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(54) **Title:** HIGH AFFINITY ANTI-PROSTATE STEM CELL ANTIGEN (PSCA) ANTIBODIES FOR CANCER TARGETING AND DETECTION

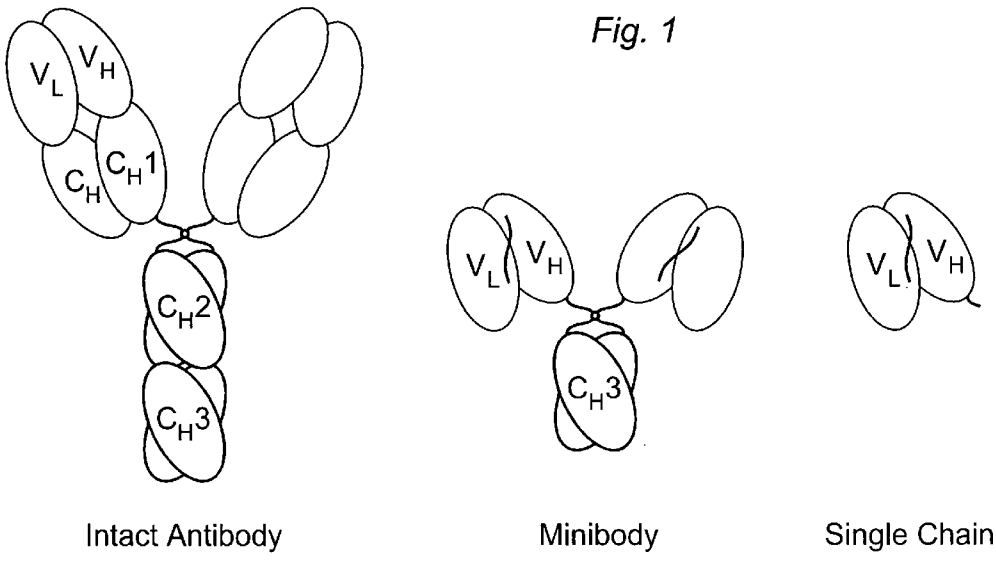

Fig. 1

(57) **Abstract:** The present invention provides novel high affinity antibodies and fragments thereof that bind to the cancer antigen PSCA. The antibodies of the present invention may be used for cancer diagnosis, prognosis, treatment, visualization, and the like. The present invention also provides methods for the detection, visualization, and treatment of various cancers expressing PSCA.

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HIGH AFFINITY ANTI-PROSTATE STEM CELL ANTIGEN (PSCA) ANTIBODIES FOR CANCER TARGETING AND DETECTION

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] NOT APPLICABLE

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with Government support of Grant No. CA092131, awarded by the NIH/NCI. The Government has certain rights in this invention.

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED ON A COMPACT DISK.

[0003] NOT APPLICABLE

BACKGROUND OF THE INVENTION

[0004] Prostate stem cell antigen (PSCA) a cell surface glycoprotein expressed in normal human prostate and bladder is over-expressed in prostate cancers (40% of primary tumors and 60-100% of lymph node and bone marrow metastases). It is also highly expressed in transitional carcinomas of the bladder and pancreatic carcinoma. 1G8, an anti-PSCA mouse monoclonal antibody specific for PSCA demonstrated anti-tumor targeting activity in vivo (Gu Z, et al. Cancer Res. 2005; 65:9495). This antibody was humanized by grafting on a human framework (Trastuzumab) and named 2B3 (Olafsen T, et al. J. Immunotherapy 2007; 30:396).

[0005] The invention addresses the need for agents that have appropriate pharmacodynamic properties to target and image tumors that express PSCA. There is a tremendous need in the field for effective agents to image cancers with sensitivity and specificity, particularly early stage tumors or ones with early metastasis not imageable by traditional means. As PSCA is highly expressed by most prostate, bladder and pancreatic tumors, it is an important target in the detection, diagnosis, prognosis, and treatment of these cancers. The current invention describes an innovative constructs with optimal characteristics for tumor imaging and

targeting. They may also be used for tumor targeting of gene therapy, radioactivity therapy, and may have therapeutic utility by themselves.

BRIEF SUMMARY OF THE INVENTION

[0006] This invention describes engineered antibodies that recognize a novel cell surface marker in prostate and other cancers with high affinity. These genetically engineered antibody fragments are tailored specifically for in vivo use for targeting and detection.

[0007] The invention addresses the need for agents that have appropriate pharmacodynamic properties to target and image tumors that express PSCA. There is a tremendous need in the field for effective agents to image cancers with sensitivity and specificity, particularly early stage tumors or ones with early metastasis not imageable by traditional means. PSCA is highly expressed by most prostate, bladder and pancreatic tumors and is a promising target. The current invention describes an innovative molecule with optimal characteristics for tumor imaging. It may also be useful for tumor targeting of gene therapy, radioactivity or may have therapeutic utility by itself.

[0008] There are multiple embodiments. For instance, there are a variety of engineered antibody formats, such as scFvs, diabodies, triabodies, minibodies, and scFv-Fc. In general, the agent should at least demonstrate bivalent, as opposed to monovalent binding. The overall size, shape, and domain composition of the agent can be varied to suit the final application. Engineered fragments that exhibit optimal targeting in humans may be slightly different from formats that are optimal in mice. Since one goal is human application, the invention incorporates a humanized set of antibody variable regions, as well as human hinge and constant regions. Additional embodiments would include fully human variable regions. The proteins can be expressed in a variety of systems, including microbial, insect cells, mammalian cell culture and transgenic animals.

[0009] For imaging purposes, a variety of radionuclides can be attached to the engineered antibodies for detection with gamma or SPECT cameras, or PET scanners. For therapy one can attach drugs, toxins, cytokines, enzymes, or other therapeutic moieties for PSCA-targeted delivery to tumors. The engineered PSCA-specific antibodies can be coupled to nanosensors for detection (in vitro or in vivo) or nanoparticles for delivery (in vivo). One can also incorporate the PSCA antibody fragments into viral vectors for targeted gene therapy of tumors.

[0010] The invention addresses the unmet need for imaging of cancer, in early diagnosis or diagnosis of metastatic disease. In particular, there is a critical need for better agents for imaging prostate cancer for detection and staging. PSCA antibody fragment imaging will be very useful for imaging bone metastases and assessing response to treatment; there are no good imaging approaches currently available. Detection of pancreatic cancer is a critical need, and an imaging agent would be useful in high-risk patients. The invention describes high-affinity, highly specific engineered antibodies tailored for in vivo targeting and detection of PSCA in prostate cancer, bladder cancer, and pancreatic cancer patients.

[0011] Accordingly, in a first aspect, the invention provides high affinity PSCA antigen binding protein constructs which can be used in the treatment and detection of cancers which overexpress PSCA. In some embodiments, these constructs are minibodies, diabodies, triabodies, ScFv, or ScFv-Fc as described further below. In one embodiment, the invention provides an antigen binding protein construct directed toward a mammalian PSCA protein (e.g., human, murine) selected from the group consisting of a minibody, a diabody, and scFv-Fc wherein the selected construct has V_L and V_H domains that are substantially identical, respectively, to the V_L domain and the V_H domain of an anti-PSCA antibody. For example, the construct can be a minibody in which the V_L and V_H chain variable domains of the anti-PSCA antibody are fused to part of the hinge region of an antibody, an amino acid linker and the C_H3 domain of an immunoglobulin molecule. In other embodiments, the construct is a diabody.

[0012] In embodiments, where the construct is a minibody or diabody or scFV-Fc, the anti-PSCA antibody can be a humanized antibody and the C_H3 domain is from a human immunoglobulin molecule. In preferred embodiments, the anti-PSCA antibody is 2B3 or 1G8. In yet other embodiments, the construct is a minibody having V_H and $V_L$domains substantially identical to an scFv fragment designated herein as A11, A2, or C5. In still further embodiments, the construct has a C_H3 domain is from a human immunoglobulin molecule. For instance, the construct may have a hinge region and C_H3 domain are the human IgG hinge region and human C_H3 domain.

[0013] In some embodiments, the anti-PSCA antibody is a monoclonal antibody designated 1G8 (ATCC No. HB-12612), 2A2 (ATCC No. HB-1203), 2H9 (ATCC No. HB-12614), 3C5 (ATCC No. HB-12616), 3E6 (ATCC No. HB12618), 3G3 (ATCC No. HB-12615), or 4A10 (ATCC No. HB-12617).

[0014] The constructs according to the invention can also be linked to therapeutic agents or detectable markers. In some embodiments, the therapeutic agent is a cytotoxic agent. For instance, the agent can be ricin, ricin A-chain, doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphteria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, arbrin A chain, modeccin A chain, alpha-sarcin, gelonin mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotin, calicheamicin, sapaonaria officinalis inhibitor, maytansinoids, or glucocorticoidricin. In other embodiments, the therapeutic agent is a radioactive isotope. The radioactive isotope can be selected, for instance, from the group consisting of ^{212}Bi, ^{131}I, ^{111}In, 90Y and ^{186}Re. In other embodiments the construct is linked to an anti-cancer pro-drug activating enzyme capable of converting a pro-drug to its active form.

[0015] In another preferred embodiments of the above, the PSCA targeted by the anti-PSCA antibody is human PSCA.

[0016] In another aspect, the invention provides an antigen binding protein construct selected from the group consisting of a minibody, a diabody, scFv and scFv-Fc wherein the selected construct has V_L and V_H domains that are substantially identical to the V_L and V_H domains of 2B3 or an scFv variant designated herein as A11, A2, or C5. In other embodiments, the binding constructs of the present invention may comprise one or more mutations found in the variant antibodies A11, A2, or C5.

[0017] In yet another aspect, the present invention provides an antigen binding protein construct selected from the group consisting of a minibody, a diabody, scFv and scFv-Fc wherein the selected construct comprises CDR regions of an anti-PSCA antibody. In certain embodiments, the anti-PSCA antibody will bind to PSCA with an affinity equal to or greater than the antibody designated 2B3. In other embodiments, the anti-PSCA antibody may be an affinity matured antibody, wherein the affinity matured antibody comprises a higher affinity for PSCA than does the parental antibody.

[0018] In another aspect, the anti-PSCA construct according to the invention is labeled with a detectable marker. The marker can be for instance, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme. Many radionuclides may be used as imaging labels, including without limitation, ^{124}I, ^{86}Y, ^{18}F, ^{94}Tc, and the like. One of skill in the art will know of other radionuclides particularly well suited for use in the present invention.

[0019] In further other embodiments of any of the above, the invention provides a pharmaceutical composition of the constructs according to the invention.

[0020] In another aspect still, the invention provides methods for treating a subject having cancer (e.g, prostate, pancreatic or bladder cancer), or inhibiting the growth of a prostate cancer cell expressing a Prostate Stem Cell Antigen (PSCA) protein comprising contacting the cancer cell (e.g., prostate, bladder, pancreatic cancer cell, with a construct according to the invention in an amount effective to inhibit the growth of the cancer cell. The method can kill the cancer cell. In some embodiments, the construct recognizes and binds the PSCA protein as shown below beginning with leucine at amino acid position 22 and ending with alanine at amino acid position 99. In additional embodiments, the method further comprises administering to a chemotherapeutic drug, radiation therapy. In some embodiments, the subject is also treated with hormone ablation therapy or hormone antagonist therapy.

[0021] The treatments may be given to the patient or subject by intravenously, intraperitoneally, intramuscularly, intratumorally, or intradermally. In some embodiments, the contacting comprises administering the construct directly into a prostate cancer, a bladder cancer, a pancreatic cancer or a metastasis thereof.

[0022] In another aspect, the invention provides methods of detecting a cancerous cell in a subject by contacting the cancer cell with a construct which bears a detectable marker. The methods can be used in screening patients at increased risk of cancer or to monitory response to therapy or to develop a prognosis for the cancer (e.g., prostate, bladder, or pancreatic cancers. The methods are particularly advantageous in detecting metastases of the cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **Figure 1:** Schematic overview of anti-PSCA minibody. A gene encoding the minibody is assembled in the order V_L-linker-V_H-hinge-C_H3, with the hinge and C_H3 domains derived from human IgGl. The protein self assembles into 80-kDa dimers.

[0024] **Figure 2:** Comparison of 2B3 ScFv variant protein sequences. The V_L sequence and part of the linker are in the top frame, the rest of the linker and V_H sequences are in the lower frame. Also indicated are the CDRs: underlined wild type sequence. The mutations are highlighted in different shading. The parental ScFv P-2B3 sequence (SEQ ID NO:4) and variant C5 (SEQ ID NO:5), A2 (SEQ ID NO:6), and A11 (SEQ ID NO:7) ScFv sequences are shown.

[0025] **Figure 3:** DNA sequence (SEQ ID NO:8) and translated protein sequence of the parental 2B3 minibody (SEQ ID NO:9). Also indicated are the starting points of the following protein segments: signal peptide (for mammalian secretion), light chain variable region (V_L), the 15 amino acid interdomain peptide linker, heavy chain variable region (V_H), human IgGl hinge sequence and 10 amino acid extension, and the human IgGl CHj domain followed by two stop codons.

[0026] **Figure 4**: SDS-PAGE analysis of the four minibodies in non-reducing conditions: parental (p), A11, A2 and C5. The arrow indicates the expected molecular weight of a minibody.

[0027] **Figure 5**: Size-exclusion HPLC of A11 minibody showing homogenous peak at expected molecular size.

[0028] **Figure 6:** Affinity ranking. (A) by Competitive ELISA binding assay: Plates were coated with CEA and biotinylated intact, chimeric anti-CEA antibody was used as probe. (B) by flow cytometry: 5pg/ml of each minibody was incubated with PSCA expressing cells. Cells were then stained with anti Human Fc PE conjugated.

[0029] **Figure 7:** Co-registered microPET/microCT scan of a nude mouse bearing LAPC-9AD (PSCA-positive human prostate cancer) xenografts. The mouse was injected with 1-1 24 radiolabeled A1 1 minibody variant and scanned serially. A, B, C; saggital sections; D, E, F; coronal sections. A, D; coregistered microPET and microCT, showing ROI (region of interest) as a white rectangle. B, E; microPET images and ROI C, F; microPET images only.

[0030] **Figure 8:** Biodistribution and microPET Imaging ranking of the 2B3 minibody variants. Biodistribution unit is a %ID/g values of weighed tissues in y- counter after 21 or 25 hours injection time. MicroPET Imaging values were obtained on the average values of 4 ROIs as shown in figure 6 and described in the materials and methods.

[0031] **Figure 9:** Summary of PSCA data and properties.

[0032] **Figure 10:** Depictions of various constructs according to the invention.

[0033] **Figure 11:** Schema for producing 2B3 variants.

[0034] **Figure 12:** MicroPET imaging and biodistribution data for various constructs.

[0035] **Figure 13:** MicroPET imaging and biodistribution results for All and parent 2B3.

[0036] **Figure 14:** MicroPET imaging and biodistribution data for pancreatic cancer Capan-1 xenographic mice using parental 2B3 and variant A11 anti-PSCA minibodies.

[0037] **Figure 15:** MicroPET imaging and biodistribution data for pancreatic cancer MIA PaCa-2 xenographic mice using parental 2B3 and variant A11 anti-PSCA minibodies.

[0038] **Figure 16:** Amino acid sequences of parental 2B3 and variant A2, A11, and C5 anti-PSCA minibodies.

DETAILED DESCRIPTION OF THE INVENTION

[0039] Prolonged clearance kinetics have hampered the development of intact antibodies as imaging and therapeutic agents, despite their ability to effectively deliver radionuclides to tumor targets i*n vivo*. Here, we also report genetically engineered antibody fragments which display rapid, high-level tumor uptake coupled with rapid clearance from the circulation in a nude mouse model to allow ready detection of tumors *in vivo*

[0040] The 2B3 antibody was humanized by grafting on a human framework (Trastuzumab) and named (Olafsen T, et al. J. Immunotherapy 2007; 30:396; which is incorporated by reference in its entirety with respect to the description, biological activity, and making of the antibody). Antibody fragments of 2B3 have been generated for PET imaging application. One of these antibody fragments, the 2B3 minibody has demonstrated rapid and specific localization to PSCA-expressing tumors in a murine model. However, humanization has decreased the affinity of the intact antibody for PSCA from a Kd of 2.6 nM to 16.7 n M. In addition, reformatting of intact antibody into antibody fragments can also affect the binding efficiency. It has been shown that quantitative tumor retention of ScFvs increases with affinity but only to a threshold close to 10^{-9} M (Adams GP, et al. Cancer Res. 2001;61:4750). There are no published data on the effect of minibody affinity on tumor targeting and imaging properties. Here, we describe the generation of three high affinity anti-PSCA ScFvs, and the subsequent generation and characterization of three high affinity anti-PSCA minibodies. We generated minibody fragments with better tumor targeting/imaging aptitude. In some embodiments, with the regard to the constructs of the invention, there is a proviso that the construct comprises a V_H or V_L domain that is not identical to a corresponding domain or the 2B3 antibody.

[0041] In one embodiment, the present invention provides antigen binding constructs selected from the group consisting of an antibody, a minibody, a diabody, an scFv, an scFv-

Fc, and the like, wherein the V_L and V_H domains are substantially identical to those found in 2B3. In a second embodiment, the antigen binding construct may comprise one or more mutations found in an antibody variant designated herein as A11, A2, or C5. In a third embodiment, the binding construct comprises at least one mutation at a residue corresponding to an amino acid of SEQ ID NO:4 selected from the group consisting of T5, S10, V15, S91, S123, S131, N179, T182, I194, A203, G213, Q228, and a combination thereof. In certain embodiments, the at least one mutation comprises a mutation corresponding to a mutation in SEQ ID NO:4 selected from the group comprising T5I, S10I, V15M, S91G, Δ123, S131Y, N179Y, T182S, I194M, A203V, E213K, Q228R, and a combination thereof. In other embodiments, the binding constructs of the invention comprise mutations corresponding to those found in variants A11, A2, or C5.

[0042] In a certain embodiment, the present invention provides a minibody wherein the V_L and V_H domains are substantially identical to those found in SEQ ID NO:10. In one embodiment, a minibody of the present invention comprises an amino acid sequence of SEQ ID NO:10. In other embodiments, the minibody comprises an amino acid sequence that is substantially identical to SEQ ID NO:10, wherein said minibody binds to PSCA with a higher affinity than a minibody of SEQ ID NO:10. In yet other embodiments, the minibody may comprise one or more mutations at a residue corresponding to an amino acid of SEQ ID NO:10 selected from the group comprising T5, S10, V15, S91, S123, S131, N179, T182, I194, A203, G213, Q228, and a combination thereof. In yet another embodiment, the construct may comprise a sequence selected from SEQ ID NOS:11, 12, and 13.

[0043] In one embodiment, the invention provides an antigen binding protein construct selected from the group consisting of a minibody, a diabody, scFv and scFv-Fc wherein the selected construct comprises CDR regions of an anti-PSCA antibody. In one embodiment, the binding protein construct will comprise at least one CDR region selected from a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, or CDR-H3 from an anti-PSCA antibody. In yet other embodiments, the protein binding construct may comprise all 3 light chain CDR regions or all three heavy chain CDR regions. In one embodiment, the protein binding constructs of the present invention may comprise all of the CDR regions of an anti-PSCA antibody. In certain embodiments, the anti-PSCA antibody will bind to PSCA with an affinity equal to or greater than the antibody designated 2B3. In other embodiments, the anti-PSCA antibody may be an affinity matured antibody, wherein the affinity matured antibody comprises a higher affinity for PSCA than does the parental antibody. In particular embodiments, the

parental anti-PSCA antibody may be selected from the group consisting of 1G8 (ATCC No. HB-12612), 2A2 (ATCC No. HB-1203), 2H9 (ATCC No. HB-12614), 3C5 (ATCC No. HB-12616), 3E6 (ATCC No. HB12618), 3G3 (ATCC No. HB-12615), 4A10 (ATCC No. HB-12617), and 2B3. In other embodiments, the CDRs may be selected from those found in SEQ ID NOS:10, 11, 12, and 13.

[0044] A "minibody" is an engineered antibody construct comprised of the variable heavy (VH) and variable light (VL) chain domains of a native antibody fused to the hinge region and to the CH3 domain of the immunoglobulin molecule (see, Figure 1). Minibodies are thus small versions of whole antibodies encoded in a single protein chain which retain the antigen binding region, and the CH3 domain which to permit assembly into a bivalent molecule and the antibody hinge to accommodate dimerization by disulfide linkages. In contrast, native antibodies are comprised of four chains, two heavy and two light. The size, valency and affinity of the minibody is particularly suited for in vivo targeting. Expression in bacterial or mammalian cells is simplified because minibodies can be produced as single amino acid chains (see, U.S. Patent No. 5,837,821) which is incorporated by reference herein in its entirety and particularly with reference to minibodies, their structure, ways of making them, and their suitable pharmaceutical formulations.

[0045] A 'diabody" comprises a first polypeptide chain which comprises a heavy (VH) chain variable domain connected to a light chain variable domain (VL) on the first polypeptide chain (VH-VL) connected by a peptide linker that is too short to allow pairing between the two domains on the first polypeptide chain and a second polypeptide chain comprising a light chain variable domain (VL) linked to a heavy chain variable domain VH on the second polypeptide chain (VL-VH) connected by a peptide linker that is too short to allow pairing between the two domains on the second polypeptide chain. The short linkages force chain pairing between the complementary domains of the first and the second polypeptide chains and promotes the assembly of a dimeric molecule with two functional antigen binding sites.

[0046] To construct bispecific diabodies the V-domains of different antibodies (e.g., antibody A and antibody B) are fused to create the two chains (e.g., VHA-VLB, VHB-VLA). Each chain is inactive in binding to antigen, but recreates the functional antigen binding sites of antibodies A and B on pairing with the other chain.

[0047] PSCA and its expression in cancer of the prostate, bladder, and pancreas is disclosed in U.S. Patent No. 6,756,036 which is incorporated by reference in its entirety. The human PSCA translated amino acid sequence is:

MKAVLLALLMAGLALQPGTALLCYSCKAQVSNEDCLQV
ENCTQLGEQCWTARIRAVGLLTVISKGCSLNCVDDS
QDYYVGKKNITCCDTDLCNASGAHALQPAAAILALLPAL
GLLLWGPGQL

(SEQ ID NO:1)

[0048] The terms "substantially identical" in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 80% identity, preferably at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity) over the referenced sequences or portions, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*see, e.g.*, NCBI web site http://www.ncbi.nlm.nih.gov/BLAST/ or the like). The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. Preferably, the sequence identity is at least 85%, 90%, 95% 97% between two referenced domains. In some embodiments, the difference in sequence is just by one, two, three or four, or from five to 12, amino acids as to referenced sequence or domain. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 15 amino acids or nucleotides in length, or more preferably over a region that is 15-50 amino acids or nucleotides in length. In other embodiments, the identity may exist over a region that is at least about 50, 100, 150, 200, or more amino acids.

[0049] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default

program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0050] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected over a specified range of residues (e.g., 20 to 50, usually about 50 to about 200, more usually about 100 to about 150) in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g., Current Protocols in Molecular Biology* (Ausubel *et al.*, eds. 1995 supplement)).

[0051] A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al., Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al., J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the

word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0052] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms capply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0053] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0054] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0055] The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan

(W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see, e.g.*, Creighton, *Proteins* (1984)).

[0056] A "label" or a "detectable moiety"or "detectable marker" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins which can be made detectable, e.g., by incorporating a radiolabel into the peptide or used to detect antibodies specifically reactive with the peptide.

[0057] "Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Typically, the antigen-binding region of an antibody will be most critical in specificity and affinity of binding.

[0058] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0059] Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see Fundamental Immunology* (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used

herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990))

[0060] For preparation of suitable antibodies or constructs of the invention and for use according to the invention, e.g., recombinant, monoclonal, or polyclonal antibodies, many techniques known in the art can be used (see, U.S. Patent Application Publication No. 20070196274 and U.S. Patent Application Publication No. 20050163782, which are each incorporated by reference in their entirities, particularly with respect to minibody and diabody design) (*see, e.g.*, Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985); Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *Antibodies, A Laboratory Manual* (1988); and Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986)). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity (*see, e.g.,* Kuby, *Immunology* (3rd ed. 1997)). Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Patent 4,946,778, U.S. Patent No. 4,816,567) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized or human antibodies (*see, e.g.*, U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, Marks *et al., Bio/Technology* 10:779-783 (1992); Lonberg *et al., Nature* 368:856-859 (1994); Morrison, *Nature* 368:812-13 (1994); Fishwild *et al., Nature Biotechnology* 14:845-51 (1996); Neuberger, *Nature Biotechnology* 14:826 (1996); and Lonberg & Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995)). Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990); Marks *et al.*, *Biotechnology* 10:779-783 (1992)). Antibodies can also be made bispecific, i.e., able to recognize two different antigens (*see, e.g.*, WO 93/08829, Traunecker *et al., EMBO J.* 10:3655-3659 (1991); and Suresh *et al., Methods in Enzymology* 121:210 (1986)).

Antibodies can also be heteroconjugates, e.g., two covalently joined antibodies, or immunotoxins (*see, e.g.*, U.S. Patent No. 4,676,980 , WO 91/00360; WO 92/200373; and EP 03089).

[0061] The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody. For example, the antibody derivatives include, without limitation, antibodies that have been modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, and the like. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-natural amino acids.

[0062] Methods for humanizing or primatizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers (*see, e.g.*, Jones *et al., Nature* 321:522-525 (1986); Riechmann *et al., Nature* 332:323-327 (1988); Verhoeyen *et al., Science* 239:1534-1536 (1988) and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0063] A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a

different or altered antigen specificity. The preferred antibodies of, and for use according to the invention include humanized and/or chimeric monoclonal antibodies.

[0064] In some embodiments, the antibody is conjugated to an "effector" moiety. The effector moiety can be any number of molecules, including labeling moieties such as radioactive labels or fluorescent labels, or can be a therapeutic moiety. In one aspect the antibody modulates the activity of the protein. Such effector moieties include, but are not limited to, an anti-tumor drug, a toxin, a radioactive agent, a cytokine, a second antibody or an enzyme. Further, the invention provides an embodiment wherein the antibody of the invention is linked to an enzyme that converts a prodrug into a cytotoxic agent.

[0065] The immunoconjugate can be used for targeting the effector moiety to a PSCA-positive cell, particularly cells, which overexpress the PCSA protein. Such differences can be readily apparent when viewing the bands of gels with approximately similarly loaded with test and controls samples.. Examples of cytotoxic agents include, but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphteria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, and glucocorticoid and other chemotherapeutic agents, as well as radioisotopes. Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme.

[0066] In some embodiments, the invention provides antigen binding protein constructs used to systemically to treat cancer (e.g., prostate, pancreatic or bladder cancer) alone or when conjugated with an effector moiety. PSCA-targeting constructs conjugated with toxic agents, such as ricin, as well as unconjugated antibodies can be useful therapeutic agents naturally targeted to PSCA bearing cancer cells. Such constructs can be useful in blocking invasiveness

[0067] Additionally, the antigen-binding protein constructs of the invention can be used to treat cancer. In such a situation, the construct is joined to at least a functionally active portion of a second protein or toxic molecule having therapeutic activity. The second protein can include, but is not limited to, an enzyme, lymphokine, oncostatin or toxin. Suitable toxins include doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D,

diphteria toxin, Pseudomonas exotoxin (PE) A, PE40, ricin, abrin, glucocorticoid and radioisotopes.

[0068] Techniques for conjugating therapeutic agents to constructs according to the invention 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); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

[0069] The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide or construct according to the invention, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions requires a construct be selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select constructs specifically immunoreactive with PSCA. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, *Using Antibodies, A Laboratory Manual* (1998) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

[0070] By "therapeutically effective dose or amount" herein is meant a dose that produces effects for which it is administered. The exact dose and formulation will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see, e.g.*, Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); *Remington: The Science and Practice of Pharmacy*, 20th Edition, Gennaro, Editor (2003), and Pickar, *Dosage Calculations* (1999)).

[0071] The term "pharmaceutically acceptable salts" or "pharmaceutically acceptable carrier" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (*see, e.g.*, Berge *et al.*, *Journal of Pharmaceutical Science* 66:1-19 (1977)). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts. Other pharmaceutically acceptable carriers known to those of skill in the art are suitable for the present invention.

[0072] The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

[0073] The methods find particular application in the diagnosis, prognosis and treatment of cancers which overexpress PSCA, for example, prostate, pancreatic and bladder cancers. In certain embodiments the methods are applied to hormone refractory or therapy resistant cancers. In certain embodiments the methods are applied to metastatic cancers.

[0074] Treatment will generally involve the repeated administration of the constructs and their immunoconjugates via an acceptable route of administration such as intravenous injection (IV), at an effective dose. Dosages will depend upon various factors generally appreciated by those of skill in the art, including without limitation the type of cancer and the severity, grade, or stage of the cancer, the binding affinity and half life of the agents used, the desired steady-state antibody concentration level, frequency of treatment, and the influence of chemotherapeutic agents used in combination with the treatment method of the invention. Typical daily doses may range from about 0.1 to 100 mg/kg. Doses in the range of 10-500 mg of the constructs or their immunoconjugates per week may be effective and well tolerated, although even higher weekly doses may be appropriate and/or well tolerated. The principal determining factor in defining the appropriate dose is the amount of a particular agent necessary to be therapeutically effective in a particular context. Repeated administrations may be required in order to achieve tumor inhibition or regression. Initial loading doses may be higher. The initial loading dose may be administered as an infusion. Periodic maintenance doses may be administered similarly, provided the initial dose is well tolerated.

[0075] Direct administration of the constructs is also possible and may have advantages in certain contexts. For example, for the treatment of bladder carcinoma, the agents may be injected directly into the bladder.

[0076] In another embodiment, the invention provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. In one embodiment, the present invention provides an expression vector encoding for an antibody or fragment thereof of the present invention. In another embodiment, the present invention provides polynucleotides encoding an antibody of the present invention for use in gene therapy or *in vivo* administration.

[0077] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, and complements thereof. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs

include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0078] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

Methods of Administration and Formulation

[0079] The constructs are administered to a subject in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration is preferred. The administration may be local or systemic.

[0080] The compositions for administration will commonly comprise an agent as described herein dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs.

[0081] Thus, a typical pharmaceutical composition for intravenous administration will vary according to the agent. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more

detail in such publications as Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa. (1980).

[0189] The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include, but are not limited to, powder, tablets, pills, capsules and lozenges. It is recognized that constructs when administered orally, should be protected from digestion. This is typically accomplished either by complexing the molecules with a composition to render them resistant to acidic and enzymatic hydrolysis, or by packaging the molecules in an appropriately resistant carrier, such as a liposome or a protection barrier. Means of protecting agents from digestion are well known in the art.

[0082] Pharmaceutical formulations, particularly, constructs and immunoconjugates and inhibitors for use with the present invention can be prepared by mixing a construct having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers. Such formulations can be lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations used. Acceptable carriers, excipients or stabilizers can be acetate, phosphate, citrate, and other organic acids; antioxidants (e.g., ascorbic acid) preservatives low molecular weight polypeptides; proteins, such as serum albumin or gelatin, or hydrophilic polymers such as polyvinylpyllolidone; and amino acids, monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents; and ionic and non-ionic surfactants (e.g., polysorbate); salt-forming counter-ions such as sodium; metal complexes (e. g. Zn-protein complexes); and/or non-ionic surfactants. The construct can be formulated at a concentration of between 0.5 - 200 mg/ml, or between 10-50 mg/ml.

[0083] The formulation may also provide additional active compounds, including, chemotherapeutic agents, cytotoxic agents, cytokines, growth inhibitory agent, and anti-hormonal agent. The active ingredients may also prepared as sustained-release preparations (e.g., semi-permeable matrices of solid hydrophobic polymers (e.g., polyesters, hydrogels (for example, poly (2-hydroxyethyl-methacrylate), or poly (vinylalcohol)), polylactides. The antibodies and immunocongugates may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin microcapsules and poly- (methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes,

albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions.

[0084] The compositions can be administered for therapeutic or prophylactic treatments. In therapeutic applications, compositions are administered to a patient suffering from a disease (e.g., cancer) in a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health. Single or multiple administrations of the compositions may be administered depending on the dosage and frequency as required and tolerated by the patient. A "patient" or "subject" for the purposes of the present invention includes both humans and other animals, particularly mammals. Thus the methods are applicable to both human therapy and veterinary applications. In the preferred embodiment the patient is a mammal, preferably a primate, and in the most preferred embodiment the patient is human. Other known cancer therapies can be used in combination with the methods of the invention. For example, the compositions for use according to the invention may also be used to target or sensitize a cell to other cancer therapeutic agents such as 5FU, vinblastine, actinomycin D, cisplatin, methotrexate, and the like.

[0085] In other embodiments, the methods of the invention may be practiced together with other cancer therapies (e.g, radical prostatectomy), radiation therapy (external beam or brachytherapy), hormone therapy (e.g., orchiectomy, LHRH-analog therapy to suppress testosterone production, anti-androgen therapy), or chemotherapy. Radical prostatectomy involves removal of the entire prostate gland plus some surrounding tissue. This treatment is used commonly when the cancer is thought not to have spread beyond the tissue. Radiation therapy is commonly used to treat prostate cancer that is still confined to the prostate gland, or has spread to nearby tissue. If the disease is more advanced, radiation may be used to reduce the size of the tumor. Hormone therapy is often used for patients whose prostate cancer has spread beyond the prostate or has recurred. The objective of hormone therapy is to lower levels of the male hormones, androgens and thereby cause the prostate cancer to shrink or grow more slowly. Luteinizing hormone-releasing hormone (LHRH) agonists decrease the production of testosterone. These agents may be injected either monthly or longer. Two such analogs are leuprolide and goserelin. Anti-androgens (e.g., flutamide, bicalutamide, and nilutamide) may also be used. Total androgen blockade refers to the use of anti-androgens in combination with orchiectomy or LHRH analogs, the s combination is called. Chemotherapy is an option for patients whose prostate cancer has spread outside of

the prostate gland and for whom hormone therapy has failed. It is not expected to destroy all of the cancer cells, but it may slow tumor growth and reduce pain. Some of the chemotherapy drugs used in treating prostate cancer that has returned or continued to grow and spread after treatment with hormonal therapy include doxorubicin (Adriamycin), estramustine, etoposide, mitoxantrone, vinblastine, and paclitaxel. Two or more drugs are often given together to reduce the likelihood of the cancer cells becoming resistant to chemotherapy. Small cell carcinoma is a rare type of prostate cancer that is more likely to respond to chemotherapy than to hormonal therapy.

[0086] The combined administrations contemplates co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

[0087] The compound of choice, alone or in combination with other suitable components, can be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

[0088] Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intratumoral, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration, oral administration, and intravenous administration are the preferred methods of administration. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

[0089] Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced by nucleic acids for *ex vivo* therapy can also be administered intravenously or parenterally as described above.

[0090] The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active

component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The composition can, if desired, also contain other compatible therapeutic agents.

[0091] Preferred pharmaceutical preparations deliver one or more constructs according to the invention, optionally in combination with one or more chemotherapeutic agents or immunotherapeutic agents, in a sustained release formulation. The construct may be administered therapeutically as a sensitizing agent that increases the susceptibility of tumor cells to other cytotoxic cancer therapies, including chemotherapy, radiation therapy, immunotherapy and hormonal therapy.

[0092] In therapeutic use for the treatment of cancer, the constructs utilized in the pharmaceutical method of the invention are administered at the initial dosage of about 0.001 mg/kg to about 1000 mg/kg daily. A daily dose range of about 0.01 mg/kg to about 500 mg/kg, or about 0.1 mg/kg to about 200 mg/kg, or about 1 mg/kg to about 100 mg/kg, or about 10 mg/kg to about 50 mg/kg, can be used. The dosages, however, may be varied depending upon the requirements of the patient, the severity of the condition being treated, and the compound being employed. For example, dosages can be empirically determined considering the type and stage of cancer diagnosed in a particular patient. The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day, if desired.

[0093] The pharmaceutical preparations for use according to the invention are typically delivered to a mammal, including humans and non-human mammals. Non-human mammals treated using the present methods include domesticated animals (*i.e.*, canine, feline, murine, rodentia, and lagomorpha) and agricultural animals (bovine, equine, ovine, porcine).

Methods of Tumor Imaging

[0094] In certain embodiments, the present invention provides methods of imaging cancer cells or tumors *in vivo* through administration of antibodies of the invention. In one embodiment, the present invention provides a method of imaging a cancer cell *in vivo*, the method comprising administering a labeled anti-PSCA antibody to a mammal and imaging the antibody *in vivo*. The methods of the present invention may be used to image a cancer cell in mammal, including without limitation, a mouse, rat, hamster, rabbit, pig, human, and the like.

[0095] Methods of *in vivo* imaging are well known in the art and include without limitation, magnetic resonance imaging (MRI), nuclear magnetic resonance (NMR) (R. Weissleder, 1999, Radiology 212:609-14), computerized axial tomography (CAT) scan, cooled charged coupled device (CCD) camera optical imaging (Honigman, et al., 2001 Mol. Ther. 4:239-249), bioluminescent optical imaging (P R Contag, et al., 1998 Nat. Med. 4:245-247), position emission tomography (PET) (M E Phelps, 1991 Neurochemical Research 16:929-994; J G Tjuvajev, et al., 1998 Cancer Res 58:4333-4341), single photon emission computed tomography (J G. Tjuvajev, et al., 1996 Cancer Res. 45:4087-4095), microPET (reviewed in McVeigh, 2006, Circ. Res. 98:879-86), and the like.

Examples

[0096] The following examples are offered to illustrate, but not limit, the claimed invention.

[0097] Example 1

[0098] This example describes the construction of a mutant scFv yeast display library and the selection of ScFv mutants with improved PSCA binding affinity.

[0099] Oligonucleotides and vectors used in the construction of the library include; Gap 5': 5'-TTAAGCTTCTGCAGGCTAGTG-3' (SEQ ID NO:2); Gap 3': 5'-GAGACCGAGGAGAGGGTTAGG-3' (SEQ ID NO:3); pYD2 inside the NcoI-Not1 restriction sites (Razai A, et al. J Mol Biol. 2005; 35 1 : 1 58).

[0100] 2B3 ScFv gene was first cloned into the yeast display vector pYD2 (Razai A, et al. J Mol. Biol. 2005; 351:158) using NcoI-Not1 restriction sites. A bacterial clone with the correct sequence was amplified and DNA extracted with QIAprep Spin Miniprep. Random mutations were introduced into the 2B3 Scfv gene using error prone PCR as follows: 2B3 ScFv gene was subject to 20 cycles of PCR with Taq in the presence of 100mM MnC12 to

generate random mutations. The PCR product was run on an agarose gel and purified using QIAquick gel extraction. The purified PCR product was re-amplified using a proof reading DNA polymerase for 35 cycles. Both PCRs were carried out with the Gap5' and Gap3' primers. The amplified 2B3 Fv gene was again run on an agarose gel and purified using QIAquick gel extraction. Mutated scFv genes and NcoI-Not1 digested pYD2 were used to transform LiAc-treated EBY 100 cells by gap repair. The resulting gene repertoire was cloned into pYD2 using gap repair to create a library of 5.9×10^5 transformants Transformation mixes were cultured and sub-cultured in SD-CAA. Library size was determined by plating serial dilutions of the transformation mixture on SD-CAA plates.

[0101] For selection, scFv display was induced by culturing in SG-CAA media plus zeocin for 24 hours at 20°C. For the first round of selection 20 million yeast (more than 30 times the library size) were washed and resuspended in FACS buffer (phosphate-buffered saline (pH 7.4), 0.5% bovine serum albumin) to which 200 nM of PSCA human Gamma 1 fusion protein was added and incubated for 1 hour at room temperature. The concentrations of PSCA human Gamma 1 used for round 2, 3 and 4 of sorting were 5 nM, 2 nM and 1nM respectively. Cells were incubated for 30 minutes with secondary antibodies at 4°C, washed once with FACS buffer, resuspended in 200-500 µl of FACS buffer and sorted on a FACSAria. Typically 1% of the PSCA binding population was gated for collection. Collected cells were grown in SD-CAA media and used for the next round of sorting after induction in SG-CAA. Twenty yeast clones from the fourth round of sorting were analyzed by flow cytometry. Eight of these clones showing strong staining were selected (A2, A4, A8, A9, A11, A12, B5 and C5) and their DNA sequenced. A2, A4, A8, A9, A12, B5 protein sequences were identical with 10 mutations, A11 had 6 mutations and C5 had 4 mutations. Protein sequence comparisons of the parental 2B3 ScFv with A2, A11 and C5 are shown in Figure 2.

[0102] Example 2

[0103] This example describes the reformatting of mutant 2B3 scFv's into minibodies.

[0104] The parental 2B3 minibody pEE12 construct (Figure 2) was used as a backbone to generate the three 2B3 minibody affinity variants where the wild type ScFv insert was replaced by each of the three 2B3 ScFv affinity variants. The parental 2B3 minibody construct is presented in Figure 1. Briefly, V_L and V_H regions were fused with a 15 residue long Gly-Ser rich linker in the V_L-V_H orientation. This ScFv is flanked by a signal peptide upstream and the human IgG1C_H3 domain via the human IgGl hinge including a 10 residue

GlySer peptide linker downstream. The final product was cloned into the PEE 12 vector of expression (Lonza Biologics, Slough, UK). This vector contains the hCMV promoter and the glutamine synthetase gene for selection (Bebbington et al., Biotechnology (N Y). 1992; 10: 169). The parental 2B3 minibody pEE12 construct was used as a backbone to generate the three 2B3 minibody affinity variants. The parental pEE12 DNA was digested with Xba I and Xho I restriction sites to remove the parental 2B3 ScFv insert that was replaced by each of the three 2B3 ScFv affinity variants. Xba I- Xho I restriction sites were added at the extremities of 2B3 ScFv affinity variants for sub-cloning in pEE12, by extension PCR.

[0105] Example 3

[0106] This example describes the expression, selection, and purification of anti-PSCA minibodies.

[0107] A total of 2 x 10^6 NS0 mouse myeloma cells were transfected with 10ug of linearized (cut with *SalI*) vector DNA by electroporation and selected in glutamine-deficient media as described (Yazaki PJ, et al. J Immunol Methods. 2001 ;253: 195). Clones were screened for expression by ELISA, whereby the desired protein was captured by goat anti-human IgG (Fc specific) and detected by alkaline phosphastase (AP)-conjugated goat anti-human IgG (Fc specific) (both from Jackson ImmunoResearch Labs, West Grove,PA). The highest producing clones were expanded and brought to terminal culture.

[0108] Soluble minibodies were purified from cell culture supernatants by Protein L chromatography using a Thermal Separations Products HPLC with an in-line UV monitor, equipped with a preparative Poros 50 A column (Applied Biosystems, Foster City, CA) and analyzed on SDS-PAGE (Figure 3). The four minibodies showed similar results. All the minibodies migrated as molecular weight species of ~95 kDa under non-reducing conditions and all showed good purity. In addition, the A11 minibody eluted at 29.5 minutes as expected when run on a calibrated size exclusion column (Figure 4).. Supernatants were loaded onto a 10x50 mm column and eluted using 0.1 M glycine pH 2.5, the pH was immediately neutralized with 2M Tris-HC1 pH 8. The purified proteins were then dialyzed against PBS using a molecular porous membrane tubing (mwco: 30,000) and concentrated with a Vivascience Vivaspin 20 (mwco: 30,000). Final protein concentrations were determined by measuring UV absorbance at 280 nm, using the parental murine antibody as the standard.

[0109] Example 4

[0110] This example describes the biochemical characterization of anti-PSCA minibodies.

[0111] Size and composition: Purified proteins were analyzed by SDS-PAGE (Figure 3) under non-reducing conditions. Native structural size was determined by size exclusion columns (Superdex 75) (Pharmacia).

[0112] The ranking of the four minibodies was determined by competition ELISA and flow cytometry (figure 5). The relative affinity as measured by competition ELISA indicated that all three affinity variants had higher affinity than the parental, with an improvement of 4.4x, 3.0x and 1.9x for A2, A11 and C5 respectively compared to the parental. Flow cytometry data also resulted in ranking the four minibodies in the same order when targeting PSCA expressed at the cell surface. In conclusion, the affinity ranking of the four minibodies was: A2>A11>C5>parental.

[0113] Competition ELISA: PSCA relative binding affinity for the minibodies was determined by competition ELISA in which microtiter plate wells were coated with purified PSCA-Fc (Olafsen T, et al. J. Immunotherapy 2007: 30:396).

[0114] Flow Cytometry : was conducted to assess cellular PSCA binding activity. An EBV transformed B-cell lymphoma cell line stably transfected with PSCA were used. Briefly, cells 5 x 10' were incubated for 30 min on ice with 100 µl of minibody at 2ug/ml concentration. Cells were washed and stained with goat anti-hFc PE conjugated antibody at 1:100 dilution.

[0115] Radioiodination: Purified minibodies were radioiodinated with the positron emitting isotope ^{124}I(sodium iodide in 0.02 M NaOH; radionuclide purity >99%) provided by Advanced Nuclide Technologies, Indianapolis, IN as previously described (Kenanova, Olafsen et al., Cancer Res. 65:622,2005). Immunoreactivity was assayed by incubating radioiodinated-minibody with an excess amount of SKW-PSCA^{+} cells for an hour at room temperature, centrifugating the cells, and counting radioactivity present into the supernatant compared to the control.

[0116] Example 5

[0117] This example describes MicroPET imaging and biodistribution studies of anti-PSCA minibodies.

[0118] All animal studies were conducted under protocols approved by the Chancellor's Animal Research Committee at the University of California, Los Angeles. Xenografts were

established in 7- to 8-week-old male nude mice (Charles River Laboratories, Wilmington, MA) by s.c. inoculation of 2 x10^6 LAPCP AD cells in the shoulder region. After 14 days, when tumor masses were in the range of 100 to 300 mg, 100 μCi of isotope ^{124}I(30-50 μg protein) was injected into the tail vein of each animal. Mice were imaged using a P4 microPET scanner (Concorde Microsystems, Inc., Knoxville, TN). To enable imaging, mice were anesthetized using 2% isoflurane, positioned in a prone position along the long axis of the microPET scanner and imaged. Acquisition time was 10 minutes (1 bed position), and images were reconstructed using a filtered backprojection reconstruction algorithm. Images were displayed and regions of interest (ROI) were drawn as described in figure 6 and quantified using AMIDE (Loening and Gambhir, Molecular Imaging 2: 13 1,2003). After scanning, tumors, liver, spleen, kidney, lung, and blood were excised, weighed and counted in a well counter (Cobra II AutoGamma, Packard, IL). Background, crossover, and decay corrections were done. Results were calculated as percentage of injected dose per gram of tissue (% ID/g).

[0119] To evaluate tumor targeting and microPET imaging efficiency, ^{124}I-labeled minibodies were injected into Nude mice bearing LAPC-9AD tumors on the right shoulder. Wholebody micoPET and CT scans were performed at 21h and/or 25h, after which the animals were sacrificed, and activity in various tissues quantified using a gamma counter. To quantify microPET imaging, four 3-dimentional ROIs were drawn in the tumors and four other ROI in soft tissues around the tumor as presented in figure 6. The ROI position and size were based on CT image information. Both biodistribution and imaging quantification are presented as a ratio of tumor signal to background. A11 minibody gave the best biodistribution and imaging data in an experiment that compared the three affinity variant (Figure 7A). Thus the affinity ranking (Figure 5) and *in vivo* tumor targeting/imaging ranking (Figure 7A) of the three affinity variant minibodies are different, suggesting that in our model *in vivo* tumor targeting/imaging effectiveness is not solely dependant on the inherent affinity of the tracer to its target. A2 which has the best affinity to PSCA did not give the best *in vivo* tumor targeting/imaging results. One possible explanation for this discordance is that A2 has a substitution of an asparagine into a tyrosine in V_L CDR2, and that iodination of this new tyrosine could affect the binding to PSCA. In a second experiment A11 minibody was compared to the parental minibody for their *in vivo* tumor targeting/imaging effectiveness. A11 showed a 20% increase in tumor targeting (n=3) and a 14 1 % increase in microPET tumor imaging (n=2) (figure 7B).

[0120] Example 6

[0121] This example describes the imaging of various pancreactic cancer tumors using anti-PSCA minibodies.

[0122] In order to evaluate the targeting and imaging potential of affinity matured anti-PSCA minibodies, ^{124}I-labeled minibodies (parental 2B3 and variant A11) were injected into athymic nude mice bearing tumors that express low levels of target PSCA antigen. Briefly, xenographic mice bearing either human Capan-1 (Figure 14) or human MIA PaCa-2 (Figure 15) pancreactic tumors were injected with either 200 or 300 μg of labeled anti-PSCA minibody. Wholebody micoPET and CT scans were performed as before, and tissue radioactivity was determined. Similarly, quantification of microPET imaging and ROI position and size were determined as in example 5.

[0123] As can be seen in Figures 14 and 15, variant A11 anti-PSCA microbodies consistently demonstrated improved tumor to muscle specificity ratios as compared to parental 2B3 minibodies. The nearly 2-fold enhancement in *in vivo* specificity suggests that these variant minibodies are better suited for use in therapeutic targeting and tumor imaging than existing anti-PSCA antibodies. Notebly, tumor uptake in Capan-1 and HPAF-11 (data not shown) was roughly 2% and less than 1% in MIA PaCa-2 tumors, suggesting specific uptake.

[0124] Related art:

[0125] 1) Sundaresan, G., Yazaki, P.J., Shively, J.E., Finn, R.D., Larson, S.M., Raubitschek, A.A., Williams, L.E., Chatziioannou, A.F., Gambhir, S.S., and Wu, A.M. (2003) Iodine-1 24 labeled engineered anti-CEA minibodies and diabodies allow highcontrast, antigen-specific small-animal PET imaging of xenografts in athymic mice. *J,* Nucl. Med., 44:1962-1969.

[0126] 2) Olafsen, T., Gu, Z., Sherman, M.A., Leyton, J.V., Witkosky, M.E., Shively, J.E.., Raubitschek, A.A., Morrison, S.L., Wu, A.M. and Reiter, R.E. (2007) Targeting, imaging, and therapy using a humanized anti-prostate stem cell antigen (PSCA) antibody. J. lmmunotherapy 30:396-405.

[0127] 3) Leyton, J.V., Olafsen, T., Sherman, M.A., Reiter, R.E., and Wu, A.M. Anti-prostate stem cell antigen (PSCA) antibody fragments for PET imaging of prostate cancer (abstract). Cancer Biotherapy & Radiopharmaceuticals 21 :391, 2006.

[0128] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes to the extent consistent with the present disclosure.

WHAT IS CLAIMED IS:

1. An antigen binding protein construct selected from the group consisting of a minibody, a diabody, scFv and scFv-Fc wherein the selected construct has V_L and V_H domains that are substantially identical to the V_L and V_H domains of a minibody or scFv designated herein as A11, A2, or C5.

2. An antigen binding protein construct selected from the group consisting of a minibody, a diabody, and a scFv-Fc, wherein the selected construct has V_L and V_H domains that are substantially identical, respectively, to the V_L domain and the V_H domain of an anti-PSCA antibody.

3. The antigen binding protein construct of claim 2, wherein said anti-PSCA antibody is an affinity matured antibody.

4. The construct of any one of claims 1 to 3, wherein the construct is a minibody in which the V_L and V_H chain variable domains of the anti-PSCA antibody are fused to the hinge region of an antibody, an amino acid linker and the C_H3 domain of an immunoglobulin molecule.

5. The construct of any one of claims 1 to 3, wherein the construct is a diabody.

6. The construct of any one of claims 1 to 5, wherein the anti-PSCA antibody is a humanized antibody and the C_H3 domain is from a human immunoglobulin molecule.

7. The construct of claim 6, wherein the hinge region is a human IgG hinge region.

8. The construct of any one of claims 2 to 7, wherein the anti-PSCA antibody is 2B3.

9. The construct of claim 2, wherein the antigen binding protein is a minibody comprising an amino acid sequence with at least 95% sequence identity to SEQ ID NO:10, wherein said minibody binds to PSCA with an affinity equal to or greater than that of antibody 2B3.

10. The construct of claim 9, wherein said minibody binds to PSCA with an affinity greater than that of antibody 2B3.

11. The minibody of claim 9, wherein said minibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:10, 11, 12, and 13.

12. The construct of claim 2 or 3, wherein the anti-PSCA antibody is a monoclonal antibody designated 1G8 (ATCC No. HB-12612), 2A2 (ATCC No. HB-1203), 2H9 (ATCC No. HB-12614), 3C5 (ATCC No. HB-12616), 3E6 (ATCC No. HB12618), 3G3 (ATCC No. HB-12615), or 4A10 (ATCC No. HB-12617).

13. An antigen binding protein construct selected from the group consisting of a minibody, a diabody, and a scFv-Fc, wherein the selected construct comprises CDR regions of an anti-PSCA antibody.

14. The construct of claim 13, wherein said anti-PSCA antibody binds to PSCA with an affinity equal to or greater than that of antibody 2B3.

15. The construct of claim 14, wherein said anti-PSCA antibody is selected from those designated 2B3, A2, A11, or C5.

16. The construct of any one of claims 13 to 15, wherein said construct is a minibody.

17. The construct of any one of claims 1 to 16, wherein the construct is conjugated to a therapeutic agent.

18. The construct of claim 17, wherein the therapeutic agent is a cytotoxic agent.

19. The construct of claim 18, wherein the cytotoxic agent is selected from a group consisting of ricin, ricin A-chain, doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphteria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, arbrin A chain, modeccin A chain, alpha-sarcin, gelonin mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotin, calicheamicin, sapaonaria officinalis inhibitor, maytansinoids, and glucocorticoidricin.

20. The construct of claim 18, wherein the therapeutic agent is a radioactive isotope.

21. The construct of claim 20, wherein the radioactive isotope is selected from the group consisting of ^{212}Bi, ^{131}I, ^{111}In, ^{90}Y and ^{186}Re.

22. The construct of any one of claims 1 to 16, wherein the construct is linked to an anti-cancer pro-drug activating enzyme capable of converting a pro-drug to its active form.

23. The construct of claim 2, wherein the PSCA is human PSCA.

24. The construct of any one of claims 1 to 16, wherein the construct is labeled with a detectable marker.

25. The construct according to claim 24, wherein the marker is a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme.

26. The construct of claim 25, wherein the marker is ^{124}I.

27. A polynucleotide encoding an anti-PSCA antibody of any one of claims 1 to 25.

28. A method for inhibiting the growth of a prostate cancer cell expressing a Prostate Stem Cell Antigen (PSCA) protein comprising contacting the cancer cell with a construct of any one of claims 1 to 23, in an amount effective to inhibit the growth of the cancer cell.

29. A method for killing a prostate cancer cell by the method of claim 28.

30. The method of claim 28, wherein the construct recognizes and binds the PSCA protein as shown in SEQ ID NO:2 beginning with leucine at amino acid position 22 and ending with alanine at amino acid position 99.

31. The method of claim 28, further comprising administering to the cell a chemotherapeutic drug.

32. The method of claim 28 further comprising administering radiation therapy to the cell.

33. A method for treating a patient suffering from a prostate, bladder, or pancreatic cancer by the method of any one of claims 28 to 32.

34. The method of claim 33, comprising administering to the patient hormone ablation therapy or hormone antagonist therapy.

35. The method of claim 34, wherein the contacting comprises administering the construct to the patient intravenously, intraperitoneally, intramuscularly, intratumorally, or intradermally.

36. The method of claim 35, wherein the contacting comprises administering the construct directly into a prostate cancer, a bladder cancer, a pancreatic cancer or a metastasis thereof.

37. A method of detecting a cancerous cell in a subject, said method comprising contacting a construct of any one of claims 24 to 26.

38. A pharmaceutical composition comprising a construct of any one of claims 2 to 1, and pharmaceutically acceptable excipient, carrier, or stabilizer.

39. The composition of claim 38, wherein the composition is a lyophilized formulations or aqueous solution.

40. A construct of any one of claims 1 to 23, for use in treating or detecting a prostate, bladder, or pancreatic cancer in a subject.

41. A method of imaging a cancer cell *in vivo*, comprising the steps of:

(a) administering to a mammal suspected of having cancer an antibody of any one of claims 24 to 26; and

(b) detecting said antibody with a method selected from the group consisting of magnetic resonance imaging (MRI), nuclear magnetic resonance (NMR), computerized axial tomography (CAT) scan, cooled charged coupled device (CCD) camera optical imaging, bioluminescent optical imaging, position emission tomography (PET), single photon emission computed tomography, and microPET, thereby imaging a cancer cell *in vivo*.

42. The method of claim 41, wherein said mammal is a mouse, rat, . hamster, rabbit, pig, or human.

Fig. 1

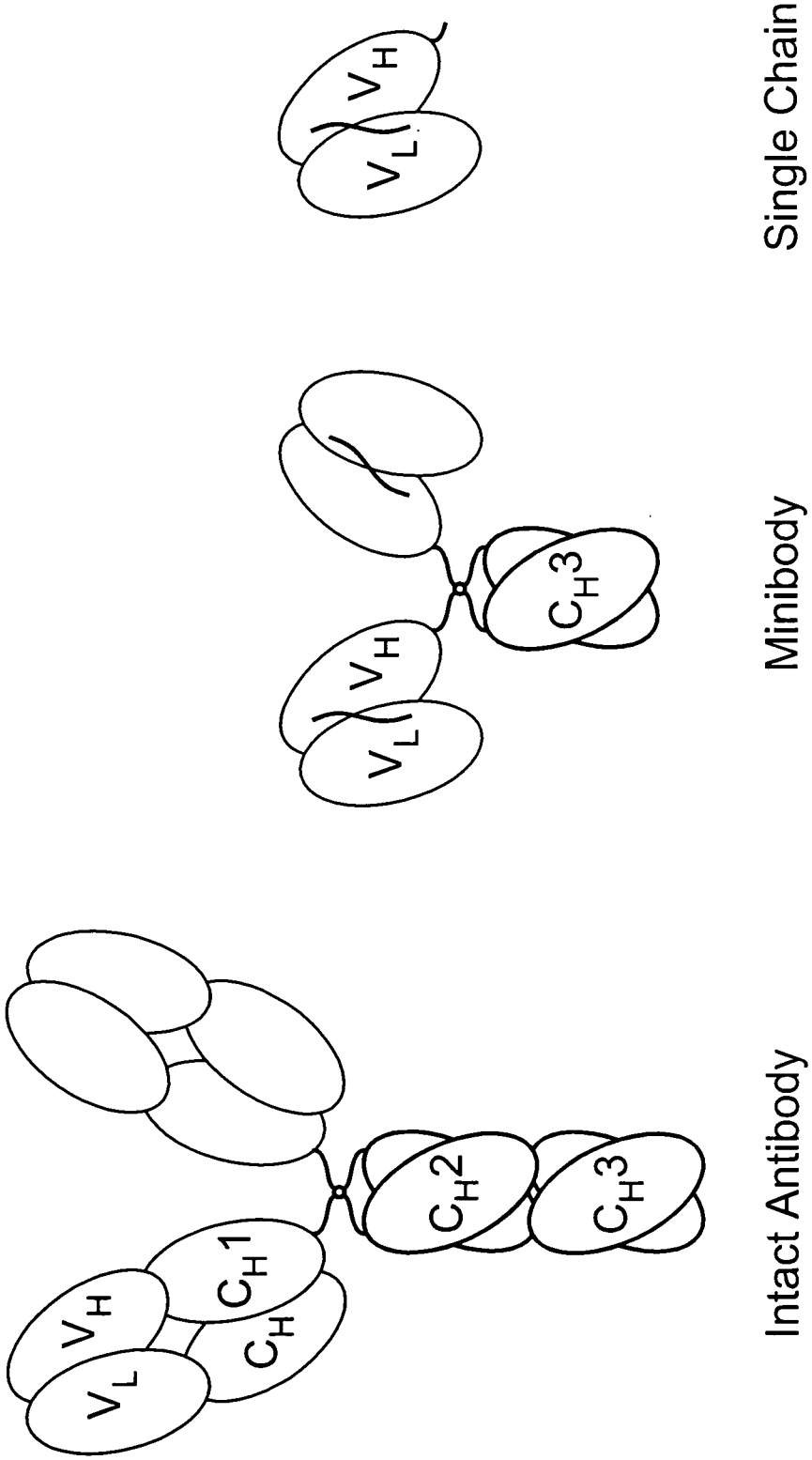
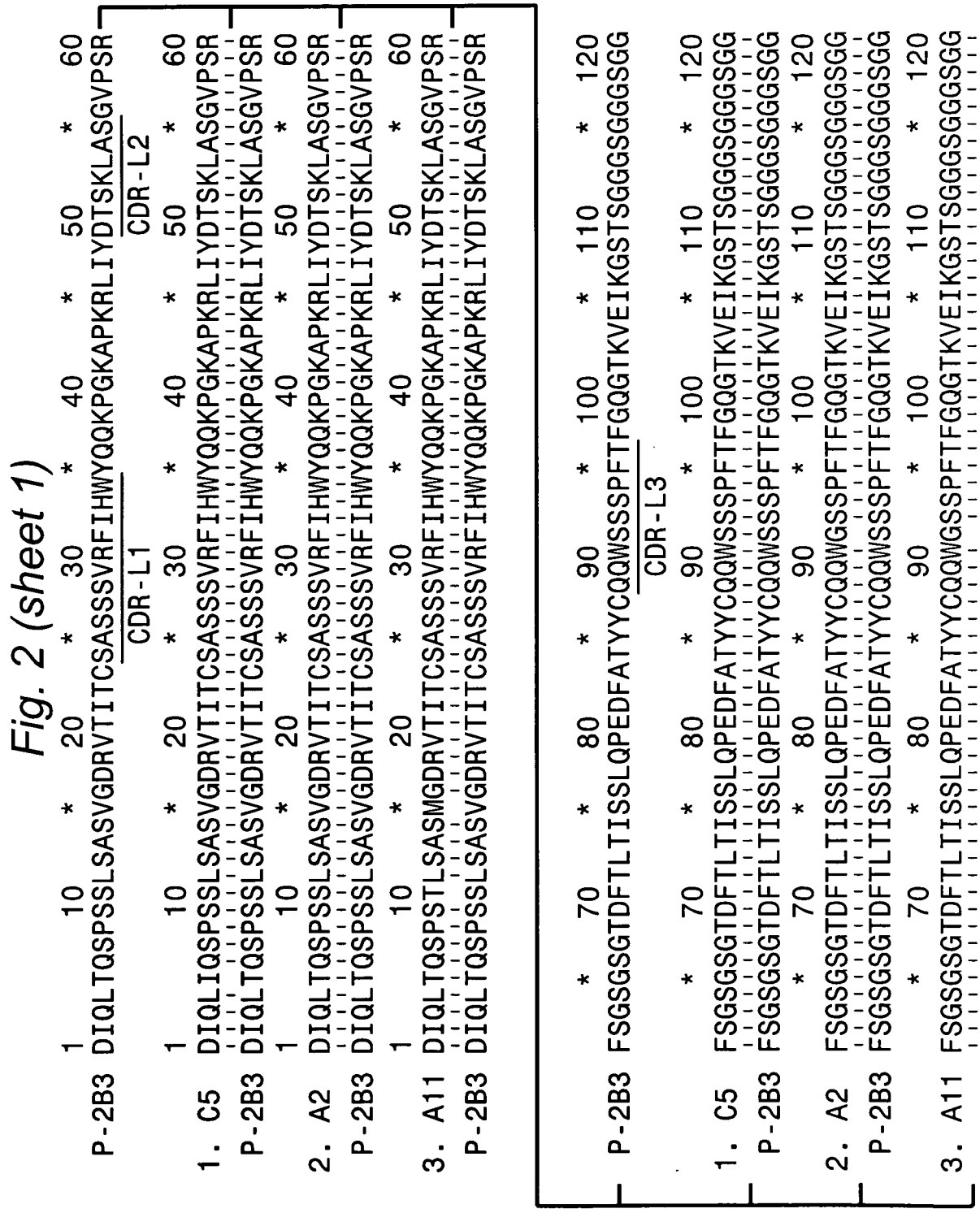


Fig. 2 (sheet 1)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CDR-L1
CDR-L2
1 10 * 20 * 30 * 40 * 50 * 60
1. C5 DIQLIQSPSSLSASVGDRVTITCSASSSVRFIHWYQQKPGKAPKRLIYDTSKLASGVPSR
P-2B3 DIQLTQSPSSLSASVGDRVTITCSASSSVRFIHWYQQKPGKAPKRLIYDTSKLASGVPSR
1 10 * 20 * 30 * 40 * 50 * 60
2. A2 DIQLTQSPSSLSASVGDRVTITCSASSSVRFIHWYQQKPGKAPKRLIYDTSKLASGVPSR
P-2B3 DIQLTQSPSSLSASVGDRVTITCSASSSVRFIHWYQQKPGKAPKRLIYDTSKLASGVPSR
1 10 * 20 * 30 * 40 * 50 * 60
3. A11 DIQLTQSPSTLSASMGDRVTITCSASSSVRFIHWYQQKPGKAPKRLIYDTSKLASGVPSR
P-2B3 DIQLTQSPSSLSASVGDRVTITCSASSSVRFIHWYQQKPGKAPKRLIYDTSKLASGVPSR
* 70 * 80 * 90 * 100 * 110 * 120
P-2B3 FSGSGSGTDFTLTISSLQPEDFATYYCQQWSSSPFTFGQGTKVEIKGSTSGGGSGGGSGG
CDR-L3
* 70 * 80 * 90 * 100 * 110 * 120
1. C5 FSGSGSGTDFTLTISSLQPEDFATYYCQQWSSSPFTFGQGTKVEIKGSTSGGGSGGGSGG
P-2B3 FSGSGSGTDFTLTISSLQPEDFATYYCQQWSSSPFTFGQGTKVEIKGSTSGGGSGGGSGG
* 70 * 80 * 90 * 100 * 110 * 120
2. A2 FSGSGSGTDFTLTISSLQPEDFATYYCQQWGSSPFTFGQGTKVEIKGSTSGGGSGGGSGG
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3. A11 FSGSGSGTDFTLTISSLQPEDFATYYCQQWGSSPFTFGQGTKVEIKGSTSGGGSGGGSGG
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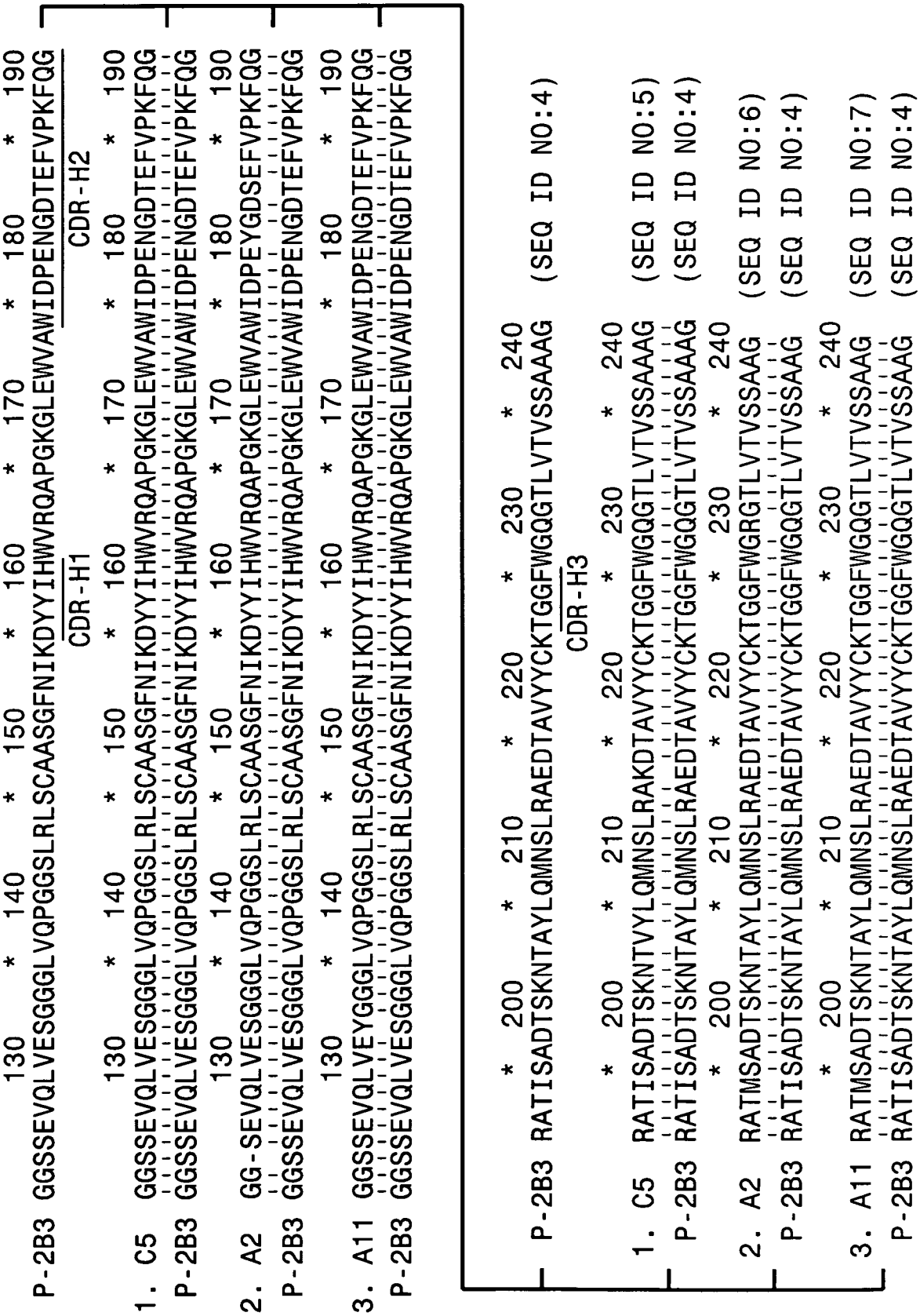
Fig. 2 (sheet 2)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CDR-H1
CDR-H2
130 * 140 * 150 * 160 * 170 * 180 * 190
1. C5 GGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGKGLEWVAWIDPENGDTEFVPKFQG
P-2B3 GGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGKGLEWVAWIDPENGDTEFVPKFQG
130 * 140 * 150 * 160 * 170 * 180 * 190
2. A2 GG-SEVQLVESGGGLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGKGLEWVAWIDPEYGDSEFVPKFQG
P-2B3 GGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGKGLEWVAWIDPENGDTEFVPKFQG
130 * 140 * 150 * 160 * 170 * 180 * 190
3. A11 GGSSEVQLVEYGGGLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGKGLEWVAWIDPENGDTEFVPKFQG
P-2B3 GGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGKGLEWVAWIDPENGDTEFVPKFQG
* 200 * 210 * 220 * 230 * 240
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CDR-H3
* 200 * 210 * 220 * 230 * 240
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P-2B3 RATISADTSKNTAYLQMNSLRAEDTAVYYCKTGGFWGQGTLVTVSSAAAG (SEQ ID NO:4)
* 200 * 210 * 220 * 230 * 240
2. A2 RATMSADTSKNTAYLQMNSLRAEDTAVYYCKTGGFWGRGTLVTVSSAAG (SEQ ID NO:6)
P-2B3 RATISADTSKNTAYLQMNSLRAEDTAVYYCKTGGFWGQGTLVTVSSAAG (SEQ ID NO:4)
* 200 * 210 * 220 * 230 * 240
3. A11 RATMSADTSKNTAYLQMNSLRAEDTAVYYCKTGGFWGQGTLVTVSSAAG (SEQ ID NO:7)
P-2B3 RATISADTSKNTAYLQMNSLRAEDTAVYYCKTGGFWGQGTLVTVSSAAG (SEQ ID NO:4)

Fig. 3 (sheet 1)

```
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signal peptide
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  T  F  G  Q  G  T  K  V  E  I  K  G  S  T  S  G  G  G  S  G
                                  linker
```

Fig. 3 (sheet 2)

V_H

```
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  Y  Y  I  H  W  V  R  Q  A  P  G  K  G  L  E  W  V  A  W  I

gatcctgagaatggtgacactgaatttgtcccgaagttccagggccgtgccactataagc
  D  P  E  N  G  D  T  E  F  V  P  K  F  Q  G  R  A  T  I  S

gatcctgagaatggtgacactgaatttgtcccgaagttccagggccgtgccactataagc
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gccgtctattattgtaaaacgggggggttctggggtcaaggaaccctggtcaccgtctcg
  A  V  Y  Y  C  K  T  G  G  F  W  G  Q  G  T  L  V  T  V  S

agcgagcccaaatcttgtgacaaaactcacacatgcccaccgtgcggcggaggtagctct
  S  E  P  K  S  C  D  K  T  H  T  C  P  P  C  G  G  G  S  S
```

hinge *extension*

Fig. 3 (sheet 3)

```
ggcggtggatccggcgggcagccccgagaaccacaggtgtacaccctgccccccatcccgg
  G   G   G   S   G   G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R
                        CHJ
gatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagc
  D   E   L   T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S
gacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcct
  D   I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T   P
cccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagc
  P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V   D   K   S
aggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccac
  R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E   A   L   H   N   H
tacacgcagaagagcctctccctgtctccgggtaaatgatag
  Y   T   Q   K   S   L   S   L   S   P   G   K   -   -
```

Fig. 4

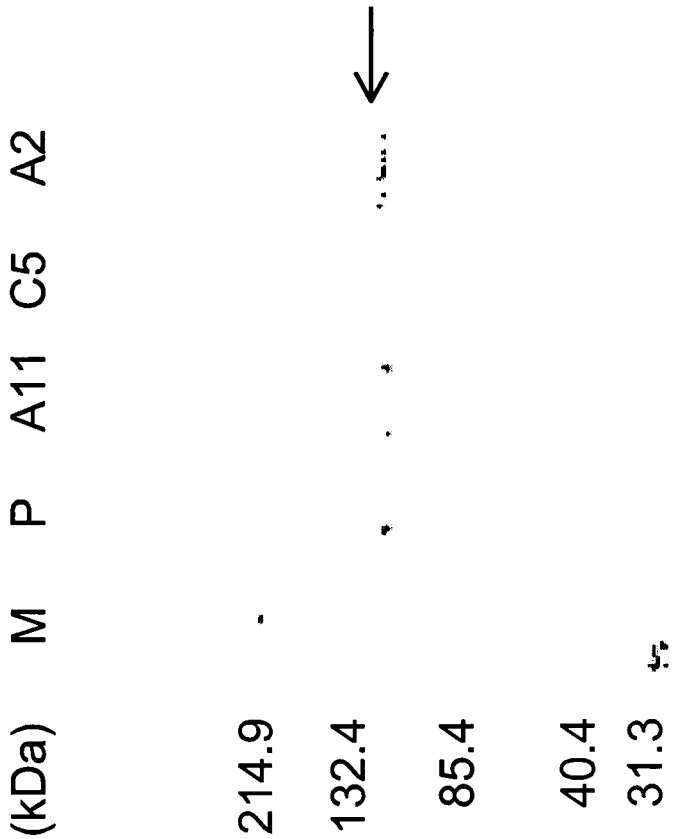


Fig. 5

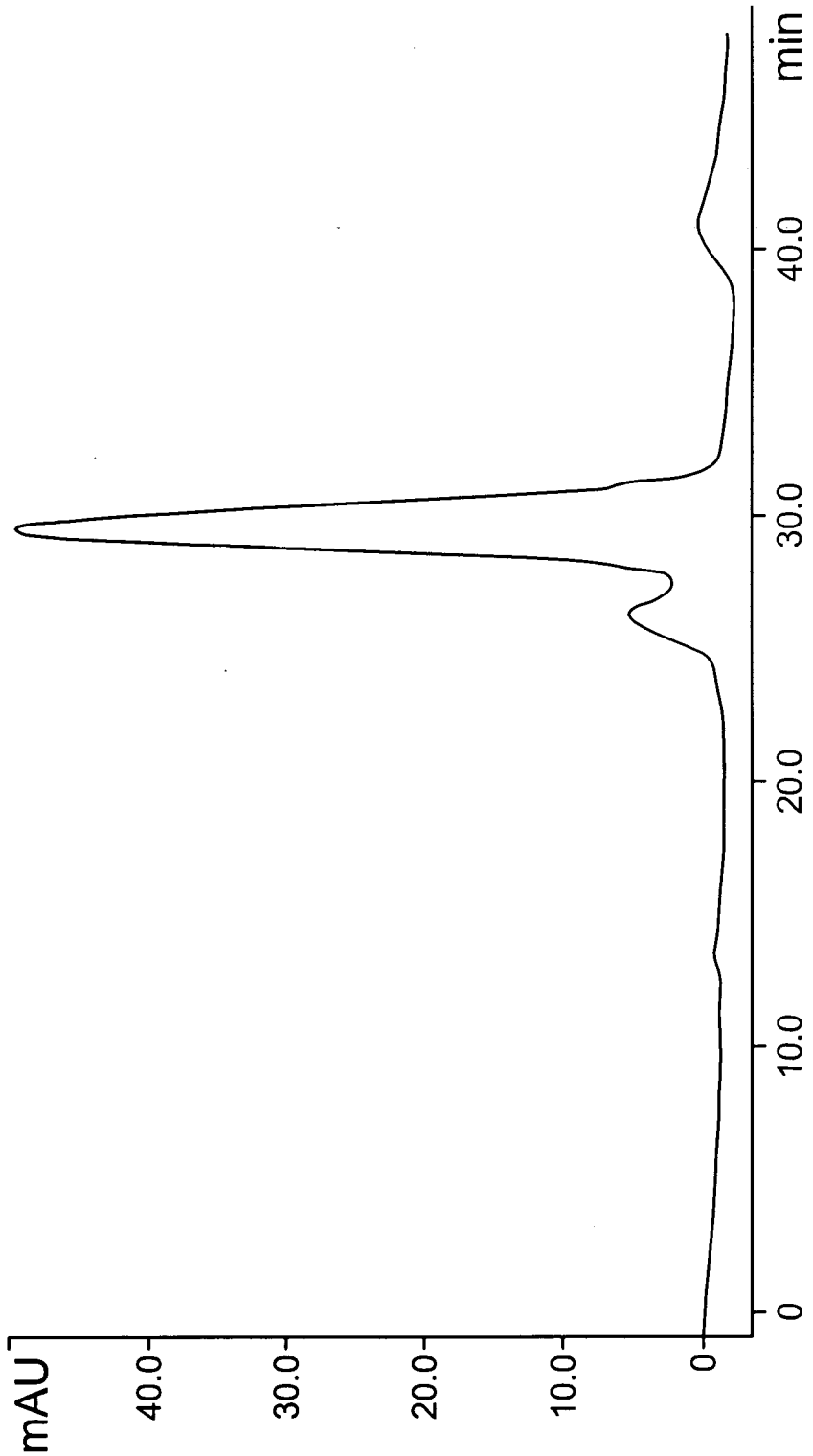

Superdex 2001030
A11Mb001:1 UV1_280nm
Superdex 2001030
A11Mb001:1 Inject
mAU
40.0
30.0
20.0
10.0
0
0
10.0
20.0
30.0
40.0
min

Fig. 6a

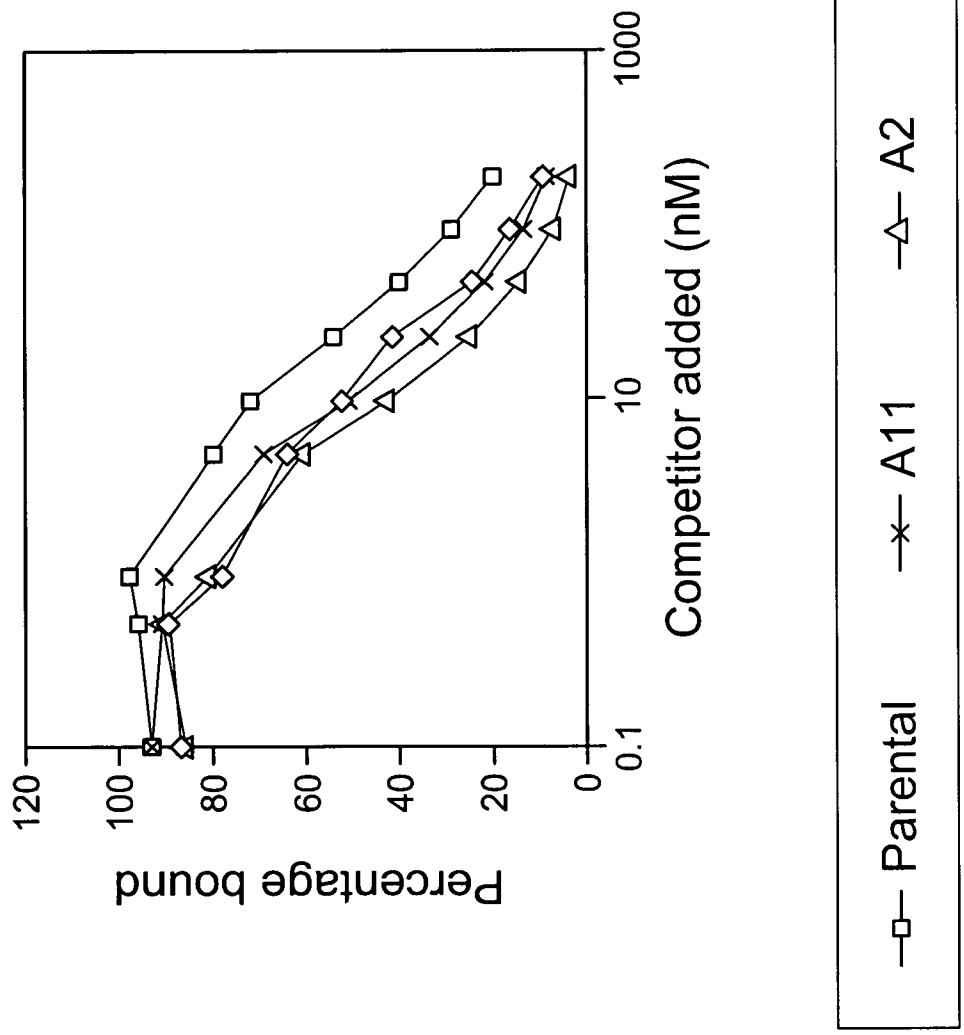

Percentage bound
120
100
80
60
40
20
0
0.1
10
1000
Competitor added (nM)
Parental
A11
A2
C5

Fig. 6b

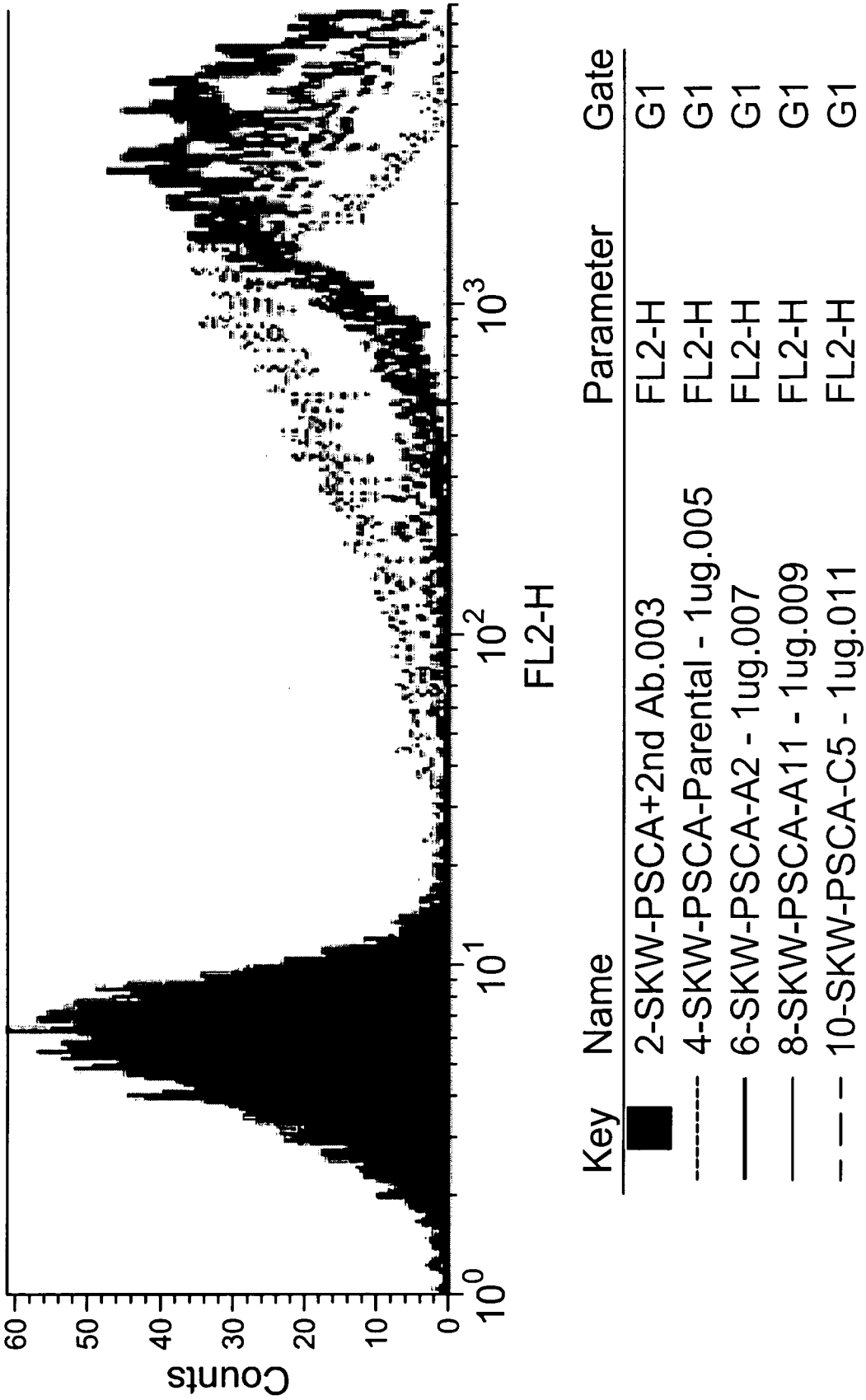

Counts
60
50
40
30
20
10
0
10^0
10^1
10^2
10^3
FL2-H
Key
Name
Parameter
Gate
2-SKW-PSCA+2nd Ab.003
FL2-H
G1
4-SKW-PSCA-Parental - 1ug.005
FL2-H
G1
6-SKW-PSCA-A2 - 1ug.007
FL2-H
G1
8-SKW-PSCA-A11 - 1ug.009
FL2-H
G1
10-SKW-PSCA-C5 - 1ug.011
FL2-H
G1

Fig. 7

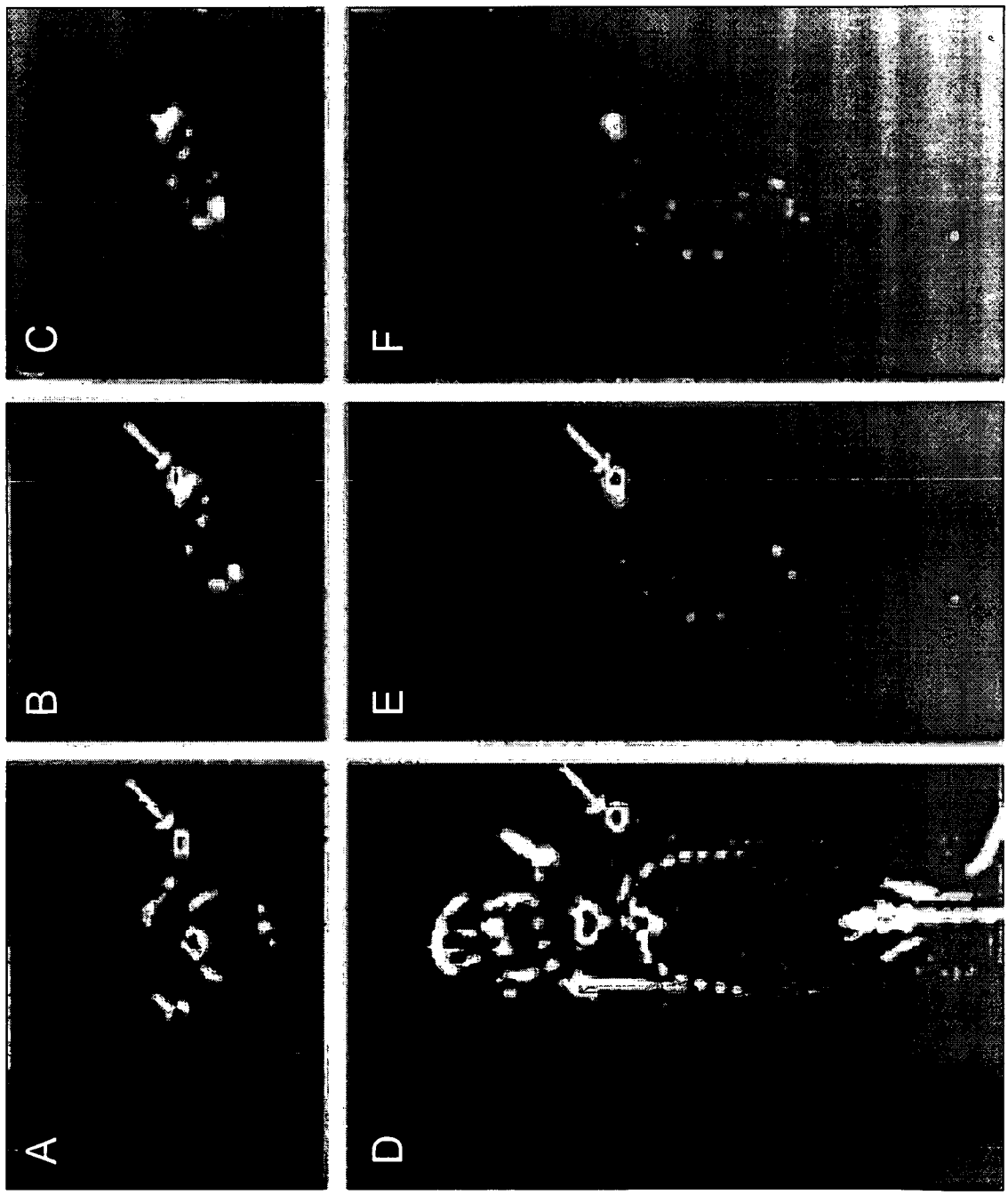


Fig. 8

	Tumor/blood Biodistribution	Tumor/background MicroPET
A2	0.67	2.9
A11	1.08	8.5
C5	0.73	2.9

	Tumor/blood Biodistribution	Tumor/background MicroPET
Parental	0.88	2.4
A11	1.11	5.8

Fig. 9

Prostate Stem Cell Antigen (PSCA)

123 a.a GPI-linked cell surface protein expressed in prostate and bladder

Over expressed in prostate cancer (40% localized, 60-100% metastatic cancer), bladder, and pancreatic cancer

Murine anti-PSCA antibody biologically active (1G8)

Gleason 4 prostate cancer stained with 1GB anti-PSCA Ab

R. Reiter et al., PNAS 1998
Saffran et al., PNAS 2000
Z Gu et al., Oncogene 2000

Fig. 10

Anti-PSCA Antibodies and Antibody Fragments

1) 1G8: mouse monoclonal antibody (150 kDa)
(Gu Z, et al., Oncogene 2000)

2) 2B3: humanized 1G8
(Olafsen T, et al. Imunother 2007)

3) 2B3 antibody fragments:

- ScVF: 28kDA
- Diabody: 55kDa
- Minibody: 80kDa
- ScFv-Fc: 110kDa

2B3 parental (P) minibody has shown better tumor targeting

Fig. 11 (sheet 1)

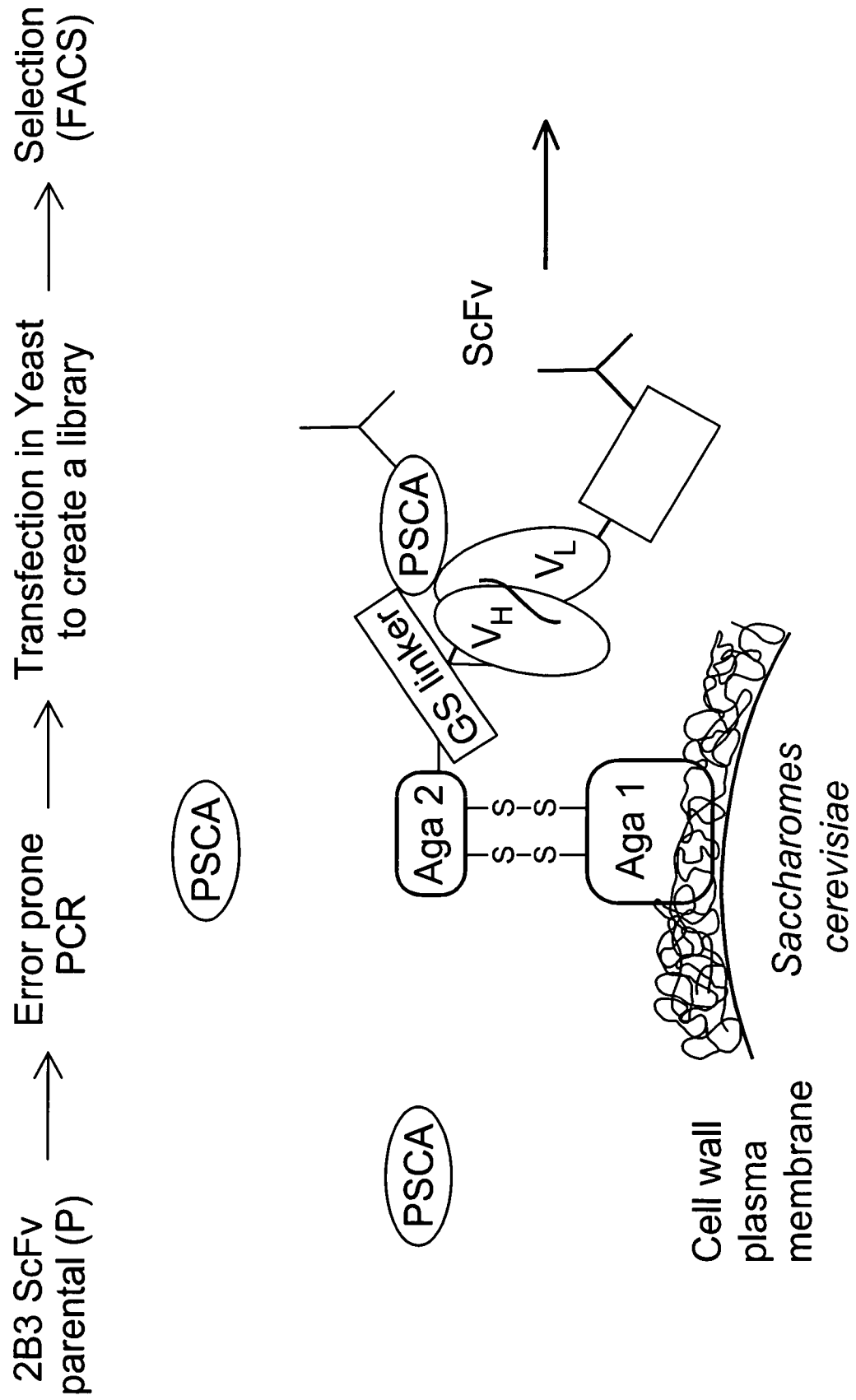


Fig. 11 (sheet 2)

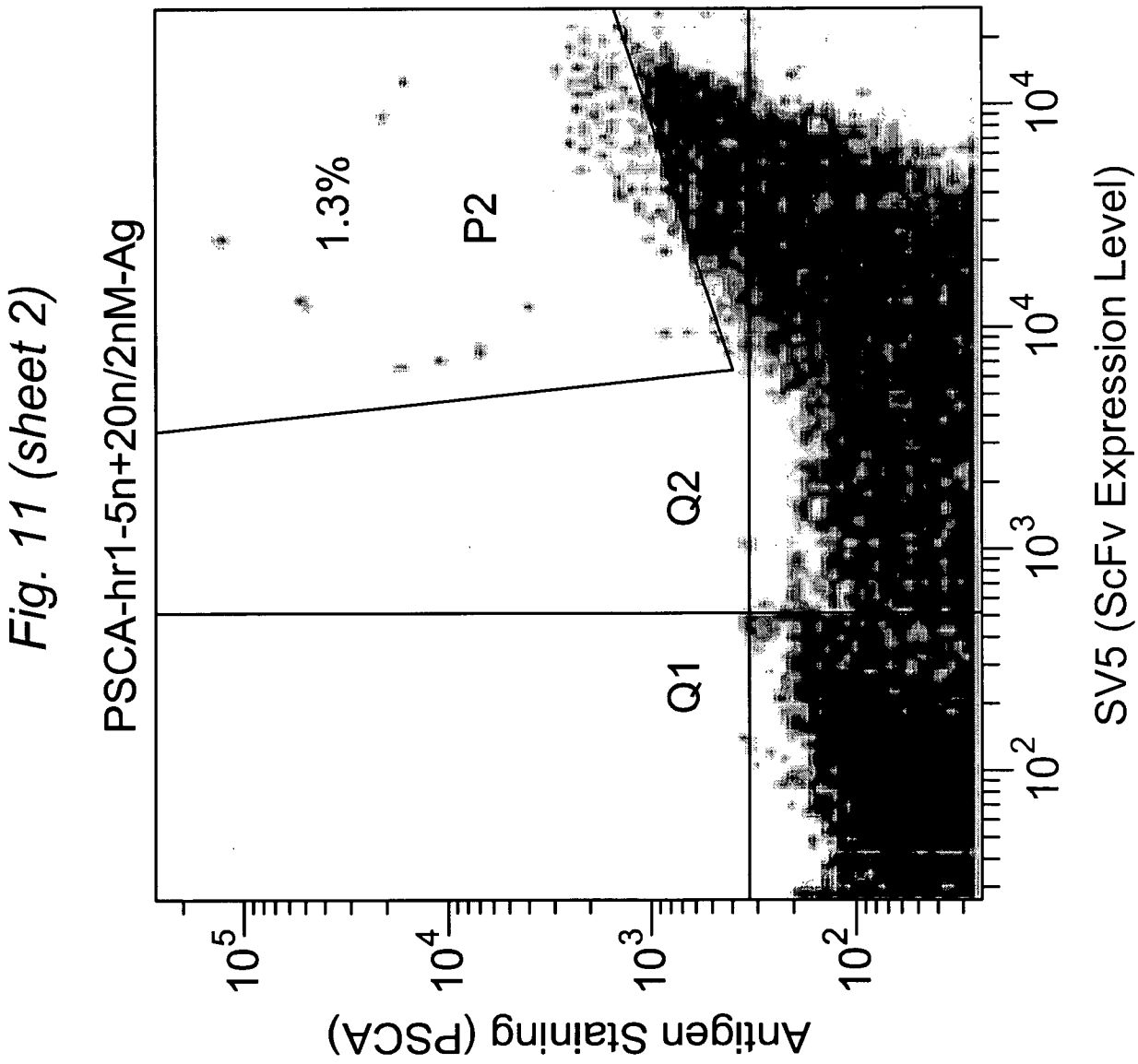


Fig. 12

MicroPET Imaging and Biodistribution:
Three 2B3 Affinity Variant Minibodies

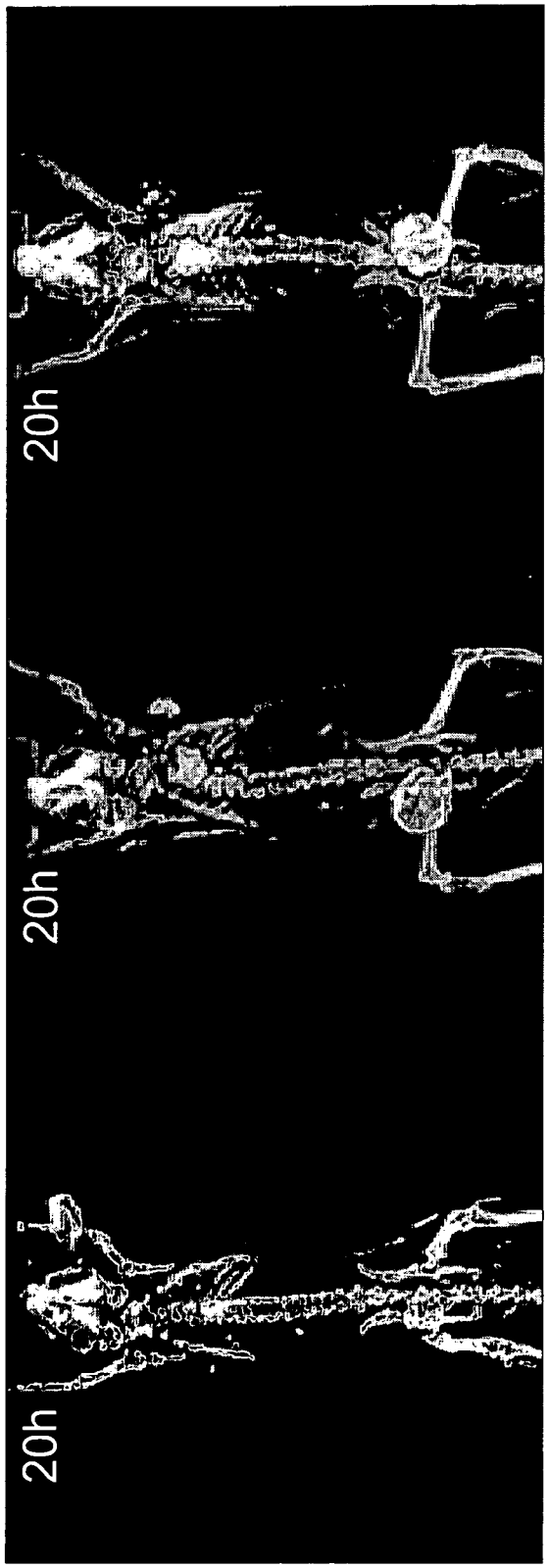


Image rendering with the same max and min threshold
(max = 2.0164e-05, min = 1.388e-05)

	A2	A11	C5
Tumor/blood (Biodistribution at 25 h)	0.67	1.08	0.73
Tumor/background MicroPET imaging at 20 h)	2.9	8.5	2.9

Fig. 13

MicroPET Imaging and Biodistribution:
Parental and A11

Image rendering with same max and min threshold (max = 11.3791e-05, min = 4.1236e-05)

	Parental	A11
Tumor/blood (Biodistribution at 29 h)	0.88	1.11
Tumor/background MicroPET imaging at 27 h)	2.4	5.8

A11 shows a 141% increase in microPET tumor imaging compared to the parental minibody

Fig. 14

PET Imaging of Capan-1 Xenografts

200 μg pMb labeled with I-124
Labeling efficiency: 89%
Specific activity: 1.3 μCi/μg
Immunoreactivity: 48%
Injected activity: 88 - 165 μCi

200 μg affMb labeled with I-124
Labeling efficiency: 85%
Specific activity: 1.6 μCi/μg
Immunoreactivity: 65%
Injected activity: 201 - 207 μCi

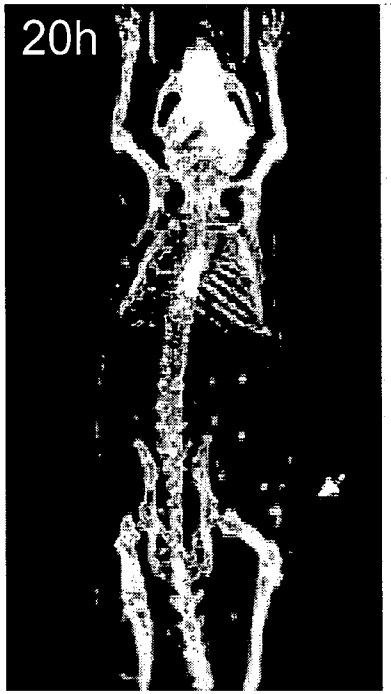
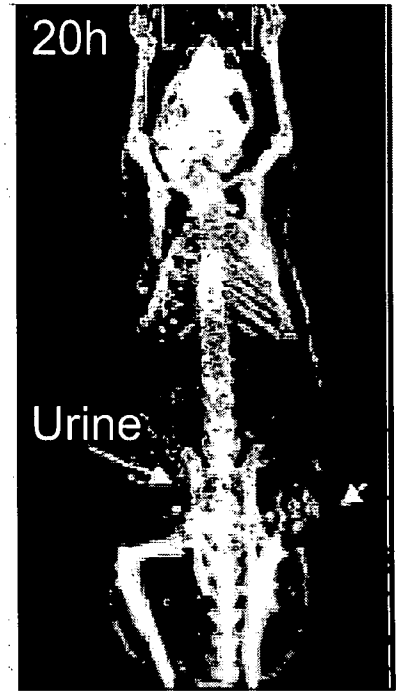
^{124}I-Parental Mb	^{124}I-Affinity matured Mb
	
Tumor uptake (n=5) 2.02(0.87)% ID/g	Tumor uptake (n=5) 2.03(0.55)% ID/g
Tumor to soft tissue ratio: 2.2	Tumor to soft tissue ratio: 4.2

Fig. 15

PET Imaging of MIA PaCa-2 Xenografts

200 μg pMb labeled with I-124
Labeling efficiency: 71.2 - 76.6%
Specific activity: 2.0 - 2.7 μCi/g
Immunoreactivity: 28%
Injected activity: 138 - 141 μCi

---

300 μg affMb labeled with I-124
Labeling efficiency: 73.3%
Specific activity: 1.9 μCi/g
Immunoreactivity: 8.2%
Injected activity: 109 - 114 μCi

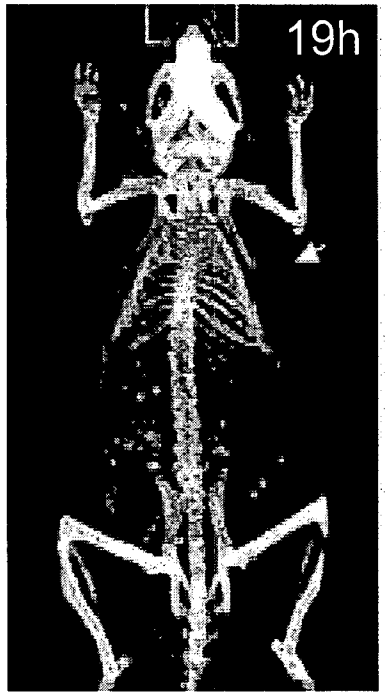
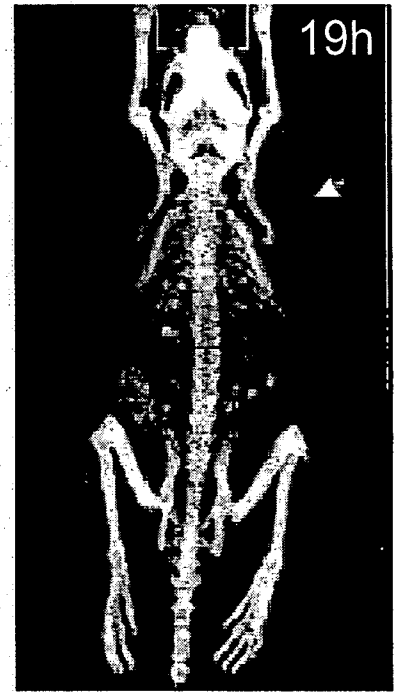
^{124}I-Parental Mb	^{124}I-Affinity matured Mb
	
Tumor uptake (n=4) 0.80(0.39)% ID/g	Tumor uptake (n=4) 0.81(0.25)% ID/g
Tumor to soft tissue ratio: 1.3	Tumor to soft tissue ratio: 2.1

Fig. 16a (sheet 1)

2B3 affinity Various Protein Sequences

2B3parental minibody sequence	2B3 A2 minibody sequence
DIQLTQSPSSLSASVGDRVTITCSASSVRFIHW	DIQLTQSPSSLSASVGDRVTITCSASSVRFIHW
YQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSG	YQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQWSSPFTFGQGT	TDFTLTISSLQPEDFATYYCQQWGSSPFTFGQG
KVEIKGSTSGGGSGGGSSEVQLVESGGG	TKVEIKGSTSGGGSGGGSGGGGS-EVQLVESGG
LVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGK	GLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPG
GLEWVAWIDPENGDTEFVPKFQGRATISADTSK	KGLEWVAWIDPEYGDSEFVPKFQGRATMSADT
NTAYLQMNSLRAEDTAVYYCKTGGFWGQGTLV	SKNTAYLQMNSLRAEDTAVYYCKTGGFWGRGT
TVSSEPKSCDKTHTCPPCGGGSSGGGSGGQPRE	LVTVSSEPKSCDKTHTCPPCGGGSSGGGSGGQP
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA	REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT	IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL	LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SPGK (SEQ ID NO:10)	SLSPGK (SEQ ID NO:11)

Fig. 16a (sheet 2)

2B3 A11 minibody sequence

DIQLTQSPSTLSASMGDRVTITCSASSSVRFIHW
YQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQWGSSPFTFGQG
TKVEIKGSTSGGGSGGGSGGGGSSEVQLVEYGG
GLVQPGGSLRLSCAASGFNIKDYYIHWVRQAPG
KGLEWVAWIDPENGDTEFVPKFQGRATMSADT
SKNTAYLQMNSLRAEDTAVYYCKTGGFWGQGT
LVTVSSEPKSCDKTHTCPPCGGGSSGGGSGGQP
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO:12)

Fig. 16b

2B3 C5 minibody sequence

DIQLIQSPSSLSASVGDRVTITCSASSSVRFIHW
YQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQWSSSPFTFGQGT
KVEIKGSTSGGGSGGGSGGGGSSEVQLVESGGG
LVQPGGSLRLSCAASGFNIKDYYIHWVRQAPGK
GLEWVAWIDPENGDTEFVPKFQGRATISADTSK
NTVYLQMNSLRAKDTAVYYCKTGGFWGQGTLV
TVSSEPKSCDKTHTCPPCGGGSSGGGSGGGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK (SEQ ID NO:13)