (B*4601); HLA-B48 (B*4801); HLA-B51 (B*51011); HLA-B52 (B*52011); HLA-B54 (B*5401); HLA-B55 (B*5502); HLA-B59 (B*5901); HLA-Cw1 (Cw*0102); HLA-Cw1 (Cw*0103); HLA-Cw3 (Cw*03031); HLA-Cw3 (Cw*03041); HLA-Cw4 (Cw*04011); HLA-Cw6 (Cw*0602); HLA-Cw7 (Cw*0702); HLA-Cw8 (Cw*0801); HLA-Cw12 (Cw*12022); HLA-Cw14 (Cw*14021); HLA-Cw14 (Cw*1403); HLA-Cw15 (Cw*15021); HLA-Cx 52 (Cw 12) (Cw*1201); HLA-Cx52 (Cw12) (Cw*1201). Human Class II HLA alleles include, without limitation, HLA-DA alpha 1-4 (pDA alpha 1-4); HLA-DA alpha 1-5 (pDA alpha 1-5); HLA-DA beta 5 (pDA beta 5); HLA-DC alpha 107 (pDC alpha 107); HLA-DO alpha 20 (pDO alpha 20); HLA-DQ beta155 (pDQ beta155); HLA-DR alpha 11 (pDR alpha 11); HLA-DR beta 134 (pDR beta 134); HLA DR beta 5 (TOK H5 DR beta); HLA-DR beta 4 (YT158); HLA-DQA1 (pgDQ4A); HLA-DQB1 (pg DQ1B); HLA-DQB1 (pg DQ1BS); HLA-DRA (DRA2EH); HLA-DPA1 (DPA 02022); HLA-DPB1 (DPB0202); HLA-DRB1 (K b DRB10803); HLA-DRB1 (K b DRB11201); HLA-DRB1 (K b DRB11302); HLA-DRB3 (DRB30301 EMJ-4); HLA-DQA1 (DQA10501 AMALA-4); HLA-DQB1 (DQB10301 AMALA-4); HLA-DQA1 (DQA10101 KAS1163-6); and HLA-DQB1 (DQB10503 EK2-4).

Activated Immune Cells

The present invention features activated immune cells capable of specifically binding to an immune response stimulating peptide when the peptide is bound to an antigen-presenting molecule (e.g., an MHC molecule (e.g., an HLA class I molecule)). In one embodiment, activated immune cells (e.g., cytotoxic T cells) may be generated *in vitro* according to the methods described in, e.g., U.S. Patent No. 6,130,087, hereby incorporated by reference. Immune cells (e.g., T cells) may be, e.g., cultured with the antigen presenting cells (e.g., dendritic cells, macrophages, monocytes, and B cells) that present the immune response stimulating peptide in complex with a histocompatibility molecule, thereby activating the immune cells such that the activated immune cells now target cells presenting the immune response stimulating peptide on its surface in a complex with a histocompatibility molecule (e.g., an HLA class I molecule). Other methods for activating immune cells are described in U.S. Patent Nos. 5,928,643, 6,074,635, and 6,210,873, hereby incorporated by reference.

Antigen-Presenting Cells

The present invention features an antigen-presenting cell that includes, on its surface, a complex between an antigen-presenting molecule and any one of the immune response stimulating peptides described herein. Antigen-presenting cells may be prepared, e.g., by contacting a cell having antigen-presenting ability (e.g., dendritic cells, macrophages, B cells, monocytes, fibroblasts, thymic epithelial cells, thyroid epithelial cells, glial cells, pancreatic beta cells, or vascular endothelial cells) with any one of the immune response stimulating peptides described herein. Such cells may be isolated from any of a variety of biological fluids, tissues, and organs (e.g., peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues, infiltrating cells, lymph nodes, spleen, skin, or umbilical cord blood).

Methods for preparing antigen-presenting cells are described in, e.g., U.S. Patent Nos. 6,787,164, hereby incorporated by reference. Briefly,

antigen-presenting cells may be engineered using gene transfer techniques (e.g., by the insertion of one or more recombinant or synthetic nucleic acid sequences encoding the immune response stimulating peptide) such that the peptides are expressed in effective amounts in the recipient host cell. By "effective amount" is meant that expression is sufficient to enable the recipient cell to provoke the desired immune response *in vivo*. For gene transfer into the cells to express the selected molecules, the nucleic acid may be directly introduced *ex vivo* in the form of "naked" nucleic acid, e.g. by microinjection, electroporation, as calcium-phosphate-DNA gels, with DEAE dextran, or in encapsulated form (e.g. in vesicles such as liposomes), or in a suitable viral vector. Expression of recombinant genes of interest after introduction into the antigen-presenting cells is confirmed by, e.g., immunoassays or biological assays for functional activity of the protein product. For example, expression of introduced molecules into cells may be confirmed by detecting the binding of labeled antibodies (specific for the immune response stimulating peptide and) to the antigen-presenting cells using assays such as, e.g., FACS or ELISA. Biological activity of the engineered cells can be verified, for example, in *in vitro* assays and in animal models (e.g., mice or non-human primates) prior to testing in humans. The ability of the engineered cells of the invention to function as desired, e.g. to process and present antigens for activating an immune response, may be tested using *in vitro* or *in vivo* assays. Immune cell activation (e.g., T cell activation) may be detected by various known methods, including measuring changes in the proliferation of immune cells, killing of target cells, and secretion of certain regulatory factors (e.g., lymphokines), expression of mRNA of certain immunoregulatory molecules, or a combination of these events.

As an alternative to gene transfer, the immune response stimulating peptides described herein may be added to antigen-presenting cells in culture and "loaded" on the antigen-presenting cell for presentation of the molecules to, e.g., T cells (see, e.g., Tykocinski et al., *Amer. J. Pathol.* 148: 1-16, 1996, hereby incorporated by reference). Peptide or protein pulsing (e.g., co-

culturing) may also be used (Inaba et al., *J. Exp. Med.* 172: 631-640, 1990). Alternatively, peptides may be introduced to cell surfaces via fusion with liposomes bearing the selected immune response stimulating peptides (Coeshott et al., *J. Immunol.* 134: 1343-1348, 1985).

The antigen-presenting cells may be suspended in any known physiologically compatible pharmaceutical carrier such as cell culture medium, physiological saline, or phosphate-buffered saline to form a physiologically acceptable, aqueous pharmaceutical composition. Parenteral vehicles include, e.g., sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, or lactated Ringer's. The antigen-presenting cells may be introduced into the subject to be treated by using one of a number of methods of administration. For example, the antigen presenting cells may be inoculated (with or without adjuvant) parenterally (including, for example, intravenous, intra-arterial, intraperitoneal, intramuscular, intradermal, and subcutaneous), by ingestion, or applied to mucosal surfaces. Alternatively, the antigen-presenting cells of the invention may be administered locally by direct injection into, e.g., a cancerous lesion or infected tissue.

The antigen-presenting cells of the invention are introduced in at least one dose into a subject in need thereof (e.g., a prostate cancer patient), with sufficient numbers of antigen-presenting cells to activate immune cells of the immune system and induce an immune response, e.g., against the cancer antigen. The cells may be administered in a single infusion containing at least, e.g., 10^6 to 10^{12} cells, or in multiple sequential infusions containing the same number of cells.

Antibodies or Antigen-Binding Fragments

Antibodies or antigen-binding fragments of the invention include, e.g., the IgG, IgA, IgM, IgD, and IgE isotypes. Antibodies or antibody fragments of the invention, as used herein, contain one or more complementarity determining regions (CDR) or binding peptides that bind to target proteins (e.g., a tumor-specific protein, such as a prostate tumor specific protein (e.g., ERG and SIM2) or

an immune response stimulating peptide). In some embodiments, the antibodies or antigen-binding fragments specifically bind to an immune response stimulating peptide when the peptide is bound to an antigen-presenting molecule (e.g., an HLA class I molecule).

Many of the antibodies, or fragments thereof, described herein can undergo non-critical amino-acid substitutions, additions or deletions in both the variable and constant regions without loss of binding specificity or effector functions, or intolerable reduction of binding affinity (e.g., below about 10^{-7} M). Usually, an antibody or antibody fragment incorporating such alterations exhibits substantial sequence identity to a reference antibody or antibody fragment from which it is derived. Occasionally, a mutated antibody or antibody fragment can be selected having the same specificity and increased affinity compared with a reference antibody or antibody fragment from which it was derived. Phage-display technology offers powerful techniques for selecting such antibodies. See, e.g., Dower et al., WO 91/17271 McCafferty et al., WO 92/01047; and Huse, WO 92/06204, hereby incorporated by reference.

Antibody fragments include separate variable heavy chains, variable light chains, Fab, Fab', $F(ab')_2$, Fabc, and scFv. Fragments can be produced by enzymatic or chemical separation of intact immunoglobulins. For example, a $F(ab')_2$ fragment can be obtained from an IgG molecule by proteolytic digestion with pepsin at pH 3.0-3.5 using standard methods such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Pubs., New York, 1988. Fab fragments may be obtained from $F(ab')_2$ fragments by limited reduction, or from whole antibody by digestion with papain in the presence of reducing agents. Fragments can also be produced by recombinant DNA techniques. Segments of nucleic acids encoding selected fragments are produced by digestion of full-length coding sequences with restriction enzymes, or by *de novo* synthesis. Often fragments are expressed in the form of phage-coat fusion proteins. This manner of expression is advantageous for affinity-sharpening of antibodies.

Methods of preparing chimeric and humanized antibodies and antibody fragments are described in, e.g., U.S. Patent Nos. 4,816,567; 5,530,101; 5,622,701; 5,800,815; 5,874,540; 5,914,110; 5,928,904; 6,210,670; 6,677,436; and 7,067,313 and U.S. Patent Application Nos. 2002/0031508; 2004/0265311; and 2005/0226876. Preparation of antibody or antigen-binding fragments thereof is further described in, e.g., U.S. Patent Nos. 6,331,415; 6,818,216; and 7,067,313.

Adjuvants

Suitable adjuvants for compositions of the present invention comprise those adjuvants that are capable of enhancing an immune response to the peptides (e.g., immune response stimulating peptides (e.g., ERG or SIM2 peptides)), antibodies or antigen-binding fragments thereof, activated immune cells, and antigen-presenting cells of the present invention. Adjuvants are well known in the art (see, e.g., *Vaccine Design-The Subunit and Adjuvant Approach*, 1995, Pharmaceutical Biotechnology, Volume 6, Eds. Powell and Newman, Plenum Press, New York and London, hereby incorporated by reference).

Preferred adjuvants for use in the compositions of the present invention include aluminum or calcium salts (e.g., hydroxide or phosphate salts). A desirable adjuvant is an aluminum hydroxide gel such as Alhydrogel™. For aluminum hydroxide gels (alum), the peptides (e.g., immune response stimulating peptides (e.g., ERG or SIM2 peptides)), antibodies or antigen-binding fragments thereof, activated immune cells, and antigen-presenting cells are admixed with the adjuvant so that between 50 to 800 μg of aluminum are present per dose, and preferably, between 400 and 600 μg are present.

Another adjuvant for use in the compositions of the present invention is an emulsion. An emulsion can be an oil-in-water emulsion or a water-in-oil emulsion. In addition to the peptides, antibodies or antigen-binding fragments thereof, activated immune cells, and antigen-presenting cells, such emulsions comprise an oil phase of, e.g., squalene or squalane and a dispersing agent. Non-ionic dispersing agents (e.g., mono- and di-C_{12}-C_{24}-fatty acid esters of

sorbitan and mannide, such as sorbitan mono-stearate, sorbitan mono-oleate, and mannide mono-oleate) may also be used.

Water-in-oil emulsions may include squalene and mannide mono-oleate (Arlacel™ A), optionally with squalane, emulsified in the composition of the invention. Well-known examples of such emulsions include Montanide™ ISA-720 and Montanide™ ISA-703 (Seppic, Castres, France), each of which is understood to contain both squalene and squalane, with squalene predominating in each, but to a lesser extent in Montanide™ ISA-703. Montanide™ ISA-720 may also be used with an oil-to-water ratio of 7:3 (w/w). Other oil-in-water emulsion adjuvants include those disclosed in WO 95/17210 and EP 0399842, hereby incorporated by reference.

The use of small molecule adjuvants is also contemplated herein. Small molecule adjuvants include 7-substituted-8-oxo- or 8-sulfo-guanosine derivatives (e.g., 7-allyl-8-oxoguanosine (loxoribine)), described in U.S. Patent Nos. 4,539,205; 4,643,992; 5,011,828; and 5,093,318; herein incorporated by reference.

Additional adjuvants include monophosphoryl lipid A (MPL) (available from Corixa Corp. (see, U.S. Patent No. 4,987,237)), CPG available from Coley Pharmaceutical Group, QS21 (available from Aquila Biopharmaceuticals, Inc.), SBAS2 (available from SmithKline Beecham), muramyl dipeptide analogues described in U.S. Patent No. 4,767,842, and MF59 (available from Chiron Corp. (see, U.S. Patent Nos. 5,709,879 and 6,086,901)). Other adjuvants include the active saponin fractions derived from the bark of the South American tree *Quillaja Saponaria Molina* (e.g., Quil™ A). Derivatives of Quil™ A, for example QS21 (an HPLC purified fraction derivative of Quil™ A), and the method of its production are disclosed in U.S. Patent No. 5,057,540. In addition to QS21 (known as QA21), other fractions such as QA17 are also disclosed.

3-De-O-acylated monophosphoryl lipid A is a well-known adjuvant manufactured by Ribi Immunochem. The adjuvant contains three components extracted from bacteria: monophosphoryl lipid (MPL) A, trehalose dimycolate

(TDM), and cell wall skeleton (CWS) in a 2% squalene/Tween™ 80 emulsion. This adjuvant can be prepared by the methods taught in GB 2122204B. A preferred form of 3-de-O-acylated monophosphoryl lipid A is in the form of an emulsion having a small particle size of less than 0.2 μm in diameter (EP 0689454 B1).

The muramyl dipeptide adjuvants include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP; U.S. Patent No. 4,606,918), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), and N-acteryl-muramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1',2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamin (CGP) 1983A, referred to as MTP-PE.

Adjuvant mixtures include, e.g., combinations of 3D-MPL and QS21 (see, e.g., EP0671948 B1), oil-in-water emulsions including 3D-MPL and QS21 (see, e.g., WO 95/17210 and PCT/EP98/05714), 3D-MPL formulated with other carriers (see, e.g., EP 0689454 B1), QS21 formulated in cholesterol-containing liposomes (see, e.g., WO 96/33739), or immunostimulatory oligonucleotides (see, e.g., WO 96/02555). Alternative adjuvants include those described in, e.g., WO 99/52549 and non-particulate suspensions of polyoxyethylene ether (see, e.g., UK Patent Application No. 9807805.8).

Adjuvants are utilized in various amounts, which can vary with the adjuvant, subject, and the components of the composition (e.g., compositions including peptides (e.g., immune response stimulating peptides (e.g., ERG or SIM2 peptides), antibodies or antigen-binding fragments thereof, activated immune cells, and antigen-presenting cells) being administered. Typical amounts can vary from about 1 μg to about 50 mg per dosage. Those skilled in the art know that appropriate concentrations or amounts can be readily determined.

Conjugation to Cytotoxic and Other Therapeutic Agents

The agents of the invention (e.g., immune response stimulating peptides and antibodies to these peptides or antigen-binding fragments thereof) may be

coupled to or administered with any known cytotoxic or therapeutic moiety to form an agent or composition of the invention, respectively, that can be administered to treat, inhibit, reduce, or ameliorate disease (e.g., prostate cancer) or one or more symptoms of disease. Examples include but are not limited to antineoplastic agents such as: Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adozelesin; Adriamycin; Aldesleukin; Altretamine; Ambomycin; A. metantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Camptothecin; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Combretestatin A-4; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; DACA (N- [2- (Dimethyl-amino) ethyl] acridine-4-carboxamide); Dactinomycin; Daunorubicin Hydrochloride; Daunomycin; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Dolasatins; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride; Ellipticine; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Ethiodized Oil I 131; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; 5-FdUMP; Flurocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Gold Au 198; Homocamptothecin; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Interferon Alfa-2a; Interferon Alfa-2b; Interferon Alfa-nl; Interferon Alfa-n3; Interferon Beta-I a; Interferon Gamma-I b; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate;

Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin;Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; PeploycinSulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Rhizoxin; Rhizoxin D; Riboprine; Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Strontium Chloride Sr 89; Sulofenur; Talisomycin; Taxane; Taxoid; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Thymitaq; Tiazofurin; Tirapazamine; Tomudex; TOP53; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine; Vinblastine Sulfate; Vincristine; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride; 2-Chlorodeoxyadenosine; 2' Deoxyformycin; 9-aminocamptothecin; raltitrexed; N-propargyl-5,8-dideazafolic acid; 2chloro-2'-arabino-fluoro-2'-deoxyadenosine; 2-chloro-2'-deoxyadenosine; anisomycin; trichostatin A; hPRL-G129R; CEP-751; linomide; sulfur mustard; nitrogen mustard (mechlor ethamine); cyclophosphamide; melphalan; chlorambucil; ifosfamide; busulfan; N-methyl-Nnitrosourea (MNU); N, N'-Bis (2-chloroethyl)-N-nitrosourea (BCNU); N- (2-chloroethyl)-N' cyclohexyl-N-nitrosourea (CCNU); N- (2-chloroethyl)-N'- (trans-4-methylcyclohexyl-N-nitrosourea (MeCCNU); N- (2-chloroethyl)-N'- (diethyl) ethylphosphonate-N-nitrosourea (fotemustine); streptozotocin; diacarbazine (DTIC); mitozolomide; temozolomide; thiotepa; mitomycin C; AZQ; adozelesin;

Cisplatin; Carboplatin; Ormaplatin; Oxaliplatin;C1-973; DWA 2114R; JM216; JM335; Bis (platinum); tomudex; azacitidine; cytarabine; gemcitabine; 6-Mercaptopurine; 6-Thioguanine; Hypoxanthine; teniposide 9-amino camptothecin; Topotecan; CPT-11; Doxorubicin; Daunomycin; Epirubicin; darubicin; mitoxantrone; losoxantrone; Dactinomycin (Actinomycin D); amsacrinc; pyrazoloacridine; all-trans retinol; 14-hydroxy-retro-retinol; all-trans retinoic acid; N- (4- Hydroxyphenyl) retinamide; 13-cis retinoic acid; 3-Methyl TTNEB; 9-cis retinoic acid; fludarabine (2-F-ara-AMP); or 2-chlorodeoxyadenosine (2-Cda).

Other therapeutic compounds include, but are not limited to, 20-pi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antincoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; argininedeaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bleomycin A2; bleomycin B2; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives (e.g., 10-hydroxy-camptothecin); canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A ; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816 ; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam;

cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; 2'deoxycoformycin (DCF); deslorelin; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9- ; dioxamycin; diphenyl spiromustine; discodermolide; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epothilones (A, R = H; B, R = Me); epithilones; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide; etoposide 4'-phosphate (etopofos); exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; homoharringtonine (HHT); hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4- ; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide + estrogen + progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maytansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; rnerbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; ifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mithracin; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal

antibody, human chorionic gonadotrophin; monophosphoryl lipid A + myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone + pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; 06-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; podophyllotoxin; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B 1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division

inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene dichloride; topotecan; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

The agents of the invention (e.g., immune response stimulating peptides or antibodies or antigen-binding fragments thereof) can also include site-specifically conjugated molecules and moieties. Site-specific conjugation allows for the controlled stoichiometric attachment of a cytotoxic or therapeutic agent to specific residues of an agent of the invention.

The agents of the invention (e.g., immune response stimulating peptides and antibodies or antigen-binding fragments thereof), or any molecule or moiety conjugated thereto, can also be coupled to a lytic peptide. Such lytic peptides induce cell death and include, but are not limited to, streptolysin O; stoichactis toxin; phallolysin; staphylococcus alpha toxin; holothurin A; digitonin; melittin; lysolecithin; cardiotoxin; and cerebratulus A toxin (Kem et al., *J. Biol. Chem.* 253(16):5752-5757, 1978). An agent of the invention can also be conjugated to a synthetic peptide that shares some sequence homology or chemical characteristics with any of the naturally occurring peptide lysins; such characteristics include, but are not limited to, linearity, positive charge, amphipathicity, and formation of alpha-helical structures in a hydrophobic environment (Leuschner et al., *Biology of*

Reproduction 73:860-865, 2005). The agents of the invention (e.g., immune response stimulating peptides and antibodies or antigen-binding fragments thereof) can also be coupled to an agent that induces complement-mediated cell lysis such as, for example, the immunoglobulin F_c subunit. The agents of the invention (e.g., immune response stimulating peptides and antibodies or antigen-binding fragments thereof) can also coupled to any member of the phospholipase family of enzymes (including phospholipase A, phospholipase B, phospholipase C, or phospholipase D) or to a catalytically-active subunit thereof.

An agent of the invention can also be coupled to a radioactive agent to form an agent that can be used for detection or therapeutic applications. Radioactive agents that can be used include but are not limited to Fibrinogen ^{125}I; Fludeoxyglucose ^{18}F; Fluorodopa ^{18}F; Insulin ^{125}I; Insulin ^{131}I; Iobenguane ^{123}I; Iodipamide Sodium ^{131}I; Iodoantipyrine ^{131}I; Iodocholesterol ^{131}I ; Iodohippurate Sodium ^{123}I; Iodohippurate Sodium ^{125}I; Iodohippurate Sodium ^{131}I; Iodopyracet ^{125}I; Iodopyracet ^{131}I; Iofetamine Hydrochloride ^{123}I; Iomethin ^{125}I; Iomethin ^{131}I; Iothalamate Sodium ^{125}I; Iothalamate Sodium ^{131}I; tyrosine ^{131}I; Liothyronine ^{125}I; Liothyronine ^{131}I; Merisoprol Acetate ^{197}Hg; Merisoprol Acetate ^{203}Hg; Merisoprol ^{197}Hg; Selenomethionine ^{75}Se; Technetium ^{99m}Tc Antimony Trisulfide Colloid; Technetium ^{99m}Tc Bicisate; Tcchnetium ^{99m}Tc Disofenin; Technetium ^{99m}Tc Etidronate; Technetium ^{99m}Tc Exametazime; Technetium ^{99m}Tc Furifosmin; Technetium ^{99m}Tc Gluceptate; Technetium ^{99m}Tc Lidofenin; Technetium ^{99m}Tc Mebrofenin; Technetium ^{99m}Tc Medronate; Technetium ^{99m}Tc Medronate Disodium; Technetium ^{99m}Tc Mertiatide; Technetium ^{99m}Tc Oxidronate; Technetium ^{99m}Tc Pentetate; Tcchnetium ^{99m}Tc Pentetate Calcium Trisodium; Technetium ^{99m}Tc Sestamibi; Technetium ^{99m}Tc Siboroxime; Technetium ^{99m}Tc; Succimer; Technetium ^{99m}Tc Sulfur Colloid; Technetium ^{99m}Tc Teboroxime; Technetium ^{99m}Tc Tetrofosmin; Technetium ^{99m}Tc Tiatide; Thyroxine ^{125}I; Thyroxine ^{131}I; Tolpovidone ^{131}I; Triolein ^{125}I; or Triolein ^{131}I.

Additionally, a radioisotope could be site-specifically conjoined to an HSA linker or HSA linker conjugate. The available reactive groups could be used to conjugate site-specific bifunctional chelating agents for labeling of

radioisotopes, including ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{99m}Tc, ^{111}In, ^{64}Cu, ^{67}Cu, ^{186}Re, ^{188}Re, ^{177}Lu, ^{90}Y, ^{77}As, ^{72}As, ^{86}Y, ^{89}Zr, ^{211}At, ^{212}Bi, ^{213}Bi, or ^{225}Ac.

The agents of the invention may also be conjugated to other, not necessarily therapeutic, moieties for the purpose of enhancing, e.g., stability or localization.

Techniques for conjugating therapeutic agents to proteins, and in particular to antibodies, are well-known (e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in Monoclonal Antibodies And Cancer Therapy (Reisfeld et al., eds., Alan R. Liss, Inc., 1985); Hellstrom et al., "Antibodies For Drug Delivery," in Controlled Drug Delivery (Robinson et al., eds., Marcel Dekker, Inc., 2nd ed. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications (Pinchera et al., eds., 1985); "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody In Cancer Therapy," in Monoclonal Antibodies For Cancer Detection And Therapy (Baldwin et al., eds., Academic Press, 1985); Thorpe et al., *Immunol. Rev.* 62:119-58 (1982); and Doronina et al., "Development of potent monoclonal antibody auristatin conjugates for cancer therapy," *Nature Biotech.* 21:(7)778-784 (2003)). See also, e.g., PCT publication WO 89/12624.

Additional Therapies

The agents of the invention (e.g., immune response stimulating peptides, activated immune cells, antigen-presenting cells, and antibodies or antigen-binding fragments thereof) may be administered alone or in combination with other known therapies for the treatment of cancer (e.g., prostate cancer). Such therapies for prostate cancer, for example, include, e.g., hormone therapy (e.g., androgen ablation (e.g., administration of luteinizing hormone-releasing hormone agonists (e.g., leuprolide, goserelin, or buserelin), antiandrogens (e.g., flutamide or nilutamide), adrenal gland inhibitors (e.g., ketoconazole or aminoglutethimide), or estrogen administration)), chemotherapy, radiation

therapy, ultrasound therapy, or surgery (e.g., pelvic lymphadenectomy, transurethral resection of the prostate, radial prostatectomy, retropubic prostatectomy, or perineal prostatectomy). Such treatments are described in, e.g., U.S. Patent Nos. 6,184,249; 6,245,807; 6,472,415; and 6,537,988, hereby incorporated by reference.

Administration and Dosage

Pharmaceutical formulations of a therapeutically effective amount of an agent of the invention (e.g., immune response stimulating peptides, activated immune cells, antigen-presenting cells, and antibodies or antigen-binding fragments thereof), or pharmaceutically acceptable salts thereof, can be administered orally, parenterally (e.g., intramuscular, intraperitoneal, intravenous, or subcutaneous injection, inhalation, intradermally, optical drops, or implant), nasally, vaginally, rectally, sublingually, or topically, in admixture with a pharmaceutically acceptable carrier adapted for the route of administration.

Methods well known in the art for making formulations are found, for example, in Remington's Pharmaceutical Sciences (18^{th} edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, PA. Compositions intended for oral use may be prepared in solid or liquid forms according to any method known to the art for the manufacture of pharmaceutical compositions. The compositions may optionally contain sweetening, flavoring, coloring, perfuming, and/or preserving agents in order to provide a more palatable preparation. Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier or excipient. These may include, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, sucrose, starch, calcium phosphate, sodium phosphate, or kaolin. Binding agents, buffering agents, and/or lubricating agents (e.g., magnesium stearate) may also be used. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and soft gelatin capsules. These forms contain inert diluents commonly used in the art, such as water or an oil medium. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying agents, and suspending agents.

Formulations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of suitable vehicles include propylene glycol, polyethylene glycol, vegetable oils, gelatin, hydrogenated naphthalenes, and injectable organic esters, such as ethyl oleate. Such formulations may also contain adjuvants, such as preserving, wetting, emulsifying, and dispersing agents. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for the agents of the invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes.

Liquid formulations can be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, or by irradiating or heating the compositions. Alternatively, they can also be manufactured in the form of sterile, solid compositions, which can be dissolved in sterile water or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories, which may contain, in addition to active substances, excipients such as coca butter or a suppository wax. Compositions for nasal or sublingual administration are also prepared with standard excipients known in the art. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops or spray, or as a gel.

The amount of active ingredient (e.g., immune response stimulating peptides, activated immune cells, antigen-presenting cells, or antibodies or antigen-binding fragments thereof) in the compositions of the invention can be varied. One skilled in the art will appreciate that the exact individual dosages may be adjusted somewhat depending upon a variety of factors, including the ingredient being administered, the time of administration, the route of administration, the nature of the formulation, the rate of excretion, the nature of the subject's conditions, and the age, weight, health, and gender of the patient. In addition, the severity of the condition targeted by an agent of the invention will also have an impact on the dosage level. Generally, dosage levels of an agent of the invention of between 0.1 μg/kg to 100 mg/kg of body weight are administered daily as a single dose or divided into multiple doses. Preferably, the general dosage range is between 250 μg/kg to 5.0 mg/kg of body weight per day. Wide variations in the needed dosage are to be expected in view of the differing efficiencies of the various routes of administration. For instance, oral administration generally would be expected to require higher dosage levels than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, which are well known in the art. In general, the precise therapeutically effective dosage will be determined by the attending physician in consideration of the above-identified factors.

An agent of the invention (e.g., .g., immune response stimulating peptides, activated immune cells, antigen-presenting cells, or antibodies or antigen-binding fragments thereof) can be administered in a sustained release composition, such as those described in, for example, U.S. Patent Nos. 5,672,659 and 5,595,760, hereby incorporated by reference. The use of immediate or sustained release compositions depends on the type of condition being treated. If the condition consists of an acute or over-acute disorder, a treatment with an immediate release form will be preferred over a prolonged release composition. Alternatively, for preventative or long-term treatments, a sustained released composition will generally be preferred.

An agent of the invention (e.g., .g., immune response stimulating peptides, activated immune cells, antigen-presenting cells, or antibodies or antigen-binding fragments thereof) can be prepared in any suitable manner. The agent may be isolated from naturally-occurring sources, recombinantly produced, or produced synthetically, identified from a library, or produced by a combination of these methods. The synthesis of short peptides is well known in the art. As described previously, a peptide portion of any of the agents of the invention can be synthesized according to standard peptide synthesis methods known in the art.

Methods for administering peptides to a subject are described, for example, in U.S. Patent Nos. 5,830,851; 5,558,085; 5,916,582; 5,960,792; and 6,720,407, hereby incorporated by reference.

Assessment of Therapy

After therapeutic treatment with the compositions of the invention described herein, the efficacy of the treatment may be assessed by a number of methods, such as assays that measure T cell proliferation, T cell cytotoxicity, antibody production, reduction in the number of antigen-positive cells or tissues, or clinical responses. An increase in the production of antibodies or immune cells recognizing the selected antigen (e.g., an ERG or SIM2 antigen) will indicate an enhanced immune response. Similarly, an increase in specific lytic activity or specific cytokine production by the subject's immune cells or tumor regression will indicate efficacy. Efficacy may also be indicated by an improvement in or resolution of the disease (e.g., prostate cancer) associated with the reduction or disappearance of the unwanted immune response or an improvement in or resolution of the disease (e.g., prostate cancer) associated with the unwanted immune response.

Diagnostics

We have shown that ERG and SIM2 are overexpressed in prostate cancer cells and not expressed in non-cancerous prostate cells. Accordingly,

the measurement of ERG and SIM2 levels can be used as a tool to diagnose prostate cancer in a subject.

The present invention features methods and compositions (e.g., immune response stimulating peptides (e.g., ERG or SIM2 peptides), activated immune cells, antigen-presenting cells, and antibodies or antigen-binding fragments thereof) for generating an immune response for the treatment of cancer (e.g., prostate cancer). The methods and compositions can include the measurement of, for example, ERG or SIM2 polypeptides, either free or bound to another molecule, or any fragments or derivatives thereof. The methods can include measurement of absolute levels of ERG or SIM2 or relative levels as compared to a normal reference. For example, a level of ERG or SIM2 in a bodily fluid of less than 5 ng/ml, 4 ng/ml, 3 ng/ml, 2 ng/ml, or less than 1 ng/ml is considered to be predictive of a low risk of prostate cancer or of a good outcome in a patient diagnosed with prostate cancer. A level of ERG or SIM2 in a bodily fluid of greater than 5 ng/ml, 10 ng/ml, 20 ng/ml, 30 ng/ml, 40 ng/ml, or 50 ng/ml is considered diagnostic of prostate cancer or of a poor outcome in a subject already diagnosed with prostate cancer.

For diagnoses based on relative levels of ERG or SIM2, a subject with prostate cancer will show an alteration (e.g., an increase of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more) in the expression of an ERG or SIM2 polypeptide (or fragment thereof) as compared to a normal reference sample or level. A normal reference sample can be, for example, a sample taken from the same subject prior to the development of prostate cancer or of symptoms suggestive of prostate cancer, a sample from a subject not having prostate cancer, or a sample of a purified reference polypeptide at a known normal concentration (i.e., not indicative of prostate cancer). By "reference standard or level" is meant a value or number derived from a reference sample. A normal reference standard or level can be a value or number derived from a normal subject.

For diagnostic assays that include measuring the amount of ERG or SIM2 polypeptide, the ERG or SIM2 polypeptide can include full-length ERG

or SIM2 polypeptide, degradation products, alternatively spliced isoforms of ERG or SIM2 polypeptide, enzymatic cleavage products of ERG or SIM2 polypeptide, and the like. In one example, an antibody that specifically binds ERG or SIM2 polypeptide is used for the diagnosis of prostate cancer.

Standard methods may be used to measure levels of ERG or SIM2 polypeptide in any cell, tissue, or bodily fluid, including, but not limited to, urine, blood, serum, plasma, saliva, or cerebrospinal fluid. Such methods include immunoassay, ELISA, Western blotting using antibodies that specifically bind to ERG or SIM2 polypeptide, and quantitative enzyme immunoassay techniques. Increases in the levels of ERG or SIM2 polypeptide, as compared to normal controls, are considered a positive indicator of prostate cancer.

ERG or SIM2 nucleic acid molecules, or substantially identical fragments thereof, or fragments or oligonucleotides of ERG or SIM2 that hybridize to ERG or SIM2 at high stringency may be used as a probe to monitor expression of ERG or SIM2 nucleic acid molecules in the diagnostic methods of the invention. Increases in the levels of ERG or SIM2 nucleic acid molecules, as compared to normal controls, are considered a positive indicator of prostate cancer.

The diagnostic methods described herein can be used individually or in combination with any other diagnostic method for a more accurate diagnosis of the presence of prostate cancer. Examples of additional methods for diagnosing prostate cancer include, e.g., the detection of prostate specific antigen (PSA) in prostate cells, digital rectal exams, trans-rectal ultrasounds, or biopsies (e.g., needle biopsies).

The invention also provides for a diagnostic test kit. For example, a diagnostic test kit can include antibodies that specifically bind to ERG or SIM2 polypeptide and components for detecting and evaluating binding between the antibodies and the ERG or SIM2 polypeptide. For detection, either the antibody or the ERG or SIM2 polypeptide is labeled and either the antibody or the ERG or SIM2 polypeptide is substrate-bound, such that the ERG or SIM2

polypeptide-antibody interaction can be established by determining the amount of label attached to the substrate following binding between the antibody and the ERG or SIM2 polypeptide. ELISA is a common, art-known method for detecting antibody-substrate interactions and can be provided with the kit of the invention. ERG or SIM2 polypeptides can be detected in virtually any bodily fluid, such as, e.g., urine, plasma, blood, blood serum, semen, or cerebrospinal fluid. A kit that determines an alteration in the level of ERG or SIM2 polypeptide relative to a reference, such as the level present in a normal control, is useful as a diagnostic kit in the methods of the invention. The kit can also contain a standard curve or a reference level or sample indicating levels of ERG or SIM2 that fall within the normal range and levels that would be considered diagnostic of prostate cancer. Desirably, the kit will contain instructions for the use of the kit. In one example, the kit contains instructions for the use of the kit for the diagnosis of prostate cancer. In yet another example, the kit contains instructions for the use of the kit to monitor therapeutic treatment or dosage regimens. The kit may also contain other diagnostics useful in diagnosing prostate cancer, or may be used in combination with known prostate cancer diagnostic measures.

Examples

Example 1. Identification of Putative Tumor-Associated Antigens (TAAs) for Prostate Cancer Immunotherapy

In an effort to identify novel putative prostate cancer tumor-associated antigens with expression specificity for prostate cancer over normal prostate or normal non-prostate tissue, we performed a genome-wide gene expression analysis of a prostate cancer and normal prostate microarray generated in our laboratory, validated the candidate TAAs in an external, published prostate cancer tissue array data set, and excluded those with detectable expression in non-prostatic adult tissues.

First, we used the Affymetrix U133 array to evaluate gene expression in cancer and normal fresh-frozen prostate tissue specimens from our tissue

repository. 250 ng of total RNA from the tissue specimens was amplified using Ambion's MessageAmp II mRNA Amplification kit. Biotin-UTP was incorporated during the overnight *in vitro* transcription step according to the manufacturer's protocol. Gene expression was assessed using Affymetrix's (Santa Clara, CA) GeneChip U133 array (Plus 2.0 chip), arrays representing whole human genome transcripts. 15 μg of cRNA was fragmented and hybridized to arrays according to the manufacturer's protocols. The image quality of scanned arrays were determined on the basis of background values, percent present calls, scaling factors, and 3'-5' ratio of β-actin and GAPDH using the BioConductor R packages. The signal value for each transcript was summarized using perfect matched (PM)-only based signal modeling algorithm described in dchip. The PM-only based signaling modeling algorithm yields fewer false positives compared to the PM-MM (mismatched) model. As such, the signal value corresponds to the absolute level of expression of a transcript. These normalized and modeled signal values for each transcript were used for further high-level bioinformatics analysis. During the calculation of model-based expression signal values, array and probe outliers were interrogated, and images spike were treated as signal outliers. The outlier detection was carried out using dchip outlier detection algorithm. A chip was considered as an outlier if the probe, single, or array outlier percentage exceeded a default threshold of 5%. When comparing two groups of samples to identify genes enriched in a given phenotype, if 90% lower confidence bound (LCB) of the fold change (FC) between the two groups was above 1.2, the corresponding gene was considered to be differentially expressed. LCB is a stringent estimate of FC and has been shown to be the better ranking statistic. It has been suggested that a criterion of selecting genes that have a LCB above 1.2 most likely corresponds to genes with an "actual" fold change of at least 2 in gene expression.

The raw gene expression data from 62 prostate cancer and 41 normal prostate specimens, published by Lapointe et al. (*Proc Natl Acad Sci USA* 101: 811-816 (2004)), were obtained from the BRB arrays archived datasets. The preprocessed data was normalized using the Z transformation. The differentially

expressed genes were identified on the basis of fold change (> 0.5) and Q value < 0.05. The analysis identified 510 differentially expressed genes.

To prioritize the biomarker and immunotherapy targets, we needed to identify genes that are not ubiquitously expressed in all normal tissues. The gene expression data for the various human normal tissues were obtained from Gene Expression Atlas of the Genomics Institute of the Novartis Research Foundation (http://symatlas.gnf.org). Using this database, MAS5 normalized expression data (along with present, absent, and marginal calls for each transcript) were obtained. On the basis of present and absent calls for each transcript, a priority value was calculated. The gene absent in all tissues was given highest priority and the gene present in all tissues was given lowest priority. To further extend the list of genes, we also obtained a list of prostate specific genes by analyzing the Novartis gene expression data (Dhanasekaran et al., *Nature* 412: 822-826 (2001)). The genes that were annotated as absent on the basis of MAS5 calls in all normal tissues except prostate were considered prostate specific genes.

The genome-wide gene expression analysis described above identified 1063 genes overexpressed in prostate cancer compared to normal prostate. Examples of the top 100 genes identified in the expression analysis include AMACR, ERG, MMP26, THBS4, and FOXD1. Next, we validated the 1063 putative TAAs by conducting a comprehensive analysis of microarray data from a previously published data set which included 41 normal prostate tissues and 62 neoplastic prostate tissues (Lapointe et al., *supra*). We looked at the genes that were significantly overexpressed in prostate cancer samples for their potential to be used as biomarkers or targets for immunotherapy. A list of 426 upregulated prostate cancer genes was obtained on the basis of the fold change (> 0.5) and FDR value < 0.05 after pre-processing and normalizing data (Z transformation). Validation of genes that were overexpressed in prostate cancer in our data set compared to the Stanford prostate cancer array dataset implicated 145 transcripts with concordant over-expression between the array datasets.

To identify prostate cancer TAAs with the greatest specificity for prostate cancer, we then sought to exclude by *in silico* analysis those genes detectable in

non-prostate normal human adult male tissues. For this purpose, gene expression data for various human tissues were obtained from the two studies conducted by Su et al. (*Proc Natl Acad Sci USA* 101: 6062-6067 (2004) and Ge et al. (*Genomics* 86: 127-141 (2005)). Genes that were annotated absent on the basis of MAS5 calls in all the normal tissues except prostate were considered as prostate specific genes. The comprehensive analysis led to the identification of 26 transcripts that are over expressed in prostate cancer samples and are highly tissue restricted (Figure 1). These transcripts correspond to 23 genes that include ERG and SIM2. The analysis also identified 17 more genes that are present in the prostate cancer samples and absent in normal tissues.

We then performed quantitative qRT-PCR targeting each of the 23 candidate antigens. 50 ng of high-quality RNA samples (by Agilent > 6.0) were reverse transcribed to obtain cDNA, and 1 µl cDNA was used for each well of RT-PCR reactions. Samples were performed in triplicates. SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) was used for two-step real-time RT-PCR analysis on Applied Biosystems 7900HT Prism instrument. PCR primers for SIM2 and GAPDH were designed as SIM2-F (5'-CTTCCCTCTGGACTCTCACG-3'), SIM2-R (5'-AGGCTGTGCCTAGCAGTGTT-3'), GAPDH-F (5'-TGCACCACCAACTGCTTAGC -3'), and GAPDH-R (5'-GGCATGGACTGTGGTCATGAG -3'). The expression value of SIM2 in a given sample was normalized to the corresponding expression of GAPDH. The $2^{-\Delta\Delta Ct}$ method was used to calculate relative expression of SIM2 (Haram et al., *Prostate* 68: 1517-1530 (2008) and Livak et al., *Methods* 25: 402-408 (2001)).

We confirmed that 15 of the candidate antigens (AMACR, BICD1, C10orf137, CDC2L6, ICA1, KIAA1661, MAP7, MYO6, OR51E2, PAICS, PCSK6, PVT1, RGS10, SGEF, and SIM2) were overexpressed in prostate cancer (Figure 2). Frequency of overexpression in prostate cancer for these antigens ranged from 57% to 86%. From among these 15 prostate cancer-specific antigens validated by qRT-PCR, the gene that was most consistently absent in normal prostate and had the highest frequency of expression in prostate cancer (Figure 2)

was the single-minded homolog gene (SIM2). SIM2 was overexpressed in 6 of 7 cancers we tested, but not in benign prostate tissue.

Example 2. ERG as a Target for Immunotherapy of Prostate Cancer

The selection of target therapeutic antigens is of paramount importance for the design of tumor vaccines (e.g., prostate cancer vaccines). Recent studies have shown that an intrachromosomal rearrangement of the prostate-specific promoter region in the 5' TMPRSS2 gene fusing in-frame with 3' ERG, leading to ERG overexpression, is present in 40-60% of human prostatectomy specimens. ERG is not normally expressed in the human prostate; the expression of ERG has been documented only in cultured endothelium or developing cells, though not detected at abundant levels in normal adult tissue. Moreover, unlike other ETS factors whose expression is maintained in various tissues through adulthood, ERG expression is subject to high tissue restriction.

In silico analyses of the ERG amino acid sequence using different algorithms that predict MHC class I-restricted epitopes identified over 50 potential binders, of which we selected 12 putative 9-mer peptides predicted to have high affinity binding to human HLA-A2.1. Three of these 9-mer ERG peptides are described by SEQ ID NOs: 2, 3, and 4. All epitopes were from the N-terminus end of the translocation that is not deleted in most TMPRSS2:ERG fusion products, and epitope sequences were 100% homologous to their corresponding mouse orthologs. Next, we screened these peptides for binding to HLA-A2.1 and found eight with significant binding affinity (Figure 3A). We then hypothesized that T cell tolerance to ERG could be overcome more easily than to other self-antigens because our *in silico* analyses identified a paucity of ERG expression in normal, human adult tissues *in situ* (data not shown). Accordingly, we used immunization studies to evaluate if these peptides could break tolerance to ERG, as measured by the induction of ERG epitope-specific, HLA-A2.1–restricted cytotoxic T lymphocytes (CTLs) in humanized HHD mice (i.e., mice with only human HLA-A2.1 class I MHC),

and observed the induction of ERG-specific CTLs, as measured by ELISPOT (Figure 3B).

In vitro studies have detected anti-ERG autoantibodies in prostate cancer patients. This was achieved by using an alternative approach to gene expression profiling for the purpose of identification of putative target prostate TAAs, mainly the detection of autoantibodies to TAA in serum of prostate cancer patients. We assembled a case-control cohort of 1000 consenting subjects who provided serum, plasma, and buffy coat. This cohort includes 35% prostate cancer cases (primary and metastatic), 55% control cases, and 10% cases with indeterminate or precancerous findings (e.g., atypia, HGPIN, or ASAP). Sera from this cohort were subjected to an initial screen using a high-density nucleic acid programmable protein array (NAPPA) to identify antigens with high incidence of autoantibodies (Figure 4A). Several candidates were selected from the top 100 antigens resulting from this first screen, including ERG, and the NAPPA data was validated using an ELISA platform. Using this methodology, the presence of anti-ERG autoantibodies in sera from 12% of prostate cancer patients we tested (data for ERG and other TAAs are illustrated in Figure 4B) was confirmed, providing evidence for the immunogenicity of ERG in prostate cancer patients.

Example 3. SIM2 as a Target for Immunotherapy of Prostate Cancer

To test whether sera from healthy individuals and prostate cancer patients harbor antibodies to SIM2, we used an ELISA system with *in vitro* expressed GST-tagged SIM2 for capture. Serum samples were collected at Harvard University and University of Michigan patient accrual sites. Written informed consent was obtained from each patient and approved by the Institutional Review Board of both institutions. All patients were over the age of 40 and were seen at the clinic because of a PSA value exceeding 2.5 ng/ml, abnormal digital rectal exam (DRE), rising PSA, or lower PSA with risk factors such as family history. The study also included men who have had previous biopsies that have not tested

positive for cancer. After enrollment and blood collection, all patients received a prostate biopsy to determine the presence or absence of cancer.

A pCR-BLUNT2-topo plasmid containing human full-length SIM2 cDNA was purchased from Open Biosystems (Huntsville, AL). SIM2 cDNA-containing plasmid (10 pg - 200 ng) went through two different PCR steps. The first PCR step contained 22.5 µl of AccuPrime Pfx Super Mix (Invitrogen) and 200 nM of each primer. The forward primers for all the genes had a shared sequence at the 5'end (AAAGCAGGCTCCACC), but had a 3' end of 22-25 nucleotides specific to each cDNA. The reverse primers also shared a sequence at the 5' end (AAAGCAGGCTCCACC), but the 3' end (21-27 nucleotides) of each primer was specific to each cDNA. The second PCR step included 22.5 µl of AccuPrime Pfx Super Mix (Invitrogen), 6.6 ml of the PCR product from the first step, and 200 nM of attF and attR primers. The cycling conditions for the two PCR steps were the same, except that the first PCR step was run for 35 cycles while the second PCR step was run for 5 cycles. The extension step was set at 1 minute/kb cDNA. The PCR product was run in a 1% agarose gel, gel purified, and cloned into pDONR plasmid (Invitrogen) to produce entry clones of each cDNA. Entry clones (130 ng) were used to produce expression clones using the pCITE-GST expression vector (130 ng), LR clonase II enzyme mix (2 µl), and TE buffer for a total volume of 10 µl. The reaction product (1 µl) was transformed into 50 µl of One Shot OmniMax™ 2T1 Phage-Resistant Cells according to the manufacturer's instructions. All of the TAAs were produced as GST recombinant proteins with GST at the C-terminus. As a negative control for serum antibody binding, a GST control vector was also produced using pDEST15 (Invitrogen) from which only GST would be expressed. The Kozak sequence was introduced into the original pDEST15 5' of GST so that it could be used in the mammalian cell free system.

This ELISA method is a single-antigen adaptation of the Nucleic Acid-Programmable Protein Array (NAPPA), which utilizes cDNA vectors coupled with a capture antibody and could be advantageous over traditional protein arrays since the proteins do not need to be purified (Figure 4A). GST-ELISA plates pre-coated with GST were purchased from GE Biosciences. The plates were blocked

overnight at 4°C with PBS, 5% milk, and 0.2% Tween-20. The different TAA-GST recombinant proteins were expressed using the rabbit reticulocyte lysate cell free expression system (Promega) for 1.5 hours at 30°C in a microcentrifuge tube according to the manufacturer's instructions. The expressed proteins were transferred to the ELISA plate and bound overnight at 4°C. The plates were washed, incubated with serum diluted 1:300 in blocking buffer for 1 hour, and then incubated for 1 hour with horseradish peroxidase linked antihuman antibodies. After washing, 100 µl of the substrate (SuperSignal ELISA Femto Maximum Sensitivity Substrate; Pierce) were added to each well, and the luminescence signal was read using Victor3 ELISA reader (no filter). Each serum was screened in duplicate. The plate also included a secondary antibody negative control and a GST control.

Significant levels of autoantibody from patient sera with specific binding to SIM2 ($P < 0.01$) were detected in two of the five evaluated prostate cancer samples (Figure 5). In contrast, autoantibodies to SIM2 were not detectable in any of the nine control patients' sera (Figure 5).

Example 4. Humanized Mouse Models as a Tool for ERG- and SIM2-Targeted Prostate Cancer Immunotherapy

To test ERG-target immunotherapeutic protocols, the HHD mouse (HLA-A*0201 transgenic mouse), the Pb-ERG mouse (probasin-ERG transgenic mouse), and the Pb-ERG/Pten$^{+/-}$ mouse are used. The HHD mouse expresses human HLA-A2.1, but lacks murine MHC I. The Pb-ERG transgenic mouse exhibits prostate-restricted ERG expression and develops mouse prostatic intraepithelial neoplasia (mPIN), a precursor lesion of prostate cancer, by 12 to 14 weeks of age. The Pb-ERG/Pten$^{+/-}$ mice showed that the combination of overexpressed ERG and reduced Pten levels are causative of multifocal prostatic adenocarcinoma with complete penetrance by 6 months of age.

Xenograft models are also used in the CTL adoptive transfer phase of ERG-based immunotherapy, as they provide a fast system for the titration and

evaluation of anti-tumor effects. This model is developed using the HLA-A2.1 (+) human LNCap cell line, stably expressing human ERG. A similar strategy is adopted for TAAs other than ERG in case of unavailability of transgenic mice.

For SIM2-targeted immunotherapy, the TRAMP-HHD hybrid mouse is used. The TRAMP mouse is a well-characterized model of prostate cancer, and we have previously utilized it as a model in several studies pertaining to prostate cancer genomics and immunity. Moreover, microarray data showed elevated levels of SIM2 in TRAMP prostate tumors.

Example 5. Characterization of Human HLA-A2.1-Restricted Epitopes of ERG That Are Suitable Targets for Immunotherapy Mediated by A2.1-Resticted CTL

The impact of developing prostate cancer on ERG-specific CTL tolerance by comparing ERG-specific CTL responsiveness in (age-matched) HHD mice to that of F1 Pb-ERG-HHD or Pb-ERG/PTEN$^{+/-}$-HHD hybrid mice before and after prostate neoplastic transformation develops is determined. Owing to the striking homology between mouse and human ERG genes, the amino acid sequences of the three epitopes we identified are 100% homologous to the murine ERG epitopes.

In preliminary data, three immunodominant epitopes of human ERG presented by human HLA-A2.1 that can break ERG-specific T cell tolerance after immunization were identified, as evidenced by induction of ERG-specific, A2.1-restricted cytotoxic T lymphocytes in humanized mice. We compare the anti-tumor efficacy of immune responses induced against each of these three immunodominant human ERG epitopes and determine whether T cell tolerance to ERG is further attenuated via androgen suppression or by inhibition of suppressive CD4/CD25 regulatory T cells by Tim-1 targeting. These preclinical studies are conducted using F1 hybrid progeny of humanized HHD mice bred with Pb-ERG mice and the PB-*ERG/Pten*$^{+/-}$ mouse. These models are mutually complementary as follows: the Pb-ERG transgenic mouse exhibits

prostate-restricted ERG expression and, despite not developing progressive cancers, these mice allow the study of how to best exploit androgen modulation to facilitate ERG-specific T cell responses. In contrast, the PB-*ERG/Pten*$^{+/-}$ mouse represents multifocal prostatic adenocarcinoma with complete penetrance by six months of age and allows us to determine which of the three immunodominant ERG epitopes shows the best ability to eradicate prostate cancer *in vivo*.

Direct comparison of the 3 dominant epitopes' ability to eliminate ERG-induced prostate cancer is performed by passive immunization and by active immunization. We and others have previously shown that adoptive transfer of CTL specific for a model prostatic oncogene (e.g., SV40Tag in TRAMP mice) can effectively treat established prostate cancers. This experiment is performed by adoptive transfer of human A2.1-restricted, ERG-specific CTL in our PB-ERG/Pten$^{+/-}$xHHD mice. Efficacy of CTL against the three immunodominant human A2.1-restricted peptides of ERG is compared. The most effective epitope is selected for phase I clinical trial IND. Active immunization studies are performed to explore avenues for optimizing *in situ* anti-tumor responsiveness in the absence of adoptive transfer in preclinical hybrid, humanized ERG/A2.1 transgenic models. Our active immunization studies focus on exploiting androgen suppression or targeting regulatory T lymphocytes (Tregs) to attenuate ERG tolerance and improve anti-tumor efficacy *in vivo*. In all immunization modalities, a CTL response to a given peptide antigen is defined by the ability of splenocytes from a peptide-immunized mouse to secrete IFN-γ (measured by ELISPOT), produce granzyme B and perforin, lyse peptide-loaded HLA-A2.1-cxpressing cells in a cytotoxicity assay, and specifically bind peptide-tetramer complexes. Anti-tumor effects are monitored by histology (for Pb-ERG mice) and prostate tumor size (for PB-*ERG/Pten*$^{+/-}$ mice).

Example 6. Identification and Characterization of Human HLA-A2.1-Restricted SIM2 Epitopes That Are Suitable Targets for Immunotherapy Mediated by A2.1-Resticted CTL

In our comprehensive analysis of microarray data from normal (41 samples) and neoplastic (62 samples) prostate tissues, we looked at the genes that are significantly overexpressed in prostate cancer for their potential to be used as biomarkers or targets for immunotherapy. A list of 343 unique prostate cancer upregulated genes was obtained on the basis of the fold change (> 0.5) and FDR value < 0.05 after pre-processing and normalizing data (Z transformation). For the purpose of this study, only those genes whose expression was completely absent in non-prostate normal human tissues were retained for further consideration. Gene expression data for various human tissues were obtained from the two studies conducted by Novartis and Stanford. MAS5 normalized expression data along with present, absent, and marginal calls for each transcript were obtained. Based on present and absent calls for each transcript, a priority value was calculated. Genes that were absent in all tissues were given highest priority. To further extend the list of genes, prostate specific genes were identified by analyzing the Stanford gene expression data. The genes that were annotated absent on the basis of MAS5 calls in all the normal tissues except prostate were considered as prostate specific genes. This comprehensive multi-step analysis led to a list of 57 genes, which included ERG. We selected the transcription factor SIM2 as our second target for its overexpression in most prostate cancer biopsies that were analyzed (Figure 6) and for the absence of expression in normal tissues. Concurrent overexpression of ERG and SIM2 was observed only in a fraction of the prostate cancer biopsies we analyzed.

To predict potential HLA-A*0201-binding epitopes, candidate target TAA protein sequences are screened against various matrix patterns, which evaluate every amino acid within octomer, nonamer or decamer peptides fitting the HLA-A*0201 motif. In addition to the widely used algorithms SYFPEITHI, BIMAS, MHCPred, and RankPep, we also utilized NetCTL,

PREDEP, ProPred-I, MAPPP, JenPep, NetMHC, and nHLApred for the purpose of our predictions.

Epitope candidates were evaluated for binding to HLA-A2.1 using an MHC stabilization assay. The assay is based on the ability of suitable peptides to stabilize MHC class I molecules from the T2 cell line. T2 cells lack a functional transporter associated with antigen presentation (TAP) and as a result accumulate empty, unstable class I molecules on the cell surface. These molecules on the cell surface dissociate rapidly unless they are stabilized by the addition of an appropriate binding peptide. In this assay, the flu M1 HLA-A*0201-binding peptide was used as a reference.

Briefly, T2 cells were cultured for 6 hours in serum-free IMDM (ATCC) prior to the addition of candidate peptides at a concentration of 50 μg/250x10^3 cells/ml and incubated overnight at 37°C. Cells were washed, and surface HLA-A2.1 molecules were stained with FITC mouse anti-human HLA-A2 mAb (clone BB7.2, Mouse IgG2b κ, BD Pharmingen) for 1 hour at 4°C. Cells were then washed three times with PBS and analyzed by flow cytometry. A negative control peptide (NEG, see Figure 6A) and the flu matrix peptide M1 binder peptide (M1, see Figure 6A) served as controls. The relative binding affinity of a given peptide was calculated as $MFI_{(peptide)}/MFI_{(negative\ peptide)}$. Only relative binding affinities of 1.5 or higher were considered for further testing.

T2 cells were incubated overnight with 50 μg/ml of each candidate peptide at 37°C in serum-free Iscove's Modified Dulbecco's Medium (IMDM). Cells were then incubated with Brefeldin A (Sigma, St. Louis, MO) at 10 μg/ml for 1 hour, washed, and incubated at 37°C for 0, 2, 4 or 6 hours in the presence of Brefeldin A (50 ng/ml). Cells were then stained with BB7.2 mAb. For each time point, peptide-induced HLA-A2.1 expression was calculated as the difference of the mean fluorescence of peptide-loaded T2 cells and mean fluorescence of negative peptide-loaded T2 cells. The rate of dissociation is reflected by the loss of A2.1 expression over time.

This MHC stabilization assay revealed nine SIM2 peptides that were able to stabilize HLA-A2.1 molecules, resulting in increased detection of surface A2.1

molecules with a specific monoclonal antibody (Figure 6A). The peptide-HLA dissociation rate correlated with time and identified weak stabilizing epitopes (epitopes 84, 199, 237 and 430) and strong stabilizing epitopes (epitopes 87, 205, 241 and 244). However, epitopes with a high dissociation rate (weak stabilizers) still showed a slight binding that was above the non-binding control epitope, even after 8 hours of incubation.

These nine SIM2 peptides were then tested for their capacity to elicit *in vivo* CTL responses in transgenic HHD mice. Ten to 12-week old male HHD mice were injected subcutaneously at the basis of the tail with 100 μg of each candidate peptide emulsified in 50 μl of Incomplete Freund's Adjuvant (IFA) and 50 μl PBS in the presence of 150 μg of the I-A^b restricted $HBVcore_{128-140}$ T helper epitope (TPPAYRPPNAPIL). Ten to twelve days post-immunization, spleens were harvested and splenocytes tested for peptide-induced specific release of IFN-γ by ELISPOT. To perform the ELISPOT assays, 96-well Millipore IP plates were coated with 100 μl/well mouse IFN-γ specific capture mAb (AN18, Mabtech Inc., Mariemont, OH) at a concentration of 10 μg/ml in PBS overnight at 4°C. Wells were washed with PBS and saturated with RPMI/10% FCS for 1 hour at 37°C. A total of 2.5 x 10^5 splenocytes were seeded in each well in four replicates and 5 x 10^4 peptide-loaded (10 μg peptide/ml, for 2 hours at 37°C) splenocytes pretreated with 50 μg/ml Mitomycin C for 1 hour were added to each well. Plates were incubated for 1-2 days at 37°C in 5% CO_2, washed 5 times with PBS, and then incubated with 1 μg/ml of biotinylated rat anti-mouse IFN-γ mAb (R4-6A2, Mabtech Inc.) for 24 hours at 4°C or at room temperature for 2 hours. The wells were washed, and incubated with 100 μl of diluted alkaline phosphatase-conjugated streptavidin for 1 hour at room temperature. Spots were developed by adding peroxidase substrates (5-bromo-4,3-indolyl phosphate and nitroblue tetrazolium) and counted using the ELR04 AID ELISPOT Reader System (Autoimmun Diagnostika GmbH , Straßberg, Germany).

We found that *in vitro* restimulation with SIM2(87) (TLDGFVFVV), SIM2(205) (YQIVGLVAV), SIM2(237) (SLDLKLIFL), SIM2(241)

(KLIFLDSRV), and SIM2 (244) (FLDSRVTEV) induced significantly ($P<.01$ for SIM2(87) and $P<.001$ for other epitopes) higher numbers of splenocytes to release IFN-γ in a peptide-specific manner in an ELISPOT assay (Figure 6B). This provides evidence that tolerance to SIM2 is circumvented through immunization of mice to these epitopes since SIM2 (and SIM1) is also expressed in other non-prostatic tissues in mice. SIM2(25) and SIM2(199) were not immunogenic, despite their ability to bind tightly to A2.1.

The potential for human HLA-A2.1-restricted, SIM2-specific immunity to impact developing prostate cancers *in vivo* will be evaluated in the TRAMP-HHD hybrid mouse. The TRAMP mouse is a well-characterized model of prostate cancer and we have previously utilized it as a model in several studies pertaining to prostate cancer genomics and immunity. In a genome-wide analysis, we have recently shown that SIM2 gene is overexpressed in TRAMP prostate tumors. We additionally have shown that SIM2 expression in LNCap cells is regulated by androgens, which offers the possibility of combining androgen manipulation with SIM2-targeted immunotherapy in this model.

Example 7. Exploitation of Androgen Ablation for Overcoming Prostate TAA-Specific Immune Tolerance

The androgen-signaling pathway is critical to the development and progression of prostate cancer. Androgen ablation is a conventional therapy for prostate cancer and believed by many to enhance immunity to tumor antigens when combined to immunotherapy. It has been shown that androgen ablation in mice attenuates tolerance and raises CD4 T cell responses to flu peptide as a model prostate tumor antigen. Our studies have extended this observation to A2.1-restricted CTL responses against PSA in probasin-PSA transgenic mice. For example, hybrid A2.1/PSA (males, castrated males, or females) transgenic and A2.1 mice were immunized with vac-prostate specific antigen (PSA), and splenocytes were restimulated with PSA protein-loaded dendritic cells and tested by ELISPOT (Figure 7A) or PSA-tetramers (Figure 7B), showing that androgen suppression attenuates prostate-specific tolerance. Additionally,

androgen ablative therapy in men has been shown to result in enhanced T cell infiltration into benign and malignant prostate tissue. We now exploit androgen responsiveness of TMPRSS2-ERG fusion and SIM2 gene (Figure 8) to attenuate ERG- and SIM2-specific immune tolerance.

Our preliminary data (Figure 8A) are consistent with the well-established positive correlation of ERG expression with androgen. We assess the effect of castration on the outcome of active immunization comparing the three immunodominant ERG peptides for their ability to induce ERG-specific CTL in Pb-ERG-HHD and to reduce tumor growth in PB-*ERG/Pten*$^{+/-}$-HHD mice.

Like ERG, SIM2 expression is dependent on androgens (Figure 8B), likely due to the location of SIM2 gene in a region rich in androgen regulatory elements on chromosome 21. Our microarray data revealed increased expression of SIM2 expression in prostate tumors from TRAMP mice. The effect of androgen ablation on immune tolerance to SIM2 and on prostate tumor growth by castration of male TRAMP-HHD hybrid mice is tested, followed by active immunization with immunogenic A2.1-restricted SIM2 peptides.

Example 8. Manipulation of the T Cell Immunoglobulin-Mucin-1 (Tim-1) Pathway for Overcoming Prostate TAA-Specific Immune Tolerance

Regulatory T lymphocytes (Tregs) severely impede anti-tumor immune responses in various cancers. To investigate the ability of stimulating T cells through the Tim-1 receptor to interfere with the rise of Treg cells and the onset of immune tolerance to TAA, we crossed the TRAMP mouse with GFP-Foxp3-KI mouse. We found that naïve CD4 T and CD8 T cells from the prostate-draining lymph nodes of TRAMP-GFP-Foxp3 hybrid mice exhibit an increased rate of conversion to Treg cells after treatment with anti-CD3/CD28+TGF-β (Figure 9A and 9B). This elevated susceptibility to TGF-β-induced differentiation is enhanced in aged mice. This novel observation might explain why prostate cancer development parallels an increase in Treg in both tumors

and peripheral blood. Depletion of Tregs prior to the administration of cancer vaccines strengthens tumor immunity in non-prostate models. T cell immunoglobulin-mucin-1 (Tim-1) stimulation with an agonistic anti-Tim 1 antibody (Ab), a Tim 4-Ig fusion protein or activation by Tim-4^+ DCs is able to polarize T cells into a TH1 and TH17 dominant phenotypes and subvert tolerance and Tregs. We first tested the ability of the anti-Tim-1 Ab by immunizing B6 mice with a recombinant, Tag-expressing vaccinia and providing different doses of the Ab at the time of immunization. We have found that agonist anti-Tim-1 Ab induced a significant increase in Tag-specific CTL as judged by Tag-tetramer specificity and IFN-γ production by CD8 cells (Figure 9C and 9D). This finding is consistent with a previous report where an agonist anti-Tim-1 antibody enhanced antigen-specific cellular proliferation and IFN-γ production in mice immunized with inactivated influenza virus. We then sought to assess the CTL enhancing ability of the Ab in adult TRAMP-GFP-Foxp3 mice using the same strategy. Similarly, Ab treatment resulted in elevated Tag-specific CTL in both spleen and prostate-draining lymph nodes (Figure 10). Together, our findings support our hypothesis that interference with the Tim-1/Tim-4 interaction by antibodies or fusion proteins will enhance responsiveness to active immunization with ERG or SIM2. The effect of Tim-1 manipulation in various mouse models of prostate cancer is tested by administering the agonist anti-Tim-1 monoclonal antibody concomitantly with antigen under an active and passive immunotherapy regimen and also in combination with androgen ablation.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

All publications, patent applications, and patents mentioned in this specification are herein incorporated by reference to the same extent as if each

independent publication, patent application, or patent was specifically and individually indicated to be incorporated by reference.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention; can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

What is claimed is:

CLAIMS

1. An isolated immune response stimulating peptide having at least 90% sequence identity to a contiguous amino acid sequence set forth in SEQ ID NO:1, wherein said peptide has at least 7 but fewer than 50 amino acid residues and is capable of activating immune cells in a mammalian host when bound to an antigen-presenting molecule.

2. The peptide of claim 1, wherein said sequence identity is 95, 96, 97, 98, or 99%.

3. The peptide of claim 1, wherein said immune response stimulating peptide has between 7 and 30 amino acid residues.

4. The peptide of claim 3, wherein said immune response stimulating peptide has between 7 and 25 amino acid residues.

5. The peptide of claim 4, wherein said immune response stimulating peptide has between 7 and 20 amino acid residues.

6. The peptide of claim 5, wherein said immune response stimulating peptide has between 7 and 15 amino acid residues.

7. The peptide of claim 6, wherein said immune response stimulating peptide has between 7 and 14 amino acid residues.

8. The peptide of claim 7, wherein said immune response stimulating peptide has between 7 and 13 amino acid residues.

9. The peptide of claim 8, wherein said immune response stimulating peptide has between 7 and 12 amino acid residues.

10. The peptide of claim 9, wherein said immune response stimulating peptide has between 7 and 11 amino acid residues.

11. The peptide of claim 10, wherein said immune response stimulating peptide has between 7 and 10 amino acid residues.

12. The peptide of claim 11, wherein said immune response stimulating peptide has 8, 9, or 10 amino acid residues.

13. The peptide of claim 12, wherein said immune response stimulating peptide has 9 amino acid residues.

14. The peptide of claim 1, wherein said immune response stimulating peptide has the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4

15. The peptide of claim 1, wherein said immune cells are cytotoxic T lymphocytes.

16. The peptide of claim 1, wherein said antigen-presenting molecule is an HLA molecule.

17. The peptide of claim 16, wherein said HLA molecule is an HLA class I molecule.

18. A composition, wherein said composition comprises a peptide of claim 1 conjugated to a heterologous compound.

19. A composition comprising the peptide of any one of claims 1-18.

20. The composition of claim 19, wherein said composition is a vaccine.

21. The composition of claim 19, further comprising an adjuvant.

22. The composition of claim 19, further comprising an additional therapeutic agent.

23. An activated immune cell capable of specifically binding to an immune response stimulating peptide of any one of claims 1-18 when said peptide is bound to an antigen-presenting molecule.

24. The activated immune cell of claim 23, wherein said immune cell is a cytotoxic T lymphocyte.

25. The activated immune cell of claim 23, wherein said antigen-presenting molecule is an HLA molecule.

26. The activated immune cell of claim 25, wherein said HLA molecule is an HLA class I molecule.

27. The activated immune cell of claim 23, wherein said peptide is bound to an antigen-presenting molecule on the surface of a cell.

28. The activated immune cell of claim 27, wherein said peptide is bound to an antigen-presenting molecule on the surface of a prostate cancer cell.

29. A composition comprising the activated immune cell of any one of claims 23-28.

30. The composition of claim 29, wherein said composition is a vaccine.

31. The composition of claim 29, further comprising an adjuvant.

32. The composition of claim 29, further comprising an additional therapeutic agent.

33. An antigen-presenting cell comprising on its surface an immune response stimulating peptide of any one of claims 1-18, wherein said peptide is bound to an antigen-presenting molecule on the surface of said antigen-presenting cell.

34. The antigen-presenting cell of claim 33, wherein said antigen-presenting cell is a dendritic cell or a macrophage.

35. The antigen-presenting cell of claim 33, wherein said antigen-presenting molecule is an HLA molecule.

36. The antigen-presenting cell of claim 33, wherein said HLA molecule is an HLA class I molecule.

37. A composition comprising the antigen-presenting cell of any one of claims 33-36.

38. The composition of claim 37, wherein said composition is a vaccine.

39. The composition of claim 37, further comprising an adjuvant.

40. The composition of claim 37, further comprising an additional therapeutic agent.

41. An antibody or antigen-binding fragment thereof that specifically binds to an immune response stimulating peptide of any one of claims 1-18 when said peptide is bound to an antigen-presenting molecule.

42. The antibody or antigen-binding fragment thereof of claim 41, wherein said antigen-presenting molecule is an HLA molecule.

43. The antibody or antigen-binding fragment thereof of claim 41, wherein said HLA molecule is an HLA class I molecule.

44. The antibody or antigen-binding fragment thereof of claim 41, wherein said antibody or antigen-binding fragment thereof is conjugated to a cytotoxic agent.

45. The antibody or antigen-binding fragment thereof of claim 44, wherein said cytotoxic agent is selected from the group consisting of the following compounds and their derivatives: ricin, doxorubicin, methotrexate, camptothecin, homocamptothecin, thiocolchicine, colchicine, combretastatin, combretastin A-4, podophyllotoxin, rhizoxin, rhizoxin-d, dolistatin, , paclitaxel, CC1065, ansamitocin p3, maytansinoid, streptolysin O, stoichactis toxin, phallolysin, staphylococcus alpha toxin, holothurin A, digitonin, melittin, lysolecithin, cardiotoxin, and cerebratulus A toxin.

46. The antibody or antigen-binding fragment thereof of claim 41, wherein said peptide is bound to an antigen-presenting molecule on the surface of a cell.

47. The antibody or antigen-binding fragment thereof of claim 42, wherein said peptide is bound to an antigen-presenting molecule on the surface of a prostate cancer cell.

48. A composition comprising the antibody or antigen-binding fragment thereof of any one of claims 42-47.

49. The composition of claim 48, wherein said composition is a vaccine.

50. The composition of claim 48, further comprising an adjuvant.

51. The composition of claim 48, further comprising an additional therapeutic agent.

52. An isolated immune response stimulating peptide having at least 90% sequence identity to a contiguous amino acid sequence set forth in SEQ ID NO:5, wherein said peptide has at least 7 but fewer than 50 amino acid residues and is capable of activating immune cells in a mammalian host when bound to an antigen-presenting molecule.

53. The peptide of claim 52, wherein said sequence identity is 95, 96, 97, 98, or 99%.

54. The peptide of claim 52, wherein said immune response stimulating peptide has between 7 and 30 amino acid residues.

55. The peptide of claim 54, wherein said immune response stimulating peptide has between 7 and 25 amino acid residues.

56. The peptide of claim 55, wherein said immune response stimulating peptide has between 7 and 20 amino acid residues.

57. The peptide of claim 56, wherein said immune response stimulating peptide has between 7 and 15 amino acid residues.

58. The peptide of claim 57, wherein said immune response stimulating peptide has between 7 and 14 amino acid residues.

59. The peptide of claim 58, wherein said immune response stimulating peptide has between 7 and 13 amino acid residues.

60. The peptide of claim 59, wherein said immune response stimulating peptide has between 7 and 12 amino acid residues.

61. The peptide of claim 60, wherein said immune response stimulating peptide has between 7 and 11 amino acid residues.

62. The peptide of claim 61, wherein said immune response stimulating peptide has between 7 and 10 amino acid residues.

63. The peptide of claim 62, wherein said immune response stimulating peptide has 8, 9, or 10 amino acid residues.

64. The peptide of claim 63, wherein said immune response stimulating peptide has 9 amino acid residues.

65. The peptide of claim 52, wherein said immune response stimulating peptide has the amino acid sequence of SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.

66. The peptide of claim 52, wherein said immune cells are cytotoxic T lymphocytes.

67. The peptide of claim 52, wherein said antigen-presenting molecule is an HLA molecule.

68. The peptide of claim 67, wherein said HLA molecule is an HLA class I molecule.

69. A composition, wherein said composition comprises a peptide of claim 52 conjugated to a heterologous compound.

70. A composition comprising the peptide of any one of claims 52-69.

71. The composition of claim 70, wherein said composition is a vaccine.

72. The composition of claim 70, further comprising an adjuvant.

73. The composition of claim 70, further comprising an additional therapeutic agent.

74. An activated immune cell capable of specifically binding to an immune response stimulating peptide of any one of claims 52-69 when said peptide is bound to an antigen-presenting molecule.

75. The activated immune cell of claim 74, wherein said immune cell is a cytotoxic T lymphocyte.

76. The activated immune cell of claim 74, wherein said antigen-presenting molecule is an HLA molecule.

77. The activated immune cell of claim 76, wherein said HLA molecule is an HLA class I molecule.

78. The activated immune cell of claim 74, wherein said peptide is bound to an antigen-presenting molecule on the surface of a cell.

79. The activated immune cell of claim 78, wherein said peptide is bound to an antigen-presenting molecule on the surface of a prostate cancer cell.

80. A composition comprising the activated immune cell of any one of claims 74-79.

81. The composition of claim 80, wherein said composition is a vaccine.

82. The composition of claim 80, further comprising an adjuvant.

83. The composition of claim 80, further comprising an additional therapeutic agent.

84. An antigen-presenting cell comprising on its surface an immune response stimulating peptide of any one of claims 52-69, wherein said peptide is bound to an antigen-presenting molecule on the surface of said antigen-presenting cell.

85. The antigen-presenting cell of claim 84, wherein said antigen-presenting cell is a dendritic cell or a macrophage.

86. The antigen-presenting cell of claim 84, wherein said antigen-presenting molecule is an HLA molecule.

87. The antigen-presenting cell of claim 84, wherein said HLA molecule is an HLA class I molecule.

88. A composition comprising the antigen-presenting cell of any one of claims 84-87.

89. The composition of claim 88, wherein said composition is a vaccine.

90. The composition of claim 88, further comprising an adjuvant.

91. The composition of claim 88, further comprising an additional therapeutic agent.

92. An antibody or antigen-binding fragment thereof that specifically binds to an immune response stimulating peptide of any one of claims 52-69 when said peptide is bound to an antigen-presenting molecule.

93. The antibody or antigen-binding fragment thereof of claim 92, wherein said antigen-presenting molecule is an HLA molecule.

94. The antibody or antigen-binding fragment thereof of claim 92, wherein said HLA molecule is an HLA class I molecule.

95. The antibody or antigen-binding fragment thereof of claim 92, wherein said antibody or antigen-binding fragment thereof is conjugated to a cytotoxic agent.

96. The antibody or antigen-binding fragment thereof of claim 95, wherein said cytotoxic agent is selected from the group consisting of the following compounds and their derivatives: ricin, doxorubicin, methotrexate, camptothecin, homocamptothecin, thiocolchicine, colchicine, combretastatin, combretastin A-4, podophyllotoxin, rhizoxin, rhizoxin-d, dolistatin, , paclitaxel, CC1065, ansamitocin p3, maytansinoid, streptolysin O, stoichactis toxin, phallolysin, staphylococcus alpha toxin, holothurin A, digitonin, melittin, lysolecithin, cardiotoxin, and cerebratulus A toxin.

97. The antibody or antigen-binding fragment thereof of claim 92, wherein said peptide is bound to an antigen-presenting molecule on the surface of a cell.

98. The antibody or antigen-binding fragment thereof of claim 92, wherein said peptide is bound to an antigen-presenting molecule on the surface of a prostate cancer cell.

99. A composition comprising the antibody or antigen-binding fragment thereof of any one of claims 92-98.

100. The composition of claim 99, wherein said composition is a vaccine.

101. The composition of claim 99, further comprising an adjuvant.

102. The composition of claim 99, further comprising an additional therapeutic agent.

103. An isolated immune response stimulating peptide having at least 90% sequence identity to a contiguous amino acid sequence set forth in any one of the following sequences: SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:26, wherein said peptide has at least 7 but fewer than 50 amino acid residues and is capable of activating immune cells in a mammalian host when bound to an antigen-presenting molecule.

104. The peptide of claim 103, wherein said sequence identity is 95, 96, 97, 98, or 99%.

105. The peptide of claim 103, wherein said immune response stimulating peptide has between 7 and 30 amino acid residues.

106. The peptide of claim 105, wherein said immune response stimulating peptide has between 7 and 25 amino acid residues.

107. The peptide of claim 106, wherein said immune response stimulating peptide has between 7 and 20 amino acid residues.

108. The peptide of claim 107, wherein said immune response stimulating peptide has between 7 and 15 amino acid residues.

109. The peptide of claim 108, wherein said immune response stimulating peptide has between 7 and 14 amino acid residues.

110. The peptide of claim 109, wherein said immune response stimulating peptide has between 7 and 13 amino acid residues.

111. The peptide of claim 110, wherein said immune response stimulating peptide has between 7 and 12 amino acid residues.

112. The peptide of claim 111, wherein said immune response stimulating peptide has between 7 and 11 amino acid residues.

113. The peptide of claim 112, wherein said immune response stimulating peptide has between 7 and 10 amino acid residues.

114. The peptide of claim 113, wherein said immune response stimulating peptide has 8, 9, or 10 amino acid residues.

115. The peptide of claim 114, wherein said immune response stimulating peptide has 9 amino acid residues.

116. The peptide of claim 103, wherein said immune cells are cytotoxic T lymphocytes.

117. The peptide of claim 103, wherein said antigen-presenting molecule is an HLA molecule.

118. The peptide of claim 117, wherein said HLA molecule is an HLA class I molecule.

119. A composition, wherein said composition comprises a peptide of claim 103 conjugated to a heterologous compound.

120. A composition comprising the peptide of any one of claims 103-119.

121. The composition of claim 120, wherein said composition is a vaccine.

122. The composition of claim 120, further comprising an adjuvant.

123. The composition of claim 120, further comprising an additional therapeutic agent.

124. An activated immune cell capable of specifically binding to an immune response stimulating peptide of any one of claims 103-119 when said peptide is bound to an antigen-presenting molecule.

125. The activated immune cell of claim 124, wherein said immune cell is a cytotoxic T lymphocyte.

126. The activated immune cell of claim 124, wherein said antigen-presenting molecule is an HLA molecule.

127. The activated immune cell of claim 126, wherein said HLA molecule is an HLA class I molecule.

128. The activated immune cell of claim 124, wherein said peptide is bound to an antigen-presenting molecule on the surface of a cell.

129. The activated immune cell of claim 128, wherein said peptide is bound to an antigen-presenting molecule on the surface of a prostate cancer cell.

130. A composition comprising the activated immune cell of any one of claims 124-129.

131. The composition of claim 130, wherein said composition is a vaccine.

132. The composition of claim 130, further comprising an adjuvant.

133. The composition of claim 130, further comprising an additional therapeutic agent.

134. An antigen-presenting cell comprising on its surface an immune response stimulating peptide of any one of claims 103-119, wherein said peptide is bound to an antigen-presenting molecule on the surface of said antigen-presenting cell.

135. The antigen-presenting cell of claim 134, wherein said antigen-presenting cell is a dendritic cell or a macrophage.

136. The antigen-presenting cell of claim 134, wherein said antigen-presenting molecule is an HLA molecule.

137. The antigen-presenting cell of claim 136, wherein said HLA molecule is an HLA class I molecule.

138. A composition comprising the antigen-presenting cell of any one of claims 134-137.

139. The composition of claim 138, wherein said composition is a vaccine.

140. The composition of claim 138, further comprising an adjuvant.

141. The composition of claim 138, further comprising an additional therapeutic agent.

142. An antibody or antigen-binding fragment thereof that specifically binds to an immune response stimulating peptide of any one of claims 103-119 when said peptide is bound to an antigen-presenting molecule.

143. The antibody or antigen-binding fragment thereof of claim 142, wherein said antigen-presenting molecule is an HLA molecule.

144. The antibody or antigen-binding fragment thereof of claim 143, wherein said HLA molecule is an HLA class I molecule.

145. The antibody or antigen-binding fragment thereof of claim 142, wherein said antibody or antigen-binding fragment thereof is conjugated to a cytotoxic agent.

146. The antibody or antigen-binding fragment thereof of claim 145, wherein said cytotoxic agent is selected from the group consisting of the following compounds and their derivatives: ricin, doxorubicin, methotrexate, camptothecin, homocamptothecin, thiocolchicine, colchicine, combretastatin, combretastin A-4, podophyllotoxin, rhizoxin, rhizoxin-d, dolistatin, , paclitaxel, CC1065, ansamitocin p3, maytansinoid, streptolysin O, stoichactis toxin, phallolysin, staphylococcus alpha toxin, holothurin A, digitonin, melittin, lysolecithin, cardiotoxin, and cerebratulus A toxin.

147. The antibody or antigen-binding fragment thereof of claim 142, wherein said peptide is bound to an antigen-presenting molecule on the surface of a cell.

148. The antibody or antigen-binding fragment thereof of claim 147, wherein said peptide is bound to an antigen-presenting molecule on the surface of a prostate cancer cell.

149. A composition comprising the antibody or antigen-binding fragment thereof of any one of claims 142-148.

150. The composition of claim 149, wherein said composition is a vaccine.

151. The composition of claim 149, further comprising an adjuvant.

152. The composition of claim 149, further comprising an additional therapeutic agent.

153. A method of treating prostate cancer in a subject comprising administering to said subject a composition comprising the peptide of any one of claims 1-18, 52-69, or 103-119 in an amount sufficient to treat said prostate cancer.

154. The method of claim 153, wherein said method further comprises administering an adjuvant.

155. The method of claim 153, wherein said method further comprises administering a cytokine.

156. The method of claim 153, wherein said method further comprises administering hormone therapy.

157. The method of claim 156, wherein said hormone therapy is androgen ablation.

158. The method of claim 153, wherein said composition comprises a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant.

159. The method of claim 153, wherein said composition is suitable for administration to a human.

160. The method of claim 153, wherein said composition is administered by injection.

161. A method of treating prostate cancer in a subject comprising administering to said subject a composition comprising the immune cell of any one of claims 1-18, 52-69, or 103-119 in an amount sufficient to treat said prostate cancer.

162. The method of claim 161, wherein said method further comprises administering an adjuvant.

163. The method of claim 161, wherein said method further comprises administering a cytokine.

164. The method of claim 161, wherein said method further comprises administering hormone therapy.

165. The method of claim 164, wherein said hormone therapy is androgen ablation.

166. The method of claim 161, wherein said composition comprises a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant.

167. The method of claim 161, wherein said composition is suitable for administration to a human.

168. The method of claim 161, wherein said composition is administered by injection.

169. A method of treating prostate cancer in a subject comprising administering to said subject a composition comprising the antigen-presenting cell of any one of claims 1-18, 52-69, or 103-119 in an amount sufficient to treat said prostate cancer.

170. The method of claim 169, wherein said method further comprises administering an adjuvant.

171. The method of claim 169, wherein said method further comprises administering a cytokine.

172. The method of claim 169, wherein said method further comprises administering hormone therapy.

173. The method of claim 172, wherein said hormone therapy is androgen ablation.

174. The method of claim 169, wherein said composition comprises a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant.

175. The method of claim 169, wherein said composition is suitable for administration to a human.

176. The method of claim 169, wherein said composition is administered by injection.

177. A method of treating prostate cancer in a subject comprising administering to said subject a composition comprising the antigen-presenting cell of any one of claims 1-18, 52-69, or 103-119 in an amount sufficient to treat said prostate cancer.

178. The method of claim 177, wherein said method further comprises administering an adjuvant.

179. The method of claim 177, wherein said method further comprises administering a cytokine.

180. The method of claim 177, wherein said method further comprises administering hormone therapy.

181. The method of claim 180, wherein said hormone therapy is androgen ablation.

182. The method of claim 177, wherein said composition comprises a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant.

183. The method of claim 177, wherein said composition is suitable for administration to a human.

184. The method of claim 177, wherein said composition is administered by injection.

185. A method of treating prostate cancer in a subject comprising administering to said subject comprising the antibody or antigen-binding fragment thereof of any one of claims 1-18, 52-69, or 103-119 in an amount sufficient to treat said prostate cancer.

186. The method of claim 185, wherein said method further comprises administering an adjuvant.

187. The method of claim 185, wherein said method further comprises administering a cytokine.

188. The method of claim 185, wherein said method further comprises administering hormone therapy.

189. The method of claim 188, wherein said hormone therapy is androgen ablation.

190. The method of claim 185, wherein said composition comprises a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant.

191. The method of claim 185, wherein said composition is suitable for administration to a human.

192. The method of claim 185, wherein said composition is administered by injection.

193. The peptide of claim 18, wherein said heterologous compound is a therapeutic or cytotoxic agent.

194. The peptide of claim 69, wherein said heterologous compound is a therapeutic or cytotoxic agent.

195. The peptide of claim 119, wherein said heterologous compound is a therapeutic or cytotoxic agent.

196. A method of diagnosing a subject as having prostate cancer, said method comprising measuring the level of an ERG or SIM2 polypeptide, or fragment thereof, in a sample from said subject and comparing it to a reference, wherein an alteration in said level compared to a reference is a diagnostic indicator of prostate cancer.

197. The method of claim 196, wherein said ERG polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, or 4.

198. The method of claim 196, wherein said SIM2 polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 5, 6, 7, 8, 9, or 10.

199. The method of claim 196, wherein said alteration in said level is an increase.

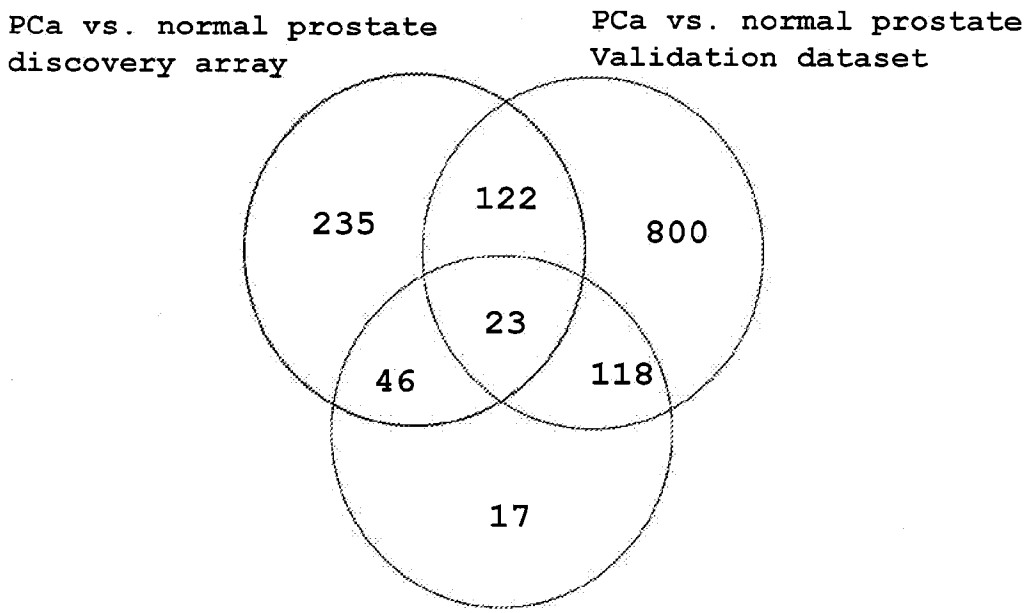
200. The method of claim 196, wherein said measuring is done on two or more occasions and an increase in said level between measurements is a diagnostic indicator of said prostate cancer.

201. The method of claim 196, wherein said sample is a bodily fluid, cell, or tissue sample from said subject in which said ERG or SIM2 polypeptide is normally detectable.

202. The method of claim 201, wherein said bodily fluid is selected from the group consisting of urine, blood, serum, plasma, and cerebrospinal fluid.

203. The method of claim 196, wherein said subject is a human.

Figure 1


PCa vs. normal prostate
discovery array
PCa vs. normal prostate
Validation dataset
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23
46
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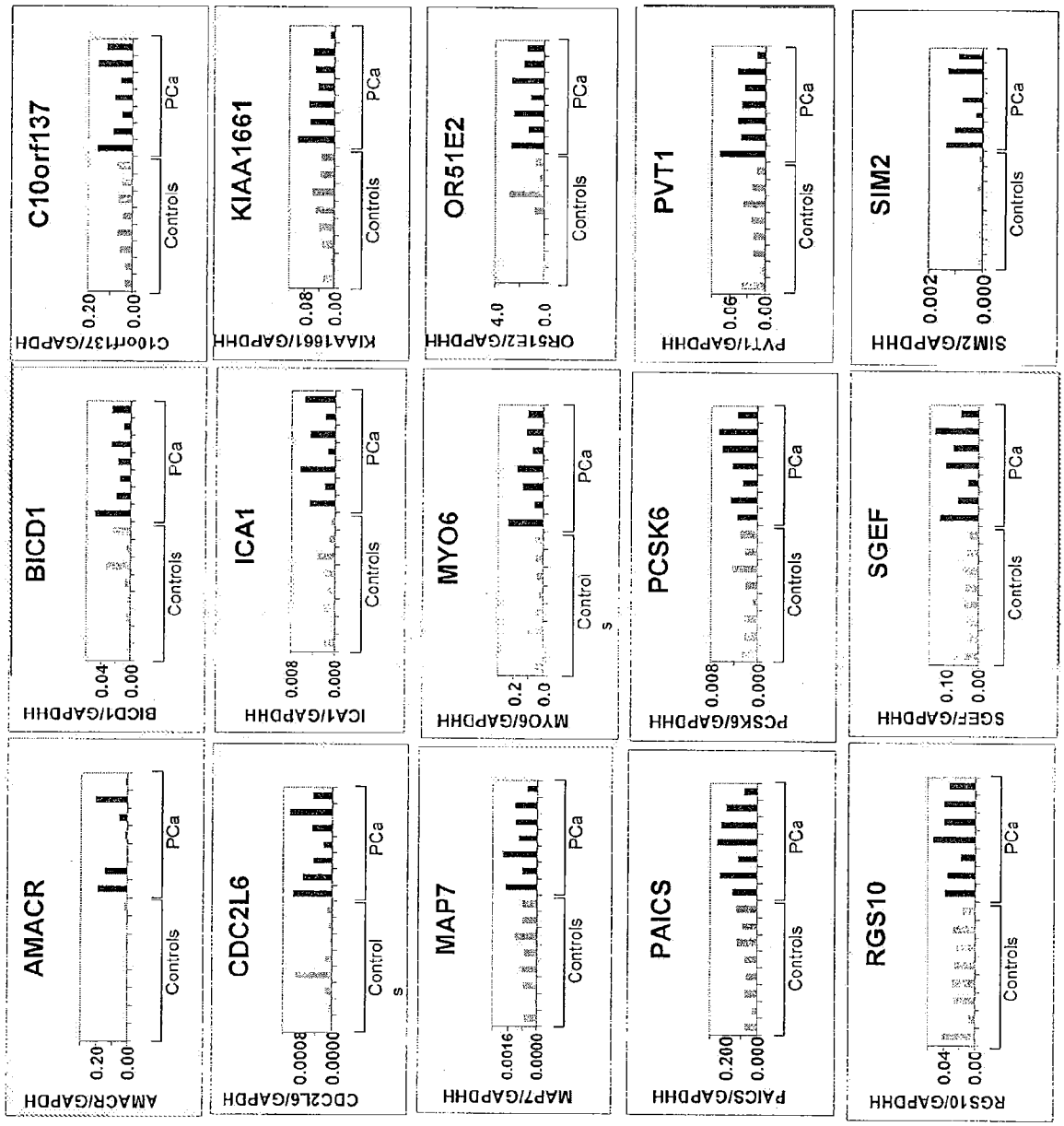
17
Genes underexpressed in extraprostatic
Human adult male tissues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BICD1/GAPDH
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0.20
0.00
CDC2L6
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0.0008
0.0000
Control
s
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0.000
KIAA1661
KIAA1661/GAPDH
0.08
0.00
MAP7
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0.0000
MYO6
MYO6/GAPDH
0.2
0.0
OR51E2
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4.0
0.0
PAICS
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0.000
PVT1
PVT1/GAPDH
0.06
0.00
RGS10
RGS10/GAPDH
0.04
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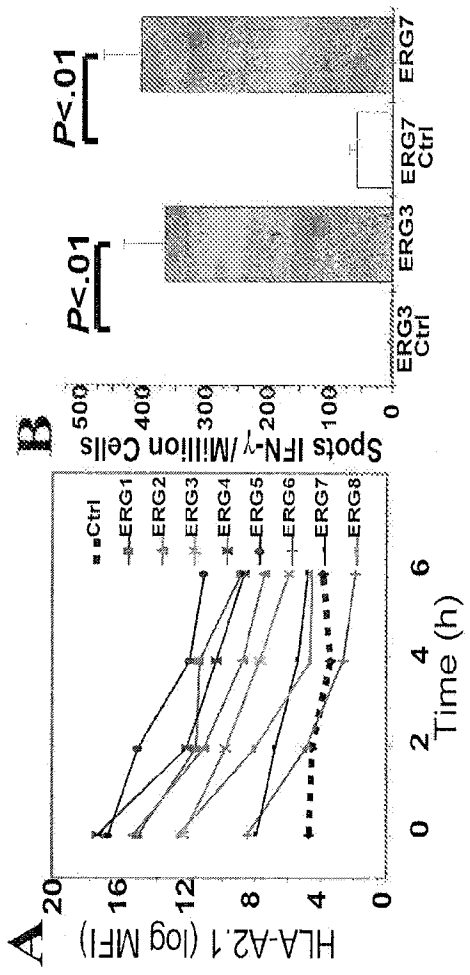
0.00
SIM2
SIM2/GAPDH
0.002
0.000

Figure 2

Figure 3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ERG2
ERG3
ERG4
ERG5
ERG6
ERG7
ERG8
B
Spots IFN-γ /Million Cells
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300
200
100
0
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P<.01
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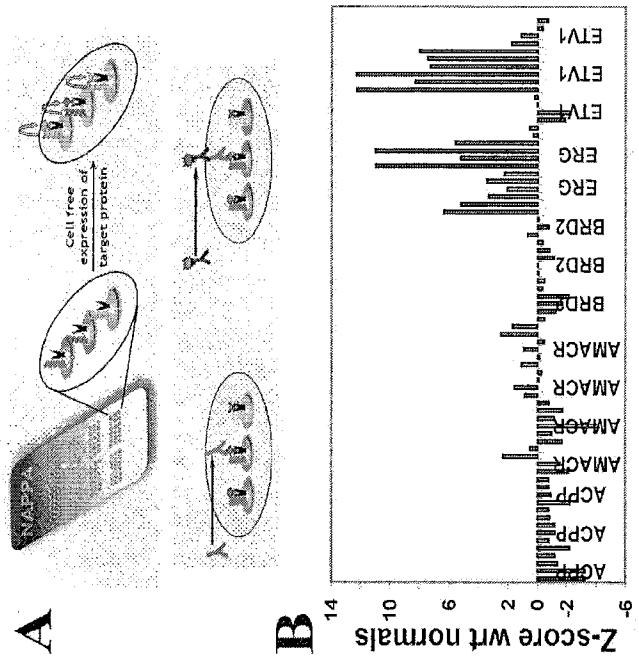
ERG3
ERG7 Ctrl
ERG7

Figure 4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10
6
2
0
-2
-6
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ETV1
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BRD2
BRD2
BRD2
AMACR
AMACR
AMACR
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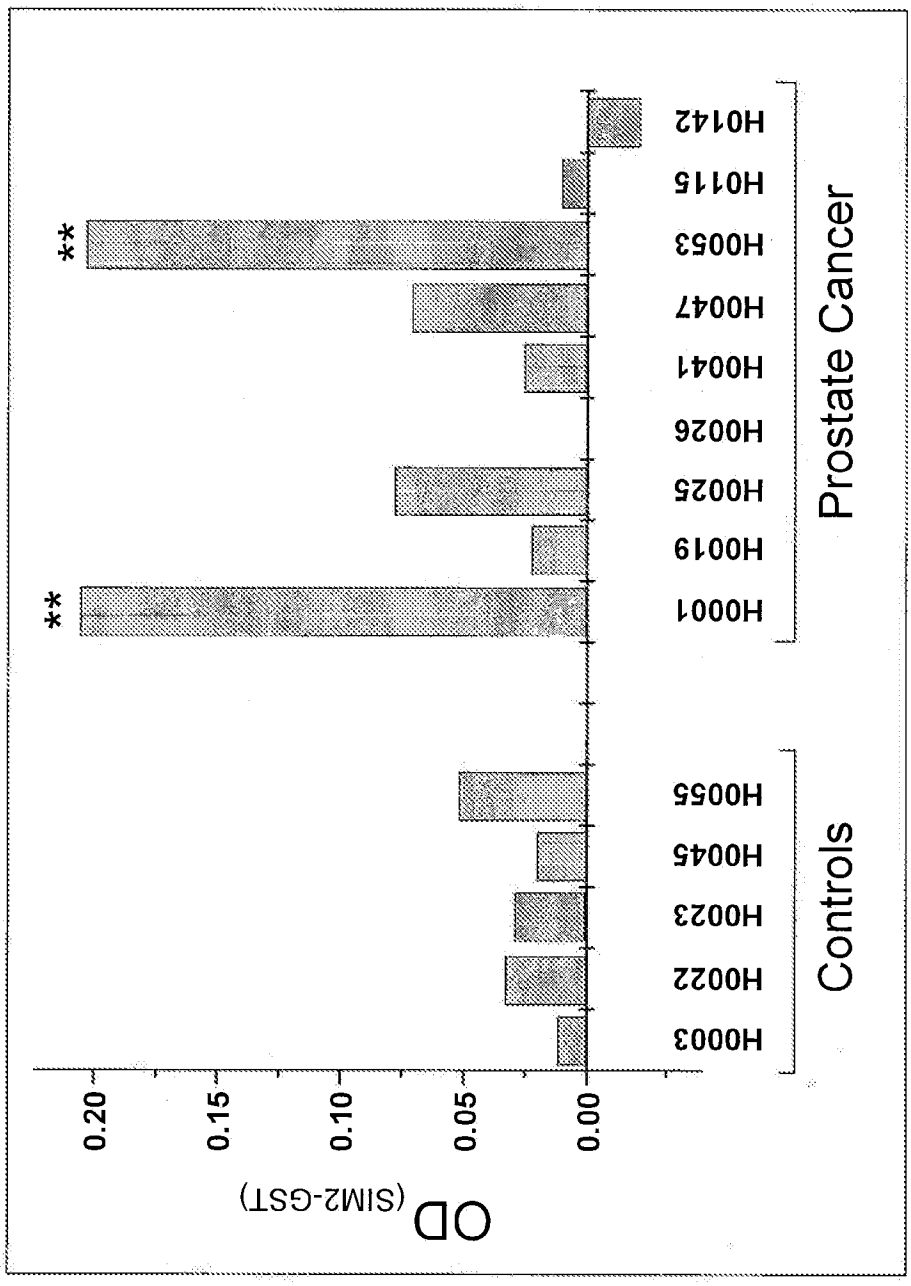
ACPP
ACPP
ACPP

Figure 5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0.05
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Controls
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H0019
H0025
H0026
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H0047
H0053
H0115
H0142
Prostate Cancer
**
**

Figure 6

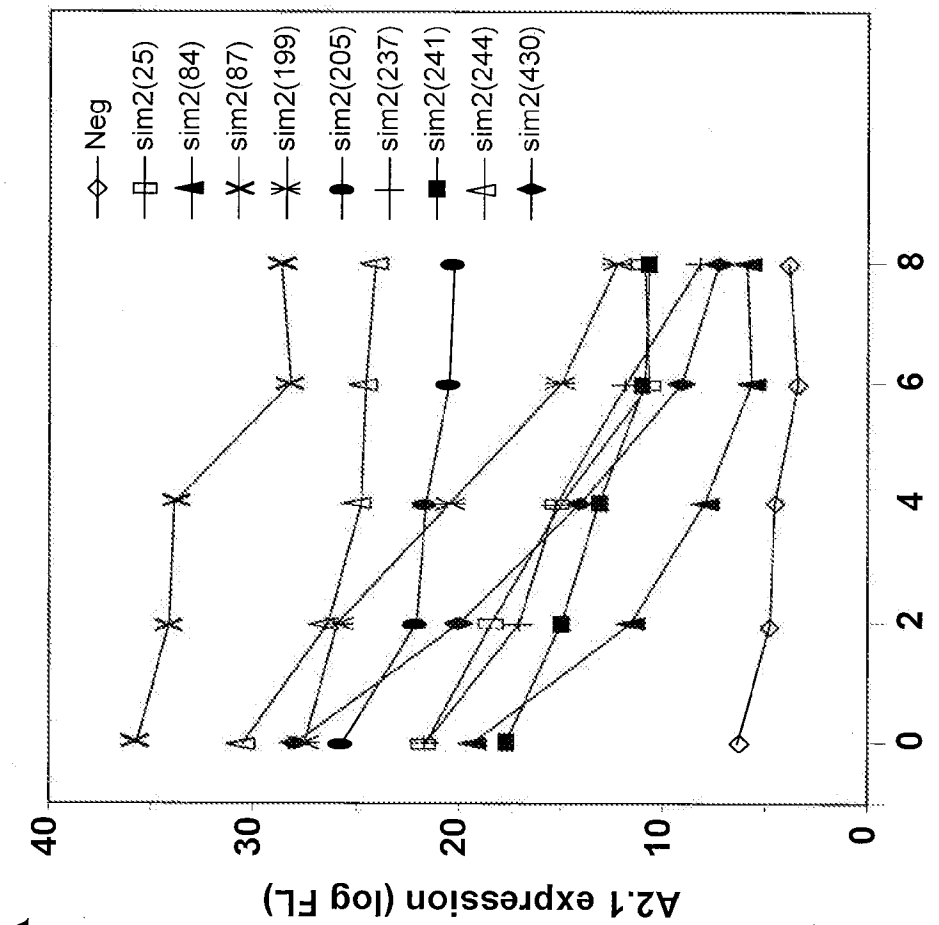

A
A2.1 expression (log FL)
40
30
20
10
0
0
2
4
6
8
Time (h)
Neg
sim2(25)
sim2(84)
sim2(87)
sim2(199)
sim2(205)
sim2(237)
sim2(241)
sim2(244)
sim2(430)

Figure 6 (continued)

B

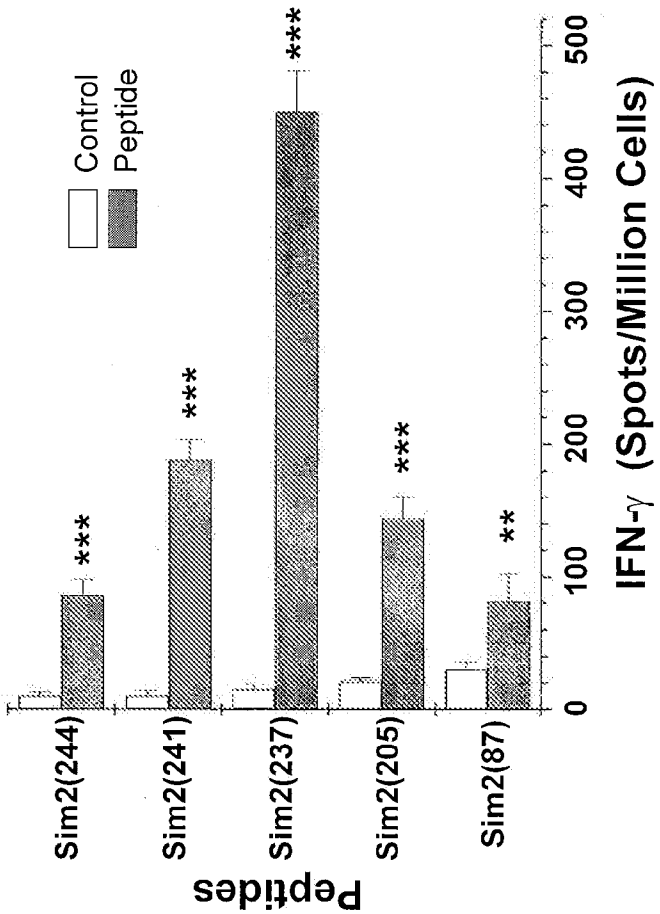


Figure 7

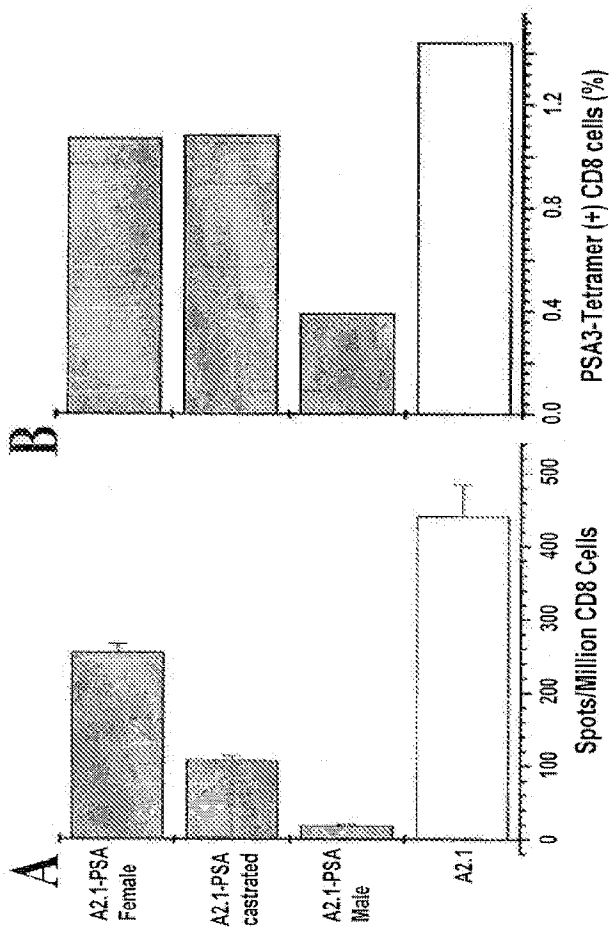

A
A2.1-PSA Female
A2.1-PSA castrated
A2.1-PSA Male
A2.1
0
100
200
300
400
500
Spots/Million CD8 Cells
B
0.0
0.4
0.8
1.2
PSA3-Tetramer (+) CD8 cells (%)

Figure 8

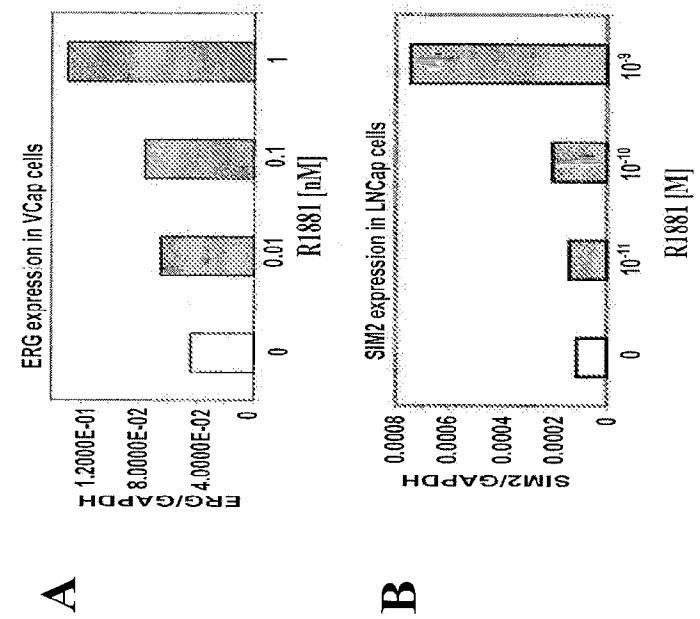
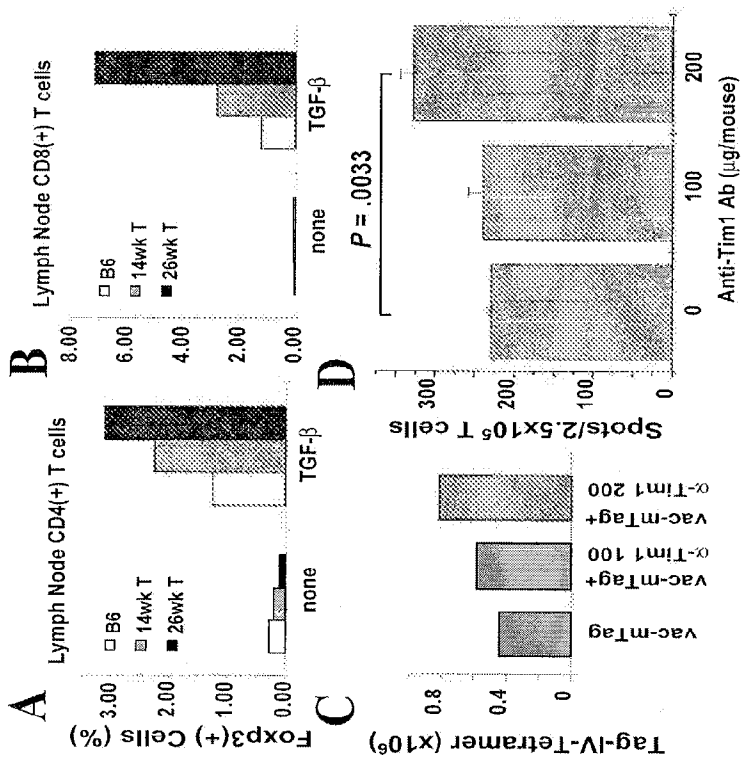

A
ERG expression in VCap cells
ERG/GAPDH
1.2000E-01
8.0000E-02
4.0000E-02
0
0
0.01
0.1
1
R1881 [nM]
B
SIM2 expression in LNCap cells
SIM2/GAPDH
0.0008
0.0006
0.0004
0.0002
0
0
10-11
10-10
10-9
R1881 [M]

Figure 9


A
Lymph Node CD4(+) T cells
B6
14wk T
26wk T
Foxp3(+) Cells (%)
3.00
2.00
1.00
0.00
none
TGF-β
B
Lymph Node CD8(+) T cells
8.00
6.00
4.00
2.00
0.00
C
Tag-IV-Tetramer (x10⁶)
0.8
0.4
0
vac-mTag
vac-mTag+ α-Tim1 100
vac-mTag+ α-Tim1 200
D
P = .0033
Spots/2.5x10⁵ T cells
300
200
100
0
0
100
200
Anti-Tim1 Ab (μg/mouse)

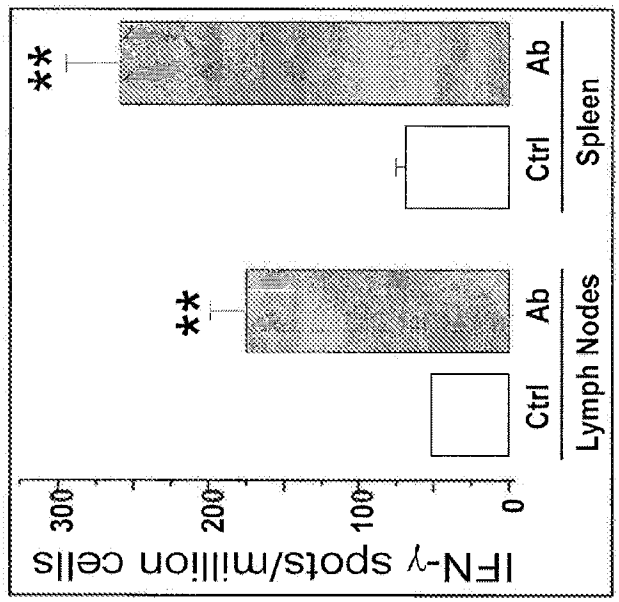

IFN-γ spots/million cells
300
200
100
0
**
**
Ctrl
Ab
Lymph Nodes
Ctrl
Ab
Spleen

Figure 10

Figure 11

MALQGISVVELSGLAPGPFCAMVLADFGARVVRVDRPGSRYDVSRLGRGKRSLV
LDLKQPRGAAVLRRLCKRSDVLLEPFRRGVMEKLQLGPEILQRENPRLIYARLS
GFGQSGSFCRLAGHDINYLALSGVLSKIGRSGENPYAPLNLLADFAGGGLMCAL
GIIMALFDRTRTGKGQVIDANMVEGTAYLSSFLWKTQKLSLWEAPRGQNMLDGG
APFYTTYRTADGEFMAVGAIEPQFYELLIKGLGLKSDELPNQMSMDDWPEMKKK
FADVFAEKTKAEWCQIFDGTDACVTPVLTFEEVVHHDHNKERGSFITSEEQDVS
PRPAPLLLNTPAIPSFKRDPFIGEHTEEILEEFGFSREEIYQLNSDKIIESNKV
KASL (SEQ ID NO:11)

Figure 12

MAAEEVLQTVDHYKTEIERLTKELTETTHEKIQAAEYGLVVLEEKLTLKQQYDELEAEYDSLKQELEQL
KEAFGQSFSIHRKVAEDGETREETLLQESASKEAYYLGKILEMQNELKQSRAVVTNVQAENERLTAVVQ
DLKENNEMVELQRIRMKDEIREYKFREARLLQDYTELEEENITLQKLVSTLKQNQVEYEGLKHEIKRFE
EETVLLNSQLEDAIRLKEIAEHQLEEALETLKNEREQKNNLRKELSQYISLNDNHISISVDGLKFAEDG
SEPNNDDKMNGHIHGPLVKLNGDYRTPTLRKGESLNPVSDLFSELNISEIQKLKQQLMQVEREKAILLA
NLQESQTQLEHTKGALTEQHERVHRLTEHVNAMRGLQSSKELKAELDGEKGRDSGEEAHDYEVDINGLE
ILECKYRVAVTEVIDLKAEIKALKEKYNKSVENYTDEKAKYESKIQMYDEQVTSLEKTTKESGEKMAHM
EKELQKMTSIANENHSTLNTAQDELVTFSEELAQLYHHVCLCNNETPNRVMLDYYRQSRVTRSGSLKGP
DDPRGLLSPRLARRGVSSPVETRTSSEPVAKESTEASKEPSPTKTPTISPVITAPPSSPVLDTSDIRKE
PMNIYNLNAIIRDQIKHLQKAVDRSLQLSRQRAAARELAPMIDKDKEALMEEILKLKSLLSTKREQIAT
LRAVLKANKQTAEVALANLKNKYENEKAMVTETMTKLRNELKALKEDAATFSSLRAMFATRCDEYVTQL
DEMQRQLAAAEDEKKTLNTLLRMAIQQKLALTQRLEDLEFDHEQSRRSKGKLGKSKIGSPKVSGEASVT
VPTIDTYLLHSQGPQTPNIRVSSGTQRKRQFSPSLCDQSRPRTSGASYLQNLLRVPPDPTSTESFLLKG
PPSMSEFIQGHRLSKEKRLTVAPPDCQQPAASVPPQCSQLAGRQDCPTVSPDTALPEEQPHSSSQCAPL
HCLSKPPHP (SEQ ID NO:12)

Figure 13

MALFLDKMGSLQKGNYSSQSGMIPGSWQHKMKLQLILKSSKAYY
VLSDAAMSLQKYGRALRYIKLALQSHDTYCCLCTNMLSEVLLFLSQYLTLCG
DIQLMLAQNANNRAAHLEEFHYQTKEDQEILHSLHRESSCQGVPQAWTTWFT
VGLCSLAHAYLSIQKRGRNIRVLIFALYLFIYFLRRSFALVAQAGVQWCNLG
SLKPPPPGFKQFSCLSLPSSWNYRHAPPCPASPPWPPKVLGLQV
(SEQ ID NO:13)

Figure 14

MDYDFKAKLAAERERVEDLFEYEGCKVGRGTYGHVYKARRKDGKDEKEYALKQIEGTGISMSACREI
ALLRELKHPNVIALQKVFLSHSDRKVWLLFDYAEHDLWHIIKFHRASKANKKPMQLPRSMVKSLLYQ
ILDGIHYLHANWVLHRDLKPANILVMGEGPERGRVKIADMGFARLFNSPLKPLADLDPVVVTFWYRA
PELLLGARHYTKAIDIWAIGCIFAELLTSEPIFHCRQEDIKTSNPFHHDQLDRIFSVMGFPADKDWE
DIRKMPEYPTLQKDFRRTTYANSSLIKYMEKHKVKPDSKVFLLLQKLLTMDPTKRITSEQALQDPYF
QEDPLPTLDVFAGCQIPYPKREFLNEDDPEEKGDKNQQQQQNQHQQPTAPPQQAAAPPQAPPPQQNS
TQTNGTAGGAGAGVGGTGAGLQHSQDSSLNQVPPNKKPRLGPSGANSGGPVMPSDYQHSSSRLNYQS
SVQGSSQSQSTLGYSSSSQQSSQYHPSHQAHRY (SEQ ID NO:14)

Figure 15

MSGHKCSYPWDLQDRYAQDKSVVNKMQQKYWETKQAFIKATGKKEDEHVVASDADLDAKLELFHSI
QRTCLDLSKAIVLYQKRICFLSQEENELGKFLRSQGFQDKTRAGKMMQATGKALCFSSQQRLALRN
PLCRFHQEVETFRHRAISDTWLTVNRMEQCRTEYRGALLWMKDVSQELDPDLYKQMEKFRKVQTQV
RLAKKNFDKLKMDVCQKVDLLGASRCNLLSHMLATYQTTLLHFWEKTSHTMAAIHESFKGYQPYEF
TTLKSLQDPMKKLVEKEEKKKINQQESTDAAVQEPSQLISLEEENQRKESSSFKTEDGKSILSALD
KGSTHTACSGPIDELLDMKSEEGACLGPVAGTPEPEGADKDDLLLLSEIFNASSLEEGEFSKEWAA
VFGDGQVKEPVPTMALGEPDPKAQTGSGFLPSQLLDQNMKDLQASLQEPAKAASDLTAWFSLFADL
DPLSNPDAVGKTDKEHELLNA (SEQ ID NO:15)

Figure 16

GDDGGGCDDGDDDGDDDGGGGDGGGDGDDGGDGGDDDGDHDD
GDGGYGGDDGDDDGDGGGDGDDDDSDDGGDDANDDGGGCHAL
LTSGKD (SEQ ID NO:16)

Figure 17

MAELGAGGDGHRGGDGAVRSETAPDSYKVQDKKNASSRPASAISGQNNNHSGNKPDPPPVLR
VDDRQRLARERREEREKQLAAREIVWLEREERARQHYEKHLEERKKRLEEQRQKEERRRAAV
EEKRRQRLEEDKERHEAVVRRTMERSQKPKQKHNRWSWGGSLHGSPSIHSAARRLQLSPWES
SVVNRLLTPTHSFLARSKSTAALSGEAASCSPIIMPYKAAHSRNSMDRPKLFVTPPEGSSRR
RIIHGTASYKKERERENVLFLTSGTRRAVSPSNPKARQPARSRLWLPSKSLPHLPGTPRPTS
SLPPGSVKAAPAQVRPPSPGNIRPVKREVKVEPEKKDPEKEPQKVANEPSLKGRAPLVKVEE
ATVEERTPAEPEVGPAAPAMAPAPASAPAPASAPAPAPVPTPAMVSAPSSTVNASASVKTSA
GTTDPEEATRLLAEKRRLAREQREKEERERREQEELERQKREELAQRVAEERTTRREEESRR
LEAEQAREKEEQLQRQAEERALREREEAERAQRQKEEEARVREEAERVRQEREKHFQREEQE
RLERKKRLEEIMKRTRRTEATDKKTSDQRNGDIAKGALTGGTEVSALPCTTNAPGNGKPVGS
PHVVTSHQSKVTVESTPDLEKQPNENGVSVQNENFEEIINLPIGSKPSRLDVTNSESPEIPL
NPILAFDDEGTLGPLPQVDGVQTQQTAEVI (SEQ ID NO:17)

Figure 18

MEDGKPVWAPHPTDGFQMGNIVDIGPDSLTIEPLNQKGKTFLAL
INQVFPAEEDSKKDVEDNCSLMYLNEATLLHNIKVRYSKDRIYTYVANILIAVNPYFD
IPKIYSSEAIKSYQGKSLGTRPPHVFAIADKAFRDMKVLKMSQSIIVSGESGAGKTEN
TKFVLRYLTESYGTGQDIDDRIVEANPLLEAFGNAKTVRNNNSSRFGKFVEIHFNEKS
SVVGGFVSHYLLEKSRICVQGKEERNYHIFYRLCAGASEDIREKLHLSSPDNFRYLNR
GCTRYFANKETDKQILQNRKSPEYLKAGSMKDPLLDDHGDFIRMCTAMKKIGLDDEEK
LDLFRVVAGVLHLGNIDFEEAGSTSGGCNLKNKSAQSLEYCAELLGLDQDDLRVSLTT
RVMLTTAGGTKGTVIKVPLKVEQANNARDALAKTVYSHLFDHVVNRVNQCFPFETSSY
FIGVLDIAGFEYFEHNSFEQFCINYCNEKLQQFFNERILKEEQELYQKEGLGVNEVHY
VDNQDCIDLIEAKLVGILDILDEENRLPQPSDQHFTSAVHQKHKDHFRLTIPRKSKLA
VHRNIRDDEGFIIRHFAGAVCYETTQFVEKNNDALHMSLESLICESRDKFIRELFESS
TNNNKDTKQKAGKLSFISVGNKFKTQLNLLLDKLRSTGASFIRCIKPNLKMTSHHFEG
AQILSQLQCSGMVSVLDLMQGGYPSRASFHELYNMYKKYMPDKLARLDPRLFCKALFK
ALGLNENDYKFGLTKVFFRPGKFAEFDQIMKSDPDHLAELVKRVNHWLTCSRWKKVQW
CSLSVIKLKNKIKYRAEACIKMQKTIRMWLCKRRHKPRIDGLVKVGTLKKRLDKFNEV
VSVLKDGKPEMNKQIKNLEISIDTLMAKIKSTMMTQEQIQKEYDALVKSSEELLSALQ
KKKQQEEEAERLRRIQEEMEKERKRREEDEKRRRKEEEERRMKLEMEAKRKQEEEERK
KREDDEKRIQAEVEAQLARQKEEESQQQAVLEQERRDRELALRIAQSEAELISDEAQA
DLALRRNDGTRPKMTPEQMAKEMSEFLSRGPAVLATKAAAGTKKYDLSKWKYAELRDT
INTSCDIELLAACREEFHRRLKVYHAWKSKNKKRNTETEQRAPKSVTDYDFAPFLNNS
PQQNPAAQIPARQREIEMNRQQRFFRIPFIRPADQYKDPQSKKKGWWYAHFDGPWIAR
QMELHPDKPPILLVAGKDDMEMCELNLEETGLTRKRGAEILPRQFEEIWERCGGIQYL
QNAIESRQARPTYATAMLQSLLK (SEQ ID NO:18)

Figure 19

MSSCNFTHATFVLIGIPGLEKAHFWVGFPLLSMYVVAMFGNCIV
VFIVRTERSLHAPMYLFLCMLAAIDLALSTSTMPKILALFWFDSREISFEACLTQMFF
IHALSAIESTILLAMAFDRYVAICHPLRHAAVLNNTVTAQIGIVAVVRGSLFFFPLPL
LIKRLAFCHSNVLSHSYCVHQDVMKLAYADTLPNVVYGLTAILLVMGVDVMFISLSYF
LIIRTVLQLPSKSERAKAFGTCVSHIGVVLAFYVPLIGLSVVHRFGNSLHPIVRVVMG
DIYLLLPPVINPIIYGAKTKQIRTRVLAMFKISCDKDLQAVGGK (SEQ ID NO:19)

Figure 20

MATAEVLNIGKKLYEGKTKEVYELLDSPGKVLLQSKDQITAGNA
ARKNHLEGKAAISNKITSCIFQLLQEAGIKTAFTRKCGETAFIAPQCEMIPIEWVCRR
IATGSFLKRNPGVKEGYKFYPPKVELFFKDDANNDPQWSEEQLIAAKFCFAGLLIGQT
EVDIMSHATQAIFEILEKSWLPQNCTLVDMKIEFGVDVTTKEIVLADVIDNDSWRLWP
SGDRSQQKDKQSYRDLKEVTPEGLQMVKKNFEWVAERVELLLKSESQCRVVVLMGSTS
DLGHCEKIKKACGNFGIPCELRVTSAHKGPDETLRIKAEYEGDGIPTVFVAVAGRSNG
LGPVMSGNTAYPVISCPPLTPDWGVQDVWSSLRLPSGLGCSTVLSPEGSAQFAAQIFG
LSNHLVWSKLRASILNTWISLKQADKKIRECNL (SEQ ID NO:20)

Figure 21

MPPRAPPAPGPRPPPRAAAATDTAAGAGGAGGAGGAGGPGFRPLAPRPWRWLLLLALPAACSAPPP
RPVYTNHWAVQVLGGPAEADRVAAAHGYLNLGQGNLEDYYHFYHSKTFKRSTLSSRGPHTFLRMDP
QVKWLQQQEVKRRVKRQVRSDPQALYFNDPIWSNMWYLHCGDKNSRCRSEMNVQAAWKRGYTGKNV
VVTILDDGIERNHPDLAPNYDSYASYDVNGNDYDPSPRYDASNENKHGTRCAGEVAASANNSYCIV
GIAYNAKIGGIRMLDGDVTDVVEAKSLGIRPNYIDIYSASWGPDDDGKTVDGPGRLAKQAFEYGIK
KGRQGLGSIFVWASGNGGREGDYCSCDGYTNSIYTISVSSATENGYKPWYLEECASTLATTYSSGA
FYERKIVTTDLRQRCTDGHTGTSVSAPMVAGIIALALEANSQLTWRDVQHLLVKTSRPAHLKASDW
KVNGAGHKVSHFYGFGLVDAEALVVEAKKWTAVPSQHMCVAASDKRPRSIPLVQVLRTTALTSACA
EHSDQRVVYLEHVVVRTSISHPRRGDLQIYLVSPSGTKSQLLAKRLLDLSNEGFTNWEFMTVHCWG
EKAEGQWTLEIQDLPSQVRNPEKQGKLKEWSLILYGTAEHPYHTFSAHQSRSRMLELSAPELEPPK
AALSPSQVEVPEDEEDYTAQSTPGSANILQTSVCHPECGDKGCDGPNADQCLNCVHFSLGSVKTSR
KCVSVCPLGYFGDTAARRCRRCHKGCETCSSRAATQCLSCRRGFYHHQEMNTCVTLCPAGFYADES
QKNCLKCHPSCKKCVDEPEKCTVCKEGFSLARGSCIPDCEPGTYFDSELIRCGECHHTCGTCVGPG
REECIHCAKNFHFHDWKCVPACGEGFYPEEMPGLPHKVCRRCDENCLSCAGSSRNCSRCKTGFTQL
GTSCITNHTCSNADETFCEMVKSNRLCERKLFIQFCCRTCLLAG (SEQ ID NO:21)

Figure 22

1 cgagcacatg ggccgcgggc cgggcgggct cggggcggcc gggacgagga ggggcgacga
61 cgagctgcga gcaaagatgt gccccgggac ccccggcacc ttccagtgga tttccttgcg
121 gaaaggatgt tggcggtccc tgtgacctgt ggagacacgg ccagatctgc cctccagcct
181 gatcttttgg ccagaaggag attaaaaaga tgcccctcaa gatggctgtg ctgtcagctg
241 catggagctt cgttcaagta ttttctgagc ctgatggatt tacagtgatg ttcagtggtc
301 tggggaataa cgctggtgga accatgcact ggaatgacac acgcccggca catttcagga
361 tactaaaagt ggttttaagg gaggctgtgg ctgaatgcct catggattct tacagcttgg
421 atgtccatgg gggacgaagg actgcagctg gctgagaggg ttgagatctc tgtttactta
481 gatctctgcc aacttccttt gggtctccct atggaatgta agaccccgac tcttcctggt
541 gaagcatctg atgcacgttc catccggcgc tcagctgggc ttgagctgac catactccct
601 ggagccttct cccgaggtgg gcgggtgacc ttggcacata cagccatcat gatggtactt
661 taagtggagg ctgaatcatc tccccttga gctgctttgg gaacgtggcc cccttggtgt
721 tccccttta ctgccaggac actgagattt ggagaggtaa gtggcttacc tgaggccatg
781 tgctaacaga gaagatgaag agatgattga aacaggccta agaccagacc taagggtctg
841 tacattttcc acatactttc catatcttta gaggcctgac caaagcagat cttttccttt
901 cttctaggta agtccaaagg cacctgcctg ctgggcccac tgttttctaa ctttcctaac
961 tttctgatcc cttggaggtg ataatcaaat attctagtct gaggcattgg gatacatggt
1021 gctaggttct gagactctgc gtcaggcctg aaccctgcat tttgtggagg tgggtgggag
1081 aatgttcccc tggggaacat gcctagacac gggggacaac agttgccctc atggggaggt
1141 acctgtttac tcgctgttat gggaccgctt tcacaaaacc actgcaggtg agtgagttcc
1201 tgctgaatat caggcctggt gtctctagac tcattattcc cccacccaac ccctatgtta
1261 gttcatctcg agccacattt ttattgccat aatccaggcc tggacaggcc aagatctttt
1321 aacaatttta attactgaaa ataataactg catttttttt taaagcccaa ctttttggta
1381 agtcagccca aaatacagtc tttgtgttgc catctgggaa ctggatttgg aattgttctt
1441 ccatgagact gcagagcaga acggcagggc cagaggtccc acgagctggt cagacccggt
1501 tctgctcctt gctggctgag tgaccttggg cattgt (SEQ ID NO:22)

Figure 23

MEHIHDSDGSSSSSHQSLKSTAKWAASLENLLEDPEGVKRFREF
LKKEFSEENVLFWLACEDFKKMQDKTQMQEKAKEIYMTFLSSKASSQVNVEGQSRLNEKI
LEEPHPLMFQKLQDQIFNLMKYDSYSRFLKSDLFLKHKRTEEEEEDLPDAQTAAKR
ASRIYNT (SEQ ID NO:23)

Figure 24

TTTTGTCACCTTTTTCCTCATTAGAAGGAAAGTAGAAAGCCTTACTTTAGGATTTTTAAAAAA
AAAATCCATCTCACCCCATATTGGTCTTAAATAAGTATAGACTAATTAACCTAAGCTACCTTT
AACAACGTAGAATTTAGATGGGTTCATATATGTGAGAAAAACCTGAATATAGGACAGGGGTCC
CACTTTTTTCCCCACCTCTGTCGCCCAGGCTAGAGTATAGTGGTGTGATCTTGGCCCACTGCA
ACCTCTGCTTCCTAGGTTCAAGTGATTCTCCTGCCTCAGCCTCCCAAGTAGCTGGGATTGTAA
GAGTATGCCACCACGCCCAGCTACTTTTTGTATTTTTAGTAGAGACAGGGTTTCATCATGTTG
GCCAGGATGGTCTCTTAACTCCTGCCCTCAAGTGATCCACCAGAGAGGAGATCCTCGGCCTCC
CCAAGTGCTGGATTATAGGCATGAGCCACCGTGCCCAGCCTACTTTCTAATTAATTAAAAAAA
AAAAAAAAAAAAAAAAAAAAAACTTCCCAAATGAGCTGATAGAAAAATGACGTGAGGCTGCTT
TGCCTTCAATAATACCTAGTTTTCAGCTGTTCCAACTCGTTTCCAAATAGAAATTAGCTGGAA
CACACTACAGTAATCTCAAGGAAGGGAAAATTAGGCCTTAAAAGATACCAAGAAGTCAGCATG
GTACCCAATTGAAACCTTTTGACCTTAGNGGGAATTCATTCTATTTGCACTAAAAGCCTTAAC
TGNTGGATTCAGAGTCCTTTTAACTGGGAGTTCTATAGAACTTTACTTTTTCCCTAGGCCCAG
AGNGGAGAAGGGTTTCTTAANAGCGGTTCATGGGA (SEQ ID NO:24)

Figure 25

CCGCGCTTCTTCTGCTTCTTGCTCTCGTCGTCCTTGTCGCGGCTGCGGGTGCTGGTGGTC
GGGGTGGAGGAGCCGGCGTCGCTGTCTCGCTTGCGCTTCCGTGATGATTTCTTCTGCCGG
ACCTCCTCTTCGATCTCCTCCAGCGTGCCCTCCTCGATGGCCTTGAGCCACTGCTTCTCC
GTCAGTGAGTCGCTGTAGTCCACCTCCTTGCGGTGGCGGGAGCCACGGCCGAACATCTTC
TCCTCCTCCTCCTCACAGGTCAGCCGCTCCACCTCCGCGTCGTCCTTGATGATCCACGAG
GGGAGCTCGTCCTCCTCCATGAGGCGCGGCTTCCGCTTGGGGTTGCGGGCCTCCTCGCGC
CTGCGGTCCAGGTCCATGCGCATGAACAGATCAAACTCCTCCTCGTGCCGGGCGATCATC
TGGTTGACGGTCTCGTCGTCGGGCACCTCGTCTTCCTCCTCATCCTGCTCCTCGTGCTCC
AGGATGGCCTGCAGGAAGGCGCGCCGCTCATGGCTGGAGGACTTCTGGTCGAACATGCCG
GCCTGNATCACCTTCTGGGTCACGTTGAGCTTGTACTTGGCTGCAGNCTAGATCTTCTNC
TNCACGCTGTNTGCGGTGCAGAGGCGGAGCACACGCACCTCGTTCTGCTGCCCCCGATGC
GTGGGGCTCGTCCTGCGCTTGCAGGTCCTGGTGAGGGATCAGTCGTGTCANAATGATCAC
AGTTCTGCCGACCTGAGTCAGCCGAGCCCCCAACCCGTTGCT (SEQ ID NO:25)

Figure 26

MSVAFAAPRQRGKGEITPAAIQKMLDDNNHLIQCIMDSQNKGKTSECSQYQQMLHTN
LVYLATIADSNQNMQSLLPAPPTQNMPMGPGGMNQSGPPPPPRSHNMPSDGMVGGGP
PAPHMQNQMNGQMPGPNHMPMQGPGPNQLNMTNSSMNMPSSSHGSMGGYNHSVPSSQ
SMPVQNQMTMSQGQPMGNYGPRPNMSMQPNQGPMMHQQPPSQQYNMPQGGGQHYQGQ
QPPMGMMGQVNQGNHMMGQRQIPPYRPPQQGPPQQYSGQEDYYGDQYSHGGQGPPEG
MNQQYYPDGNSQYGQQQDAYQGPPPQQGYPPQQQQYPGQQGYPGQQQGYGPSQGGPG
PQYPNYPQGQGQQYGGYRPTQPGPPQPPQQRPYGYDQGQYGNYQQ
(SEQ ID NO:26)

Figure 27

miqtvpdpaahikealsvvsedqslfecaygtphlaktemtassssdy
gqtskmsprvpqqdwlsqpparvtikmecnpsqvngsrnspdecsvak
ggkmvgspdtvgmnygsymeekhmpppnmttnerrvivpadptlwstd
hvrqwlewavkeyglpdvnillfqnidgkelckmtkddfqrltpsyna
dillshlhylretplphltsddvdkalqnsprlmharntggaafifpn
tsvypeatqrittrpdlpyeppprrsawtghghptpqskaaqpspstvp
ktedqrpqldpyqilgptssrlanpgsgqiqlwqfllellsdssnssc
itwegtngefkmtdpdevarrwgerkskpnmnydklsralryyydkni
mtkvhgkryaykfdfhgiaqalqphppesslykypsdlpymgsyhahp
qkmnfvaphppalpvtsssffaapnpywnsptggiypntrlptshmps
hlgtyy (SEQ ID NO:1)

Figure 28

mkeksknaaktrrekengefyelaklplpsaitsqldkasiirlttsyl
kmravfpeglgdawgqpsragpldgvakelgshllqtldgfvfvvasdgk
imyisetasvhlglsqveltgnsiyeyihpsdhdemtavltahqplhhhl
lqeyeiersfflrmkcvlakrnagltcsgykvihcsgylkirqymldmsl
ydscyqivglvavgqslppsaiteiklysnmfmfrasldlklifldsrvt
evtgyepqdliektlyhhvhgcdvfhlryahhlllvkgqvttkyyrllsk
rggwvwvqsyatvvhnsrssrphcivsvnyvlteieykelqlsleqvsta
ksqdswrtalstsqetrklvkpkntkmktklrtnpyppqqyssfqmdkle
cgqlgnwrasppasaaappelqphsessdllytpsyslpfsyhyghfpld
shvfsskkpmlpakfgqpqgspcevarfflstlpasgecqwhyanplvps
ssspaknppeppantarhslvpsyeapaaavrrfgedtappsfpscghyr
eepalgpakaarqaardgarlalaraapeccapptpeapgapaqlpfvll
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aplphylgasviitngr (SEQ ID NO:5)