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(54) Title: HUMAN MONOCLONAL ANTIBODIES TO PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA)

(57) Abstract: Isolated human monoclonal antibodies which bind to PSMA, and related antibody-based compositions and molecules, are disclosed. The human antibodies can be produced in a nonhuman transgenic animal, e.g., a transgenic mouse, capable of producing multiple isotypes of human monoclonal antibodies by undergoing V-D-J recombination and isotype switching. Also disclosed are pharmaceutical compositions comprising the human antibodies, nonhuman transgenic animals and hybridomas which produce the human antibodies, and therapeutic and diagnostic methods for using the human antibodies.

HUMAN MONOCLONAL ANTIBODIES TO PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA)

Related Applications

The present application claims priority to U.S. Utility Application Serial No. 10/059,989 filed on January 28, 2002, which is a continuation-in-part of PCT International Application PCT/US00/20247 filed 26 July 2000, which claims priority to U.S. Provisional Application Serial No. 60/146,285 filed 29 July 1999, U.S. Provisional Application Serial No. 60/158,759 filed 12 October 1999 and U.S. Provisional Application Serial No. 60/188,087 filed 09 March 2000. The entire contents of each of these applications are hereby incorporated herein by reference.

Background of the Invention

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Prostate cancer is a leading cause of morbidity and mortality among men. Treatments for prostate cancer include surgery, hormones, radiation, and chemotherapy. There is little effective treatment for metastatic prostate disease. Therefore, the identification of genes and/or gene products that represent diagnostic and prognostic markers, as well as targets for therapy, is critical. Prostate specific antigen (PSA) is one such cancer marker which is useful in the clinical diagnosis and staging of prostate cancer. However, PSA cannot differentiate benign prostatic hyperplasia (BPH) from prostatitis or prostate cancer in the range of 4-10 ng/ml, thus, necessitating a cytologic and/or histologic assessment to confirm the proper diagnosis (Barren, R.J. *et al.* (1998) Prostate 36:181-188).

Prostate specific membrane antigen (PSMA) is a 750 amino acid, type II transmembrane glycoprotein of approximately 110 kD that has 54% homology to the transferrin receptor. PSMA has 3 structural domains, including a 19 amino acid intracellular domain, a 24 amino acid transmembrane domain, and a 707 amino acid extracellular domain. The PSMA protein displays neurocarboxypeptidase and folate hydrolase activity and is reported to be involved in the neuroendocrine regulation of prostate growth and differentiation (Heston, W.D. (1996) Urologe-Ausgabe A. 35:400-

407). PSM' is an alternatively spliced form of PSMA which is localized in the cytoplasm. PSMA is predominantly expressed by prostatic epithelial cells. The expression of PSMA is increased in prostate cancer, especially in poorly differentiated, metastatic, and hormone refractory carcinomas (Gregorakis, A.K. *et. al.* (1998) Seminars in Urologic Oncology 16:2-12; Silver, D.A. (1997) Clinical Cancer Research 3:81-85). Low level expression of PSMA is observed in extraprostatic tissues such as the small bowel, salivary gland, duodenal mucosa, proximal renal tubules, and brain (Silver, D.A. (1997) Clinical Cancer Research 3:81-85). PSMA is also expressed in endothelial cells of capillary vessels in peritumoral and endotumoral areas of certain malignancies, including renal cell carcinomas, and colon carcinomas, but not in blood vessels from normal tissues. In addition, PSMA is reported to be related to tumor angiogenesis (Silver, D.A. (1997) Clinical Cancer Research 3:81-85).

Accordingly, PSMA represents a valuable target for the treatment of prostate cancer and a variety of other diseases characterized by PSMA expression.

Summary of the Invention

The present invention provides isolated human monoclonal antibodies which bind to human Prostate Specific Membrane Antigen (PSMA), as well as immunoconjugates, bispecific molecules, and other therapeutic compositions containing such antibodies, alone or in combination with additional therapeutic agents. In particular, human antibodies of the present invention bind to a native protein epitope on human PSMA (*e.g.* an epitope located in the extracellular domain of human PSMA) and inhibit the growth and/or mediate killing of cells which express PSMA (*e.g. via* lysis or phagocytosis) in the presence of human effector cells, *e.g.*, polymorphonuclear cells, monocytes, macrophages, and dendritic cells. Accordingly, the antibodies can be used in a variety of methods for diagnosing, treating, and/or preventing diseases related to the expression of PSMA, particularly PSMA-expressing tumors and cancers, such as prostate cancer, colon cancer, and renal carcinoma.

In a first aspect, the invention provides an isolated human monoclonal antibody that binds to human PSMA, wherein the antibody comprises a human heavy chain variable region derived from the human heavy chain VH_{5-51} germline sequence (SEQ ID NO: 54).

In a second aspect, the invention provides an isolated human monoclonal antibody that competes for binding to PSMA with the human monoclonal antibody of the first aspect.

In a third aspect, the invention provides a pharmaceutical composition comprising the human antibody of the invention and a pharmaceutically acceptable carrier.

In a fourth aspect, the invention provides an immunoconjugate comprising the human antibody of the invention linked to a therapeutic agent.

In a fifth aspect, the invention provides a pharmaceutical composition comprising the immunoconjugate of the fourth aspect and a pharmaceutically acceptable carrier.

In a sixth aspect, the invention provides an isolated nucleic acid molecule encoding the human antibody of the invention.

In a seventh aspect, the invention provides a transfectoma comprising the isolated nucleic acid of the sixth aspect.

In an eighth aspect, the invention provides a transgenic nonhuman animal which expresses the human antibody of the invention, wherein the transgenic non-human animal has a genome comprising a human heavy chain transgene or transchromosome and a human light chain transgene or transchromosome.

In a ninth aspect, the invention provides use of an antibody according to the invention in the manufacture of a medicament for inhibiting growth of a cell expressing PSMA, comprising contacting the cell with an effective amount of the antibody, such that the growth of the cell is inhibited.

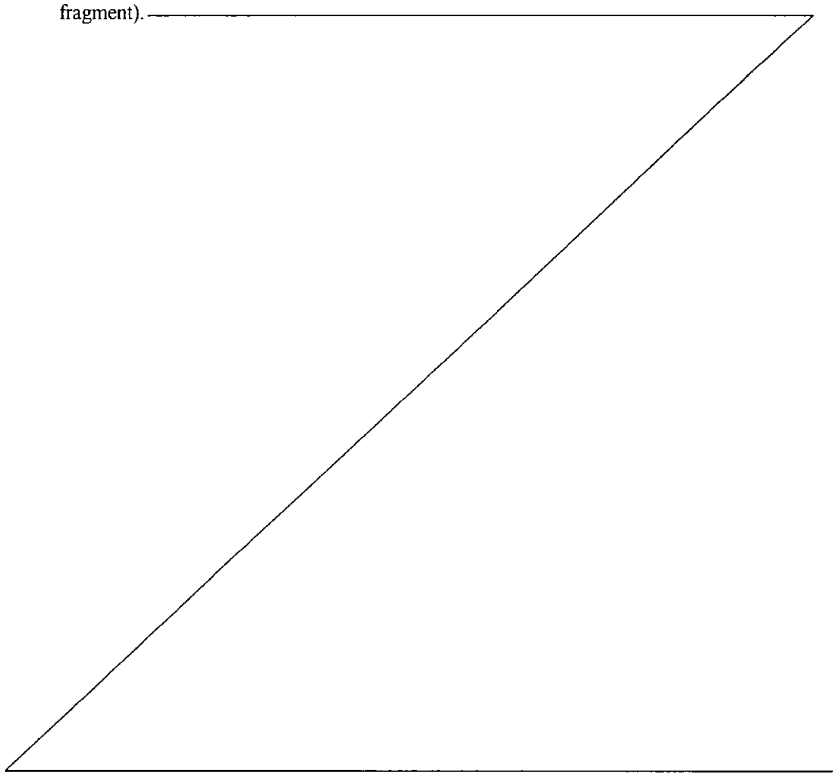
In a tenth aspect, the invention provides use of the human antibody of the invention in the manufacture of a medicament for treating or preventing a disease characterized by growth of tumor cells expressing PSMA.

In an eleventh aspect, the invention provides a method for inhibiting growth of a cell expressing PSMA, comprising contacting the cell with an effective amount of an antibody according to the invention.

In a twelfth aspect, the invention provides a method of treating or preventing a disease characterized by growth of tumor cells expressing PSMA, comprising administering to a subject the human antibody of the invention, in an amount effective to treat or prevent the disease.

Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

Isolated human antibodies of the invention include a variety of antibody isotypes, such as IgG1, (*e.g.*, IgG1k), IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, and IgE. The antibodies can be full-length andibodies (*e.g.*, IgG1 or IgG3) or can include only an antigen-binding portion (*e.g.*, a Fab, F(ab')2, Fv, or a single chain Fv fragment).



Particular therapeutic antibodies of the invention include human monoclonal antibody (HuMAb) 4A3, 7F12, 8A11, 8C12, 16F9, and functionally equivalent antibodies which, for example, (a) are encoded by human heavy chain and human light chain nucleic acids comprising nucleotide sequences in their variable regions as set forth in SEQ ID NOs: 1, 3, 5, 7 or 9 and SEQ ID NOs: 2, 4, 6, 8, or 10, respectively, and conservative modifications thereof, and/or (b) include heavy chain and light chain variable regions which comprise the amino acid sequence as set forth in SEQ ID NOs:11, 12, 13, 14, or 15, and SEQ ID NOs:16, 17, 18, 19, or 20, respectively, and conservative modifications thereof.

Still other particular human antibodies of the invention include those which comprise a CDR domain having a human heavy and light chain CDR1 region, a human heavy and light chain CDR2 region, and a human heavy and light chain CDR3 region, wherein

(a) the CDR1, CDR2, and CDR3 of the human heavy chain regions comprise an amino acid sequence selected from the group consisting of the amino acid sequences of the CDR1, CDR2, and CDR3 regions shown in Figure 19 (SEQ ID NOs:21-35), and conservative sequence modifications thereof, and

(b) the CDR1, CDR2, and CDR3 of the human light chain regions comprise an amino acid sequence selected from the group consisting of the amino acid sequences of the CDR1, CDR2, and CDR3 regions shown in Figures 22 and 23 (SEQ ID NOs:36-50), and conservative sequence modifications thereof.

Other particular antibodies of the invention include human monoclonal antibodies which bind to an epitope defined by antibody 4A3, 7F12, 8A11, 8C12, or 16F9, and/or which compete for binding to PSMA with antibody 4A3, 7F12, 8A11, 8C12, or 16F9, or which have other functional binding characteristics exhibited by antibody 4A3, 7F12, 8A11, 8C12, or 16F9. Such antibodies include, for example, those which bind to PSMA with a dissociation constant (K_D) of 10^{-7} M or less, such as of 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, or even lower (e.g., 10-^{11} M or less). Such antibodies further include those which cross react with murine anti-PSMA antibody 3C6 (ATCC Accession Number HB 12491), but exhibit no cross reactivity with murine anti-PSMA antibodies 4D4 (ATCC Accession Number HB 12493) or 1G9 (ATCC Accession Number HB 12495).

In yet another aspect of the invention, the human anti-PSMA antibodies are derivatized, linked to or co-expressed with another functional molecule, *e.g.*, another peptide or protein (*e.g.*, an Fab' fragment). For example, an antibody or antigen-binding portion of the invention can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (*e.g.*, to produce a bispecific or a multispecific antibody), a cytotoxin, a cellular ligand or an antigen. Accordingly, present invention encompasses a large variety of antibody conjugates, bi- and multispecific molecules, and fusion proteins, all of which bind to PSMA expressing cells and which target other molecules to the cells, or which bind to PSMA and to other molecules or cells.

In a particular embodiment, the invention includes a bispecific or multispecific molecule comprising at least one binding specificity for PSMA which is a human anti-PSMA antibody (or fragment or mimetic thereof), and a second binding specificity for an Fc receptor, *e.g.*, human FcγRI or a human Fcα receptor, or another antigen on an antigen presenting cell (APC). The second binding specificity can also be an antibody or fragment thereof (*e.g.*, an Fab, Fab', $F(ab')_2$, Fv, or a single chain Fv), such as a human antibody or a portion thereof, or a "chimeric" or a "humanized" antibody or a portion thereof (*e.g.*, has a variable region, or at least a complementarity determining region (CDR), derived from a nonhuman antibody (*e.g.*, murine) with the remaining portion(s) being human in origin).

Accordingly, the present invention includes bispecific and multispecific molecules that bind to both human PSMA and to an Fc receptor, *e.g.*, a human IgG receptor, *e.g.*, an Fc-gamma receptor (FcγR), such as FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). Other Fc receptors, such as human IgA receptors (*e.g.* FcαRI), also can be targeted. The Fc receptor is preferably located on the surface of an effector cell, *e.g.*, a monocyte, macrophage or an activated polymorphonuclear cell. In a preferred embodiment, the bispecific and multispecific molecules bind to an Fc receptor at a site which is distinct from the immunoglobulin Fc (*e.g.*, IgG or IgA) binding site of the receptor. Therefore, the binding of the bispecific and multispecific molecules is not blocked by physiological levels of immunoglobulins.

In another embodiment, the present invention provides an immunoconjugate, *e.g.*, an immunotoxin, which includes a fully human anti-PSMA antibody conjugated to a therapeutic agent, *e.g.*, a cytotoxic drug, an enzymatically active toxin, or a fragment thereof, a radioisotope, or a small molecule anti-cancer drug.

Alternatively, human antibodies of the invention can be co-administered with such therapeutic and cytotoxic agents, but not linked to them. They can be coadministered simultaneously with such agents (*e.g.*, in a single composition or separately) or can be administered before or after administration of such agents. Such agents can include chemotherapeutic agents, such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, cyclophosphamide hydroxyurea and combinations thereof. Human antibodies of the invention also can be administered in conjunction with radiation therapy.

In another embodiment, the present invention provides compositions, *e.g.*, pharmaceutical and diagnostic compositions/kits, comprising a pharmaceutically acceptable carrier and at least one human anti-PSMA antibody, or an antigen-binding portion thereof. In one embodiment, the composition comprises a combination of human antibodies or antigen-binding portions thereof, preferably each of which binds to a distinct epitope. For example, a pharmaceutical composition comprising a human monoclonal antibody that mediates highly effective killing of target cells in the presence of effector cells can be combined with another human monoclonal antibody that inhibits the growth of cells expressing PSMA. Thus, the combination provides multiple therapies tailored to provide the maximum therapeutic benefit. Compositions, *e.g.*, pharmaceutical compositions, comprising a combination of at least one human anti-PSMA antibody, or antigen-binding portion thereof, and at least one bispecific or multispecific molecule of the invention, are also within the scope of the invention.

In yet another embodiment, the present invention provides a method for inhibiting the proliferation and/or growth of a cell expressing PSMA, and/or inducing killing of a cell expressing PSMA, by contacting the cells with (e.g., administering to a subject) one or more human antibodies of the invention and/or related therapeutic compositions, derivatives etc. containing the antibodies as described above. In a particular embodiment, the method comprises contacting cells expressing PSMA either *in vitro* or *in vivo* with one or a combination of human anti-PSMA antibodies of the invention in the presence of a human effector cell. The method can be employed in

culture, *e.g. in vitro* or *ex vivo* (*e.g.*, cultures comprising cells expressing PSMA and effector cells). For example, a sample containing cells expressing PSMA and effector cells can be cultured *in vitro*, and combined with an antibody of the invention. Alternatively, the method can be performed in a subject, *e.g.*, as part of an *in vivo* (*e.g.*, therapeutic or prophylactic) protocol.

For use in *in vivo* treatment and prevention of PSMA mediated diseases, human antibodies of the present invention are administered to patients (*e.g.*, human subjects) at therapeutically effective dosages (e.g., to inhibit, eliminate or prevent growth of cells expressing PSMA) using any suitable route of administration for antibody-based clinical products as are well known in the art, such as by injection or infusion.

Accordingly, human antibodies of the present invention can be used to treat and/or prevent a variety of diseases characterized by PSMA expression by administering a suitable dosage (or series of dosages) of the antibodies to patients suffering from such diseases. Exemplary diseases that can be treated (*e.g.*, ameliorated) or prevented using the methods and compositions of the invention include, but are not limited to, cancers, such as prostate cancer, colon cancer, and renal carcinoma.

In a particular embodiment of the invention, the patient can be additionally treated with a chemotherapeutic agent, radiation, or an agent that modulates, *e.g.*, enhances, the expression or activity of an Fc receptor, *e.g.*, an Fcα receptor or an Fcγ receptor, such as a cytokine. Typical cytokines for administration during treatment include granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), and tumor necrosis factor (TNF). Typical therapeutic agents include, among others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, and cyclophosphamide hydroxyurea.

In another embodiment, the present invention provides a method for detecting *in vitro* or *in vivo* the presence of PSMA or PSMA expressing cells, *e.g.*, for diagnosing a PSMA-related disease. This can be achieved by, for example, contacting a sample to be tested, optionally along with a control sample, with a human monoclonal antibody of the invention (or an antigen-binding portion thereof) under conditions that allow for formation of a complex between the antibody and PSMA. Complex formation is then detected (*e.g.*, using an ELISA). When using a control

sample along with the test sample, complex is detected in both samples and any statistically significant difference in the formation of complexes between the samples is indicative of the presence of PSMA in the test sample.

In yet another aspect, the present invention provides a transgenic nonhuman animal, such as a transgenic mouse (also referred to herein as a "HuMAb mouse"), which expresses a fully human monoclonal antibody that binds to PSMA. In a particular embodiment, the transgenic nonhuman animal is a transgenic mouse having a genome comprising a human heavy chain transgene and a human light chain transgene encoding all or a portion of an anti-PSMA antibody of the invention. To generate human anti-PSMA antibodies, the transgenic nonhuman animal can be immunized with a purified or enriched preparation of PSMA antigen and/or cells expressing PSMA. Preferably, the transgenic nonhuman animal, *e.g.*, the transgenic mouse, is capable of producing multiple isotypes of human monoclonal antibodies to PSMA (*e.g.*, IgG, IgA and/or IgM) by undergoing V-D-J recombination and isotype switching. Isotype switching may occur by, *e.g.*, classical or non-classical isotype switching.

Accordingly, in another embodiment, the invention provides isolated B-cells derived from a transgenic nonhuman animal as described above, *e.g.*, a transgenic mouse, which express human anti-PSMA antibodies. The isolated B-cells can then be immortalized to by fusion to an immortalized cell to provide a source (*e.g.*, a hybridoma) of human anti-PSMA antibodies. Such hybridomas (*i.e.*, which produce human anti-PSMA antibodies) are also included within the scope of the invention.

As exemplified herein, human anti-PSMA antibodies can be obtained directly from hybridomas which express the antibody, or can be cloned and recombinantly expressed in a host cell, such as a transfectoma (*e.g.*, a transfectoma consisting of immortalized CHO cells or lymphocytic cells). Accordingly, the present invention provides methods for producing human monoclonal antibodies which bind to human PSMA. In a particular embodiment, the method includes immunizing a transgenic nonhuman animal, *e.g.*, a transgenic mouse, as previously described (*e.g.*, having a genome comprising a human heavy chain transgene and a human light chain transgene encoding all or a portion of an anti-PSMA antibody), with a purified or enriched preparation of human PSMA antigen and/or cells expressing human PSMA. B cells (*e.g.*, splenic B cells) of the animal are then obtained and fused with myeloma

cells to form immortal, hybridoma cells that secrete human monoclonal antibodies against PSMA.

In yet another aspect, the invention provides nucleic acid molecules encoding all or a portion of a human monoclonal anti-PSMA antibody (e.g., which encode at least one light or heavy chain of the antibody), as well as recombinant expression vectors which include such nucleic acids, and host cells transfected with such vectors. Methods of producing the antibodies by culturing such host cells are also encompassed by the invention. Particular nucleic acids provided by the invention comprise the nucleotide sequences shown in SEQ ID NOs:1, 3, 5, 7, or 9 and SEQ ID NOs:2, 4, 6, 8, or 10, which encode the heavy and light chains, respectively, of human anti-PSMA antibodies (HuMAbs) 4A3, 7F12, 8A11, 8C12, and 16F9.

Other features and advantages of the instant invention be apparent from the following detailed description and examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Brief Description of the Drawings

Figure 1 is a bar graph showing the reactivity (solid phase ELISA) of HuMAb 11C10 with full length PSMA and bacterially expressed fusion proteins containing PSMA fragments corresponding to amino acids 1-173, 134-437, and 438-750. The assays were conducted using the murine 7E11 antibody as a control.

Figure 2 is a graph showing the reactivity (solid phase ELISA) of human anti-PSMA monoclonal antibodies with membrane fractions from human prostatic adenocarcinoma LNCaP and PC3 cells. Background absorbance at 405 nm was 0.05.

Figure 3 is a bar graph showing the effect of heat denaturation of isolated PSMA on antibody binding. Purified PSMA, with and without heat denaturation, was coated onto 96-well plates and treated with the indicated antibodies. Bound antibody was detected by ELISA.

Figure 4 shows immunoprecipitation of PSMA from LNCaP cell detergent lysates using HuMAbs. Immunoprecipitated protein was separated by SDS gel electrophoresis, blotted onto PVDF membranes, and probed with the murine anti-PSMA 4D8 antibody (lanes 2-7). Lane 1 shows total LNCaP cell lysate. Lanes 2-7 show immunoprecipitation with the following antibodies, respectively: irrelevant human IgG1, 4A3, 7F12, 8A11, 8C12 and 16F9. The positions of PSMA and PSM' are indicated by arrows.

Figure 5 shows graphs measuring the antibody dependent cellular cytotoxicity (ADCC) response of HuMAbs 4A3, 7F12, 8A11, 8C12, and 16F9 using LNCaP cell targets with PBMC's from two donors (Panels A and B), each at an E:T ratio of 100:1.

Figure 6 shows a fully human bispecific molecule, 14A8 x 8C12, which binds to CD89 (FcαR) and to PSMA. The molecule contains an anti-CD89 F ab' antibody fragment (derived from human monoclonal anti-CD89 antibody, 14A8) chemically linked by disulfide bond to an anti-PSMA F ab' antibody fragment (derived from human monoclonal anti-PSMA antibody, 8C12).

Figure 7, Panel A is a graph showing monocyte-mediated antibody dependent cell cytotoxicity (ADCC) of PSMA-expressing cells via the 14A8 x 8C12 bispecific molecule shown in Figure 6 as a function bispecific molecule concentration. Results were measured as a percent of specific cell lysis using no added inhibitor, 50 μg/ml free anti-FcRαR (14A8) F(ab')2 and 50 μg/ml free anti-FcγRI (H22) F(ab')2; *Panel B* is a graph showing monocyte-mediated antibody dependent cell cytotoxicity (ADCC) of LNCaP cells via the bispecific molecule 14A8 x 8C12 and monoclonal antibody 8C12 at an effector:target ratio of 100:1; *Panel C* is a graph showing monocyte-mediated antibody dependent cell cytotoxicity (ADCC) of LNCaP cells via the 14A8 x 8C12 bispecific molecule in the absence of inhibitor, or in the presence of excess amounts of 14A8 F(ab')2 or H22 F(ab')2, and at an effector:target ratio of 100:1.

Figure 8, Panel A is a graph showing neutrophil-mediated antibody dependent cell cytotoxicity (ADCC) of PSMA-expressing cells via the 14A8 x 8C12 bispecific molecule shown in Figure 6 as a function bispecific molecule concentration. Results were measured as a percent of specific cell lysis using no added inhibitor, 25 μg/ml free anti-FcRαR (14A8) F(ab')2 and 25 μg/ml free anti-FcγRI (H22) F(ab')2; *Panel B* is a graph showing neutrophil-mediated antibody dependent cell cytotoxicity (ADCC) of LNCaP cells via the 14A8 x 8C12 bispecific molecule in the absence of inhibitor, or in the presence of excess amounts of 14A8 F(ab')2 or H22 F(ab')2, and at an effector:target ratio of 200:1.

Figure 9, Panel A is a graph showing whole blood-mediated antibody dependent cell cytotoxicity (ADCC) of PSMA-expressing cells via the 14A8 x 8C12 bispecific molecule shown in Figure 6 as a function bispecific molecule concentration. Results were measured as a percent of specific cell lysis using no added inhibitor, 25 μg/ml free anti-FcRαR (14A8) F(ab')2 and 25 μg/ml free anti-FcγRI (H22) F(ab')2; *Panel B* is a graph showing whole blood-mediated antibody dependent cell cytotoxicity (ADCC) of LNCaP cells via the 14A8 x 8C12 bispecific molecule in the absence of inhibitor, or in the presence of excess amounts of 14A8 F(ab')2 or H22 F(ab')2.

Figure 10 is a graph showing bispecific molecule (14A8 x 8C12) - mediated phagocytosis of PSMA expressing (LNCaP) cells by monocyte derived macrophages (MDM) (circles). Results were measured as a percent of phagocytosis both in the presence and absence of excess 14A8 antibody as an inhibitor (squares) and H22 antibody as a control (diamonds).

Figure 11 is a graph showing bispecific molecule (14A8 x 8C12)- mediated phagocytosis and antibody (8C12)- mediated phagocytosis of LNCaP tumor cells by monocyte derived macrophages (MDM).

Figure 12 is a graph showing bispecific molecule (14A8 x 8C12)- mediated phagocytosis of LNCaP tumor cells by monocyte derived macrophages (MDM) (circles). The inset is a graph showing phagocytosis mediated by the 14A8 x

8C12 bispecific molecule (1 μg/ml) in the presence of excess 14A8 F(ab')2 or H22 F(ab')2 .

Figure 13 is a bar graph showing the biodistribution of ^{125}I-4A3 in nude mice with LNCaP cell tumors. Animals were injected with 100μg of ^{125}I-4A3 through the tail vein and sacrificed at 0.25 and 24 hours after injection. The amount of radioactivity present in each tissue was determined. The data shows results from duplicate animals at each time point.

Figure 14 is a graph showing the internalization and processing of ^{125}I-labeled HuMAb by LNCaP cells in culture. LNCaP cells were labeled with iodinated antibody, washed extensively, and the amount of cell surface bound label internalized and converted to TCA soluble products was determined at the indicated times. Results are shown for three HuMAbs that retained antigen binding properties after iodination, as well as irrelevant human IgG_1 as a negative control.

Figure 15 is a graph showing the effect of iodination with ^{125}I on the antigen binding ability of certain anti-PSMA HuMAbs. The results show the amount of ^{125}I-labeled HuMAb bound to immobilized native purified LNCaP PSMA, as a function of the dilution factor of the antibody.

Figure 16 include graphs showing the effect of DOTA-labeling on the antigen binding ability of certain anti-PSMA HuMAbs. The results show the amount of DOTA-labeled HuMAb, or unconjugated antibody, bound to PSMA, as measured by ELISA, as a function of the titration of the amount of antibody (in μg/ml).

Figure 17A and B shows the nucleotide sequence sequences of the V_H- and V_L-regions, respectively, from each HuMAb 4A3, 7F12, 8C12, 8A11, and 16F9, (SEQ ID NOS: 1-10).

Figure 18 is an alignment comparison of the nucleotide sequence of the heavy chain V regions of HuMAbs 4A3, 7F12, 8A11, 8C12, 16F9 (SEQ ID NOS: 1, 3, 5, 7 and 9), and the corresponding chain V region of the germline nucleotide sequence, (SEQ ID NO: 54)

Figure 19 is an alignment comparison of the amino acid sequence of the heavy chain V region of HuMAbs 4A3, 7F12, 8A11, 8C12, 16F9 (SEQ ID NOS 11-15), and the corresponding chain V region of the germline amino acid sequence (SEQ ID NO: 51).

Figure 20 is an alignment comparison of the nucleotide sequence of the light (kappa) chain V region of HuMAbs 4A3, 7F12, 8C12 (SEQ ID NOS: 2, 4 and 8), and the corresponding chain V region of the germline nucleotide sequence (SEQ ID NO: 55).

Figure 21 is an alignment comparison of the nucleotide sequence of the light (kappa) chain V region of HuMAbs 8A11, 16F9 (SEQ ID NOS: 6 and 10), and the corresponding chain V region of the germline nucleotide sequence (SEQ ID NO: 56).

Figure 22 is an alignment comparison of the amino acid sequence of the light (kappa) chain V region of HuMAbs 4A3, 7F12, 8C12 (SEQ ID NOS: 16, 17 and 19), and the corresponding chain V region of the germline amino acid sequence (SEQ ID NO: 52).

Figure 23 is an alignment comparison of the amino acid sequence of the light (kappa) chain V region of HuMAbs 8A11, 16F9 (SEQ ID NOS: 18 and 20), and the corresponding chain V region of the germline amino acid sequence (SEQ ID NO: 53).

Detailed Description of the Invention

The present invention provides novel antibody-based therapies for treating and diagnosing diseases characterized by expression of Prostate Specific Membrane Antigen (referred to herein as "PSMA"). Therapies of the invention employ isolated human monoclonal antibodies and/or related compositions containing the antibodies which bind to an epitope present on PSMA. In a particular embodiment exemplified herein, the human antibodies are produced in a nonhuman transgenic animal, *e.g.*, a transgenic mouse, capable of producing multiple isotypes of human monoclonal antibodies to PSMA (*e.g.*, IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching. Accordingly, aspects of the invention include not only antibodies, antibody fragments, and pharmaceutical compositions thereof, but also nonhuman transgenic animals, B-cells and hybridomas which produce monoclonal antibodies. Methods of

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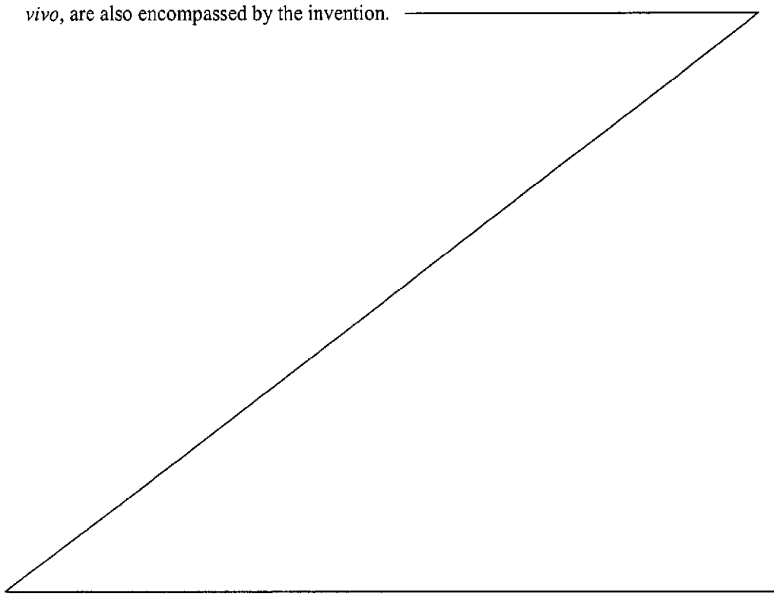
using the antibodies of the invention to detect a cell expressing PSMA, or to inhibit growth, differentiation and/or motility of a cell expressing PSMA, either *in vitro* or *in vivo*, are also encompassed by the invention.



In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The term "Prostate Specific Membrane Antigen," "PSMA," and "PSMA antigen" are used interchangeably herein, and include variants, isoforms and species homologs of human PSMA. Accordingly, human antibodies of the invention may, in certain cases, cross-react with PSMA from species other than human, or other proteins which are structurally related to human PSMA (e.g., human PSMA homologs). In other cases, the antibodies may be completely specific for human PSMA and not exhibit species or other types of cross-reactivity.

As used herein, the term "inhibits growth" (*e.g.*, referring to cells) is intended to include any measurable decrease in the growth of a cell when contacted with an anti-PSMA antibody as compared to the growth of the same cell not in contact with an anti-PSMA antibody, *e.g.*, the inhibition of growth of a cell by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99%, or 100%.

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chain thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_{H1}, C_{H2} and C_{H3}. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, PSMA). It has been shown that 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 portion" of an antibody include (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 fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR). 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. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883).* Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

The term "native conformational epitope" or "native protein epitope" are used interchangeably herein, and include protein epitopes resulting from conformational folding of the PSMA molecule which arise when amino acids from differing portions of the linear sequence of the PSMA molecule come together in close proximity in 3-dimensional space. Such conformational epitopes are distributed on the extracellular side of the plasma membrane.

The term "bispecific molecule" is intended to include any agent, *e.g.*, a protein, peptide, or protein or peptide complex, which has two different binding specificities. For example, the molecule may bind to, or interact with, (a) a cell surface antigen and (b) an Fc receptor on the surface of an effector cell. The term "multispecific molecule" or "heterospecific molecule" is intended to include any agent, *e.g.*, a protein, peptide, or protein or peptide complex, which has more than two different binding specificities. For example, the molecule may bind to, or interact with, (a) a cell surface antigen, (b) an Fc receptor on the surface of an effector cell, and (c) at least one other component. Accordingly, the invention includes, but is not limited to, bispecific, trispecific, tetraspecific, and other multispecific molecules which are directed to cell surface antigens, such as PSMA, and to other targets, such as Fc receptors on effector cells.

The term "bispecific antibodies" also includes diabodies. Diabodies are bivalent, bispecific antibodies in which the V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123).

The term "human antibody derivatives" refers to any modified form of the antibody, *e.g.*, a conjugate of the antibody and another agent or antibody.

As used herein, a human antibody is "derived from" a particular germline sequence if the antibody is obtained from a system using human immunoglobulin sequences, *e.g.*, by immunizing a transgenic mouse carrying human immunoglobulin genes or by screening a human immunoglobulin gene library. A human antibody that is "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequence of human germline immunoglobulins. A selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99%

identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

As used herein, the term "heteroantibodies" refers to two or more antibodies, antibody binding fragments (*e.g.*, Fab), derivatives therefrom, or antigen binding regions linked together, at least two of which have different specificities. These different specificities include a binding specificity for an Fc receptor on an effector cell, and a binding specificity for an antigen or epitope on a target cell, *e.g.*, a tumor cell. The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic

or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further in Section I, below), (b) antibodies isolated from a host cell transformed to express the antibody, *e.g.*, from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

As used herein, a "heterologous antibody" is defined in relation to the transgenic nonhuman organism producing such an antibody. This term refers to an antibody having an amino acid sequence or an encoding nucleic acid sequence corresponding to that found in an organism not consisting of the transgenic nonhuman animal, and generally from a species other than that of the transgenic nonhuman animal.

As used herein, a "heterohybrid antibody" refers to an antibody having a light and heavy chains of different organismal origins. For example, an antibody having a human heavy chain associated with a murine light chain is a heterohybrid antibody. Examples of heterohybrid antibodies include chimeric and humanized antibodies, discussed supra.

An "isolated antibody," as used herein, is intended to refer to an antibody which is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds to PSMA is substantially free of antibodies that specifically bind antigens other than PSMA). An isolated antibody that specifically binds to an epitope, isoform or variant of human PSMA may, however, have cross-reactivity to other related antigens, *e.g.*, from other species (*e.g.*, PSMA species homologs). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals. In one embodiment of the invention, a combination of "isolated" monoclonal antibodies having different specificities are combined in a well defined composition.

As used herein, "specific binding" refers to antibody binding to a predetermined antigen. Typically, the antibody binds with a dissociation constant (K_D) of 10^{-7} M or less , and binds to the predetermined antigen with a K_D that is at least two-fold less than its K_D for binding to a non-specific antigen (*e.g.*, BSA, casein) other than the predetermined antigen or a closely-related antigen. The phrases "an antibody recognizing an antigen" and " an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen".

As used herein, the term "high affinity" for an IgG antibody refers to an antibody having a K_D of 10^{-8} M or less, more preferably 10^{-9} M or less and even more preferably 10^{-10} M or less. binding affinity of at least about 10^7M^{-1}, preferably at least about 10^9M^{-1}, more preferably at least about $10^{10}M^{-1}$,$10^{11}M^{-1}$, $10^{12}M^{-1}$ or greater, *e.g.*, up to$10^{13}M^{-1}$ or greater. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a KD of 10^{-7} M or less, more preferably 10^{-8} M or less..

The term "K_{assoc}" or "K_a", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (*i.e*,. K_d/K_a) and is expressed as a molar concentration (M).

As used herein, "isotype" refers to the antibody class (*e.g.*, IgM or IgGl) that is encoded by heavy chain constant region genes.

As used herein, "isotype switching" refers to the phenomenon by which the class, or isotype, of an antibody changes from one Ig class to one of the other Ig classes.

As used herein, "nonswitched isotype" refers to the isotypic class of heavy chain that is produced when no isotype switching has taken place; the CH gene encoding the nonswitched isotype is typically the first CH gene immediately downstream from the functionally rearranged VDJ gene. Isotype switching has been classified as classical or non-classical isotype switching. Classical isotype switching occurs by recombination events which involve at least one switch sequence region in the transgene. Non-classical isotype switching may occur by, for example, homologous

recombination between human σ_μ and human $\sum_\mu$ (δ-associated deletion). Alternative non-classical switching mechanisms, such as intertransgene and/or interchromosomal recombination, among others, may occur and effectuate isotype switching.

As used herein, the term "switch sequence" refers to those DNA sequences responsible for switch recombination. A "switch donor" sequence, typically a μ switch region, will be 5' (*i.e.*, upstream) of the construct region to be deleted during the switch recombination. The "switch acceptor" region will be between the construct region to be deleted and the replacement constant region (*e.g.*, γ, ε, etc.). As there is no specific site where recombination always occurs, the final gene sequence will typically not be predictable from the construct.

As used herein, "glycosylation pattern" is defined as the pattern of carbohydrate units that are covalently attached to a protein, more specifically to an immunoglobulin protein. A glycosylation pattern of a heterologous antibody can be characterized as being substantially similar to glycosylation patterns which occur naturally on antibodies produced by the species of the nonhuman transgenic animal, when one of ordinary skill in the art would recognize the glycosylation pattern of the heterologous antibody as being more similar to said pattern of glycosylation in the species of the nonhuman transgenic animal than to the species from which the CH genes of the transgene were derived.

The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "rearranged" as used herein refers to a configuration of a heavy chain or light chain immunoglobulin locus wherein a V segment is positioned immediately adjacent to a D-J or J segment in a conformation encoding essentially a complete V_H or V_L domain, respectively. A rearranged immunoglobulin gene locus can be identified by comparison to germline DNA; a rearranged locus will have at least one recombined heptamer/nonamer homology element.

The term "unrearranged" or "germline configuration" as used herein in reference to a V segment refers to the configuration wherein the V segment is not recombined so as to be immediately adjacent to a D or J segment.

The term "nucleic acid molecule", as used herein, is intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule," as used herein in reference to nucleic acids encoding antibodies or antibody portions (*e.g.*, V_H, V_L, CDR3) that bind to PSMA, is intended to refer to a nucleic acid molecule in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than PSMA, which other sequences may naturally flank the nucleic acid in human genomic DNA. In one embodiment, the human anti-PSMA antibody, or portion thereof, includes the nucleotide or amino acid sequence of 4A3, 7F12, 8A11, 8C12, or 16F9, as well as heavy chain (V_H) and light chain (V_L) variable regions having the sequences shown in SEQ ID NOs:1, 3, 5, 7, or 9 and 2, 4, 6, 8, or 10, respectively.

As disclosed and claimed herein, the sequences set forth in SEQ ID NOs: 1-58 include "conservative sequence modifications", *i.e.*, nucleotide and amino acid sequence modifications which do not significantly affect or alter the binding characteristics of the antibody encoded by the nucleotide sequence or containing the amino acid sequence. Such conservative sequence modifications include nucleotide and amino acid substitutions, additions and deletions. Modifications can be introduced into SEQ ID NOs: 1-58 by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a human anti-PSMA antibody is preferably replaced with another amino acid residue from the same side chain family.

Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a anti-PSMA antibody coding sequence, such as by saturation mutagenesis, and the resulting modified anti-PSMA antibodies can be screened for binding activity.

Accordingly, antibodies encoded by the (heavy and light chain variable region) nucleotide sequences disclosed herein and/or containing the (heavy and light chain variable region) amino acid sequences disclosed herein (*i.e.*, SEQ ID NOs: 1-50) include substantially similar antibodies encoded by or containing similar sequences which have been conservatively modified. Further discussion as to how such substantially similar antibodies can be generated based on the partial (*i.e.*, heavy and light chain variable regions) sequences disclosed herein as SEQ ID NOs: 1-50 is provided below.

For nucleic acids, the term "substantial homology" indicates that two nucleic acids, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate nucleotide insertions or deletions, in at least about 80% of the nucleotides, usually at least about 90% to 95%, and more preferably at least about 98% to 99.5% of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to the complement of the strand.

The percent identity between two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue

table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. *See*, F. Ausubel, *et al.*, ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987).

The nucleic acid compositions of the present invention, while often in a native sequence (except for modified restriction sites and the like), from either cDNA, genomic or mixtures may be mutated, thereof in accordance with standard techniques to provide gene sequences. For coding sequences, these mutations, may affect amino acid sequence as desired. In particular, DNA sequences substantially homologous to or derived from native V, D, J, constant, switches and other such sequences described

herein are contemplated (where "derived" indicates that a sequence is identical or modified from another sequence).

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. With respect to transcription regulatory sequences, operably linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. For switch sequences, operably linked indicates that the sequences are capable of effecting switch recombination.

The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within

the scope of the term "host cell" as used herein. Recombinant host cells include, for example, CHO cells and lymphocytic cells.

As used herein, the term "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as nonhuman primates, sheep, dog, cow, chickens, amphibians, reptiles, etc.

The terms "transgenic, nonhuman animal" refers to a nonhuman animal having a genome comprising one or more human heavy and/or light chain transgenes or transchromosomes (either integrated or non-integrated into the animal's natural genomic DNA) and which is capable of expressing fully human antibodies. For example, a transgenic mouse can have a human light chain transgene and either a human heavy chain transgene or human heavy chain transchromosome, such that the mouse produces human anti-PSMA antibodies when immunized with PSM antigen and/or cells expressing PSMA. The human heavy chain transgene can be integrated into the chromosomal DNA of the mouse, as is the case for transgenic, *e.g.*, HuMAb mice, or the human heavy chain transgene can be maintained extrachromosomally, as is the case for transchromosomal (*e.g.*, KM) mice as described in WO 02/43478. Such transgenic and transchromosomal mice are capable of producing multiple isotypes of human monoclonal antibodies to PSMA (*e.g.*, IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching.

Various aspects of the invention are described in further detail in the following subsections.

I. Production of Human Antibodies to PSMA

Human monoclonal antibodies (mAbs) of the present invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology *e.g.*, the standard somatic cell hybridization technique of Kohler and Milstein (1975) Nature 256: 495. Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibody can be employed *e.g.*, viral or oncogenic transformation of B lymphocytes.

The preferred animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for

fusion are known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known.

In a preferred embodiment, human monoclonal antibodies directed against PSMA can be generated using transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse system. These transgenic and transchromosomic mice include mice referred to herein as HuMAb mice and KM mice, respectively, and are collectively referred to herein as "transgenic mice."

The HuMAb mouse contains a human immunoglobulin gene miniloci that encodes unrcarranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg, *et al.* (1994) *Nature* 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal (Lonberg, N. *et al.* (1994), *supra*; reviewed in Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* Vol. 13: 65-93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci* 764:536-546). The preparation of HuMAb mice is described in detail Section II below and in Taylor, L. *et al.* (1992) *Nucleic Acids Research* 20:6287-6295; Chen, J. *et al.* (1993) *International Immunology* 5: 647-656; Tuaillon *et al.* (1993) *Proc. Natl. Acad. Sci USA* 90:3720-3724; Choi *et al.* (1993) *Nature Genetics* 4:117-123; Chen, J. *et al.* (1993) *EMBO J.* 12: 821-830; Tuaillon *et al.* (1994) *J. Immunol.* 152:2912-2920; Lonberg *et al.*, (1994) *Nature* 368(6474): 856-859; Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Taylor, L. *et al.* (1994) *International Immunology* 6: 579-591; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* Vol. 13: 65-93; Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci* 764:536-546; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851, the contents of all of which are hereby incorporated by reference in their entirety. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay, and GenPharm International; U.S. Patent No. 5,545,807 to Surani *et al.*; International Publication Nos. WO 98/24884, published on June 11, 1998; WO 94/25585, published November 10, 1994; WO 93/1227, published June 24, 1993; WO 92/22645, published December 23, 1992; WO 92/03918, published

March 19, 1992, the disclosures of all of which are hereby incorporated by reference in their entity. Alternatively, the HCO12 transgenic mice described in Example 2, can be used to generate human anti-PSMA antibodies.

Immunizations

To generate fully human monoclonal antibodies to PSMA, HuMAb mice can be immunized with a purified or enriched preparation of PSMA antigen and/or cells expressing PSMA, as described by Lonberg, N. *et al.* (1994) *Nature* 368(6474): 856-859; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851 and WO 98/24884. Preferably, the mice will be 6-16 weeks of age upon the first infusion. For example, a purified or enriched preparation (5-20 μg) of PSMA antigen (*e.g.*, purified from PSMA-expressing LNCaP cells) can be used to immunize the HuMAb mice intraperitoneally. In the event that immunizations using a purified or enriched preparation of PSMA antigen do not result in antibodies, mice can also be immunized with cells expressing PSMA, *e.g.*, a tumor cell line, to promote immune responses.

Cumulative experience with various antigens has shown that the HuMAb transgenic mice typically respond best when initially immunized intraperitoneally (IP) with antigen in complete Freund's adjuvant, followed by every other week i.p. immunizations (up to a total of 6) with antigen in incomplete Freund's adjuvant, followed by every other week IP/SC immunizations (up to a total of 10) with antigen in incomplete Freund's adjuvant. The immune response can be monitored over the course of the immunization protocol with plasma samples being obtained by retroorbital bleeds. The plasma can be screened by ELISA (as described below), and mice with sufficient titers of anti-PSMA human immunoglobulin can be used for fusions. Mice can be boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. It is expected that 2-3 fusions for each antigen may need to be performed. Several mice will be immunized for each antigen. For example, a total of twelve HuMAb mice of the HC07 and HC012 strains can be immunized.

Generation of Hybridomas Producing Human Monoclonal Antibodies to PSMA

To generate hybridomas producing human monoclonal antibodies to PSMA, splenocytes and lymph node cells from immunized mice can be isolated and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The

resulting hybridomas can be screened for the production of antigen-specific antibodies. For example, single cell suspensions of splenic lymphocytes from immunized mice can be fused to one-sixth the number of P3X63-Ag8.653 nonsecreting mouse myeloma cells (ATCC, CRL 1580) with 50% PEG. Cells are plated at approximately 2 x 10^5 in flat bottom microtiter plate, followed by a two week incubation in selective medium containing 20% fetal Clone Serum, 18% "653" conditioned media, 5% origen (IGEN), 4 mM L-glutamine, 1 mM L~glutamine, 1 mM sodium pyruvate, 5mM HEPES, 0.055 mM 2-mercaptoethanol, 50 units/ml penicillin, 50 mg/ml streptomycin, 50 mg/ml gentamycin and 1X HAT (Sigma; the HAT is added 24 hours after the fusion). After approximately two weeks, cells can be cultured in medium in which the HAT is replaced with HT. Individual wells can then be screened by ELISA for human anti-PSMA monoclonal IgM and IgG antibodies. Once extensive hybridoma growth occurs, medium can be observed usually after 10-14 days. The antibody secreting hybridomas can be replated, screened again, and if still positive for human IgG, anti-PSMA monoclonal antibodies, can be subcloned at least twice by limiting dilution. The stable subclones can then be cultured *in vitro* to generate small amounts of antibody in tissue culture medium for characterization.

Generation of Transfectomas Producing Human Monoclonal Antibodies to PSMA

Human antibodies of the invention also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (*e.g.*, Morrison, S. (1985) Science 229:1202).

For example, to express the antibodies, or antibody fragments thereof, DNAs encoding partial or full-length light and heavy chains, can be obtained by standard molecular biology techniques (*e.g.*, PCR amplification, site directed mutagenesis) and can be inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the

antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the V_H segment is operatively linked to the C_H segment(s) within the vector and the V_I, segment is operatively linked to the C_L segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, CA (1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc*. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)) and polyoma. Alternatively, nonviral regulatory sequences may be used, such as the ubiquitin promoter or β-globin promoter.

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, *e.g.*, U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Prokaryotic expression of antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss, M. A. and Wood, C. R. (1985) Immunology Today 6:12-13).

Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R. J. Kaufman and P. A. Sharp (1982) Mol. Biol. 159:601-621), NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another preferred expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more

preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Use of Partial Antibody Sequences to Express Intact Antibodies

Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, *e.g.*, Riechmann, L. *et al.*, 1998, *Nature* 332:323-327; Jones, P. *et al.*, 1986, *Nature* 321:522-525; and Queen, C. *et al.*, 1989, *Proc. Natl. Acad. See.* U.S.A. 86:10029-10033). Such framework sequences can be obtained from public DNA databases that include germline antibody gene sequences. These germline sequences will differ from mature antibody gene sequences because they will not include completely assembled variable genes, which are formed by V(D)J joining during B cell maturation. Germline gene sequences will also differ from the sequences of a high affinity secondary repertoire antibody at individual evenly across the variable region. For example, somatic mutations are relatively infrequent in the amino-terminal portion of framework region. For example, somatic mutations are relatively infrequent in the amino terminal portion of framework region 1 and in the carboxy-terminal portion of framework region 4. Furthermore, many somatic mutations do not significantly alter the binding properties of the antibody. For this reason, it is not necessary to obtain the entire DNA sequence of a particular antibody in order to recreate an intact recombinant antibody having binding properties similar to those of the original antibody (see PCT/US99/05535 filed on March 12, 1999, which is herein incorporated by referenced for all purposes). Partial heavy and light chain sequence spanning the CDR regions is typically sufficient for this purpose. The partial sequence is used to determine which germline variable and joining gene segments contributed to the recombined antibody variable genes. The germline sequence is then

used to fill in missing portions of the variable regions. Heavy and light chain leader sequences are cleaved during protein maturation and do not contribute to the properties of the final antibody. For this reason, it is necessary to use the corresponding germline leader sequence for expression constructs. To add missing sequences, cloned cDNA sequences cab be combined with synthetic oligonucleotides by ligation or PCR amplification. Alternatively, the entire variable region can be synthesized as a set of short, overlapping, oligonucleotides and combined by PCR amplification to create an entirely synthetic variable region clone. This process has certain advantages such as elimination or inclusion or particular restriction sites, or optimization of particular codons.

The nucleotide sequences of heavy and light chain transcripts from a hybridomas are used to design an overlapping set of synthetic oligonucleotides to create synthetic V sequences with identical amino acid coding capacities as the natural sequences. The synthetic heavy and kappa chain sequences can differ from the natural sequences in three ways: strings of repeated nucleotide bases are interrupted to facilitate oligonucleotide synthesis and PCR amplification; optimal translation initiation sites are incorporated according to Kozak's rules (Kozak, 1991, J. Biol. Chem. 266:19867-19870); and, HindIII sites are engineered upstream of the translation initiation sites.

For both the heavy and light chain variable regions, the optimized coding, and corresponding non-coding, strand sequences are broken down into 30 – 50 nucleotide approximately the midpoint of the corresponding non-coding oligonucleotide. Thus, for each chain, the oligonucleotides can be assemble into overlapping double stranded sets that span segments of 150 – 400 nucleotides. The pools are then used as templates to produce PCR amplification products of 150 – 400 nucleotides. Typically, a single variable region oligonucleotide set will be broken down into two pools which are separately amplified to generate two overlapping PCV products. These overlapping products are then combined by PCT amplification to form the complete variable region. It may also be desirable to include an overlapping fragment of the heavy or light chain constant region (including the BbsI site of the kappa light chain, or the AgeI site if the gamma heavy chain) in the PCR amplification to generate fragments that can easily be cloned into the expression vector constructs.

The reconstructed heavy and light chain variable regions are then combined with cloned promoter, translation initiation, constant region, 3' untranslated, polyadenylation, and transcription termination, sequences to form expression vector constructs. The heavy and light chain expression constructs can be combined into a single vector, co-transfected, serially transfected, or separately transfected into host cells which are then fused to form a host cell expressing both chains.

Plasmids for use in construction of expression vectors for human IgGκ are described below. The plasmids were constructed so that PCR amplified V heavy and V kappa light chain cDNA sequences could be used to reconstruct complete heavy and light chain minigenes. These plasmids can be used to express completely human, or chimeric $IgG_1\kappa$ or $IgG_4\kappa$ antibodies. Similar plasmids can be constructed for expression of other heavy chain isotypes, or for expression of antibodies comprising lambda light chains.

Thus, in another aspect of the invention, the structural features of an human anti-PSMA antibodies of the invention, 4A3, 7F12, 8A11, 8C12 or 16F9, are used to create structurally related human anti-PSMA antibodies that retain at least one functional property of the antibodies of the invention, such as binding to PSMA. More specifically, one or more CDR regions of 4A3, 7F12, 8A11, 8C12 or 16F9 can be combined recombinantly with known human framework regions and CDRs to create additional, recombinantly-engineered, human anti-PSMA antibodies of the invention.

Accordingly, in another embodiment, the invention provides a method for preparing an anti-PSMA antibody comprising:

preparing an antibody comprising (1) human heavy chain framework regions and human heavy chain CDRs, wherein at least one of the human heavy chain CDRs comprises an amino acid sequence selected from the amino acid sequences of CDRs shown in Figure 19 (SEQ ID NOs: 21-35); and (2) human light chain framework regions and human light chain CDRs, wherein at least one of the human heavy chain CDRs comprises an amino acid sequence selected from the amino acid sequences of CDRs shown in Figures 22 and 23 (SEQ ID NOs: 36-50);

wherein the antibody retains the ability to bind to PSMA.

The ability of the antibody to bind PSMA can be determined using standard binding assays, such as those set forth in the Examples (*e.g.*, an ELISA).

Since it is well known in the art that antibody heavy and light chain CDR3 domains play a particularly important role in the binding specificity/affinity of an antibody for an antigen, the recombinant antibodies of the invention prepared as set forth above preferably comprise the heavy and light chain CDR3s of 4A3, 7F12, 8A11, 8C12 or 16F9. The antibodies further can comprise the CDR2s of 4A3, 7F12, 8A11, 8C12 or 16F9. The antibodies further can comprise the CDR1s of 4A3, 7F12, 8A11, 8C12 or 16F9. Accordingly, the invention further provides anti-PSMA antibodies comprising: (1) human heavy chain framework regions, a human heavy chain CDR1 region, a human heavy chain CDR2 region, and a human heavy chain CDR3 region, wherein the human heavy chain CDR3 region is selected from the CDR3s of 4A3, 7F12, 8A11, 8C12 and 16F9 as shown in Figure 19 (SEQ ID NOs: 23, 26, 29, 32, or 35); and (2) human light chain framework regions, a human light chain CDR1 region, a human light chain CDR2 region, and a human light chain CDR3 region, wherein the human light chain CDR3 region is selected from the CDR3s of 4A3, 7F12, 8A11, 8C12 and 16F9 as shown in Figures 22 and 23 (SEQ ID NOs: 38, 41, 44, 47, or 50), wherein the antibody binds PSMA. The antibody may further comprise the heavy chain CDR2 and/or the light chain CDR2 of 4A3, 7F12, 8A11, 8C12 or 16F9. The antibody may further comprise the heavy chain CDR1 and/or the light chain CDR1 of 4A3, 7F12, 8A11, 8C12 or 16F9.

Preferably, the CDR1, 2, and/or 3 of the engineered antibodies described above comprise the exact amino acid sequence(s) as those of 4A3, 7F12, 8A11, 8C12 or 16F9 disclosed herein. However, the ordinarily skilled artisan will appreciate that some deviation from the exact CDR sequences of 4A3, 7F12, 8A11, 8C12 and 16F9 may be possible while still retaining the ability of the antibody to bind PSMA effectively (*e.g.*, conservative substitutions). Accordingly, in another embodiment, the engineered antibody may be composed of one or more CDRs that are, for example, 90%, 95%, 98% or 99.5% identical to one or more CDRs of 4A3, 7F12, 8A11, 8C12 or 16F9

In addition to simply binding PSMA, engineered antibodies such as those described above may be selected for their retention of other functional properties of antibodies of the invention, such as:

1) binding to live cells expressing human PSMA;
2) binding to human PSMA with a K_D of 10^{-8} M or less (e.g., 10^{-9} M or 10^{-10} M or less);

3) binding to a unique epitope on PSMA (to eliminate the possibility that monoclonal antibodies with complimentary activities when used in combination would compete for binding to the same epitope);
4) growth inhibition of PSMA expressing tumor cells *in vivo*; and/or
5) phagocytosis and/or killing of cells expressing PSMA in the presence of human effector cells (e.g., in an ADCC assay).

Characterization of Binding of Human Monoclonal Antibodies to PSMA

Human monoclonal antibodies of the invention can be tested for binding to PSMA by, for example, standard ELISA. Briefly, microtiter plates are coated with purified PSMA at 0.25 μg/ml in PBS, and then blocked with 5% bovine serum albumin in PBS. Dilutions of plasma from PSMA-immunized mice are added to each well and incubated for 1-2 hours at 37°C. The plates are washed with PBS/Tween and then incubated with a goat-anti-human IgG Fc-specific polyclonal reagent conjugated to alkaline phosphatase for 1 hour at 37°C. After washing, the plates are developed with pNPP substrate (1 mg/ml), and analyzed at OD of 405-650. Preferably, mice which develop the highest titers will be used for fusions.

An ELISA assay as described above can also be used to screen for hybridomas that show positive reactivity with PSMA immunogen. Hybridomas that bind with high avidity to PSMA will be subcloned and further characterized. One clone from each hybridoma, which retains the reactivity of the parent cells (by ELISA), can be chosen for making a 5-10 vial cell bank stored at -140 °C, and for antibody purification.

To purify human anti-PSMA antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, NJ). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD_{280} using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80 °C.

To determine if the selected human anti-PSMA monoclonal antibodies bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, IL). Competition studies using unlabeled monoclonal

antibodies and biotinylated monoclonal antibodies can be performed using PSMA coated-ELISA plates as described above. Biotinylated mAb binding can be detected with a strep-avidin-alkaline phosphatase probe.

To determine the isotype of purified antibodies, isotype ELISAs can be performed. Wells of microtiter plates can be coated with 10 μg/ml of anti-human Ig overnight at 4°C. After blocking with 5% BSA, the plates are reacted with 10 μg/ml of monoclonal antibodies or purified isotype controls, at ambient temperature for two hours. The wells can then be reacted with either human IgGl or human IgM-specific alkaline phosphatase-conjugated probes. Plates are developed and analyzed as described above.

In order to demonstrate binding of monoclonal antibodies to live cells expressing the PSMA, flow cytometry can be used. Briefly, cell lines expressing PSMA (grown under standard growth conditions) are mixed with various concentrations of monoclonal antibodies in PBS containing 0.1% Tween 80 and 20% mouse serum, and incubated at 37°C for 1 hour. After washing, the cells are reacted with Fluorescein-labeled anti-human IgG antibody under the same conditions as the primary antibody staining. The samples can be analyzed by FACScan instrument using light and side scatter properties to gate on single cells. An alternative assay using fluorescence microscopy may be used (in addition to or instead of) the flow cytometry assay. Cells can be stained exactly as described above and examined by fluorescence microscopy. This method allows visualization of individual cells, but may have diminished sensitivity depending on the density of the antigen.

Anti-PSMA human IgGs can be further tested for reactivity with PSMA antigen by Western blotting. Briefly, cell extracts from cells expressing PSMA can be prepared and subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens will be transferred to nitrocellulose membranes, blocked with 20% mouse serum, and probed with the monoclonal antibodies to be tested. Human IgG binding can be detected using anti-human IgG alkaline phosphatase and developed with BCIP/NBT substrate tablets (Sigma Chem. Co., St. Louis, MO).

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Phagocytic and Cell Killing Activities of Human Monoclonal Antibodies to PSMA

In addition to binding specifically to PSMA, human monoclonal anti-PSMA antibodies can be tested for their ability to mediate phagocytosis and killing of cells expressing PSMA. The testing of monoclonal antibody activity *in vitro* will provide an initial screening prior to testing *in vivo* models. Briefly, polymorphonuclear cells (PMN), or other effector cells, from healthy donors can be purified by Ficoll Hypaque density centrifugation, followed by lysis of contaminating erythrocytes. Washed PMNs, can be suspended in RPMI supplemented with 10% heat-inactivated fetal calf serum and mixed with ^{51}Cr labeled cells expressing PSMA, at various ratios of effector cells to tumor cells (-effector cells:tumor cells). Purified human anti-PSMA IgGs can then be added at various concentrations. Irrelevant human IgG can be used as negative control. Assays can be carried out for 0-120 minutes at 37°C. Samples can be assayed for cytolysis by measuring ^{51}Cr release into the culture supernatant. Anti-PSMA monoclonal can also be tested in combinations with each other to determine whether cytolysis is enhanced with multiple monoclonal antibodies.

Human monoclonal antibodies which bind to PSMA also can be tested in an *in vivo* model (*e.g.*, in mice) to determine their efficacy in mediating phagocytosis and killing of cells expressing PSMA, *e.g.*, tumor cells. These antibodies can be selected, for example, based on the following criteria, which are not intended to be exclusive:

1) binding to live cells expressing PSMA;

2) high affinity of binding to PSMA;

3) binding to a unique epitope on PSMA (to eliminate the possibility that monoclonal antibodies with complimentary activities when used in combination would compete for binding to the same epitope);

4) opsonization of cells expressing PSMA;

5) mediation of growth inhibition, phagocytosis and/or killing of cells expressing PSMA in the presence of human effector cells.

Preferred human monoclonal antibodies of the invention meet one or more, and preferably all, of these criteria. In a particular embodiment, the human monoclonal antibodies are used in combination, *e.g.*, as a pharmaceutical composition comprising two or more anti-PSMA monoclonal antibodies or fragments thereof. For example, human anti-PSMA monoclonal antibodies having different, but

complementary activities can be combined in a single therapy to achieve a desired therapeutic or diagnostic effect. An illustration of this would be a composition containing an anti-PSMA human monoclonal antibody that mediates highly effective killing of target cells in the presence of effector cells, combined with another human anti-PSMA monoclonal antibody that inhibits the growth of cells expressing PSMA.

II. Production of Transgenic Nonhuman Animals Which Generate Human Monoclonal Anti-PSMA Antibodies

In yet another aspect, the invention provides transgenic and transchromosomal nonhuman animals, such as transgenic or transchromosomal mice, which are capable of expressing human monoclonal antibodies that specifically bind to PSMA. In a particular embodiment, the invention provides a transgenic or transchromosomal mouse having a genome comprising a human heavy chain transgene, such that the mouse produces human anti-PSMA antibodies when immunized with PSMA and/or cells expressing PSMA. The human heavy chain transgene can be integrated into the chromosomal DNA of the mouse, as is the case for transgenic, *e.g.*, HuMAb mice, as described in detail herein and exemplified. Alternatively, the human heavy chain transgene can be maintained extrachromosomally, as is the case for transchromosomal (*e.g.*, KM) mice as described in WO 02/43478. Such transgenic and transchromosomal animals are capable of producing multiple isotypes of human monoclonal antibodies to PSMA (*e.g.*, IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching. Isotype switching may occur by, *e.g.*, classical or non-classical isotype switching.

The design of a transgenic or transchromosomal nonhuman animal that responds to foreign antigen stimulation with a heterologous antibody repertoire, requires that the heterologous immunoglobulin transgenes contain within the transgenic animal function correctly throughout the pathway of B-cell development. This includes, for example, isotype switching of the heterologous heavy chain transgene. Accordingly, transgenes are constructed so as to produce isotype switching and one or more of the following: (1) high level and cell-type specific expression, (2) functional gene rearrangement, (3) activation of and response to allelic exclusion, (4) expression of a sufficient primary repertoire, (5) signal transduction, (6) somatic hypermutation, and (7) domination of the transgene antibody locus during the immune response.

Not all of the foregoing criteria need be met. For example, in those embodiments wherein the endogenous immunoglobulin loci of the transgenic animal are functionally disrupted, the transgene need not activate allelic exclusion. Further, in those embodiments wherein the transgene comprises a functionally rearranged heavy and/or light chain immunoglobulin gene, the second criteria of functional gene rearrangement is unnecessary, at least for that transgene which is already rearranged. For background on molecular immunology, see, Fundamental Immunology, 2nd edition (1989), Paul William E., ed. Raven Press, N.Y., which is incorporated herein by reference.

In certain embodiments, the transgenic or transchromosomal nonhuman animals used to generate the human monoclonal antibodies of the invention contain rearranged, unrearranged or a combination of rearranged and unrearranged heterologous immunoglobulin heavy and light chain transgenes in the germline of the transgenic animal. Each of the heavy chain transgenes comprises at least one C_H gene. In addition, the heavy chain transgene may contain functional isotype switch sequences, which are capable of supporting isotype switching of a heterologous transgene encoding multiple C_H genes in the B-cells of the transgenic animal. Such switch sequences may be those which occur naturally in the germline immunoglobulin locus from the species that serves as the source of the transgene C_H genes, or such switch sequences may be derived from those which occur in the species that is to receive the transgene construct (the transgenic animal). For example, a human transgene construct that is used to produce a transgenic mouse may produce a higher frequency of isotype switching events if it incorporates switch sequences similar to those that occur naturally in the mouse heavy chain locus, as presumably the mouse switch sequences are optimized to function with the mouse switch recombinase enzyme system, whereas the human switch sequences are not. Switch sequences may be isolated and cloned by conventional cloning methods, or may be synthesized *de novo* from overlapping synthetic oligonucleotides designed on the basis of published sequence information relating to immunoglobulin switch region sequences (Mills *et al.*, *Nucl. Acids Res.* 15:7305-7316 (1991); Sideras *et al.*, *Intl. Immunol.* 1:631-642 (1989), which are incorporated herein by reference).

For each of the foregoing transgenic animals, functionally rearranged heterologous heavy and light chain immunoglobulin transgenes are found in a significant fraction of the B-cells of the transgenic animal (at least 10 percent).

The transgenes used to generate the transgenic animals of the invention include a heavy chain transgene comprising DNA encoding at least one variable gene segment, one diversity gene segment, one joining gene segment and at least one constant region gene segment. The immunoglobulin light chain transgene comprises DNA encoding at least one variable gene segment, one joining gene segment and at least one constant region gene segment. The gene segments encoding the light and heavy chain gene segments are heterologous to the transgenic nonhuman animal in that they are derived from, or correspond to, DNA encoding immunoglobulin heavy and light chain gene segments from a species not consisting of the transgenic nonhuman animal. In one aspect of the invention, the transgene is constructed such that the individual gene segments are unrearranged, *i.e.*, not rearranged so as to encode a functional immunoglobulin light or heavy chain. Such unrearranged transgenes support recombination of the V, D, and J gene segments (functional rearrangement) and preferably support incorporation of all or a portion of a D region gene segment in the resultant rearranged immunoglobulin heavy chain within the transgenic nonhuman animal when exposed to PSMA antigen.

In an alternate embodiment, the transgenes comprise an unrearranged "mini-locus." Such transgenes typically comprise a substantial portion of the C, D, and J segments as well as a subset of the V gene segments. In such transgene constructs, the various regulatory sequences, *e.g.* promoters, enhancers, class switch regions, splice-donor and splice-acceptor sequences for RNA processing, recombination signals and the like, comprise corresponding sequences derived from the heterologous DNA. Such regulatory sequences may be incorporated into the transgene from the same or a related species of the nonhuman animal used in the invention. For example, human immunoglobulin gene segments may be combined in a transgene with a rodent immunoglobulin enhancer sequence for use in a transgenic mouse. Alternatively, synthetic regulatory sequences may be incorporated into the transgene, wherein such synthetic regulatory sequences are not homologous to a functional DNA sequence that is known to occur naturally in the genomes of mammals. Synthetic regulatory sequences are designed according to consensus rules, such as, for example, those specifying the permissible sequences of a splice-acceptor site or a promoter/enhancer motif. For example, a minilocus comprises a portion of the genomic immunoglobulin locus having at least one internal (*i.e.*, not at a terminus of the portion) deletion of a non-essential

DNA portion (*e.g.*, intervening sequence; intron or portion thereof) as compared to the naturally-occurring germline Ig locus.

In a preferred embodiment of the invention, the transgenic or transchromosomal animal used to generate human antibodies to PSMA contains at least one, typically 2-10, and sometimes 25-50 or more copies of the transgene described in Example 12 of WO 98/24884 (*e.g.*, pHC1 or pHC2) bred with an animal containing a single copy of a light chain transgene described in Examples 5, 6, 8, or 14 of WO 98/24884, and the offspring bred with the J_H deleted animal described in Example 10 of WO 98/24884, the contents of which are hereby expressly incorporated by reference. Animals are bred to homozygosity for each of these three traits. Such animals have the following genotype: a single copy (per haploid set of chromosomes) of a human heavy chain unrearranged mini-locus (described in Example 12 of WO 98/24884), a single copy (per haploid set of chromosomes) of a rearranged human K light chain construct (described in Example 14 of WO 98/24884), and a deletion at each endogenous mouse heavy chain locus that removes all of the functional J_H segments (described in Example 10 of WO 98/24884). Such animals are bred with mice that are homozygous for the deletion of the J_H segments (Examples 10 of WO 98/24884) to produce offspring that are homozygous for the J_H deletion and hemizygous for the human heavy and light chain constructs. The resultant animals are injected with antigens and used for production of human monoclonal antibodies against these antigens.

B cells isolated from such an animal are monospecific with regard to the human heavy and light chains because they contain only a single copy of each gene. Furthermore, they will be monospecific with regards to human or mouse heavy chains because both endogenous mouse heavy chain gene copies are nonfunctional by virtue of the deletion spanning the J_H region introduced as described in Example 9 and 12 of WO 98/24884. Furthermore, a substantial fraction of the B cells will be monospecific with regards to the human or mouse light chains because expression of the single copy of the rearranged human κ light chain gene will allelically and isotypically exclude the rearrangement of the endogenous mouse κ and lambda chain genes in a significant fraction of B-cells.

Preferred transgenic and transchromosomal nonhuman animals, *e.g.*, mice, will exhibit immunoglobulin production with a significant repertoire, ideally substantially similar to that of a native mouse. Thus, for example, in embodiments

where the endogenous Ig genes have been inactivated, the total immunoglobulin levels will range from about 0.1 to 10 mg/ml of serum, preferably 0.5 to 5 mg/ml, ideally at least about 1.0 mg/ml. When a transgene capable of effecting a switch to IgG from IgM has been introduced into the transgenic mouse, the adult mouse ratio of serum IgG to IgM is preferably about 10:1. The IgG to IgM ratio will be much lower in the immature mouse. In general, greater than about 10%, preferably 40 to 80% of the spleen and lymph node B cells express exclusively human IgG protein.

The repertoire will ideally approximate that shown in a native mouse, usually at least about 10% as high, preferably 25 to 50% or more. Generally, at least about a thousand different immunoglobulins (ideally IgG), preferably 10^4 to 10^6 or more, will be produced, depending primarily on the number of different V, J and D regions introduced into the mouse genome. These immunoglobulins will typically recognize about one-half or more of highly antigenic proteins, *e.g.*, staphylococcus protein A. Typically, the immunoglobulins will exhibit an affinity for preselected antigens of at least about $10^7 M^{-1}$, preferably at least about $10^9 M^{-1}$, more preferably at least about $10^{10} M^{-1}$,$10^{11} M^{-1}$, $10^{12} M^{-1}$, or greater, *e.g.*, up to$10^{13} M^{-1}$ or greater.

In some embodiments, it may be preferable to generate nonhuman animals with predetermined repertoires to limit the selection of V genes represented in the antibody response to a predetermined antigen type. A heavy chain transgene having a predetermined repertoire may comprise, for example, human V_H genes which are preferentially used in antibody responses to the predetermined antigen type in humans. Alternatively, some V_H genes may be excluded from a defined repertoire for various reasons (*e.g.*, have a low likelihood of encoding high affinity V regions for the predetermined antigen; have a low propensity to undergo somatic mutation and affinity sharpening; or are immunogenic to certain humans). Thus, prior to rearrangement of a transgene containing various heavy or light chain gene segments, such gene segments may be readily identified, *e.g.* by hybridization or DNA sequencing, as being from a species of organism other than the transgenic animal.

The transgenic and transchromosomal nonhuman animals, e.g., mice, as described above can be immunized with, for example, a purified or recombinant preparation of PSMA and/or cells expressing PSMA as described previously. Alternatively, the transgenic animals can be immunized with DNA encoding human PSMA. The animals will then produce B cells which undergo class-switching via

intratransgene switch recombination (cis-switching) and express immunoglobulins reactive with PSMA. The immunoglobulins can be human antibodies (also referred to as "human sequence antibodies"), wherein the heavy and light chain polypeptides are encoded by human transgene sequences, which may include sequences derived by somatic mutation and V region recombinatorial joints, as well as germline-encoded sequences; these human antibodies can be referred to as being substantially identical to a polypeptide sequence encoded by a human V_L or V_H gene segment and a human J_L or J_L segment, even though other non-germline sequences may be present as a result of somatic mutation and differential V-J and V-D-J recombination joints. The variable regions of each antibody chain are typically at least 80 percent encoded by human germline V, J, and, in the case of heavy chains, D, gene segments; frequently at least 85 percent of the variable regions are encoded by human germline sequences present on the transgene; often 90 or 95 percent or more of the variable region sequences are encoded by human germline sequences present on the transgene. However, since non-germline sequences are introduced by somatic mutation and VJ and VDJ joining, the human sequence antibodies will frequently have some variable region sequences (and less frequently constant region sequences) which are not encoded by human V, D, or J gene segments as found in the human transgene(s) in the germline of the mice. Typically, such non-germline sequences (or individual nucleotide positions) will cluster in or near CDRs, or in regions where somatic mutations are known to cluster.

Human antibodies which bind to the predetermined antigen can result from isotype switching, such that human antibodies comprising a human sequence γ chain (such as γl, γ2, γ3, or γ4) and a human sequence light chain (such as K) are produced. Such isotype-switched human sequence antibodies often contain one or more somatic mutation(s), typically in the variable region and often in or within about 10 residues of a CDR) as a result of affinity maturation and selection of B cells by antigen, particularly subsequent to secondary (or subsequent) antigen challenge. These high affinity human sequence antibodies may have K_Ds of 10^{-7} M or less, such as 10^{-8} M or less, 10^{-9} M or less, or 10^{-10} M or less, or even lower.

Another aspect of the invention pertains to the B cells derived from transgenic or transchromosomal nonhuman animals as described herein. The B cells can be used to generate hybridomas expressing human monoclonal antibodies which bind with high affinity (*e.g.*, a K_D of 10^{-7} M or less) to PSMA. Thus, in another embodiment,

the invention provides a hybridoma which produces a human antibody having a K_D of 10^{-7} M or less, such as 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, or even lower, , wherein the antibody comprises:

a human sequence light chain composed of (1) a light chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human V_L gene segment and a human J_L segment, and (2) a light chain constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human C_L gene segment; and

a human sequence heavy chain composed of a (1) a heavy chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human V_H gene segment, optionally a D region, and a human J_H segment, and (2) a constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human C_H gene segment.

The development of high affinity human monoclonal antibodies against PSMA is facilitated by a method for expanding the repertoire of human variable region gene segments in a transgenic mouse having a genome comprising an integrated human immunoglobulin transgene, said method comprising introducing into the genome a V gene transgene comprising V region gene segments which are not present in said integrated human immunoglobulin transgene. Often, the V region transgene is a yeast artificial chromosome comprising a portion of a human V_H or V_L (V_K) gene segment array, as may naturally occur in a human genome or as may be spliced together separately by recombinant methods, which may include out-of-order or omitted V gene segments. Often at least five or more functional V gene segments are contained on the YAC. In this variation, it is possible to make a transgenic mouse produced by the V repertoire expansion method, wherein the mouse expresses an immunoglobulin chain comprising a variable region sequence encoded by a V region gene segment present on the V region transgene and a C region encoded on the human Ig transgene. By means of the V repertoire expansion method, transgenic mice having at least 5 distinct V genes can be generated; as can mice containing at least about 24 V genes or more. Some V gene segments may be non-functional (*e.g.*, pseudogenes and the like); these segments may be retained or may be selectively deleted by recombinant methods available to the skilled artisan, if desired.

Once the mouse germline has been engineered to contain a functional YAC having an expanded V segment repertoire, substantially not present in the human Ig transgene containing the J and C gene segments, the trait can be propagated and bred into other genetic backgrounds, including backgrounds where the functional YAC having an expanded V segment repertoire is bred into a mouse germline having a different human Ig transgene. Multiple functional YACs having an expanded V segment repertoire may be bred into a germline to work with a human Ig transgene (or multiple human Ig transgenes). Although referred to herein as YAC transgenes, such transgenes when integrated into the genome may substantially lack yeast sequences, such as sequences required for autonomous replication in yeast; such sequences may optionally be removed by genetic engineering (*e.g.*, restriction digestion and pulsed-field gel electrophoresis or other suitable method) after replication in yeast in no longer necessary (*i.e.*, prior to introduction into a mouse ES cell or mouse prozygote). Methods of propagating the trait of human sequence immunoglobulin expression, include breeding a transgenic mouse having the human Ig transgene(s), and optionally also having a functional YAC having an expanded V segment repertoire. Both V_H and V_L gene segments may be present on the YAC. The transgenic mouse may be bred into any background desired by the practitioner, including backgrounds harboring other human transgenes, including human Ig transgenes and/or transgenes encoding other human lymphocyte proteins. The invention also provides a high affinity human sequence immunoglobulin produced by a transgenic mouse having an expanded V region repertoire YAC transgene. Although the foregoing describes a preferred embodiment of the transgenic animal of the invention, other embodiments are contemplated which have been classified in four categories:

I. Transgenic animals containing an unrearranged heavy and rearranged light immunoglobulin transgene;

II. Transgenic animals containing an unrearranged heavy and unrearranged light immunoglobulin transgene;

III. Transgenic animal containing rearranged heavy and an unrearranged light immunoglobulin transgene; and

IV. Transgenic animals containing rearranged heavy and rearranged light immunoglobulin transgenes.

Of these categories of transgenic animal, the preferred order of preference is as follows II > I > III > IV where the endogenous light chain genes (or at least the K gene) have been knocked out by homologous recombination (or other method) and I > II > III >IV where the endogenous light chain genes have not been knocked out and must be dominated by allelic exclusion.

III. Bispecific/ Multispecific Molecules Which Bind to PSMA

In yet another embodiment of the invention, human monoclonal antibodies to PSMA, or antigen-binding portions thereof, can be derivatized or linked to another functional molecule, *e.g.*, another peptide or protein (*e.g.*, an Fab' fragment) to generate a bispecific or multispecific molecule which binds to multiple binding sites or target epitopes. For example, an antibody or antigen-binding portion of the invention can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic.

Accordingly, the present invention includes bispecific and multispecific molecules comprising at least one first binding specificity for PSMA and a second binding specificity for a second target epitope. In a particular embodiment of the invention, the second target epitope is an Fc receptor, *e.g.*, human FcγRI (CD64) or a human Fcα receptor (CD89). Therefore, the invention includes bispecific and multispecific molecules capable of binding both to FcγR, FcαR or FcεR expressing effector cells (*e.g.*, monocytes, macrophages or polymorphonuclear cells (PMNs)), and to target cells expressing PSMA. These bispecific and multispecific molecules target PSMA expressing cells to effector cell and, like the human monoclonal antibodies of the invention, trigger Fc receptor-mediated effector cell activities, such as phagocytosis of a PSMA expressing cells, antibody dependent cell-mediated cytotoxicity (ADCC), cytokine release, or generation of superoxide anion.

Bispecific and multispecific molecules of the invention can further include a third binding specificity, in addition to an anti-Fc binding specificity and an anti-PSMA binding specificity. In one embodiment, the third binding specificity is an anti-enhancement factor (EF) portion, *e.g.*, a molecule which binds to a surface protein involved in cytotoxic activity and thereby increases the immune response against the target cell. The "anti-enhancement factor portion" can be an antibody, functional

antibody fragment or a ligand that binds to a given molecule, *e.g.*, an antigen or a receptor, and thereby results in an enhancement of the effect of the binding determinants for the F_c receptor or target cell antigen. The "anti-enhancement factor portion" can bind an F_c receptor or a target cell antigen. Alternatively, the anti-enhancement factor portion can bind to an entity that is different from the entity to which the first and second binding specificities bind. For example, the anti-enhancement factor portion can bind a cytotoxic T-cell (*e.g.* via CD2, CD3, CD8, CD28, CD4, CD40, ICAM-1 or other immune cell that results in an increased immune response against the target cell).

In one embodiment, the bispecific and multispecific molecules of the invention comprise as a binding specificity at least one antibody, or an antibody fragment thereof, including, *e.g.*, an Fab, Fab', $F(ab')_2$, Fv, or a single chain Fv. The antibody may also be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct as described in Ladner *et al.* U.S. Patent No. 4,946,778, issued August 7, 1990, the contents of which is expressly incorporated by reference.

In one embodiment bispecific and multispecific molecules of the invention comprise a binding specificity for an FcγR or an FcαR present on the surface of an effector cell, and a second binding specificity for a target cell antigen, *e.g.*, PSMA.

In one embodiment, the binding specificity for an Fc receptor is provided by a human monoclonal antibody, the binding of which is not blocked by human immunoglobulin G (IgG). As used herein, the term "IgG receptor" refers to any of the eight γ-chain genes located on chromosome 1. These genes encode a total of twelve transmembrane or soluble receptor isoforms which are grouped into three Fcγ receptor classes: FcγRI (CD64), FcγRII(CD32), and FcγRIII (CD16). In one preferred embodiment, the Fcγ receptor a human high affinity FcγRI. The human FcγRI is a 72 kDa molecule, which shows high affinity for monomeric IgG (10^8 - $10^9 M^{-1}$).

The production and characterization of these preferred monoclonal antibodies are described by Fanger *et al.* in PCT application WO 88/00052 and in U.S. Patent No. 4,954,617, the teachings of which are fully incorporated by reference herein. These antibodies bind to an epitope of FcγRI, FcγRII or FcγRIII at a site which is distinct from the Fcγ binding site of the receptor and, thus, their binding is not blocked substantially by physiological levels of IgG. Specific anti-FcγRI antibodies useful in this invention are mAb 22, mAb 32, mAb 44, mAb 62 and mAb 197. The hybridoma

producing mAb 32 is available from the American Type Culture Collection, ATCC Accession No. HB9469. In other embodiments, the anti-Fcγ receptor antibody is a humanized form of monoclonal antibody 22 (H22). The production and characterization of the H22 antibody is described in Graziano, R.F. *et al.* (1995) J. Immunol 155 (10): 4996-5002 and PCT/US93/10384. The H22 antibody producing cell line was deposited at the American Type Culture Collection under the designation HA022CL1 and has the accession no. CRL 11177.

In still other preferred embodiments, the binding specificity for an Fc receptor is provided by an antibody that binds to a human IgA receptor, *e.g.*, an Fc-alpha receptor (FcαRI (CD89)), the binding of which is preferably not blocked by human immunoglobulin A (IgA). The term "IgA receptor" is intended to include the gene product of one α-gene (FcαRI) located on chromosome 19. This gene is known to encode several alternatively spliced transmembrane isoforms of 55 to 110 kDa. FcαRI (CD89) is constitutively expressed on monocytes/macrophages, eosinophilic and neutrophilic granulocytes, but not on non-effector cell populations. FcαRI has medium affinity ($\approx 5 \times 10^7\ M^{-1}$) for both IgA1 and IgA2, which is increased upon exposure to cytokines such as G-CSF or GM-CSF (Morton, H.C. *et al.* (1996) Critical Reviews in Immunology 16:423-440). Four FcαRI-specific monoclonal antibodies, identified as A3, A59, A62 and A77, which bind FcαRI outside the IgA ligand binding domain, have been described (Monteiro, R.C. *et al.*, 1992, *J. Immunol.* 148:1764).

FcαRI and FcγRI are preferred trigger receptors for use in the invention because they are (1) expressed primarily on immune effector cells, *e.g.*, monocytes, PMNs, macrophages and dendritic cells; (2) expressed at high levels (*e.g.*, 5,000-100,000 per cell); (3) mediators of cytotoxic activities (*e.g.*, ADCC, phagocytosis); (4) mediate enhanced antigen presentation of antigens, including self-antigens, targeted to them.

In other embodiments, bispecific and multispecific molecules of the invention further comprise a binding specificity which recognizes, *e.g.*, binds to, a target cell antigen, *e.g.*, PSMA. In a preferred embodiment, the binding specificity is provided by a human monoclonal antibody of the present invention.

An "effector cell specific antibody" as used herein refers to an antibody or functional antibody fragment that binds the Fc receptor of effector cells. Preferred antibodies for use in the subject invention bind the Fc receptor of effector cells at a site which is not bound by endogenous immunoglobulin.

As used herein, the term "effector cell" refers to an immune cell which is involved in the effector phase of an immune response, as opposed to the cognitive and activation phases of an immune response. Exemplary immune cells include a cell of a myeloid or lymphoid origin, *e.g.*, lymphocytes (*e.g.*, B cells and T cells including cytolytic T cells (CTLs)), killer cells, natural killer cells, macrophages, monocytes, eosinophils, neutrophils, polymorphonuclear cells, granulocytes, mast cells, and basophils. Some effector cells express specific Fc receptors and carry out specific immune functions. In preferred embodiments, an effector cell is capable of inducing antibody-dependent cell-mediated cytotoxicity (ADCC), *e.g.*, a neutrophil capable of inducing ADCC. For example, monocytes, macrophages, which express FcR are involved in specific killing of target cells and presenting antigens to other components of the immune system, or binding to cells that present antigens. In other embodiments, an effector cell can phagocytose a target antigen, target cell, or microorganism. The expression of a particular FcR on an effector cell can be regulated by humoral factors such as cytokines. For example, expression of FcγRI has been found to be up-regulated by interferon gamma (IFN-γ). This enhanced expression increases the cytotoxic activity of FcγRI-bearing cells against targets. An effector cell can phagocytose or lyse a target antigen or a target cell.

"Target cell" shall mean any undesirable cell in a subject (*e.g.*, a human or animal) that can be targeted by a composition (*e.g.*, a human monoclonal antibody, a bispecific or a multispecific molecule) of the invention. In preferred embodiments, the target cell is a cell expressing or overexpressing PSMA. Cells expressing PSMA typically include tumor cells, such as bladder, breast, colon, kidney, ovarian, prostate, renal cell, squamous cell, lung (non-small cell), and head and neck tumor cells. Other target cells include synovial fibroblast cells.

While human monoclonal antibodies are preferred, other antibodies which can be employed in the bispecific or multispecific molecules of the invention are murine, chimeric and humanized monoclonal antibodies.

Chimeric mouse-human monoclonal antibodies (*i.e.*, chimeric antibodies) can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted. (see Robinson *et al.*, International Patent Publication PCT/US86/02269; Akira, *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, International Application WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988 Science 240:1041-1043); Liu *et al.* (1987) PNAS 84:3439-3443; Liu *et al.*, 1987, J. Immunol. 139:3521-3526; Sun *et al.* (1987) PNAS 84:214-218; Nishimura *et al.*, 1987, Canc. Res. 47:999-1005; Wood *et al.* (1985) Nature 314:446-449; and Shaw *et al.*, 1988, J. Natl Cancer Inst. 80:1553-1559).

The chimeric antibody can be further humanized by replacing sequences of the Fv variable region which are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General reviews of humanized chimeric antibodies are provided by Morrison, S. L., 1985, *Science* 229:1202-1207 and by Oi *et al.*, 1986, *BioTechniques* 4:214. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from 7E3, an anti-$GPIIb_bIII_a$ antibody producing hybridoma. The recombinant DNA encoding the chimeric antibody, or fragment thereof, can then be cloned into an appropriate expression vector. Suitable humanized antibodies can alternatively be produced by CDR substitution U.S. Patent 5,225,539; Jones *et al.* 1986 Nature 321:552-525; Verhoeyan *et al.* 1988 Science 239:1534; and Beidler *et al.* 1988 J. Immunol. 141:4053-4060.

All of the CDRs of a particular human antibody may be replaced with at least a portion of a nonhuman CDR or only some of the CDRs may be replaced with nonhuman CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to the Fc receptor.

An antibody can be humanized by any method, which is capable of replacing at least a portion of a CDR of a human antibody with a CDR derived from a nonhuman antibody. Winter describes a method which may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987), the contents of which is expressly incorporated by reference. The human CDRs may be replaced with nonhuman CDRs using oligonucleotide site-directed mutagenesis as described in International Application WO 94/10332 entitled, *Humanized Antibodies to Fc Receptors for Immunoglobulin G on Human Mononuclear Phagocytes*.

Also within the scope of the invention are chimeric and humanized antibodies in which specific amino acids have been substituted, deleted or added. In particular, preferred humanized antibodies have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, in a humanized antibody having mouse CDRs, amino acids located in the human framework region can be replaced with the amino acids located at the corresponding positions in the mouse antibody. Such substitutions are known to improve binding of humanized antibodies to the antigen in some instances. Antibodies in which amino acids have been added, deleted, or substituted are referred to herein as modified antibodies or altered antibodies.

The term modified antibody is also intended to include antibodies, such as monoclonal antibodies, chimeric antibodies, and humanized antibodies which have been modified by, *e.g.*, deleting, adding, or substituting portions of the antibody. For example, an antibody can be modified by deleting the constant region and replacing it with a constant region meant to increase half-life, *e.g.*, serum half-life, stability or affinity of the antibody. Any modification is within the scope of the invention so long as the bispecific and multispecific molecule has at least one antigen binding region specific for an FcγR and triggers at least one effector function.

Bispecific and multispecific molecules of the present invention can be made using chemical techniques (see *e.g.*, D. M. Kranz *et al.* (1981) *Proc. Natl. Acad. Sci.* USA 78:5807), "polydoma" techniques (See U.S. Patent 4,474,893, to Reading), or recombinant DNA techniques.

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In particular, bispecific and multispecific molecules of the present invention can be prepared by conjugating the constituent binding specificities, *e.g.*, the anti-FcR and anti-PSMA binding specificities, using methods known in the art and described in the examples provided herein. For example, each binding specificity of the bispecific and multispecific molecule can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohaxane-1-carboxylate (sulfo-SMCC) (see *e.g.*, Karpovsky *et al.* (1984) J. Exp. Med. 160:1686; Liu, MA *et al.* (1985) Proc. Natl. Acad. Sci. USA 82:8648). Other methods include those described by Paulus (Behring Ins. Mitt. (1985) No. 78, 118-132); Brennan *et al.* (Science (1985) 229:81-83), and Glennie *et al.* (J. Immunol. (1987) 139: 2367-2375). Preferred conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, IL).

When the binding specificities are antibodies (*e.g.*, two humanized antibodies), they can be conjugated via sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains. In a particularly preferred embodiment, the hinge region is modified to contain an odd number of sulfhydryl residues, preferably one, prior to conjugation.

Alternatively, both binding specificities can be encoded in the same vector and expressed and assembled in the same host cell. This method is particularly useful where the bispecific and multispecific molecule is a mAb x mAb, mAb x Fab, Fab x $F(ab')_2$ or ligand x Fab fusion protein. A bispecific and multispecific molecule of the invention, *e.g.*, a bispecific molecule can be a single chain molecule, such as a single chain bispecific antibody, a single chain bispecific molecule comprising one single chain antibody and a binding determinant, or a single chain bispecific molecule comprising two binding determinants. Bispecific and multispecific molecules can also be single chain molecules or may comprise at least two single chain molecules. Methods for preparing bi- and multspecific molecules are described for example in U.S. Patent Number 5,260,203; U.S. Patent Number 5,455,030; U.S. Patent Number 4,881,175; U.S. Patent Number 5,132,405; U.S. Patent Number 5,091,513; U.S. Patent

Number 5,476,786; U.S. Patent Number 5,013,653; U.S. Patent Number 5,258,498; and U.S. Patent Number 5,482,858.

Binding of the bispecific and multispecific molecules to their specific targets can be confirmed by enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay (RIA), FACS analysis, a bioassay (*e.g.*, growth inhibition), or a Western Blot Assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.*, an antibody) specific for the complex of interest. For example, the FcR-antibody complexes can be detected using *e.g.*, an enzyme-linked antibody or antibody fragment which recognizes and specifically binds to the antibody-FcR complexes. Alternatively, the complexes can be detected using any of a variety of other immunoassays. For example, the antibody can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography.

IV. Antibody Conjugates/Immunotoxins

In another aspect, the present invention features a human anti-PSMA monoclonal antibody, or a fragment thereof, conjugated to another therapeutic moiety, such as a cytotoxin, a drug or a radioisotope. When conjugated to a cytotoxin, these antibody conjugates are referred to as "immunotoxins." A cytotoxin or cytotoxic agent includes any agent that is detrimental to (*e.g.*, kills) cells or which inhibits their growth. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-

dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine). Other examples of therapeutic cytotoxins that can be conjugated to an antibody of the invention include calicheamicins and duocarmycins.

Human antibodies of the present invention also can be conjugated to a radioisotope, *e.g.*, radioactive iodine, to generate cytotoxic or non-cytotoxic radiopharmaceuticals for treating or diagnosing PSMA-related disorders (e.g., tumors).

Antibody conjugates of the invention can be used to modify a given biological response, and the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor or interferon-γ; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (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.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).

V. Pharmaceutical Compositions

In another aspect, the present invention provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of human monoclonal antibodies, or antigen-binding portion(s) thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier. Such compositions may include one or a combination of (*e.g.*, two or more different) human antibodies of the invention.

In one embodiment, the invention provides a therapeutic composition comprising a combination of human anti-PSMA antibodies which bind to different epitopes on human PSMA and have complementary activities, *e.g.*, as a pharmaceutical composition. For example, a human monoclonal antibody that mediates highly effective killing of target cells in the presence of effector cells can be combined with another human monoclonal antibody that inhibits the growth of cells expressing PSMA.

In another embodiment, the therapeutic composition comprises one or a combination of immunoconjugates or bispecific (or multispecific) molecules of the invention.

Pharmaceutical compositions of the invention also can be administered in combination therapy, *i.e.*, combined with other agents. For example, the combination therapy can include a composition of the present invention with at least one anti-tumor agent or other conventional therapy.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, antibody, bispecific and multispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see *e.g.*, Berge, S.M., *et al.* (1977) J. Pharm. Sci. 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as

from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A composition of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

To administer a compound of the invention by certain routes of administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.* (1984) *J. Neuroimmunol.* 7:27).

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

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Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular

therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

For the therapeutic compositions, formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredient, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate. Dosage forms for the topical or transdermal administration of compositions of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac,

intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given alone or as a pharmaceutical composition containing, for example, 0.01 to 99.5% (more preferably, 0.1 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention

employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a compositions of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. It is preferred that administration be intravenous, intramuscular, intraperitoneal, or subcutaneous, preferably administered proximal to the site of the target. If desired, the effective daily dose of a therapeutic compositions may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for

continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

In certain embodiments, the human monoclonal antibodies of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (*see, e.g.*, V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134), different species of which may comprise the formulations of the inventions, as well as components of the invented molecules; p120 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994) *Immunomethods* 4:273. In one embodiment of the invention, the therapeutic compounds of the invention are formulated in liposomes; in a more preferred embodiment, the liposomes include a targeting moiety. In a most preferred embodiment, the therapeutic compounds in the liposomes are delivered by bolus injection to a site proximal to the tumor or infection. The composition must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi.

A "therapeutically effective dosage" preferably inhibits tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit cancer can be evaluated in an animal

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model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

The composition must be sterile and fluid to the extent that the composition is deliverable by syringe. In addition to water, the carrier can be an isotonic buffered saline solution, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition. Long-term absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

When the active compound is suitably protected, as described above, the compound may be orally administered, for example, with an inert diluent or an assimilable edible carrier.

VI. Uses and Methods of the Invention

Human monoclonal anti-PSMA antibodies and related derivatives/conjugates and compositions of the present invention have a variety of *in vitro* and *in vivo* diagnostic and therapeutic utilities. For example, these molecules can be administered to cells in culture, *e.g. in vitro* or *ex vivo*. Alternatively, they can be administered to a subject, *e.g., in vivo,* to treat, prevent or diagnose a variety of PSMA-related disorders. As used herein, the term "subject" is intended to include both human and nonhuman animals. Preferred subjects include human patients exhibiting disorders characterized by expression of PSMA, typically aberrant expression (*e.g.*, overexpression) of PSMA. Accordingly, the methods and compositions of the present invention can be used to treat subjects with tumorigenic disorders characterized by the

presence of tumor cells expressing PSMA including, for example, prostate cancer, colon cancer, and renal carcinoma. The term "nonhuman animals" of the invention includes all vertebrates, *e.g.*, mammals and non-mammals, such as nonhuman primates, sheep, dog, cow, chickens, amphibians, reptiles, etc.

In a preferred embodiment, the invention provides a method for treating prostate cancer in a subject comprising administering to the subject one of the anti-PSMA antibodies of the invention. In another embodiment, the anti-PSMA antibody is administered as a conjugate, wherein the antibody is linked to, for example, a radioactive agent or a cytotoxic drug. In yet another embodiment, the anti-PSMA antibody is administered as a bispecific molecule, *e.g.*, linked to anti-FcγRI or anti-Fcα.

Human antibodies of the invention can be initially tested for binding activity associated with therapeutic or diagnostic use *in vitro*. For example, compositions of the invention can be tested using the ELISA and flow cytometric assays described in the Examples below. Moreover, the activity of these molecules in triggering at least one effector-mediated effector cell activity, including cytolysis of cells expressing PSMA can be assayed. Protocols for assaying for effector cell-mediated phagocytosis are described in the Examples below.

Human antibodies of the invention also have additional utility in therapy and diagnosis of PSMA-related diseases. For example, the human monoclonal antibodies, the multispecific or bispecific molecules can be used, for example, to elicit *in vivo* or *in vitro* one or more of the following biological activities: to opsonize a cell expressing PSMA; to mediate phagocytosis or cytolysis of a cell expressing PSMA in the presence of human effector cells; or to inhibit the growth of a cell expressing PSMA.

Suitable methods for administering antibodies and compositions of the present invention are well known in the art. Suitable dosages also can be determined within the skill in the art and will depend on the age and weight of the subject and the particular drug used.

Human anti-PSMA antibodies of the invention also can be co-administered with other therapeutic agents, *e.g.*, a chemotherapeutic agent, or can be co-administered with other known therapies, *e.g.*, an anti-cancer therapy, *e.g.*, radiation. Such therapeutic agents include, among others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which

are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/m^2 dose once every four weeks and adriamycin is intravenously administered as a 60-75 mg/m^2 dose once every 21 days. Co-administration of the human anti-PSMA antibodies, or antigen binding fragments thereof, of the present invention with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them unreactive with the antibody.

Target-specific effector cells, *e.g.*, effector cells linked to human antibodies, multispecific or bispecific molecules of the invention, also can also be used as therapeutic agents. Effector cells for targeting can be human leukocytes such as macrophages, neutrophils or monocytes. Other cells include eosinophils, natural killer cells and other IgG- or IgA-receptor bearing cells. If desired, effector cells can be obtained from the subject to be treated. The target-specific effector cells, can be administered as a suspension of cells in a physiologically acceptable solution. The number of cells administered can be in the order of 10^8-10^9 but will vary depending on the therapeutic purpose. In general, the amount will be sufficient to obtain localization at the target cell, *e.g.*, a tumor cell expressing PSMA, and to effect cell killing by, *e.g.*, phagocytosis. Routes of administration can also vary.

Therapy with target-specific effector cells can be performed in conjunction with other techniques for removal of targeted cells. For example, anti-tumor therapy using the compositions (*e.g.*, human antibodies, multispecific and bispecific molecules) of the invention and/or effector cells armed with these compositions can be used in conjunction with chemotherapy. Additionally, combination immunotherapy may be used to direct two distinct cytotoxic effector populations toward tumor cell rejection. For example, anti-PSMA antibodies linked to anti-Fc-gammaRI or anti-CD3 may be used in conjunction with IgG- or IgA-receptor specific binding agents.

Bispecific and multispecific molecules of the invention can also be used to modulate FcγR or FcαR levels on effector cells, such as by capping and elimination of receptors on the cell surface. Mixtures of anti-Fc receptors can also be used for this purpose.

The compositions (*e.g.*, human antibodies, multispecific and bispecific molecules) of the invention which have complement binding sites, such as portions from IgG1, -2, or -3 or IgM which bind complement, can also be used in the presence of complement. In one embodiment, *ex vivo* treatment of a population of cells comprising target cells with a binding agent of the invention and appropriate effector cells can be supplemented by the addition of complement or serum containing complement. Phagocytosis of target cells coated with a binding agent of the invention can be improved by binding of complement proteins. In another embodiment target cells coated with the compositions (*e.g.*, human antibodies, multispecific and bispecific molecules) of the invention can also be lysed by complement. In yet another embodiment, the compositions of the invention do not activate complement.

The compositions (*e.g.*, human antibodies, multispecific and bispecific molecules) of the invention can also be administered together with complement. Accordingly, within the scope of the invention are compositions comprising human antibodies, multispecific or bispecific molecules and serum or complement. These compositions are advantageous in that the complement is located in close proximity to the human antibodies, multispecific or bispecific molecules. Alternatively, the human antibodies, multispecific or bispecific molecules of the invention and the complement or serum can be administered separately.

Also within the scope of the invention are kits comprising the compositions (*e.g.*, human antibodies, multispecific and bispecific molecules) of the invention and instructions for use. The kit can further contain a least one additional reagent, such as complement, or one or more additional human antibodies of the invention (*e.g.*, a human antibody having a complementary activity which binds to an epitope in PSMA antigen distinct from the first human antibody).

In other embodiments, the subject can be additionally treated with an agent that modulates, *e.g.*, enhances or inhibits, the expression or activity of Fcγ or Fcα receptors by, for example, treating the subject with a cytokine. Preferred cytokines for administration during treatment with the multispecific molecule include of granulocyte colony-stimulating factor (G-CSF), granulocyte- macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), and tumor necrosis factor (TNF).

The compositions (*e.g.*, human antibodies, multispecific and bispecific molecules) of the invention can also be used to target cells expressing FcγR or PSMA, for example for labeling such cells. For such use, the binding agent can be linked to a molecule that can be detected. Thus, the invention provides methods for localizing *ex vivo* or *in vitro* cells expressing Fc receptors, such as FcγR, or PSMA. The detectable label can be, *e.g.*, a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor.

Human antibodies of the invention also can be used to detect the presence of PSMA antigen in a sample, or to measure the amount of PSMA antigen in a sample, by contacting the sample (e.g., along with a control sample) with the human monoclonal antibody under conditions that allow for formation of a complex between the antibody and PSMA. The formation of a complex is then detected, wherein a difference complex formation between the sample compared to the control sample is indicative the presence of PSMA antigen in the sample.

In still another embodiment, the invention provides a method for detecting the presence or quantifying the amount of Fc-expressing cells *in vivo* or *in vitro*. The method comprises (i) administering to a subject a composition (*e.g.*, a multi- or bispecific molecule) of the invention or a fragment thereof, conjugated to a detectable marker; (ii) exposing the subject to a means for detecting said detectable marker to identify areas containing Fc-expressing cells.

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

Methods and Materials

Screening assay for PSMA-specific monoclonal antibodies: PSMA-HuMAbs were detected using a solid-phase ELISA-based assay. Immunoaffinity purified PSMA from LNCaP cells, or bacterially-expressed fusion proteins containing PSMA derived fragments, were coated onto Maxi-Sorp (Nunc, Rochester, NY) 96-well plates with an overnight incubation at 4°C. The plates were washed with PBS-O.2%

Tween-20 and blocked with 5% BSA in PBS for 1 hour at room temperature. Fifty µl of supernatant from the hybridoma cultures was added to the PSMA-coated wells and the plates were incubated for 2 hours at room temperature. The plates were washed as above and 50µl of 1:1000 diluted rabbit-anti-human IgG H&L chain (ICN, Costa Mesa, CA) was added to each well. Following a one-hour incubation at room temperature, the plates were washed as above and 50µl of a 1:2000 dilution of HRP-conjugated Protein-A (Sigma, St. Louis, MO) was added to each well. Following a one-hour incubation at room temperature, the plates were washed as above and 100 µl of ABTS (150 mg 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid in 500 ml of 0.1 M citric acid, pH 4. 35)/H2O2 (10 µl 30% H2O2 per 10 ml of ABTS solution) chromagen/substrate solution was added to each well. After a five minute incubation the reaction was stopped with the addition of 100 µl of stop solution (SDS/dimethylformamide) and the absorbance at 405 nm was read in a microplate reader. The hybridoma cells producing supernatants with high A^{405} values were cloned by limiting dilution and subjected to additional analysis.

Isolation of antibody protein: Monoclonal antibodies were isolated from a Cellmax bioreactor (Cellco, Laguna Hills, CA) using RPMI-1640 medium containing 1- to 5% Fetalclone (Hyclone, Logan, UT). Monoclonal antibodies were purified by chromatography on a Protein A-Agarose column according to manufacturer's specifications (KPL, Gaithersburg, MD).

Preparation of LNCaP cell membranes: LNCaP cells were scraped from plastic dishes, washed extensively in PBS, resuspended in 10 volumes of deionized water, and homogenized by three strokes with a Dounce homogenizer. The membrane fraction was isolated by centrifugation at 15,000 x g for 45 minutes and the pellet resuspended in PBS. Protein concentration of the membrane pellet was determined using the Pierce (Rockford, IL) BCA kit.

Heat denaturation experiments: An aliquot of immunoaffinity purified PSMA from LNCaP cells (40 µg/ml in PBS) was heat denatured by boiling for 10 minutes and cooled on ice. This, and an identical aliquot which was not heat denatured, were diluted 1:4 in PBS and 50 µl was added to wells of a 96-well Maxi-Sorp plate (Nunc) and coated overnight at 4°. After coating, all plates were blocked with 5% BSA in PBS for one hour, washed in PBS and subjected to a standard sandwich ELISA using the indicated primary antibodies as described above.

Western blot analysis: Western blot analysis following SDS-PAGE of PSMA containing fractions and transfer to PVDF membranes. The blots were blocked overnight in TBS containing 5% nonfat milk and incubated with purified antibody present at a concentration of 5 μg/ml in TBS for one hour. The blots were washed 5 times with TBS containing 0.5% Tween-20 (TBS-T), the blots were developed using the LumiGLO chemiluminescent substrate kit (KPL, Gaithersburg, MD), and visualized by exposing x-ray film.

Immunoprecipitation studies: A detergent lysate of LNCaP cells was prepared by adding PBS containing 1% NP-40 to LNCaP cells, incubating for one hour, and centrifugation to remove particulate material. The lysate was pre-cleared by adding 150 μg irrelevant human IgG_1 per ml of lysate, incubating for one hour at room temperature, followed by the addition of 150 μl of packed Protein G-Sepharose beads per ml lystae. The supernatant fraction was used after centrifugation to remove the beads. Aliquots, 100 μl each, of the pre-cleared lysate were mixed with 5 μg of antibody protein and incubated overnight at 4°C. At the end of this period, 20 μl of packed Protein G-Sepharose beads were added to each tube and the tubes were incubated for one hour at 4°C. Following extensive washing with lysis buffer, 50 μl of Laemmli sample buffer (Bio-Rad) was added to each sample and the tubes were heated for 10 minutes at 95°C. The tubes were centrifuged for two minutes and 25 μl of each sample was loaded onto an SDS-PAGE gel and electrophoresed at 175 volts for 60 minutes. The electrophoresed samples were electroblotted onto PVDF membranes for Western blot analysis using the murine anti-PSMA antibody 4D8 (5 μg/ml) and developed as described above.

Flow cytometry: LNCaP and PC-3 cells were freshly harvested from tissue culture flasks and a single cell suspension prepared. LNCaP cell suspensions were either stained with primary antibody directly or after fixation with 1% paraformaldehyde in PBS. Approximately one million cells were resuspended in PBS containing 0.5% BSA and 50-200 μg/ml of primary antibody and incubated on ice for 30 minutes. The cells were washed twice with PBS containing 0.1% BSA, 0.01% NaN_3, resuspended in 100 μl of 1:100 diluted FITC-conjugated goat-anti-human IgG (Jackson ImmunoResearch, West Grove, PA), and incubated on ice for an additional 30 minutes. The cells were again washed twice, resuspended in 0.5 ml of wash buffer and analyzed

for fluorescent staining on a FACSCalibur cytometer (Becton-Dickinson, San Jose, CA) with CellQuest acquisition software.

FITC labeling of monoclonal antibodies: Purified monoclonal antibodies were first extensively dialyzed against O.3M sodium carbonate buffer, pH 9.5. A stock fluorescein isothiocyanate (FITC) solution was prepared by dissolving 1 mg solid FITC in 1 ml of DMSO. Stock FITC was added dropwise with constant mixing in an amount to provide 50 μg FITC per mg of antibody protein. Once added, the solution was incubated in the dark at room temperature for 1-3 hours. FITC-labeled antibody was isolated by gel filtration on a Sephadex G-I0 column equilibrated in PBS.

DOTA labeling of 4A3 and 7F12: Five milligrams of 4A3 and 7F12 antibody protein was DOTA labeled via direct coupling of one of the four carboxylic acid groups of DOTA to amino groups of the antibody protein. DOTA (tetraazacyclododecanetetraacidic acid) is a common chelator which can be used for complexing radionuclides. The protein in approximately 1.5 ml of PBS was first washed in a centrifugal concentrator with a M_r 25,000 cut-off using 5 x 4ml of 1 % DTPA (diethylenetriaminepentaacetic acid), pH 5.0 over a 24 hour period. The antibody buffer was then changed to 0.1M phosphate, pH 7.0 using the same procedure. An active ester of DOTA was created by dissolving 30 mg of DOTA (0.072 mmol) in 0.4 ml water and the pH was adjusted to 7.3 with NaOH. Ten mg of l-ethyl-3-(3-dimethylaminopropyl)carbodiimide was then added and the mixture cooled on ice for one hour and added to the antibody solution and stirred at 4°C overnight. The resultant DOTA-antibody conjugate was separated from excess DOTA and other reactants by repeated washing with 0.3 M NH_4Oac and centrifugal concentration.

Antibody inhibition studies: Approximately one million LNCaP cells were initially treated with 200 μg/ml purified 4A3, 7F12, 8A11, 8C12, 16F9, or irrelevant human IgG_1 antibody in PBS for one hour on ice. After washing, the cells were incubated with 50 μg/ml FITC conjugated human or murine anti-PSMA monoclonal antibody for one hour on ice. After washing, the cells were stained with 10 μg/ml propidium iodide and analyzed by flow cytometry on a FACsCalibur with CellQuest software.

Biodistribution of ^{125}I-labeled HuMAb in nude mice bearing LNCaP cell tumors: LNCaP cells, 2x106 in 50% Matrigel (Becton-Dickinson (150 μl total volume), were injected subcutaneously in nude mice. When tumors reached a size of

approximately 0.5 cm in diameter, the animals were subjected to *in vivo* labeling with ^{125}I-labeled antibody via i. v. administration through the tail vein of 100 μg of antibody (containing 5- to 35 μCi of ^{125}I). After varying time points, the animals were sacrificed and the level of ^{125}I-label present in individual normal organs and tissues, as well as tumor tissue, was determined.

One milligram of antibody protein was iodinated with 1- to 1.5 mCi of ^{125}I using Iodobeads (Pierce) according to manufacturer's instructions.

Internalization of ^{125}I-labeled HuMAbs: LNCaP cells were plated into 6-well plates and allowed to reach near confluency. The medium was then removed and the wells washed with PBS to remove non-adherant cells. The cells were then labeled with ^{125}I-labeled HuMAb or isotype matched irrelevant human IgG present at a concentration of 10 μg/ml in a total volume of 1.5 ml in fresh culture medium and incubated in a 37°C incubator for 10 minutes. At the end of this period the medium was removed, the cells washed extensively with PBS to remove unbound labeled antibody, 1.5 ml of culture medium was added, and the plates returned to the 37° incubator for the desired incubation time. At 0 (immediately after addition of the culture medium), 4, 18, and 28 hours of incubation the culture medium was removed, centrifuged to eliminate non-adherent cells. The supernatant fraction was cooled on ice, and subjected to TCA precipitation by addition of 100% TCA to yield a 10% final TCA concentration. After incubating on ice for 10 minutes, the fraction was centrifuged for 10 minutes at 1000 xg and the supernatant removed. The amount of radioactivity present in both the TCA soluble and insoluble fractions was determined in a gamma counter. The adherent cells present in the wells after removal of the medium for TCA precipitation were released using trypsin and placed in a tube for counting along with a 1ml wash with 0.1N NaOH. The radioactivity bound to the cells was also determined in a gamma counter. The proportion of the total counts originally bound to the cells which was internalized, processed, and distributed into the TCA soluble fraction with time was plotted.

Purified antibody protein, 0.5 mg, was iodinated with 1 mCi of ^{125}I as described previously. Labeling of each antibody was between 0.4 and 1.6 μCi/μg antibody protein.

ADCC and CDC screening of HuMAbs: ADCC and CDC tests were four hour ^{51}Cr release assays using LNCaP cells as target cells. Assays were conducted in 96-well plates with 2500 targets per well in triplicate using an effector to target cell ratio (E:T ratio) of 100:1. Effector cells were PBMC's isolated from one male and one female donor. For CDC assays, fresh human plasma at a final concentration of 1:200 was used as a complement source.

Tissue cross reactivity screening of HuMAbs by IHC: Conditions for IHC cross reactivity screening were first optimized by use of a fixed concentration of purified unconjugated human monoclonal antibody (5 μg/ml) and varying concentrations of biotinylated goat-anti-human IgGl secondary antibody on frozen tissue sections fixed by treatment with acetone for 10 minutes after cryotomy or with 10% neutral-buffered formalin for 10 seconds immediately prior to staining. Based upon these conditions, a second test was conducted with the optimized secondary antibody concentration with varying concentrations of the HuMAb primary antibody.

Based on these results, tissue screening was conducted on human frozen sections after fixation in acetone for 10 minutes at the time of cryotomy followed by 10 seconds in 10% neutral-buffered formalin immediately prior to staining. Staining was done with primary antibody at a concentration of 5 μg/ml containing 1.5 mg/ml excess carrier IgG followed by 7.5 μg/ml biotinylated goat-anti-human IgG secondary antibody containing 1.5 mg/ml excess carrier IgG. Heat aggregated rabbit IgG (1 mg/ml), 5% normal goat serum, and 1% BSA were included in the protein block of the sections.

Example 1 Generation of Cmu targeted mice for the production of anti-PSMA human antibodies

Construction of a CMD targeting vector: The plasmid pICEmu contains an EcoRI/XhoI fragment of the murine Ig heavy chain locus, spanning the mu gene, that was obtained from a Balb/C genomic lambda phage library (Marcu *et al.* Cell 22: 187, 1980). This genomic fragment was subcloned into the XhoI/EcoRI sites of the plasmid pICEMI9H (Marsh et al; Gene 32, 481-485, 1984). The heavy chain sequences included in pICEmu extend downstream of the EcoRI site located just 3' of the mu intronic enhancer, to the XhoI site located approximately 1 kb downstream of the last

transmembrane exon of the mu gene; however, much of the mu switch repeat region has been deleted by passage in *E. coli*.

The targeting vector was constructed as follows. A 1.3 kb HindIII/SmaI fragment was excised from pICEmu and subcloned into HindIII/SmaI digested pBluescript (Stratagene, La Jolla, CA). This pICEmu fragment extends from the HindIII site located approximately 1 kb 5' of Cmu1 to the SmaI site located within Cmu1. The resulting plasmid was digested with SmaI/SpeI and the approximately 4 kb SmaI/XbaI fragment from pICEmu, extending from the SmaI site in Cmu1 3' to the XbaI site located just downstream of the last Cmu exon, was inserted. The resulting plasmid, pTAR1, was linearized at the SmaI site, and a neo expression cassette inserted. This cassette consists of the neo gene under the transcriptional control of the mouse phosphoglycerate kinase (pgk) promoter (XbaI/TaqI fragment; Adra *et al.* (1987) Gene 60: 65-74) and containing the pgk polyadenylation site (PvuII/HindIII fragment; Boer *et al.* (1990) Biochemical Genetics 28: 299-308). This cassette was obtained from the plasmid pKJ1 (described by Tybulewicz *et al.* (1991) Cell 65: 1153-1163) from which the neo cassette was excised as an EcoRI/HindIII fragment and subcloned into EcoRI/HindIII digested pGEM-7Zf (+) to generate pGEM-7 (KJ1). The neo cassette was excised from pGEM-7 (KJ1) by EcoRI/SalI digestion, blunt ended and subcloned into the SmaI site of the plasmid pTAR1, in the opposite orientation of the genomic Cmu sequences. The resulting plasmid was linearized with Not I, and a herpes simplex virus thymidine kinase (tk) cassette was inserted to allow for enrichment of ES clones bearing homologous recombinants, as described by Mansour *et al.* (1988) Nature 336: 348-352. This cassette consists of the coding sequences of the tk gene bracketed by the mouse pgk promoter and polyadenylation site, as described by Tybulewicz *et al.* (1991) Cell 65: 1153-1163. The resulting CMD targeting vector contains a total of approximately 5.3 kb of homology to the heavy chain locus and is designed to generate a mutant mu gene into which has been inserted a neo expression cassette in the unique SmaI site of the first Cmu exon. The targeting vector was linearized with PvuI, which cuts within plasmid sequences, prior to electroporation into ES cells.

Generation and analysis of targeted ES cells: AB-1 ES cells (McMahon, A. P. and Bradley, A., (1990) Cell 62: 1073-1085) were grown on mitotically inactive SNL76/7 cell feeder layers (ibid.) essentially as described (Robertson, E. J. (1987) in Teratocarcinomas and Embryonic Stem Cells: a Practical Approach (E. J. Robertson,

ed.) Oxford: IRL Press, p. 71-112). The linearized CMD targeting vector was electroporated into AB-1 cells by the methods described Hasty *et al.* (Hasty, P. R. *et al.* (1991) Nature 350: 243-246). Electroporated cells were plated into 100 mm dishes at a density of 1-2 x 10^6 cells/dish. After 24 hours, G418 (200 micrograms/ml of active component) and FIAU (5 x 10^{-7} M) were added to the medium, and drug-resistant clones were allowed to develop over 8-9 days. Clones were picked, trypsinized, divided into two portions, and further expanded. Half of the cells derived from each clone were then frozen and the other half analyzed for homologous recombination between vector and target sequences.

DNA analysis was carried out by Southern blot hybridization. DNA was isolated from the clones as described Laird *et al.* (Laird, P. W. *et al.*, (1991) Nucleic Acids Res. 19 : 4293). Isolated genomic DNA was digested with SpeI and probed with a 915 bp SacI fragment, probe A (see Figure 1), which hybridizes to a sequence between the mu intronic enhancer and the mu switch region. Probe A detects a 9.9 kb SpeI fragment from the wild type locus, and a diagnostic 7.6 kb band from a mu locus which has homologously recombined with the CMD targeting vector (the neo expression cassette contains a SpeI site). Of 1132 G418 and FIAU resistant clones screened by Southern blot analysis, 3 displayed the 7.6 kb SpeI band indicative of homologous recombination at the mu locus. These 3 clones were further digested with the enzymes BglI, BstXI, and EcoRI to verify that the vector integrated homologously into the mu gene. When hybridized with probe A, Southern blots of wild type DNA digested with BglI, BstXI, or EcoRI produce fragments of 15.7, 7.3, and 12.5 kb, respectively, whereas the presence of a targeted mu allele is indicated by fragments of 7.7, 6.6, and 14.3 kb, respectively. All 3 positive clones detected by the SpeI digest showed the expected BglI, BstXI, and EcoRI restriction fragments diagnostic of insertion of the neo cassette into the Cmu1 exon.

Generation of mice bearing the mutated mu gene: The three targeted ES clones, designated number 264, 272, and 408, were thawed and injected into C57BL/6J blastocysts as described by Bradley (Bradley, A. (1987) in Teratocarcinomas and Embryonic Stem Cells: a Practical Approach. (E. J. Robertson, ed.) Oxford: IRL Press, p. 113-151). Injected blastocysts were transferred into the uteri of pseudopregnant females to generate chimeric mice representing a mixture of cells derived from the input ES cells and the host blastocyst. The extent of ES cell contribution to the chimera can

be visually estimated by the amount of agouti coat coloration, derived from the ES cell line, on the black C57BL/6J background. Clones 272 and 408 produced only low percentage chimeras (*i.e.* low percentage of agouti pigmentation) but clone 264 produced high percentage male chimeras. These chimeras were bred with C57BL/6J females and agouti offspring were generated, indicative of germline transmission of the ES cell genome. Screening for the targeted mu gene was carried out by Southern blot analysis of BglI digested DNA from tail biopsies (as described above for analysis of ES cell DNA). Approximately 50% of the agouti offspring showed a hybridizing BglI band of 7.7 kb in addition to the wild type band of 15.7 kb, demonstrating a germline transmission of the targeted mu gene.

Analysis of transgenic mice for functional inactivation of mu gene: To determine whether the insertion of the neo cassette into Cmu1 has inactivated the Ig heavy chain gene, a clone 264 chimera was bred with a mouse homozygous for the JHD mutation, which inactivates heavy chain expression as a result of deletion of the JH gene segments (Chen et al, (1993) Immunol. 5: 647-656). Four agouti offspring were generated. Serum was obtained from these animals at the age of 1 month and assayed by ELISA for the presence of murine IgM. Two of the four offspring were completely lacking IgM (see Table 1). Genotyping of the four animals by Southern blot analysis of DNA from tail biopsies by BglI digestion and hybridization with probe A (see Figure 1), and by StuI digestion and hybridization with a 475 bp EcoRI/StuI fragment (ibid.) demonstrated that the animals which fail to express serum IgM are those in which one allele of the heavy chain locus carries the JHD mutation, the other allele the Cmu1 mutation. Mice heterozygous for the JHD mutation display wild type levels of serum Ig. These data demonstrate that the Cmu1 mutation inactivates expression of the mu gene.

TABLE 1

Mouse	Serum IgM (micrograms/ml)	Ig H chain genotype
42	<0.002	CMD/JHD
43	196	+/JHD
44	<0.002	CMD/JHD
45	174	+/JHD
129 x BL6 F1	153	+/+
JHD	<0.002	JHD/JHD

Table 1 shows the levels of serum IgM, detected by ELISA, for mice carrying both the CMD and JHD mutations (CMD/JHD), for mice heterozygous for the JHD mutation (+/JHD), for wild type (129Sv x C57BL/6J)F1 mice (+/+), and for B cell deficient mice homozygous for the JHD mutation (JHD/JHD).

Example 2 Generation of HCO12 transgenic mice for the production of anti-PSMA human antibodies

The HCO12 human heavy chain transgene: The HCO12 transgene was generated by coinjection of the 80 kb insert of pHC2 (Taylor *et al.*, 1994, Int. Immunol., 6: 579-591) and the 25 kb insert of pVx6. The plasmid pVx6 was constructed as described below.

An 8.5 kb HindIII/SalI DNA fragment, comprising the germline human V_H1-18 (DP-14) gene together with approximately 2.5 kb of 5' flanking, and 5 kb of 3' flanking genomic sequence was subcloned into the plasmid vector pSP72 (Promega, Madison, WI) to generate the plasmid p343.7.16. A 7 kb BamHI/HindIII DNA fragment, comprising the germline human V_H5-51 (DP-73) gene together with approximately 5 kb of 5' flanking and 1 kb of 3' flanking genomic sequence, was cloned into the pBR322 based plasmid cloning vector pGP1f (Taylor *et al.* 1992, Nucleic Acids Res. 20: 6287-6295), to generate the plasmid p251f. A new cloning vector derived from pGP1f, pGP1k, was digested with EcoRV/BamHI, and ligated to a 10 kb EcoRV/BamHI DNA fragment, comprising the germline human V_H3-23 (DP47) gene together with approximately 4 kb of 5' flanking and 5 kb of 3' flanking genomic sequence. The resulting plasmid, p112.2RR.7, was digested with BamHI/SalI and ligated with the 7 kb purified BamHI/SalI insert of p251f. The resulting plasmid, pVx4, was digested with XhoI and ligated with the 8.5 kb XhoI/SalI insert of p343.7.16.

A clone was obtained with the V_H1-18 gene in the same orientation as the other two V genes. This clone, designated pVx6, was then digested with NotI and the purified 26 kb insert coinjected (together with the purified 80 kb NotI insert of pHC2 at a 1:1 molar ratio) into the pronuclei of one-half day (C57BL/6J x DBA/2J)F2 embryos as described by Hogan *et al.* (B. Hogan *et al.*, Manipulating the Mouse Embryo, A Laboratory Manual, 2nd edition, 1994, Cold Spring Harbor Laboratory Press, Plainview

NY). Three independent lines of transgenic mice comprising sequences from both Vx6 and HC2 were established from mice that developed from the injected embryos. These lines are designated (HCO12)14881, (HCO12)15083, and (HCO12)15087. Each of the three lines were then bred with mice comprising the CMD mutation described in Example 1, the JKD mutation (Chen *et al.* 1993, EMBO J. 12: 811-820), and the (KCo5)9272 transgene (Fishwild *et al.* 1996, Nature Biotechnology 14: 845-851). The resulting mice express human immunoglobulin heavy and kappa light chain transgenes in a background homozygous for disruption of the endogenous mouse heavy and kappa light chain loci.

Example 3 Production of Human Monoclonal Antibodies and Bispecifics Against PSMA

Antigen: Antigen (Northwest Biotherapeutics, Inc) was provided in two forms: (1) cell membranes and (2) purified protein (PSMA) isolated from LNCaP cells (Cat#CRL-1740; American Type Culture Collection, Rockville, MD). With purified antigen (1.05 mg/ml), one immunization and the final tail vein boosts were performed. The monoclonal antibody 7Ell.C5 was obtained from Cytogen, Inc, Princeton, NJ.

Soluble PSMA and membranes from LNCaP cells were mixed with either complete or incomplete Freunds adjuvant (CFA and IFA). Mice were injected with 0.2cc prepared antigen into the intraperitoneal cavity. Final tail vein immunizations were performed with soluble PSMA in sterile PBS.

Transgenic Mice: Mice were housed in filter cages and were evaluated to be in good physical condition on dates of immunization, bleeds and the day of the fusion. Hybridomas 4A3, 7F12, 8A11, 8C12, and 16F9 were produced by a male mouse ID#17018 of the (CMD)++; (HCo12) 15087+; (JKD) ++; (KCo5) 9272+ genotype. Individual transgene designations are in parentheses, followed by line numbers for randomly integrated transgenes. The symbols ++ and + indicate homozygous or hemizygous; however, because the mice are routinely screened using a PCR-based assay that does not allow distinction between heterozygosity and homozygosity for the randomly integrated human Ig transgenes, a + designation may be given to mice that are actually homozygous for these elements.

Immunization Procedure: The immunization schedule is listed in Table 2. Mouse #17018 was fused on Day 112 included among a cohort of ten mice from HCo7 and HCo12 genotypes. All immunizations were injected into the intraperitoneal cavity. Three and two days prior to fusion, IV boosts were performed.

TABLE 2

Date of activity	Immunization: adjuvant, antigen	Bleed and titer*
Day 1	CFA, membranes	
Day 13	IFA, PSMA (~ 50μg)	
Day 27	IFA, membranes	
Day 38		Titer
Day 40	IFA, PSMA (~ 50μg)	
Day 48		Titer
Day 55	IFA, PSMA (50μg)	
Day 62		Titer
Day 70	IFA, membranes	
Day 80		Titer
Day 84	IFA, membranes	
Day 94		Titer
	IV boost day – 3 & day – 2 prior to fusion. Fusion performed Day 112	

*For titers, see Table 3.

Hybridoma Preparation: The P3 X63 ag8.653 myeloma cell line (ATCC CRL 1580, lot F-15183) was used for the fusions. The original ATCC vial was thawed and expanded in culture. A seed stock of frozen vials was prepared from this expansion. Cells are maintained in culture for 3–6 months, passed twice a week. P388D1 (ATCC TIB-63 FL) was expanded to 200mLs and exhausted. The supernatant was spun and filtered and used as a media addition for the hybridomas. This cell line is passed for 3-6 months when a new vial is thawed.

High Glucose DMEM: (Mediatech Cellgro, # 1001233) containing 10% FBS and Penicillin-Streptomycin (Gibco, #11K1763), was used to culture P388D1 cells and myeloma cells. Additional media supplements were added to the Hybridoma growth media.

The spleen from mouse number #17018 was normal in size and yielded 1.78 x 10^8 viable cells. The splenocytes were fused.

The initial ELISA screen for human IgG,κ antibodies was performed 7-10 days post fusion. Human IgG,κ positive wells were then screened on soluble PSMA coated ELISA plates. Antigen positive hybridomas were then transferred to 24 well plates, and eventually to tissue culture flasks. PSMA specific hybridomas were subcloned by limiting dilution to assure monoclonality. Antigen positive hybridomas were preserved at several stages in the development process by freezing cells in DMEM, 50% FBS plus 10% DMSO (Sigma, D2650).

The titers for mouse #17018 are shown below in the table. The titers are Hu- antigen specific-γ. The response to the antigen after repeated immunizations show a robust response level and the mouse was prepared for fusion.

TABLE 3

Date	**Titer**
Day 38	100
Day 48	50
Day 62	50
Day 80	1600
Day 94	3200

The fusion resulted in 38 Hu-γ,κ hybridomas that were re-screened on antigen. Following the screen on antigen (ELISA based) five antigen specific hybridomas were identified. These were retested on antigen and all five clones were confirmed positive for the target: 4A3, 7F12, 8A11, 8C12 and 16F9. Supernatants of these five hybridomas were further evaluated. Antibodies from these five clones bound to the native form of PSMA expressed on LNCaP cells. All five antibodies are γ1,κ isotype.

The bispecific molecule designated 14A8 x 8C12 was made by chemical conjugation of the Fab'_2 fragments from the human anti-CD89 antibody 14A8 or a subclone of the 14A8 antibody, designated 14A8, and the human anti-PSMA antibody, 8C12, via disulfide bonds using standard cross-linking procedures (Figure 6).

Example 4 Binding Characteristics of Human anti-PSMA Antibodies

Solid phase ELISA studies: Binding characteristics of anti-PSMA specific antibodies were studied by comparing reactivities (solid phase ELISA) against full length PSMA and bacterially expressed fusion proteins containing portions of the PSMA protein. HuMAbs 4A3, 7F12, 8A11, 8C12, and 16F9 reacted with purified PSMA but were unreactive with any fusion protein containing a portion of the PSMA sequence (results not shown). In contrast, the HuMAb 11C10 reacted strongly with both full length PSMA and the fusion protein containing the PSMA amino acid 1-173 sequence (Figure 1). A lower level of binding of the 11C10 antibody was also observed to the amino acid 134-437 PSMA fragment.

Binding characteristics of human anti-PSMA specific antibodies were also studied by solid phase ELISA using plasma membrane fractions derived from both LNCaP and PC3 cells. Membrane fractions were serially diluted in 96-well plates and air dried. The plates were blocked with 5% BSA and treated with 5 μg/ml antibody in PBS for one hour prior to detection using standard ELISA procedures.

The results presented in Figure 2 show results for HuMAbs 4A3, 7F12, 8A11, 8C12, and 16F9 demonstrate high specificity for LNCaP cell membranes over a range of antigen concentrations. Little or no antibody binding above background was observed to PC3 cell membranes. Bispecific molecule 14A8 x 8C12 was also tested for the ability to bind to PSMA-expressing LNCaP cells and CD89-expressing U937 cells using similar assays. The bispecific molecule binds both LNCaP and U937 cells in a dose-dependent fashion.

The ELISA results with native PSMA and bacterially expressed PSMA fusion protein fragments show that all of the HuMAbs, except 11C10, are specific for PSMA when present in a native conformation. To confirm this observation, antibody binding to native and heat denatured PSMA was tested to determine the importance of protein conformation on binding specificity. Figure 3 shows results from a solid phase ELISA with native and heat denatured PSMA. The murine anti-PSMA monoclonal antibody 7E11, specific for an epitope composed of the first six amino acids from the N-terminal of the protein, was used as a positive control. The results indicate that heat denaturation has no impact on 7E11 binding, consistent with its recognition of a linear sequence epitope. In contrast to these results, antibodies 4A3, 7F12, 8A11,

8C12, and 16F9 all strongly bound to native purified PSMA. However, heat denaturation of PSMA virtually abolished antibody binding indicating a native protein conformation is required for antibody binding. Consistent with this result, antibodies 4A3, 7F12, 8A11, 8C12, and 16F9 were ineffective in detecting PSMA in a Western blot analysis (results not shown).

Accordingly, HuMAbs 4A3, 7F12, 8A11, 8C12, and 16F9 do not recognize linear amino acid sequence epitopes but instead bind to protein conformational epitopes, *i.e.*, native protein epitopes resulting from conformational folding of the PSMA molecules which arise when amino acids from differing portions of the linear sequence come together in close proximity in 3-dimensional space. Such conformational epitopes are distributed on the extracellular side of the plasma membrane.

Immunoprecipitation of PSMA from LNCaP Cells: The binding specificity of antibodies 4A3, 7F12, 8A11, 8C12, and 16F9 was studied by immunoprecipitation of protein derived from a 1% NP-40 detergent lysate of LNCaP cells. The lysate was treated with antibody followed by addition of Protein G-Sepharose beads. The beads were washed extensively and the bound immune complex subjected to SDS gel electrophoresis and Western blotting with the murine linear PSMA sequence epitope specific antibody 4D8. The results are shown in Figure 4. Lane 1 shows Western blot reactivity of PSMA and PSM' (an alternate splice variant missing the first 57 amino acids from the N-terminal) present in LNCaP cell lysate. Lane 2 shows results from immunoprecipitation with an isotype matched (IgG_1) irrelevant human antibody. Lanes 3 through 7 show results from immunoprecipitation with antibodies 4A3, 7F12, 8A11, 8C12, and 16F9, respectively. In each case, intense bands corresponding to both PSMA and PSM' are observed indicating these antibodies bind to protein epitopes present within the extracellular domain of the protein.

HuMAb binding to PSMA expressed on live LNCaP cells: Antibody binding to viable and non-viable (fixed) LNCaP cells was studied by flow cytometry using irrelevant human IgG_1 antibody as a control. Viable cells are a propidium iodide negative cell population. Fixed cells were treated with 1% paraformaldehyde in PBS prior to primary antibody treatment. Strong binding of antibodies 4A3, 7F12, 8A11, 8C12, and 16F9 was observed to both live and fixed LNCaP cells. Negative staining

was observed with PC3 cells or when an isotype matched irrelevant human antibody was used with LNCaP cells.

By comparison, antibody binding results with murine linear epitope-specific antibodies using the same preparation of viable and fixed cells and irrelevant murine antibody control demonstrated significantly lower binding to live cells. Binding to fixed cells was higher, however, no linear epitope antibody was comparable under any condition to the binding observed with conformational HuMAbs found using irrelevant human IgG_1 antibody as a control. No binding of either murine or human conformational or linear epitope antibodies was detected in experiments using PSMA negative PC3 cells (results not shown).

Accordingly, HuMAbs exhibit strong antibody binding to live LNCaP cells and bind to an epitope related to that bound by the murine conformational antibody 3C6.

FACS analysis of antibody binding competition: Antibody binding competition studies were conducted to address whether each antibody bound to a similar or distinct epitope on PSMA. In these experiments, LNCaP cells were first treated with either irrelevant human IgG_1, or PSMA-specific HuMAbs, extensively washed, and labeled with FITC-labeled individual HuMAbs prior to analysis by flow cytometry. The ability of unlabeled HuMAbs to block binding of FITC-labeled HuMAb was tested. Strong binding of FITC-labeled antibody was found in each case with cells pre-treated with irrelevant human IgG_1. In contrast, pretreatment with anti-PSMA specific HuMAbs gave rise to substantial inhibition of FITC-labeled antibody binding in each case. Taken together, the data shows the competitive binding behavior of 7F12 and 16F9 with the other HuMAbs are most similar. Slight variations in extents of binding inhibition are seen with differing antibody pairings. For example, 8C12 effectively inhibits 4A3, 8A11, and 8C12 binding but has much less of an effect on 7F12. Overall, slightly different, but closely distributed conformational epitopes are recognized by these antibodies.

HuMAb binding competition with murine PSMA conformational antibodies: Murine PSMA-specific antibodies designated 1G9, 3C6, and 4D4 were developed which are also directed toward protein conformational epitopes. Antibody competition studies, as measured by flow cytometry, were conducted with antibodies 1G9, 3C6, and 4D4 to determine if the HuMAbs recognize epitopes in common with the

murine antibodies. The ability of unlabeled HuMAbs to block binding of FITC-labeled 1G9, 3C6, and 4D4 was tested. Pretreatment of LNCaP cells with HuMAbs, followed by labeling with FITC-murine 4D4 and 1G9, indicated similar results with little or no apparent inhibition. In contrast, significant inhibition of FITC-3C6 was observed by HuMAbs 4A3, 7F12, 8A11, 8C12, and 16F9 indicating each binds to a similar or closely distributed epitope as recognized by 3C6.

Binding affinity of conformational HuMAbs to PSMA on LNCaP cells: PSMA HuMAbs are highly sensitive to the native conformation of PSMA. Numerous experiments to determine affinity constants using purified PSMA failed to provide reliable or reproducible results. Experiments were conducted to obtain some affinity binding information from native PSMA as expressed in viable LNCaP cells. To test the binding affinity of each antibody to native PSMA, a flow cytometric assay was used in which the primary antibody concentration was varied for a fixed number of LNCaP cells (1×10^6) with excess FITC-labeled secondary antibody. The data in Table 4 shows results expressed as the antibody concentration required to give half maximal shift in cell labeling intensity. These results demonstrate that the highest binding affinities were found with antibodies 4A3, 7F12, and 16F9. Antibody 8C12 had about a 3-fold lower binding affinity followed by antibody 8A11 with a binding affinity approximately 20-fold lower than the high affinity antibodies.

TABLE 4

Antibody	**Antibody Required for ½ Maximal Shift (μ/ml)**
7F12	7
4A3	9
16F9	10
8C12	28
8A11	195

Example 5 ADCC and CDC activity of Human anti-PSMA Antibodies and Bispecifics

I. Antibody dependent cell-mediated cytotoxicity (ADCC) activity of human anti-PSMA antibodies: The ability of anti-PSMA HuMAbs to mediate antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) was tested for each conformational antibody described in the examples above in experiments using PBMC's from two donors. The results shown in Figure 5A and B indicate strong ADCC for each HuMAb. Each HuMAb has similar titer and has reactivity similar to Herceptin as a positive control (Figure 5B). No CDC activity was observed for any HuMAb (data not shown),

II. Antibody dependent cell-mediated cytotoxicity (ADCC) activity of human anti-PSMA bispecific antibodies: Bispecific molecule 14A8 x 8C12 (shown in Figure 6) and the monoclonal antibody 8C12 were tested for polymorphonuclear cell-mediated ADCC killing of labeled PSMA-expressing tumor cells.

In particular, mononuclear cells (monocytes and neutrophils), as well as whole blood, were isolated from healthy donors and incubated with ^{51}Cr labeled PSMA expressing tumor cells in the presence of bispecific molecule 14A8 x 8C12. After approximately 4 hours, the culture supernatant from the wells was harvested and ^{51}Cr release measured on a gamma counter. The percent specific lysis was determined according the following formula: (experimental CPM – target leak CPM)/(detergent lysis CPM – target leak CPM) × 100%. The results, shown in Figures 7A, 8A and 9A, demonstrate that 14A8 x 8C12 mediates dose dependent lysis of tumor cells by monocytes and neutrophils and whole blood, respectively, as compared to a control antibody.

Mononuclear cells and whole blood were also incubated with ^{51}Cr labeled LNCaP tumor cells in the presence of either bispecific molecule 14A8 x 8C12 or monoclonal antibody 8C12 (Figures 7B, 7C, 8B and 9B). LNCaP cells were labeled with 100 μCi of ^{51}Cr for 1 hour at 37°C (5% CO_2) prior to incubation with mononuclear cells and whole blood, along with various concentrations of bispecific or monoclonal antibody. After an incubation of 16 hours, the supernatant was harvested and analyzed for radioactivity as described above.

Monocyte Induced ADCC: As shown in Figure 7A, bispecific molecule 14A8 × 8C12 mediated cell killing of tumor cells expressing PSMA by monocytes in a dose dependent fashion. Addition of 50 μg/ml of 14A8 Fab'_2 completely blocked ADCC of the tumor cells by 1 μg/ml of the bispecific molecule 14A8 × 8C12, demonstrating that targeted cell killing was mediated exclusively by CD89 on the effector cells. As shown in Figure 7B, bispecific molecule 14A8 x 8C12 and monoclonal antibody 8C12 also mediated dose dependent lysis by monocytes of LNCaP tumor cells. Moreover, the addition of excess 14A8 $F(ab)'_2$ completely inhibited ADCC of the tumor cells by bispecific molecule 14A8 x 8C12 as compared to H22 $F(ab)'_2$ (humanized anti-FcγRI), indicating that targeted cell killing was mediated through CD89 (see Figure 7C).

Neutrophil Induced ADCC: As shown in Figure 8A, bispecific molecule 14A8 × 8C12 mediated cell killing of tumor cells expressing PSMA by neutrophils in a dose dependent fashion. Addition of 25 μg/ml of 14A8 Fab'_2 significantly blocked ADCC of the tumor cells by the bispecific molecule, demonstrating that targeted cell killing was mediated specifically by CD89 binding to the effector cells. As shown in Figure 8B, bispecific molecule 14A8 x 8C12 also mediated dose dependent lysis by neutrophils of LNCaP tumor cells. The addition of excess 14A8 $F(ab)'_2$ completely inhibited ADCC of the tumor cells by 14A8 x 8C12 as compared to H22 $F(ab)'_2$ (humanized anti-FcγRI), indicating that targeted cell killing was mediated through CD89.

Whole Blood Induced ADCC: As shown in Figure 9A, bispecific molecule 14A8 × 8C12 mediated cell killing of tumor cells expressing PSMA by whole-blood in a dose dependent fashion. Addition of 25 μg/ml of 14A8 Fab'_2 significantly blocked ADCC of the tumor cells by the bispecific molecule, again demonstrating that targeted cell killing was mediated specifically by CD89 binding to the effector cells. Similarly, as shown in Figure 9B, bispecific molecule 14A8 x 8C12 also mediated dose dependent lysis by whole blood of LNCaP tumor cells. The addition of excess 14A8 $F(ab)'_2$ completely inhibited ADCC of the tumor cells by 14A8 x 8C12 as compared to H22 $F(ab)'_2$ (humanized anti-FcγRI), indicating that targeted cell killing was mediated through CD89.

III. Human anti-PSMA antibodies and bispecific antibodies mediate phagocytosis and killing of tumor cells expressing PSMA in the presence of human effector cells: Bispecific molecule 14A8 x 8C12 and the monoclonal antibody 8C12 were tested for their ability to mediate phagocytosis of labeled PSMA-expressing tumor cells (LNCaP cells) alone, as well as in the presence of excess 14A8 (human anti-FcαR) Fab'_2 antibody or excess H22 (humanized anti-FcγRI) Fab'_2 antibody as a control.

Briefly, bispecific-mediated phagocytosis of LNCaP cells by monocyte derived macrophages (MDM) was examined by a modification of the method described Munn *et al.* (1990) *J. Exp. Med.* 172:231-237. Monocytes, purified from normal adult source leukopacs (ABI), were differentiated in 24-well plates (1 x 10^6/ml) in macrophage serum free medium (Gibco, Grand Island, NY) supplemented with 10% FBS and 10ng/ml of M-CSF for 7-12 days. LNCaP cells were labeled with the lipophilic red fluorescent dye, PKH 26 (Sigma, St. Louis, MO). The labeled LNCaP cells were added to the wells containing MDM in the absence or presence of bispecific antibody (or control antibody) and incubated at 37^0 C for 5-24 hours (5% CO_2). MDM and non-phagocytized LNCaP cells were recovered with trypsin, and stained with a FITC-labeled anti-CD33 mAb (251) and an anti-CD14 mAb (AML-2-23) for 1 hour on ice (4°C). Cells were washed and analyzed by two color fluorescence using the FACScan. Percent phagocytosis was calculated as the number of dual-positive target cells (ingested by MDM) divided by the total number of target cells x 100%.

As shown in Figure 10, bispecific molecule 14A8 x 8C12 mediated increasing specific phagocytosis of tumor cells in a dose-dependent fashion. Addition of 14A8 Fab'_2 significantly blocked phagocytosis of the tumor cells by the bispecific molecule, again demonstrating that targeted phagocytosis was mediated specifically by CD89 binding to the effector cells. Similarly, as shown in Figure 11, bispecific molecule 14A8 x 8C12 and monoclonal antibody 8C12 also mediated phagocytosis of LNCaP cells in a dose dependent fashion. Figure 12 shows that 14A8 x 8C12 mediated phagocytosis of LNCaP tumor cells was mediated through CD89, as it was inhibited by the addition of excess 14A8 $F(ab)'_2$, as compared to H22 $F(ab)'_2$ (humanized anti-FcγRI) (see inset, Figure 12).

The foregoing Examples demonstrate the generation of human monoclonal antibodies and bispecifics that specifically react with high affinity to PSMA. These antibodies and bispecifics recognize native conformational protein

epitopes present in the extracellular domain of the molecule, rather than epitopes defined by a linear amino acid sequence. In addition, the human anti-PSMA antibodies and bispecific molecules thereof mediate cell killing and phagocytosis in the presence of effector cells against human tumor cells expressing high levels of PSMA.

Example 6 Biodistribution of ^{125}I-labeled Human anti-PSMA Antibodies to LNCaP Cell Tumors in Nude Mice

Biodistribution of ^{125}I-labled HuMAb in nude mice bearing LNCaP cell tumors was tested by following time-dependent uptake of labeled antibody into normal and tumor tissues. Results were obtained for two HuMAbs, 4A3 and 7F12. Antibody 7F12 was used initially based upon binding affinity studies and availability of adequate antibody protein. Subsequent experiments, however, demonstrated that iodination of 7F12 virtually abolished the ability of this antibody to bind antigen. Thus, useful data was obtained only for 4A3. The results for 4A3 are shown in Figure 13 and indicate that the labeled antibody is initially predominantly in blood and highly vascularized tissues. This diminishes as tumor uptake occurs and tumor labeling is highest compared to normal tissues (2-fold or greater labeling compared to normal tissues) after 24 hours. Thus, significant biodistribution to tumor tissue occurs. The extent of tumor labeling with time after uptake may be, in part, a function of diminished levels of circulating antibody and antibody internalization of bound antibody with the resultant release from the cell of labeled antibody protein fragments.

Example 7 Analysis of Internalization of ^{125}I-labeled Human anti-PSMA Antibodies by LNCaP Cells

Internalization of ^{125}I-labeled HuMAb protein was monitored by analyzing the time-dependent release into the culture supernatant of TCA soluble ^{125}I counts. LNCaP cells, surface labeled with iodinated antibody, were incubated at 37°C and the culture supernate removed, TCA precipitated, and the amount of ^{125}I label present in the supernatant fraction determined at the times shown in Figure 14. The results indicate that iodinated antibodies 4A3, 16F9, and 8A11 effectively labeled LNCaP cells at zero time and were efficiently internalized into the cells, degraded, and

protein fragments released into the culture supernatant. Approximately 50% of the originally bound antibody was recovered in a TCA soluble fraction after an 18 hour incubation period with each of these antibodies.

Differing cell binding and internalization results were obtained for iodinated HuMAbs 7F12 and 8C12. In particular, significantly lower total LNCaP cell labeling was found using iodinated 7F12 and 8C12 antibodies suggesting iodination may have an impact on the ability of the labeled antibodies to bind antigen. To test this hypothesis, a solid phase binding assay was conducted using immobilized native purified LNCaP cell PSMA and iodinated HuMAb. The results shown in Figure 15 confirm binding of the positive control iodinated 4A3 antibody to PSMA and also demonstrate that iodination abolished the ability of both 7F12 and 8C12 to bind antigen. Thus, internalization rates for antibodies 7F12 and 8C12 cannot be assessed using ^{125}I-labeled 7F12 and 8C12 antibodies.

Example 8 Effect of DOTA Labeling of HuMAbs 4A3 and 7F12 on Binding to PSMA

Antibodies were DOTA-labeled and the effect on antibody binding to antigen tested by ELISA. DOTA (tetraazacyclododecanetetraacidic acid) is a common chelator which can be used for complexing radionuclides. The results in Figure 16 demonstrate that DOTA-labeled antibodies retain high antigen binding capability indicating these antibodies will be useful in forming radiometal chelates.

Example 9 Tissue Reactivity of Human anti-PSMA Antibodies binding to Normal and Malignant Human Tissues by Immunohistochemistry

Immunohistochemical analysis of the binding of five HuMAbs specific for conformational epitopes present in PSMA was applied to a cross reactivity screen with frozen sections of normal and malignant human tissues. The results from a single specimen of each tissue type demonstrate strong binding to prostatic epithelium and tumor vascular endothelium of non-prostatic malignancies or of normal tissues. No staining of vascular endothelium within prostatic cancer was observed. Other weaker reactivity was observed including varying reactivity of glandular epithelium from non-

neoplastic tissues, potential cross reactivity in brain tissues, and staining of lymphocytes of the jejunum and Kuppfer cells in the liver. There is some question concerning non-prostatic staining results given that some of these "normal" tissues were obtained from the same donor that showed strong tumor vascular staining from adjacent or nearby tumors. Lymphocyte staining may be due to antigen uptake from the primary tumor. Other tissues and elements were negative with these antibodies. No significant differences were observed in tissue reactivity *via* IHC between the five HuMAbs tested.

Example 10 Binding Affinity

Binding affinity of anti-PSMA HuMAbs was determined using flow cytometry with LNCaP cells wherein the HuMAb was diluted out over a series of tubes of cells. The amount of antibody bound was detected using an FITC-labeled secondary antibody present in saturating amounts. The data, analyzed as the amount of antibody protein required for ½ maximal shift (the shift seen with saturating HuMAb), was as follows:

TABLE 5

Antibody	Antibody Required for ½ Maximal Shift (μ/ml)
7F12	7
4A3	9
16F9	10
8C12	28
8A11	195

Further affinity studies on HuMAbs 4A3 and 7F12 utilized radiolabeled antibody (^{111}In-DOTA-labeled antibody) and binding to a fixed number of LNCaP cells. Scatchard analysis was performed with the resulting antibody binding data. The results for both antibodies (4A3 and 7F12) demonstrated similar affinity constants with a K_D= 0.5 ± 0.1 nM or 10^{-10} M).

Example 11 V region cloning

PolyA$^+$ mRNA and first strand cDNA were prepared from the anti-PSMA hybridomas utilizing mRNA isolation and cDNA synthesis kits (Invitrogen, Carlsbad, CA). The 4A3, 7F12 and 8C12 V regions were amplified by polymerase chain reaction (PCR) utilizing a panel of 5' primers that correspond to human V_H and V_L (or V_K) signal sequences. The 8A11 and 16F9 V regions were amplified using primers that bind to the 5' end of the mature V region sequences in framework 1. The 3' V_H and V_K PCR primers contained the following sequences, respectively: TGCCAGGGGGAAGACCGATGG (SEQ ID NO: 57) and CGGGAAGATGAAGACAGATG (SEQ ID NO: 58). The PCR, cloning and sequencing were performed in duplicate in order to monitor for potential PCR-introduced changes in the sequences.

Conclusion

The foregoing examples demonstrate the production of five fully human monoclonal antibodies specific for conformational epitopes on human PSMA, as well as therapeutic bispecific agents containing the antibodies. All five antibodies had high antigen specificity and reactivity with native, but not denatured, PSMA. The antibodies are efficiently internalized into PSMA expressing cells, have strong antibody-dependent cell mediated cytotoxicity (ADCC) activity, bio-distribute to PSMA expressing tumors in animal models and have similar performance in immuno-histochemical studies of human tissues, supporting their therapeutic and diagnostic utility in human treatment.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Incorporation by Reference

All patents, pending patent applications and other publications cited herein are hereby incorporated by reference in their entirety.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. An isolated human monoclonal antibody that binds to human PSMA, wherein the antibody comprises a human heavy chain variable region derived from the human heavy chain VH_{5-51} germline sequence (SEQ ID NO:54).

2. The isolated human monoclonal antibody of claim 1, wherein the human heavy chain variable region CDR3 sequence is selected from the group consisting of SEQ ID NOs: 23, 26, 29, 32, and 35, and conservative modifications thereof.

3. The isolated human monoclonal antibody of claim 1 or 2, wherein the human heavy chain variable region CDR2 sequence is selected from the group consisting of SEQ ID NOs: 22, 25, 28, 31, and 34, and conservative modifications thereof.

4. The isolated human monoclonal antibody of any one of claims 1-3, wherein the human heavy chain variable region CDR1 sequence is selected from the group consisting of SEQ ID NOs: 21, 24, 27, 30, and 33, and conservative modifications thereof.

5. The isolated human monoclonal antibody of any one of claims 1-4, further comprising a human light chain variable region derived from the human light chain L6 (SEQ ID NO: 55) or 04/014 (SEQ ID NO: 56) germline sequence.

6. The isolated human monoclonal antibody of claim 5, wherein the human light chain variable region CDR3 sequence is selected from the group consisting of SEQ ID NOs: 38, 41, 44, 47, and 50, and conservative modifications thereof.

7. The isolated human antibody of claim 5 or 6, wherein the human light chain variable region CDR2 sequence is selected from the group consisting of SEQ ID NOs: 37, 40, 43, 46, and 49, and conservative modifications thereof.

8. The isolated human antibody of any one of claims 5-7, wherein the human light chain variable region CDR1 sequence is selected from the group consisting of SEQ ID NOs: 36, 39, 42, 45, and 48, and conservative modifications thereof.

9. The isolated human monoclonal antibody of any one of the preceding claims, comprising a human heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 11, 12, 13, 14, 15, and sequences that are at least 80% homologous to SEQ ID NOs: 11, 12, 13, 14, and 15.

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10. The isolated human monoclonal antibody of any one of the preceding claims, comprising a human light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 17, 18, 19, 20, and sequences that are at least 80% homologous to SEQ ID NOs: 16, 17, 18, 19, and 20.

11. The isolated human monoclonal antibody of any one of the preceding claims, wherein the human antibody binds to human PSMA with a K_D of 10^{-8} M or less.

12. The isolated human antibody of any one of the preceding claims, which binds to human PSMA with a K_D of 10^{-9} M or less.

13. The isolated human monoclonal antibody of any one of the preceding claims, wherein the human antibody mediates lysis of PSMA+ tumor cells in an antibody dependent cellular cytotoxicity (ADCC) assay.

14. The isolated human antibody of any one of the preceding claims, wherein the antibody binds to human PSMA with a K_D of 10^{-9}M or less.

15. The isolated human monoclonal antibody of claim 1, comprising human heavy chain and human light chain variable regions comprising the amino acid sequences shown in SEQ ID NO: 11 and SEQ ID NO: 16, respectively.

16. The isolated human monoclonal antibody of claim 1, comprising human heavy chain and human light chain variable regions comprising the amino acid sequences shown in SEQ ID NO: 12 and SEQ ID NO: 17, respectively.

17. The isolated human monoclonal antibody of claim 1, comprising human heavy chain and human light chain variable regions comprising the amino acid sequences shown in SEQ ID NO: 13 and SEQ ID NO: 18, respectively.

18. The isolated human monoclonal antibody of claim 1, comprising human heavy chain and human light chain variable regions comprising the amino acid sequences shown in SEQ ID NO: 14 and SEQ ID NO: 19, respectively.

19. The isolated human monoclonal antibody of claim 1, comprising human heavy chain and human light chain variable regions comprising the amino acid sequences shown in SEQ ID NO: 15 and SEQ ID NO: 20, respectively.

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20. The isolated human antibody of any one of the preceding claims produced by a hybridoma, wherein the hybridoma is prepared from a B cell obtained from a transgenic non-human animal having a genome comprising a human heavy chain transgene or transchromosome and a human light chain transgene or transchromosome, fused to an immortalized cell.

21. The human antibody of any one of the preceding claims which binds to a tumor cell selected from the group consisting of bladder, breast, colon, kidney, ovarian, prostate, renal cell, squamous cell, lung (non-small cell), and head and neck tumor cells.

22. The human antibody of any one of the preceding claims, comprising a human IgG heavy chain and a human kappa light chain.

23. The human antibody of any one of the preceding claims, comprising an IgG1 or IgG3 heavy chain.

24. A pharmaceutical composition comprising the human antibody of any one of the preceding claims and a pharmaceutically acceptable carrier.

25. An immunoconjugate comprising the human antibody according to any one of claims 1 to 23 linked to a therapeutic agent.

26. The immunoconjugate of claim 25 wherein the therapeutic agent is a cytotoxin.

27. The immunoconjugate of claim 25, wherein the therapeutic agent is a radioisotope.

28. A pharmaceutical composition comprising the immunoconjugate of any one of claims 25-27 and a pharmaceutically acceptable carrier.

29. An isolated nucleic acid molecule encoding the human antibody of any one of claims 1 to 23.

30. The isolated nucleic acid molecule of claim 29, wherein the nucleic acid molecule is incorporated into an expression vector.

31. A transfectoma comprising the isolated nucleic acid of claim 29 or 30.

32. A transgenic nonhuman animal which expresses the human antibody of any one of claims 1 to 23, wherein the transgenic non-human animal has a genome comprising a human heavy chain transgene or transchromosome and a human light chain transgene or transchromosome.

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33. Use of an antibody according to any one of claims 1 to 23 in the manufacture of a medicament for inhibiting growth of a cell expressing PSMA, comprising contacting the cell with an effective amount of the antibody, such that the growth of the cell is inhibited.

34. Use of the human antibody of any one of claims 1 to 23 in the manufacture of a medicament for treating or preventing a disease characterized by growth of tumor cells expressing PSMA.

35. The use of claim 34, wherein the disease is cancer.

36. The use of claim 35, wherein the cancer is selected from the group consisting of prostate cancer, colon cancer, and renal carcinoma.

37. The use of claim 36, wherein the cancer is prostate cancer.

38. The use of claim 33, wherein the human antibody is conjugated to a therapeutic agent.

39. The use of claim 38, wherein the therapeutic agent is a cytotoxin.

40. The use of claim 38, wherein the therapeutic agent is a radioisotope.

41. A method for inhibiting growth of a cell expressing PSMA, comprising contacting the cell with an effective amount of an antibody according to any one of claims 1 to 23, the pharmaceutical composition according to claim 24 or the immunoconjugate according to claim 25.

42. A method of treating or preventing a disease characterized by growth of tumor cells expressing PSMA, comprising administering to a subject the human antibody of any one of claims 1 to 23, the pharmaceutical composition according to claim 24 or the immunoconjugate according to claim 25.

43. An isolated human monoclonal antibody that binds to human PSMA, substantially as herein described with reference to any one or more of the examples but excluding comparative examples.

44. A pharmaceutical composition, an immunoconjugate, an isolated nucleic acid molecule, a transfectoma, or a transgenic nonhuman animal, substantially as herein described with reference to any one or more of the examples but excluding comparative examples.

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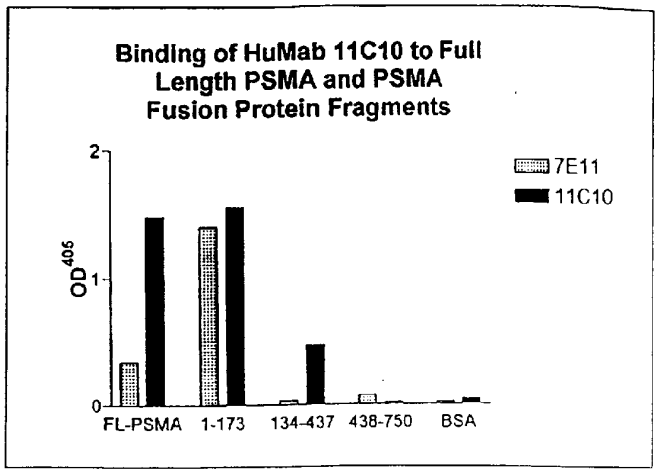
45. Use of an antibody or human antibody in the manufacture of a medicament; a method for inhibiting growth of a cell expressing PSMA; or a method of treating or preventing a disease characterized by growth of a tumor cells expressing PSMA, substantially as herein described with reference to any one or more of the examples but excluding comparative examples.

Figure 1


Binding of HuMab 11C10 to Full Length PSMA and PSMA Fusion Protein Fragments
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2
1
0
7E11
11C10
FL-PSMA
1-173
134-437
438-750
BSA

Figure 2

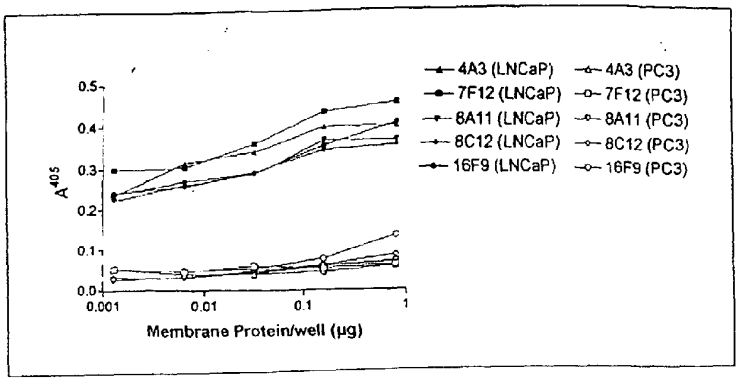

4A3 (LNCaP)
7F12 (LNCaP)
8A11 (LNCaP)
8C12 (LNCaP)
16F9 (LNCaP)
4A3 (PC3)
7F12 (PC3)
8A11 (PC3)
8C12 (PC3)
16F9 (PC3)
A405
0.5
0.4
0.3
0.2
0.1
0.0
0.001
0.01
0.1
1
Membrane Protein/well (µg)

Figure 3

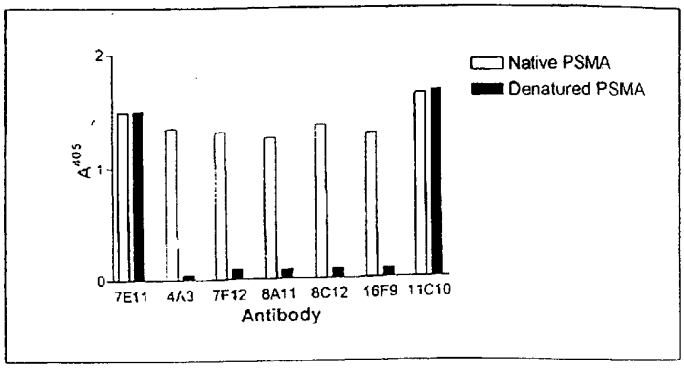
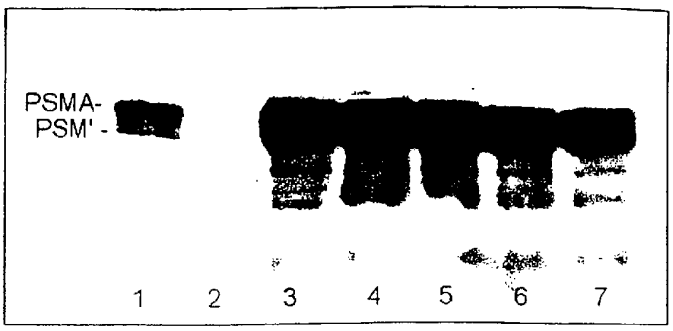

Native PSMA
Denatured PSMA
A405
2
1
0
7E11
4A3
7F12
8A11
8C12
16F9
11C10
Antibody

Figure 4



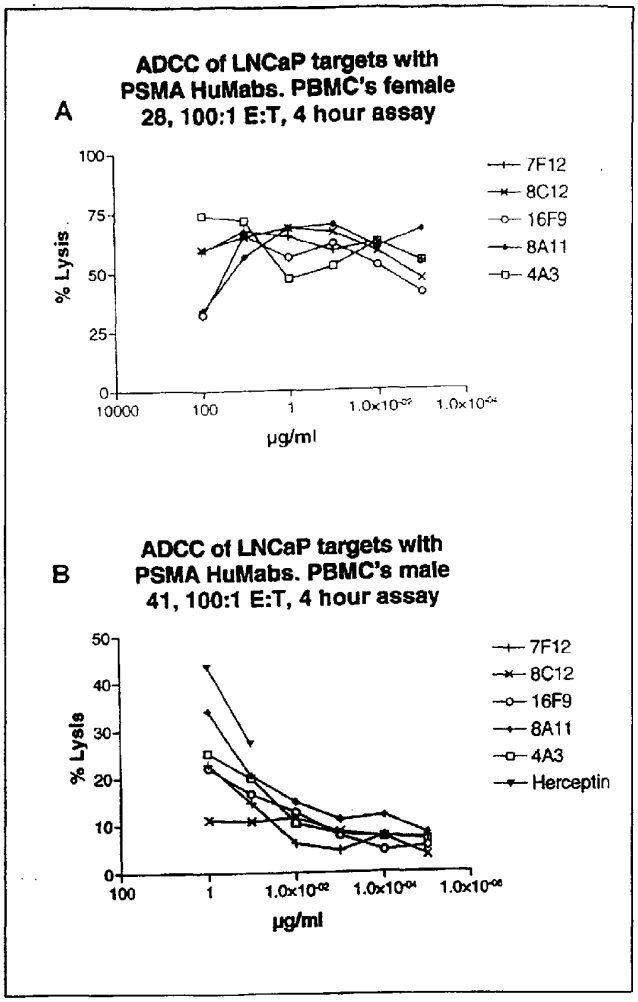

A
ADCC of LNCaP targets with PSMA HuMabs. PBMC's female 28, 100:1 E:T, 4 hour assay
% Lysis
100
75
50
25
0
10000
100
1
1.0x10-02
1.0x10-04
µg/ml
7F12
8C12
16F9
8A11
4A3
B
ADCC of LNCaP targets with PSMA HuMabs. PBMC's male 41, 100:1 E:T, 4 hour assay
% Lysis
50
40
30
20
10
0
100
1
1.0x10-02
1.0x10-04
1.0x10-06
µg/ml
7F12
8C12
16F9
8A11
4A3
Herceptin

Figure 5

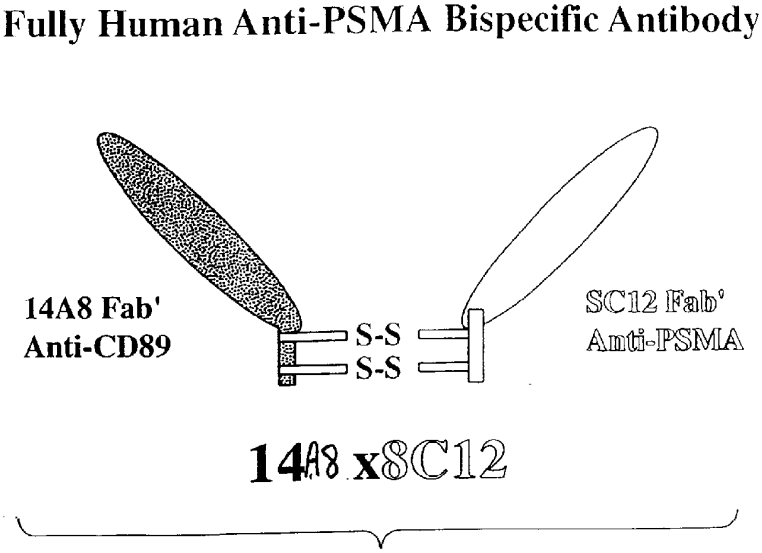

Fully Human Anti-PSMA Bispecific Antibody
14A8 Fab'
Anti-CD89
S-S
S-S
SC12 Fab'
Anti-PSMA
14A8 x8C12

Figure 6

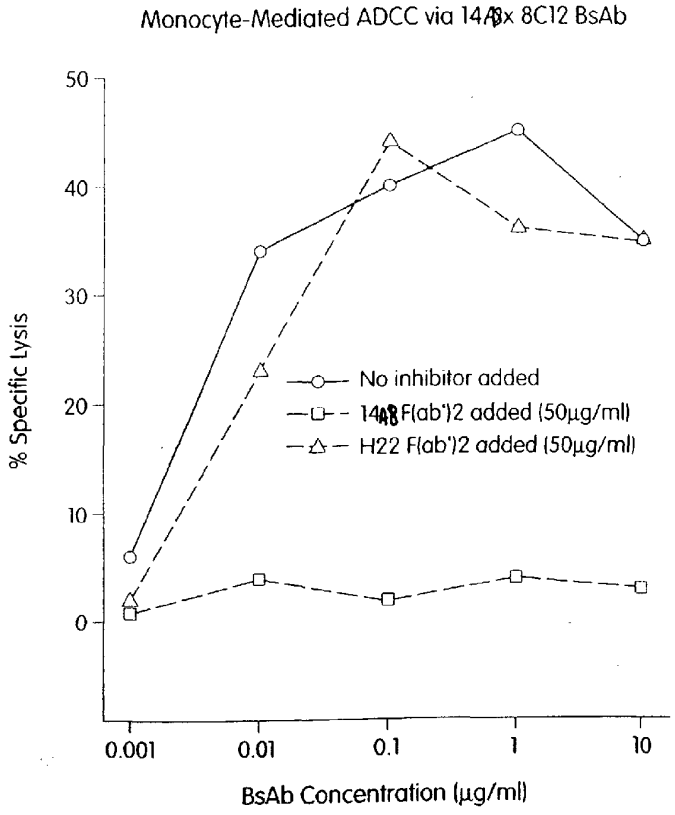

Monocyte-Mediated ADCC via 14.8x 8C12 BsAb
% Specific Lysis
50
40
30
20
10
0
0.001
0.01
0.1
1
10
BsAb Concentration (µg/ml)
No inhibitor added
14.8 F(ab')2 added (50µg/ml)
H22 F(ab')2 added (50µg/ml)

Figure 7A

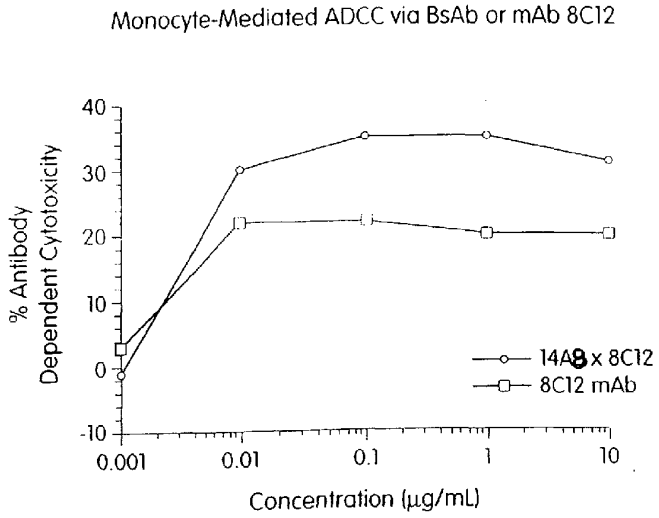

Monocyte-Mediated ADCC via BsAb or mAb 8C12
% Antibody Dependent Cytotoxicity
40
30
20
10
0
-10
0.001
0.01
0.1
1
10
Concentration (µg/mL)
14A8 x 8C12
8C12 mAb

Figure 7B

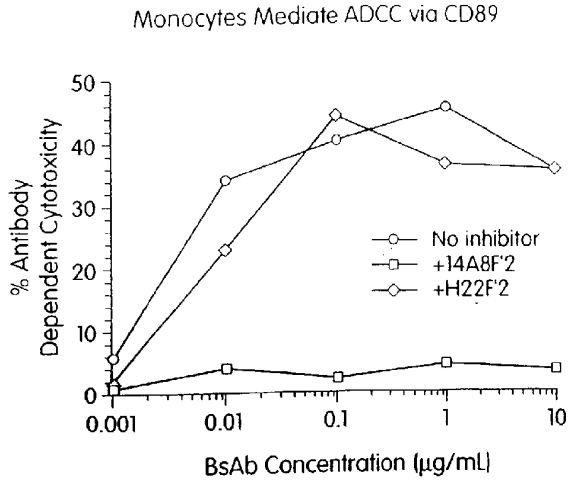

Monocytes Mediate ADCC via CD89
% Antibody Dependent Cytotoxicity
50
40
30
20
10
0
0.001
0.01
0.1
1
10
BsAb Concentration (µg/mL)
No inhibitor
+14A8F'2
+H22F'2

Figure 7C

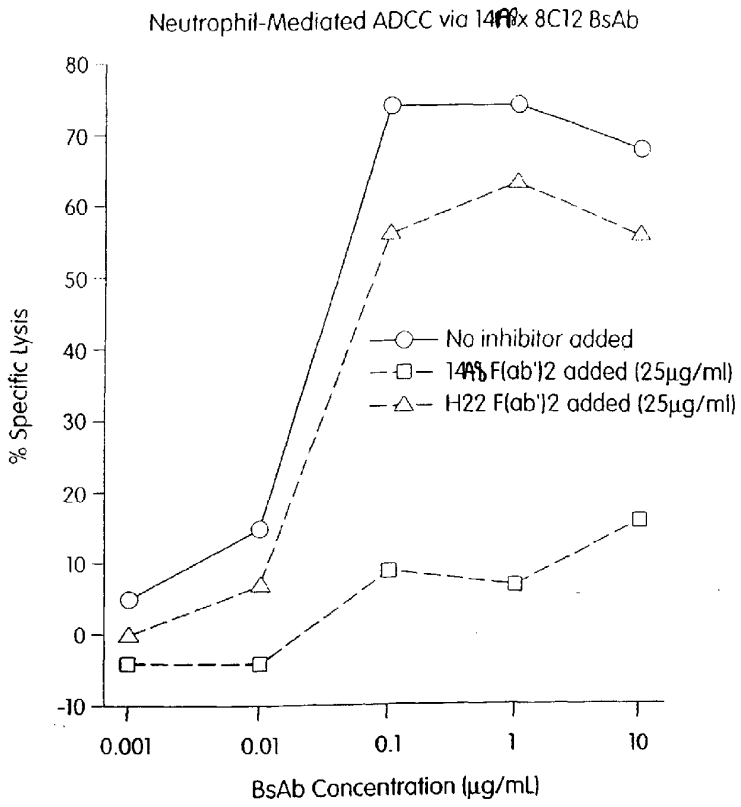

Neutrophil-Mediated ADCC via 14[illegible]x 8C12 BsAb
80
70
60
50
40
30
20
10
0
-10
% Specific Lysis
No inhibitor added
14[illegible] F(ab')2 added (25µg/ml)
H22 F(ab')2 added (25µg/ml)
0.001
0.01
0.1
1
10
BsAb Concentration (µg/mL)

Figure 8A

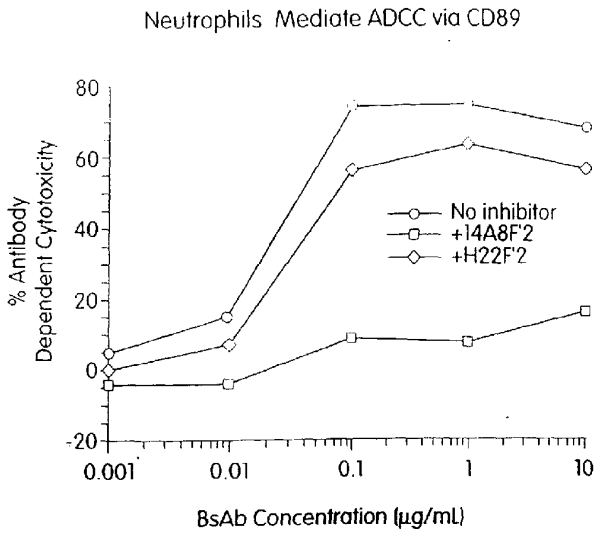

Neutrophils Mediate ADCC via CD89
% Antibody Dependent Cytotoxicity
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
BsAb Concentration (µg/mL)
No inhibitor
+14A8F'2
+H22F'2

Figure 8B

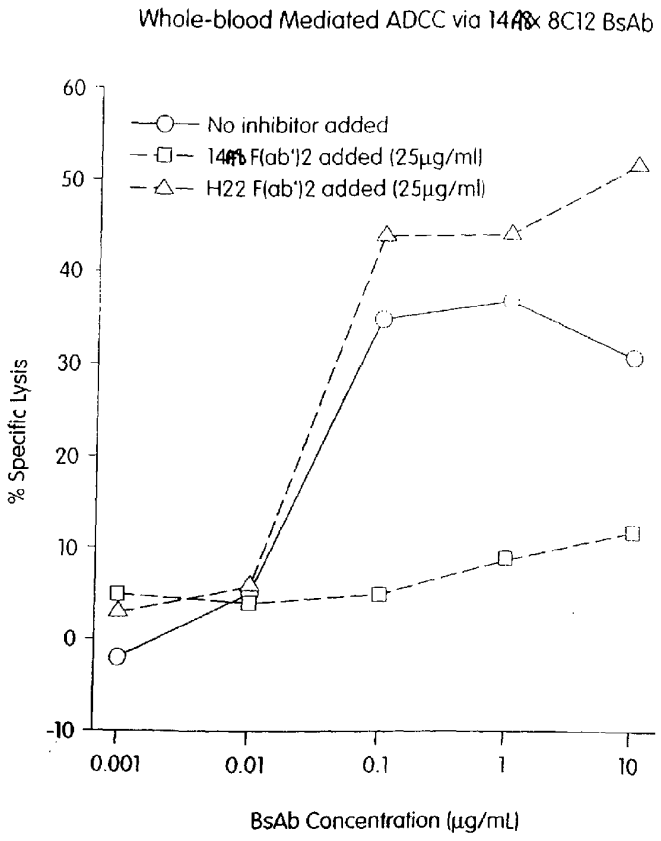

Whole-blood Mediated ADCC via 14.8x 8C12 BsAb
No inhibitor added
14.8 F(ab')2 added (25µg/ml)
H22 F(ab')2 added (25µg/ml)
% Specific Lysis
60
50
40
30
20
10
0
-10
0.001
0.01
0.1
1
10
BsAb Concentration (µg/mL)

Figure 9A

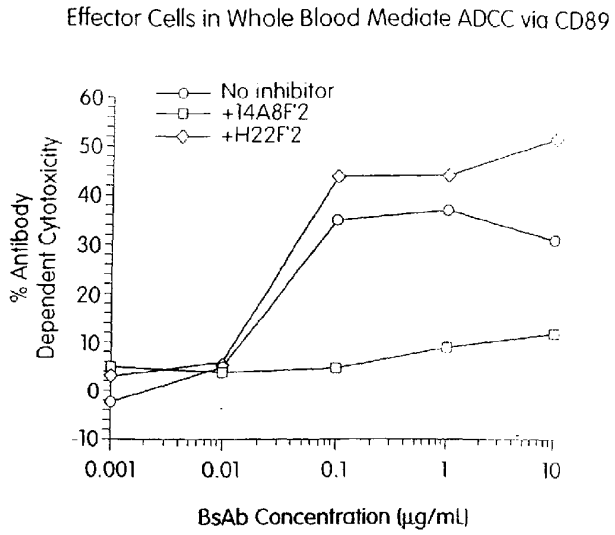

Effector Cells in Whole Blood Mediate ADCC via CD89
No inhibitor
+14A8F'2
+H22F'2
% Antibody Dependent Cytotoxicity
60
50
40
30
20
10
0
-10
0.001
0.01
0.1
1
10
BsAb Concentration (µg/mL)

Figure 9B

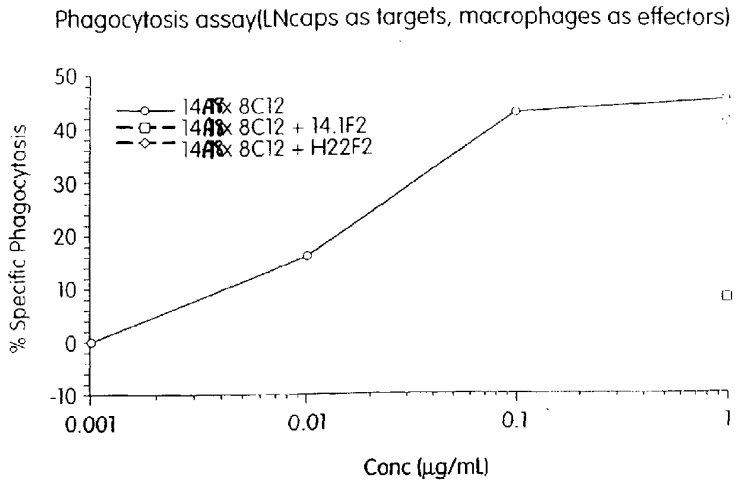

Phagocytosis assay(LNcaps as targets, macrophages as effectors)
% Specific Phagocytosis
50
40
30
20
10
0
-10
0.001
0.01
0.1
1
Conc (µg/mL)
14A1 x 8C12
14A1 x 8C12 + 14.1F2
14A1 x 8C12 + H22F2

Figure 10

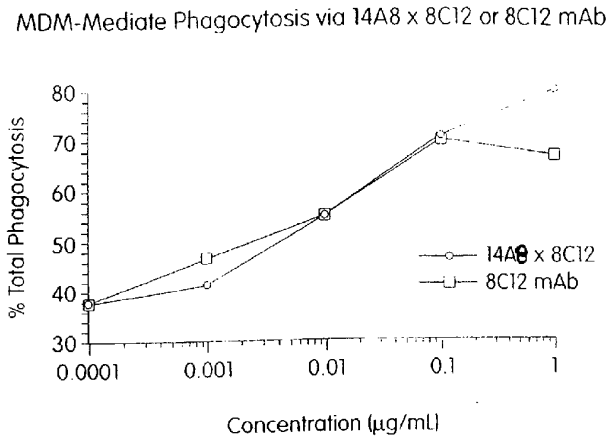

MDM-Mediate Phagocytosis via 14A8 x 8C12 or 8C12 mAb
% Total Phagocytosis
80
70
60
50
40
30
0.0001
0.001
0.01
0.1
1
Concentration (µg/mL)
14A8 x 8C12
8C12 mAb

Figure 11

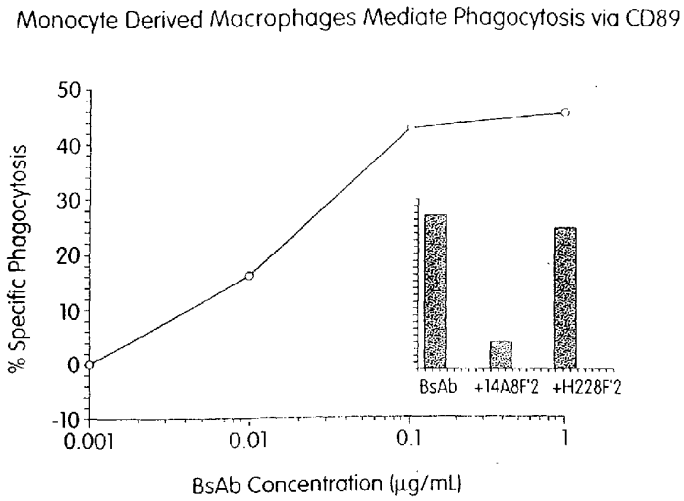

Monocyte Derived Macrophages Mediate Phagocytosis via CD89
% Specific Phagocytosis
50
40
30
20
10
0
-10
0.001
0.01
0.1
1
BsAb
+14A8F'2
+H228F'2
BsAb Concentration (µg/mL)

Figure 12

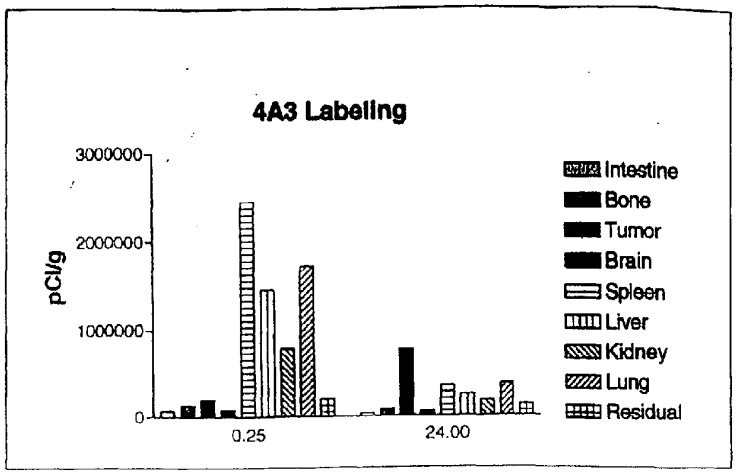

4A3 Labeling
3000000
2000000
1000000
0
pCi/g
0.25
24.00
Intestine
Bone
Tumor
Brain
Spleen
Liver
Kidney
Lung
Residual

Figure 13

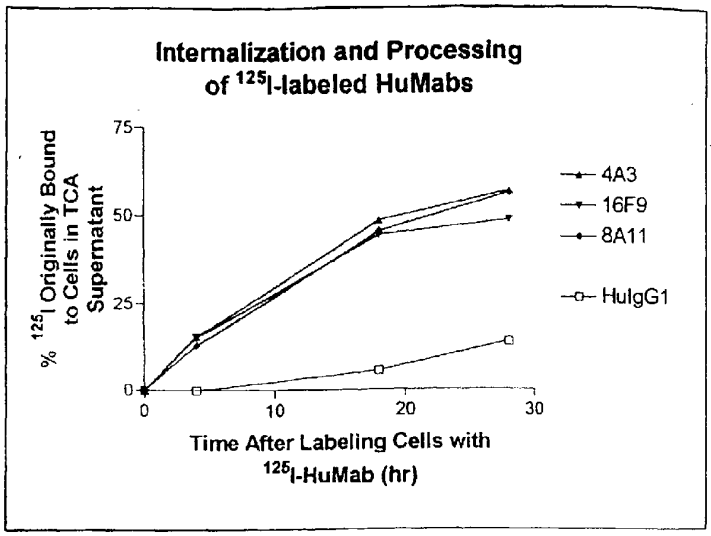

Internalization and Processing
of 125I-labeled HuMabs
% 125I Originally Bound to Cells in TCA Supernatant
75
50
25
0
0
10
20
30
Time After Labeling Cells with 125I-HuMab (hr)
4A3
16F9
8A11
HuIgG1

Figure 14

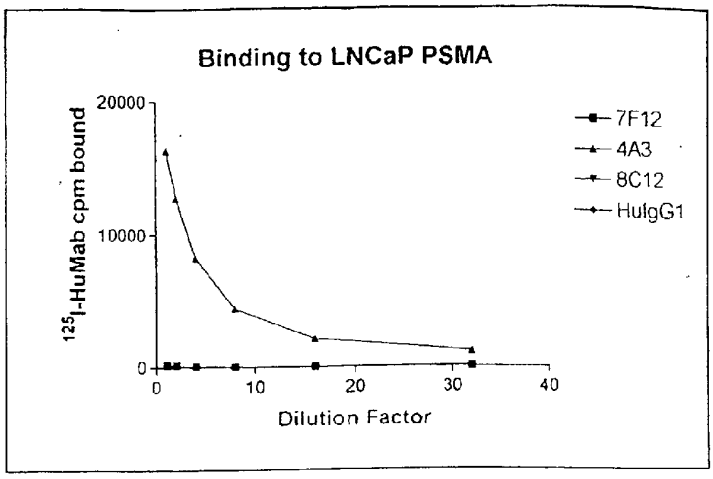

Binding to LNCaP PSMA
^{125}I-HuMab cpm bound
20000
10000
0
0
10
20
30
40
Dilution Factor
7F12
4A3
8C12
HulgG1

Figure 15

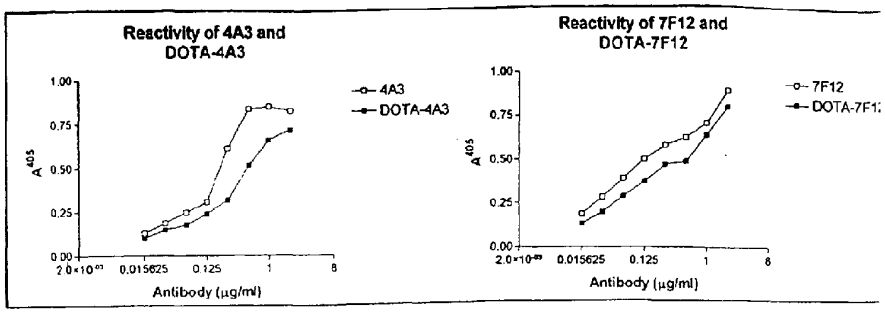

Reactivity of 4A3 and DOTA-4A3
4A3
DOTA-4A3
A405
1.00
0.75
0.50
0.25
0.00
0.015625
0.125
1
8
Antibody (µg/ml)
Reactivity of 7F12 and DOTA-7F12
7F12
DOTA-7F1
A405
1.00
0.75
0.50
0.25
0.00
0.015625
0.125
1
8
Antibody (µg/ml)

Figure 16

Fig. 17A

4A3 VH
GAGGTGCAGTTGGTGCAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTGAAGAT
CTCCTGTAAGGGTTCTGGATACAGTTTTACCAGCTACTGGATCGGCTGGGCGCGCCAGA
TGCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGA
TACAGCCCGTCCTTCCAAGGCCAGGTCACCATCTCAGCCGACAAGTCCATCAGCACCGC
CTACCTGCAGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTTCGGCCG
CTAATTCTTCTCACTGGTACTTCGATCTCTGGGGCCGTGGCACCCTGGTCACTGTCTCC
TCA

4A3 VK
GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCAC
CCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCTACTTAGCCTGGTTCCAACAGAAAC
CTGGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCA
GCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGA
GCCTGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCAACTGGCTCATGTACACTT
TTGGCCAGGGGACCAAGCTGGAGATCAAAC

7F12 VH
GCGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTGAAGAT
CTCCTGTAAGGGTTCTGGATATAGTTTTACCAGCTTCTGGATCGGCTGGGCGCGCCAGA
TGCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGA
TACAGCCCGTCCTTCCAAGGCCAGGTCACCATCTCAGCCGACAAGTCCATCAGCACCGC
CTACCTGCAGTGGAGTAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGACCG
CTAACTCCTCTTTCTGGAATTTCGATCTCTGGGGCCGTGGCACCCTGGTCACTGTCTCC
TCA

7F12 VK
GAAATTGTGTTGACaCAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCAC
CCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCTACTTAGCCTGGTACCAACAGAAAC
CTGGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCA
GCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGA
GCCTGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCAACTGGCTCATGTACACTT
TTGGCCAGGGGACCAAGCTGGAGATCAAAC

8C12 VH
GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAACGCCCGGGGAGTCTCTGAAGAT
CTCCTGTAAGGGCTCTGGATACACCTTTACCAGCTACTGGATCGGCTGGGTGCGCCAGA
TGCCCGGGAAAGGCCCGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGA
TACAGCCCGTCCTTCCAAGGCCAGGTCACCTTCTCAGCCGACAAGTCCATCAGCACCGC
CTACCTGCAGTGGAACAGCCTGAAGACCTCGGACACCGCCATGTATTACTGTGCGACCG
CTAACCCCTCTTATTGGTATTTCGATCTCTGGGGCCGTGGCACCCTGGTCACTGTCTCC
TCA

Figure 17B

8C12 VK
GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGAAAGAGCC
ACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCTACTTAGCCTGGTACCAACAG
AAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAGGGCCACTGGC
ATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGC
AGCCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAGCAGCGTAGCGACTGGCTC
ATGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAAC

8A11 VH
AGTCTGGAGCAGAGGTGAAAACGCCCGGGGAGTCTCTGAAGATCTCCTGTAAGGGCT
CTGGATACACCTTTACCAACTACTGGATCGGCTGGGTGCGCCAGATGCCCGGGAAAG
GCCCGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGATACAGCCCGT
CCTTCCAAGGCCAGGTCACCTTCTCAGCCGACAAGTCCATCAGCACCGCCTACCTGC
AGTGGAGCAGCCTGAAGACCTCGGACACCGCCATGTATTACTGTGCGACCGCTAACC
CCTCTTATTGGTATTTCGATCTCTGGGGCCGTGGCACCCTGGTCACTGTCTCCTCA

8A11 VK
TCCATCCTCCCTGTCTGCATCTGTAGGAGACAGCGTCACCATCACTTGCCGGGTGAG
TCAGGGCATTAGCAGTTATTTAAATTGGTATCGGCAGAAACCAGGGAAAGTTCCTAA
GCTCCTGATCTATAGTGCATCCAATTTGCAACCTGGAGTCCCATCTCGGTTCAGTGG
CAGTGGATCTGGGACAGATTTCACTCTCACTATCAACAGCCTGCAGCCTGAAGATGT
TGCAACTTATTACGGTCAACGGACTTACAATGCCCCATTCACTTTCGGCCCTGGGAC
CAAAGTGGATATCAAAC

16F9 VH
AGTCTGGAGCAGAACTGAAAAAGCCCGGGGAGTCTCTGAAGATCTCCTGTAAGGGTT
CTGGATACAGTTTTACCAACTACTGGATCGGCTGGGCGCGCCAGATGCCCGGGAAAG
GCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCAGATACAGTCCGT
CCTTCCAAGGCCAGGTCACCATCTCTGCCGACAAGTCCGTCAGCACCGCCTACCTGC
AGTGGAACAGTCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGACCGCTAACT
CCTCTTTCTGGAACTTCGATCTCTGGGGCCGTGGCACCCTGGTCACTGTCTCCTCA

16F9 VK
TCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGTGAG
TCAGGGCATTAGCAGTTATTTAAATTGGTATCGGCAGAAACCAGGGAAAGTTCCTAA
GCTCCTGATGTATAGTGCATCCAATTTGCAATCTGGAGTCCCATCTCGGTTCAGTGG
CAGTGGATCTGGGACAGATTTCACTCTCACTATCAGCAGCCTGCAGCCTGAAGATGT
TGCAACTTATTACGGTCAACGGACTTACAATGCCCCATTCACTTTCGGCCCTGGGAC
CAAAGTGGATATCAAAC

Figure 18

Anti-PSMA VH Regions

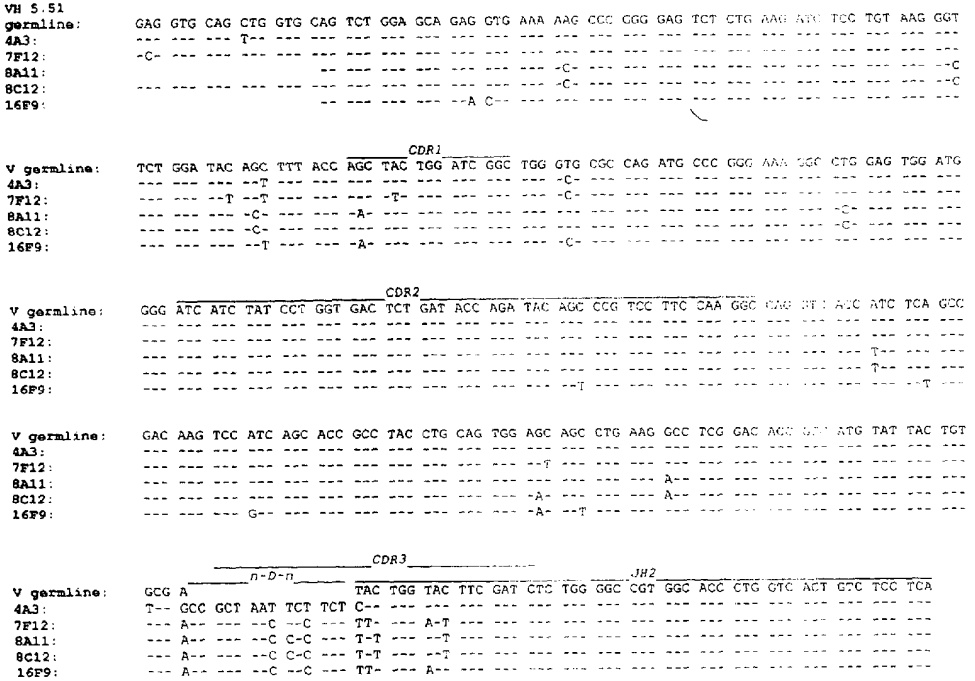


Fig. 19

Anti-PSMA VH Regions

```
                                                                    CDR1
5-51 germline  E V Q L V Q S G A E V K K P G E S L K I S C K G S G Y S F T  S Y W I G
4A3            - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  - - - - -
7F12           A - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  - F - - -
8C12           - - - - - - - - - - - - T - - - - - - - - - - - - - - T - -  - - - - -
8A11                       - - - - - - T - - - - - - - - - - - - - - T - -  N - - - -
16F9                       - - - - L - - - - - - - - - - - - - - - - - - -  N - - - -

                                                         CDR2
5-51 germline  W V R Q M P G K G L E W M G  I I Y P G D S D T R Y S P S F Q G  Q V T I
4A3            - A - - - - - - - - - - - -  - - - - - - - - - - - - - - - - -  - - - -
7F12           - A - - - - - - - - - - - -  - - - - - - - - - - - - - - - - -  - - - -
8C12           - - - - - - - - - P - - - -  - - - - - - - - - - - - - - - - -  - - - F
8A11           - - - - - - - - - P - - - -  - - - - - - - - - - - - - - - - -  - - - F
16F9           - A - - - - - - - - - - - -  - - - - - - - - - - - - - - - - -  - - - -

                                                                   CDR3
5-51 germline  S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R
4A3            - - - - - - - - - - - - - - - - - - - - - - - - - - S A  A N S S H W Y F D L
7F12           - - - - - - - - - - - - - - - - - - - - - - - - - - - T  - - - - F - N - - -
8C12           - - - - - - - - - - - - - N - - - T - - - - - - - - - T  - - P - Y - - - - -
8A11           - - - - - - - - - - - - - - - - - T - - - - - - - - - T  - - P - Y - - - - -
16F9           - - - - - V - - - - - - - N - - - - - - - - - - - - - T  - - - - F - N - - -

4A3            W G R G T L V T V S S
7F12           - - - - - - - - - - -
8C12           - - - - - - - - - - -
8A11           - - - - - - - - - - -
16F9           - - - - - - - - - - -
```

Figure 20

Anti-PSMA VK Regions

```
L6 germline: GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA GCC ACC CTC
4A3:         --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
7F12:        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
8C12:        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                  CDR1
L6 germline: TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC CAA CAG AAA CCT GGC CAG
4A3:         --- --- --- --- --- --- --- --- --- --- --- --- --- --- -T- --- --- --- --- --- ---
7F12:        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
8C12:        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                                  CDR2
L6 germline: GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG GCC ACT GGC ATC CCA GCC AGG TTC AGT
4A3:         --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
7F12:        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
8C12:        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

L6 germline: GGC AGT GGG TCT GGG ACA GAC TTC ACT CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA
4A3:         --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
7F12:        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
8C12:        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                      CDR3                                  JK2
L6 germline: GTT TAT TAC TGT CAG CAG CGT AGC AAC TGG C                      ACT TTT GGC CAG GGG ACC AAG CTG
4A3:         --- --- --- --- --- --- --- --- --- --- -TC ATG TAC ACT TTT GGC CAG GGG ACC AAG CTG
7F12:        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
8C12:        --- --- --- --- --- --- --- --- --- G-- --- --- --- --- --- --- --- --- --- --- ---

4A3:         GAG ATC AAA C
7F12:        --- --- --- -
8C12:        --- --- --- -
```

Figure 21

Anti-PSMA VK Regions

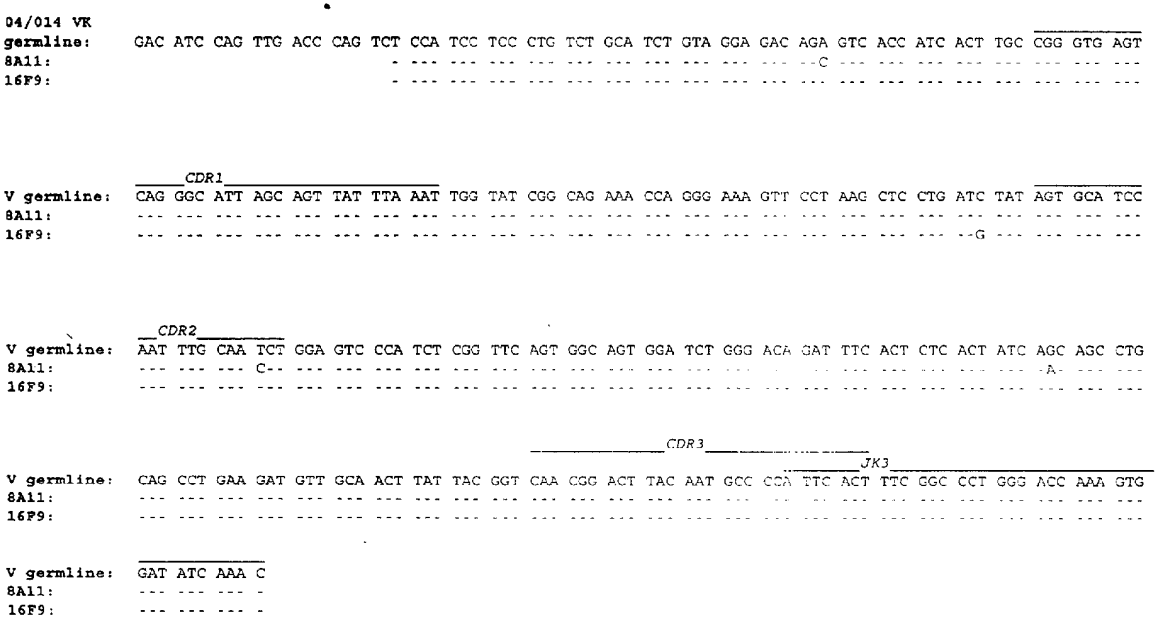


Figure 22

Anti-PSMA VK Regions

```
                                                                      CDR1
L6 germline:  E I V L T Q S P A T L S L S P G E R A T L S C   R A S Q S V S S Y L A
4A3:          - - - - - - - - - - - - - - - - - - - - - - -   - - - - - - - - - - -
7F12:         - - - - - - - - - - - - - - - - - - - - - - -   - - - - - - - - - - -
8C12:         - - - - - - - - - - - - - - - - - - - - - - -   - - - - - - - - - - -

                                                CDR2
L6 germline:  W Y Q Q K P G Q A P R L L I Y   D A S N R A T   G I P A R F S G S G S
4A3:          - F - - - - - - - - - - - - -   - - - - - - -   - - - - - - - - - - -
7F12:         - - - - - - - - - - - - - - -   - - - - - - -   - - - - - - - - - - -
8C12:         - - - - - - - - - - - - - - -   - - - - - - -   - - - - - - - - - - -

L6 germline:  G T D F T L T I S S L E P E D F A V Y Y C
4A3:          - - - - - - - - - - - - - - - - - - - - -
7F12:         - - - - - - - - - - - - - - - - - - - - -
8C12:         - - - - - - - - - - - - - - - - - - - - -

                    CDR3
L6 germline:  Q Q R S N W
4A3:          - - - - - - L M Y T   F G Q G T K L E I K
7F12:         - - - - - - - - - -   - - - - - - - - - -
8C12:         - - - - D - - - - -   - - - - - - - - - -
```

Fig. 23

Anti-PSMA VK Regions

```
                                                                    CDR1
VK 04/014
germline:   D I Q L T Q S P S S L S A S V G D R V T I T C   R V S Q G I S S Y L N
8A11:                     - - - - - - - - - - S - - - - -   - - - - - - - - - - -
16F9:                     - - - - - - - - - - - - - - - -   - - - - - - - - - - -

                                                    CDR2
014 germline: W Y R Q K P G K V P K L L I Y   S A S N L Q S   G V P S R F S G S G S
8A11:         - - - - - - - - - - - - - - -   - - - - - - -   - - - - - - - - - - -
16F9:         - - - - - - - - - - - - - M -   - - - - - - -   - - - - - - - - - - -

                                                               CDR3
014 germline: G T D F T L T I S S L Q P E D V A T Y Y G   Q R T Y N A P F T
8A11:         - - - - - - - - N - - - - - - - - - - - -   - - - - - - - - - F G P G
16F9:         - - - - - - - - - - - - - - - - - - - - -   - - - - - - - - - - - - -

8A11:              T K V D I K
16F9:              - - - - - -
```

SEQUENCE LISTING

<110> Medarex, Inc. et al.

<120> HUMAN MONOCLONAL ANTIBODIES TO PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA)

<130> MXI-163CPPC

<140> PCT/US2003/002448
<141> 2003-01-28

<150> 10/059989
<151> 2002-01-28

<160> 60

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 357
<212> DNA
<213> Homo sapiens

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tcctgtaagg gttctggata cagttttacc agctactgga tcggctgggc gcgccagatg 120
cccgggaaag gcctggagtg gatggggatc atctatcctg gtgactctga taccagatac 180
agcccgtcct tccaaggcca ggtcaccatc tcagccgaca agtccatcag caccgcctac 240
ctgcagtgga gcagcctgaa ggcctcggac accgccatgt attactgttc ggccgctaat 300
tcttctcact ggtacttcga tctctggggc cgtggcaccc tggtcactgt ctcctca 357

<210> 2
<211> 325
<212> DNA
<213> Homo sapiens

<400> 2
gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccagggga aagagccacc 60
ctctcctgca gggccagtca gagtgttagc agctacttag cctggttcca acagaaacct 120
ggccaggctc ccaggctcct catctatgat gcatccaaca gggccactgg catcccagcc 180
aggttcagtg gcagtgggtc tgggacagac ttcactctca ccatcagcag cctagagcct 240
gaagattttg cagtttatta ctgtcagcag cgtagcaact ggctcatgta cacttttggc 300
caggggacca agctggagat caaac 325

<210> 3
<211> 357
<212> DNA
<213> Homo sapiens

<400> 3
gcggtgcagc tggtgcagtc tggagcagag gtgaaaaagc ccggggagtc tctgaagatc 60
tcctgtaagg gttctggata tagttttacc agcttctgga tcggctgggc gcgccagatg 120
cccgggaaag gcctggagtg gatggggatc atctatcctg gtgactctga taccagatac 180
agcccgtcct tccaaggcca ggtcaccatc tcagccgaca agtccatcag caccgcctac 240

ctgcagtgga gtagcctgaa ggcctcggac accgccatgt attactgtgc gaccgctaac 300
tcctctttct ggaatttcga tctctggggc cgtggcaccc tggtcactgt ctcctca 357

<210> 4
<211> 325
<212> DNA
<213> Homo sapiens

<400> 4
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ctctcctgca gggccagtca gagtgttagc agctacttag cctggtacca acagaaacct 120
ggccaggctc ccaggctcct catctatgat gcatccaaca gggccactgg catcccagcc 180
aggttcagtg gcagtgggtc tgggacagac ttcactctca ccatcagcag cctagagcct 240
gaagattttg cagtttatta ctgtcagcag cgtagcaact ggctcatgta cacttttggc 300
caggggacca agctggagat caaac 325

<210> 5
<211> 341
<212> DNA
<213> Homo sapiens

<400> 5
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gatacacctt taccaactac tggatcggct gggtgcgcca gatgcccggg aaaggcccgg 120
agtggatggg gatcatctat cctggtgact ctgataccag atacagcccg tccttccaag 180
gccaggtcac cttctcagcc gacaagtcca tcagcaccgc ctacctgcag tggagcagcc 240
tgaagacctc ggacaccgcc atgtattact gtgcgaccgc taacccctct tattggtatt 300
tcgatctctg gggccgtggc accctggtca ctgtctcctc a 341

<210> 6
<211> 302
<212> DNA
<213> Homo sapiens

<400> 6
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gatctatagt gcatccaatt tgcaacctgg agtcccatct cggttcagtg gcagtggatc 180
tgggacagat ttcactctca ctatcaacag cctgcagcct gaagatgttg caacttatta 240
cggtcaacgg acttacaatg ccccattcac tttcggccct gggaccaaag tggatatcaa 300
ac 302

<210> 7
<211> 357
<212> DNA
<213> Homo sapiens

<400> 7
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tcctgtaagg gctctggata caccttacc agctactgga tcggctgggt gcgccagatg 120
cccgggaaag gcccggagtg gatggggatc atctatcctg gtgactctga taccagatac 180
agcccgtcct tccaaggcca ggtcaccttc tcagccgaca agtccatcag caccgcctac 240
ctgcagtgga acagcctgaa gacctcggac accgccatgt attactgtgc gaccgctaac 300
ccctcttatt ggtatttcga tctctggggc cgtggcaccc tggtcactgt ctcctca 357

<210> 8
<211> 325

<212> DNA
<213> Homo sapiens

<400> 8
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ggccaggctc ccaggctcct catctatgat gcatccaaca gggccactgg catcccagcc 180
aggttcagtg gcagtgggtc tgggacagac ttcactctca ccatcagcag cctagagcct 240
gaagattttg cagtttatta ctgtcagcag cgtagcgact ggctcatgta cacttttggc 300
caggggacca agctggagat caaac 325

<210> 9
<211> 341
<212> DNA
<213> Homo sapiens

<400> 9
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gatacagttt taccaactac tggatcggct gggcgcgcca gatgcccggg aaaggcctgg 120
agtggatggg gatcatctat cctggtgact ctgataccag atacagtccg tccttccaag 180
gccaggtcac catctctgcc gacaagtccg tcagcaccgc ctacctgcag tggaacagtc 240
tgaaggcctc ggacaccgcc atgtattact gtgcgaccgc taactcctct ttctggaact 300
tcgatctctg gggccgtggc accctggtca ctgtctcctc a 341

<210> 10
<211> 302
<212> DNA
<213> Homo sapiens

<400> 10
tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc atcacttgcc gggtgagtca 60
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gatgtatagt gcatccaatt tgcaatctgg agtcccatct cggttcagtg gcagtggatc 180
tgggacagat ttcactctca ctatcagcag cctgcagcct gaagatgttg caacttatta 240
cggtcaacgg acttacaatg ccccattcac tttcggccct gggaccaaag tggatatcaa 300
ac 302

<210> 11
<211> 119
<212> PRT
<213> Homo sapiens

<400> 11
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
 1   5   10   15
Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr
            20   25   30
Trp Ile Gly Trp Ala Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35   40   45
Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
    50   55   60
Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65   70   75   80
Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
            85   90   95
Ser Ala Ala Asn Ser Ser His Trp Tyr Phe Asp Leu Trp Gly Arg Gly
            100   105   110

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Thr Leu Val Thr Val Ser Ser
        115


<210> 12
<211> 119
<212> PRT
<213> Homo sapiens

<400> 12
Ala Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
 1               5                  10                  15
Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Phe
            20                  25                  30
Trp Ile Gly Trp Ala Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45
Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
    50                  55                  60
Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80
Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95
Ala Thr Ala Asn Ser Ser Phe Trp Asn Phe Asp Leu Trp Gly Arg Gly
            100                 105                 110
Thr Leu Val Thr Val Ser Ser
        115


<210> 13
<211> 113
<212> PRT
<213> Homo sapiens

<400> 13
Ser Gly Ala Glu Val Lys Thr Pro Gly Glu Ser Leu Lys Ile Ser Cys
 1               5                  10                  15
Lys Gly Ser Gly Tyr Thr Phe Thr Asn Tyr Trp Ile Gly Trp Val Arg
            20                  25                  30
Gln Met Pro Gly Lys Gly Pro Glu Trp Met Gly Ile Ile Tyr Pro Gly
        35                  40                  45
Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln Gly Gln Val Thr Phe
    50                  55                  60
Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu Gln Trp Ser Ser Leu
65                  70                  75                  80
Lys Thr Ser Asp Thr Ala Met Tyr Tyr Cys Ala Thr Ala Asn Pro Ser
                85                  90                  95
Tyr Trp Tyr Phe Asp Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser
            100                 105                 110
Ser


<210> 14
<211> 119
<212> PRT
<213> Homo sapiens
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<400> 14
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Thr Pro Gly Glu
1 5 10 15
Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Pro Glu Trp Met
35 40 45
Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
50 55 60
Gln Gly Gln Val Thr Phe Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80
Leu Gln Trp Asn Ser Leu Lys Thr Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95
Ala Thr Ala Asn Pro Ser Tyr Trp Tyr Phe Asp Leu Trp Gly Arg Gly
100 105 110
Thr Leu Val Thr Val Ser Ser
115

<210> 15
<211> 113
<212> PRT
<213> Homo sapiens

<400> 15
Ser Gly Ala Glu Leu Lys Lys Pro Gly Glu Ser Leu Lys Ile Ser Cys
1 5 10 15
Lys Gly Ser Gly Tyr Ser Phe Thr Asn Tyr Trp Ile Gly Trp Ala Arg
20 25 30
Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly Ile Ile Tyr Pro Gly
35 40 45
Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln Gly Gln Val Thr Ile
50 55 60
Ser Ala Asp Lys Ser Val Ser Thr Ala Tyr Leu Gln Trp Asn Ser Leu
65 70 75 80
Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala Thr Ala Asn Ser Ser
85 90 95
Phe Trp Asn Phe Asp Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser
100 105 110
Ser

<210> 16
<211> 108
<212> PRT
<213> Homo sapiens

<400> 16
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30
Leu Ala Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

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Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Leu Met
                85                  90                  95
Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105


<210> 17
<211> 108
<212> PRT
<213> Homo sapiens

<400> 17
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1               5                  10                  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Leu Met
                85                  90                  95
Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105


<210> 18
<211> 100
<212> PRT
<213> Homo sapiens

<400> 18
Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Ser Val Thr Ile Thr Cys
 1               5                  10                  15
Arg Val Ser Gln Gly Ile Ser Ser Tyr Leu Asn Trp Tyr Arg Gln Lys
            20                  25                  30
Pro Gly Lys Val Pro Lys Leu Leu Ile Tyr Ser Ala Ser Asn Leu Gln
        35                  40                  45
Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
    50                  55                  60
Thr Leu Thr Ile Asn Ser Leu Gln Pro Glu Asp Val Ala Thr Tyr Tyr
65                  70                  75                  80
Gly Gln Arg Thr Tyr Asn Ala Pro Phe Thr Phe Gly Pro Gly Thr Lys
                85                  90                  95
Val Asp Ile Lys
            100


<210> 19
<211> 108
<212> PRT
<213> Homo sapiens
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<400> 19
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1   5   10   15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
            20   25   30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35   40   45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50   55   60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65   70   75   80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asp Trp Leu Met
                85   90   95
Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100   105

<210> 20
<211> 100
<212> PRT
<213> Homo sapiens

<400> 20
Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
 1   5   10   15
Arg Val Ser Gln Gly Ile Ser Ser Tyr Leu Asn Trp Tyr Arg Gln Lys
            20   25   30
Pro Gly Lys Val Pro Lys Leu Leu Met Tyr Ser Ala Ser Asn Leu Gln
        35   40   45
Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
    50   55   60
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Val Ala Thr Tyr Tyr
65   70   75   80
Gly Gln Arg Thr Tyr Asn Ala Pro Phe Thr Phe Gly Pro Gly Thr Lys
                85   90   95
Val Asp Ile Lys
            100

<210> 21
<211> 5
<212> PRT
<213> Homo sapiens

<400> 21
Ser Tyr Trp Ile Gly
 1   5

<210> 22
<211> 17
<212> PRT
<213> Homo sapiens

<400> 22
Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln
 1   5   10   15

Gly

<210> 23
<211> 10
<212> PRT
<213> Homo sapiens

<400> 23
Ala Asn Ser Ser His Trp Tyr Phe Asp Leu
1 5 10

<210> 24
<211> 5
<212> PRT
<213> Homo sapiens

<400> 24
Ser Phe Trp Ile Gly
1 5

<210> 25
<211> 17
<212> PRT
<213> Homo sapiens

<400> 25
Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln
1 5 10 15
Gly

<210> 26
<211> 10
<212> PRT
<213> Homo sapiens

<400> 26
Ala Asn Ser Ser Phe Trp Asn Phe Asp Leu
1 5 10

<210> 27
<211> 5
<212> PRT
<213> Homo sapiens

<400> 27
Asn Tyr Trp Ile Gly
1 5

<210> 28

<211> 17
<212> PRT
<213> Homo sapiens

<400> 28
Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln
 1   5   10   15
Gly

<210> 29
<211> 10
<212> PRT
<213> Homo sapiens

<400> 29
Ala Asn Pro Ser Tyr Trp Tyr Phe Asp Leu
 1   5   10

<210> 30
<211> 5
<212> PRT
<213> Homo sapiens

<400> 30
Ser Tyr Trp Ile Gly
 1   5

<210> 31
<211> 17
<212> PRT
<213> Homo sapiens

<400> 31
Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln
 1   5   10   15
Gly

<210> 32
<211> 10
<212> PRT
<213> Homo sapiens

<400> 32
Ala Asn Pro Ser Tyr Trp Tyr Phe Asp Leu
 1   5   10

<210> 33
<211> 5
<212> PRT
<213> Homo sapiens

<400> 33
Asn Tyr Trp Ile Gly
1 5

<210> 34
<211> 17
<212> PRT
<213> Homo sapiens

<400> 34
Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe Gln
1 5 10 15
Gly

<210> 35
<211> 10
<212> PRT
<213> Homo sapiens

<400> 35
Ala Asn Ser Ser Phe Trp Asn Phe Asp Leu
1 5 10

<210> 36
<211> 11
<212> PRT
<213> Homo sapiens

<400> 36
Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 37
<211> 7
<212> PRT
<213> Homo sapiens

<400> 37
Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 38
<211> 10
<212> PRT
<213> Homo sapiens

<400> 38
Gln Gln Arg Ser Asn Trp Leu Met Tyr Thr
1 5 10

<210> 39
<211> 11
<212> PRT
<213> Homo sapiens

<400> 39
Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 40
<211> 7
<212> PRT
<213> Homo sapiens

<400> 40
Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 41
<211> 10
<212> PRT
<213> Homo sapiens

<400> 41
Gln Gln Arg Ser Asn Trp Leu Met Tyr Thr
1 5 10

<210> 42
<211> 11
<212> PRT
<213> Homo sapiens

<400> 42
Arg Val Ser Gln Gly Ile Ser Ser Tyr Leu Asn
1 5 10

<210> 43
<211> 7
<212> PRT
<213> Homo sapiens

<400> 43
Ser Ala Ser Asn Leu Gln Ser
1 5

<210> 44
<211> 9
<212> PRT
<213> Homo sapiens

<400> 44

Gln Arg Thr Tyr Asn Ala Pro Phe Thr
1 5

<210> 45
<211> 11
<212> PRT
<213> Homo sapiens

<400> 45
Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 46
<211> 7
<212> PRT
<213> Homo sapiens

<400> 46
Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 47
<211> 10
<212> PRT
<213> Homo sapiens

<400> 47
Gln Gln Arg Ser Asp Trp Leu Met Tyr Thr
1 5 10

<210> 48
<211> 11
<212> PRT
<213> Homo sapiens

<400> 48
Arg Val Ser Gln Gly Ile Ser Ser Tyr Leu Asn
1 5 10

<210> 49
<211> 7
<212> PRT
<213> Homo sapiens

<400> 49
Ser Ala Ser Asn Leu Gln Ser
1 5

<210> 50
<211> 9
<212> PRT

<213> Homo sapiens

<400> 50
Gln Arg Thr Tyr Asn Ala Pro Phe Thr
1 5

<210> 51
<211> 98
<212> PRT
<213> Homo sapiens

<400> 51
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15
Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr
20 25 30
Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
50 55 60
Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80
Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95
Ala Arg

<210> 52
<211> 94
<212> PRT
<213> Homo sapiens

<400> 52
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp
85 90

<210> 53
<211> 97
<212> PRT
<213> Homo sapiens

<400> 53
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

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Asp Arg Val Thr Ile Thr Cys Arg Val Ser Gln Gly Ile Ser Ser Tyr
            20                  25                  30
Leu Asn Trp Tyr Arg Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Ser Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Val Ala Thr Tyr Tyr Gly Gln Arg Thr Tyr Asn Ala Pro Phe
                85                  90                  95
Thr
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<210> 54
<211> 343
<212> DNA
<213> Homo sapiens

<400> 54
gaggtgcagc tggtgcagtc tggagcagag gtgaaaaagc ccggggagtc tctgaagatc 60
tcctgtaagg gttctggata cagctttacc agctactgga tcggctgggt gcgccagatg 120
cccgggaaag gcctggagtg gatggggatc atctatcctg gtgactctga taccagatac 180
agcccgtcct tccaaggcca ggtcaccatc tcagccgaca agtccatcag caccgcctac 240
ctgcagtgga gcagcctgaa ggcctcggac accgccatgt attactgtgc gatactggta 300
cttcgatctc tggggccgtg gcaccctggt cactgtctcc tca 343

<210> 55
<211> 283
<212> DNA
<213> Homo sapiens

<400> 55
gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccagggga aagagccacc 60
ctctcctgca gggccagtca gagtgttagc agctacttag cctggtacca acagaaacct 120
ggccaggctc ccaggctcct catctatgat gcatccaaca gggccactgg catcccagcc 180
aggttcagtg gcagtgggtc tgggacagac ttcactctca ccatcagcag cctagagcct 240
gaagattttg cagtttatta ctgtcagcag cgtagcaact ggc 283

<210> 56
<211> 322
<212> DNA
<213> Homo sapiens

<400> 56
gacatccagt tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgcc gggtgagtca gggcattagc agttatttaa attggtatcg gcagaaacca 120
gggaaagttc ctaagctcct gatctatagt gcatccaatt tgcaatctgg agtcccatct 180
cggttcagtg gcagtggatc tgggacagat ttcactctca ctatcagcag cctgcagcct 240
gaagatgttg caacttatta cggtcaacgg acttacaatg ccccattcac tttcggccct 300
gggaccaaag tggatatcaa ac 322

<210> 57
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 57
tgccaggggg aagaccgatg g 21

<210> 58
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 58
cgggaagatg aagacagatg 20

<210> 59
<211> 302
<212> DNA
<213> Homo sapiens

<400> 59
tccatcctcc ctgtctgcat ctgtaggaga cagcgtcacc atcacttgcc gggtgagtca 60
gggcattagc agttatttaa attggtatcg gcagaaacca gggaaagttc ctaagctcct 120
gatctatagt gcatccaatt tgcaacctgg agtcccatct cggttcagtg gcagtggatc 180
tgggacagat ttcactctca ctatcaacag cctgcagcct gaagatgttg caacttatta 240
cggtcaacgg acttacaatg ccccattcac tttcggccct gggaccaaag tggatatcaa 300
ac 302

<210> 60
<211> 302
<212> DNA
<213> Homo sapiens

<400> 60
tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc atcacttgcc gggtgagtca 60
gggcattagc agttatttaa attggtatcg gcagaaacca gggaaagttc ctaagctcct 120
gatgtatagt gcatccaatt tgcaatctgg agtcccatct cggttcagtg gcagtggatc 180
tgggacagat ttcactctca ctatcagcag cctgcagcct gaagatgttg caacttatta 240
cggtcaacgg acttacaatg ccccattcac tttcggccct gggaccaaag tggatatcaa 300
ac 302