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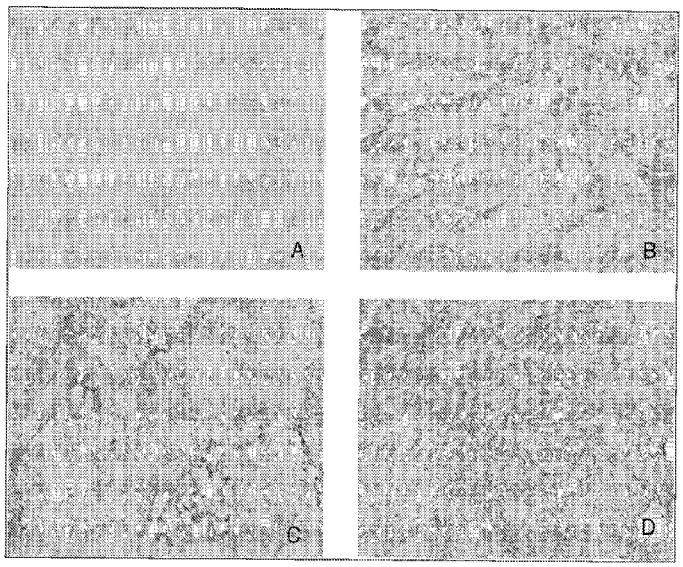

Figure 5

(57) Abstract: The androgen receptor (AR) is the key driver of prostate differentiation and prostate cancer (PC) progression, and androgen ablation is the cornerstone of advanced PC treatment. Prostate-specific membrane antigen (PSMA) represents another target of interest in PC. Previous publications have reported inconsistent associations between androgen levels and PSMA expression. Using a panel of prototypical human PC cell lines, this relationship is clarified. PSMA is a biomarker that distinguishes AR-positive/PSMA-positive adenocarcinomas from AR- negative variants. PSMA is a cell surface barometer of androgen activity that can be readily identified by immunohistochemistry and/or in vivo imaging. Given that anti-androgen therapy is likely to remain a cornerstone of PC treatment, the associated up-regulation of PSMA, as well as its other characteristics, makes it a compelling target opportunity in PC.

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PSMA AS A BIOMARKER FOR ANDROGEN ACTIVITY IN PROSTATE CANCER

BACKGROUND

[0001] The androgen receptor (AR) is the key driver of prostate epithelial differentiation and prostate cancer (PC) progression, and androgen ablation is the cornerstone of advanced PC treatment. Recently, more potent anti-androgenic agents capable of virtual complete suppression of endocrine and intracrine androgen synthesis and signaling have demonstrated clinical benefit (Morris *et al.*, JCO 2010; 28(9): 1496-1501; and Scher *et al.* Lancet 2010; 375:1437-46). But many patients manifest *de novo* or acquired resistance to these therapies, suggesting the continuing need to develop additional therapeutic targets and agents. And currently, the only biomarker utilized to measure androgen action is serum prostate specific antigen (PSA); there is no way to measure androgen axis activity at the level of the cell or lesion *in vivo*.

[0002] Prostate-specific membrane antigen (PSMA) represents another molecule of interest in PC. PSMA has many features that make it an attractive and valuable target: (1) its expression is highly specific for prostatic epithelium; (2) it is significantly up-regulated in PC (Israeli *et al.*, Can Res 1994; 54:1807-11; Wright *et al.*, Urol Oncol 1995; 1:118-28; Troyer *et al.*, Int J Can 1995; 62: 552-58; and Sokoloff *et al.*, The Prostate 2000; 4: 3150-57); (3) it is expressed by virtually all PCs (Wright *et al.*, Urol Oncol 1995; 1:118-28; Sweat *et al.*, Urol 1998; 52: 637-40; Bostwick *et al.*, Cancer 1998; 82: 2256-61; Mannweiler *et al.*, Pathol Oncol Res 2009; 15: 167-72; Kusumi *et al.*, Pathol Int 2008; 58: 687-94; Ananias *et al.*, Prostate 2009; 69: 1101-8); (4) its expression increases directly with tumor grade and clinical aggressiveness (Wright *et al.*, Urol Oncol 1995; 1:118-28); and (5) it functions as an internalizing cell surface receptor. Previous publications have run the gamut with respect to showing an association between androgen levels and PSMA expression. On the one hand, Israeli (Israeli *et al.*, Can Res 1994; 54:1807-11) noted PSMA down-regulation in the LNCaP cell line in the presence of

androgens and Wright (Wright *et al.*, Urology 1996; 48:326-334) found that 55% (11 of 20) of primary PCs expressed higher levels of PSMA after hormonal therapy. On the other hand, Chang reported no increase in PSMA expression when comparing prostatectomy specimens from patients undergoing 3 months of neo-adjuvant androgen ablation versus those who did not (Chang *et al.*, Cancer 2000; 88: 407–415). Kusumi reported that PSMA expression was decreased by hormonal therapy and, noting the conflicting literature, suggested further study was necessary (Kusumi *et al.*, Pathol Int 2008; 58: 687-94).

[0003] Given that androgen inhibition will almost certainly remain a critical component of any PC therapy plus previous observations of a possible relationship between androgen activity and PSMA expression, clarification of this relationship was needed.

BRIEF SUMMARY OF THE INVENTION

[0004] When a panel of prototypical human PC cell lines was examined, PSMA expression consistently co-typed with those lines that were AR-positive, some of which also expressed PSA. Two prototypic AR-negative lines, PC3 and DU145, were PSMA-negative and PSA-negative. In this panel of cell lines, PSMA was tightly and more faithfully linked to AR expression than PSA. This suggests that classical prostatic adenocarcinoma bears the phenotype of AR^{+}, $PSMA^{+}$, $PSA^{+/-}$ whereas the triple-negative (AR^{-}, $PSMA^{-}$, PSA^{-}) phenotype is characteristic of small cell or other variants (Tai *et al.*, Prostate 2011; 71:1668-1679; and Beltran *et al.*, Cancer Discovery 2011; 1:487-495). This *in vitro* finding is consistent with multiple publications that report PSMA expression by approximately 95% of PC cases (Wright *et al.*, Urol Oncol 1995; 1:118-28.; Sweat *et al.*, Urol 1998; 52: 637-40; Bostwick *et al.*, Cancer 1998; 82: 2256-61; Mannweiler *et al.*, Pathol Oncol Res 2009; 15: 167-72; Kusumi *et al.*, Pathol Int 2008; 58: 687-94; and Ananias *et al.*, Prostate 2009; 69: 1101-8), reflecting the known preponderance of adenocarcinomas relative to small cell variants. PSMA, therefore, is a

biomarker that can be easily identified by immunohistochemistry, circulating tumor cell (CTC) analysis, and/or *in vivo* imaging to identify and distinguish AR-positive /PSMA-positive adenocarcinomas from AR-negative variants.

[0005] It was discovered that androgen withdrawal from AR-positive lines, in all cases, led to as much as a 10-fold increase in PSMA expression relative to its level in physiological concentrations of DHT. Similarly, silencing the AR gene, in all cases, led to increased PSMA expression whereas increasing AR expression via transfection led to decreased PSMA expression. These findings suggest that the effect of androgen withdrawal on PSMA is mediated via AR and that PSMA is an AR-regulated (repressed) gene. It is likely that liganded AR suppresses PSMA expression via its known binding to regulatory sequences of the PSMA gene (Noss *et al.*, Gene 2002; 285(1-2):247-256; Watt *et al.*, Genomics 2001; 73:243–54; and Evans et al., PNAS 2011; 108:9578-9582). If AR activity is decreased due to pathophysiologic or pharmacologic reasons, the PSMA gene is de-repressed. Nevertheless, while the directional changes in PSMA expression associated with changes in androgen level were identical among the different lines, the various cell lines expressed widely different levels of PSMA even under identical concentrations of DHT. This suggests that PSMA expression is not a first order stoichiometric event based purely on androgen level but other factors are involved. PSMA, nevertheless, represents a useful cellular biomarker to aid in interrogating AR gene regulation. A static reading of PSMA level will be less informative than a comparison of readings pre- and post- therapeutic intervention. Importantly, our findings indicate that the time to peak PSMA expression is approximately 2 weeks after complete hormonal withdrawal. Use of shorter intervals for assessment may cause underestimation of actual hormonal effects. The nature of the therapeutic intervention itself may effect the time to peak expression and should be determined for each form of intervention.

[0006] PSA and PSMA both represent biomarkers of androgen activity, albeit the former is stimulated while the latter is suppressed by androgens. In addition, while PSA may be sampled in plasma or serum and represents the average output of all lesions, the absolute level as well as changes in PSMA expression can be used as a pharamcodynamic biomarker of androgen activity at the level of the individual cell or lesion. For example, *ex vivo* analysis of captured CTCs or *in vivo* patient imaging with PSMA-targeted agents can identify PSMA up-regulation indicating suppression of androgen activity (Evans *et al.*, PNAS 2011; 108:9578-9582) or *vice versa*. Lack of PSMA would suggest a non-adenocarcinoma variant particularly if found in association with a low/absent PSA and direct therapies away from hormonal manipulation to more appropriate approaches. Clinical trials using ^{89}Zirconium-J591, a PSMA-targeted immunoPET agent capable of quantitative reporting of PSMA levels *in vivo*, would corroborate these findings (Holland *et al.*, J Nucl Med 2010; 51: 1293-1300).

[0007] Lastly, the obligate expression of AR in prostate adenocarcinomas, the tight linkage between AR and PSMA expression, the central role of anti-androgen therapy in PC coupled with the effect of anti-androgen therapy to increase PSMA expression make AR and PSMA a compelling coordinate target combination. This paradigm may be incorporated into PSMA-targeted antibody therapy trials.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] **Figure 1: Co-expression of PSMA and AR by cell lines.** Eight cell lines were evaluated by western blot for the expression of PSMA and AR. Six of these lines were $AR^+/PSMA^+$; 1 cell line (PC3) was $AR^-/PSMA^-$. One line (PC3-PSMA) represents the PC3 line transfected with PSMA (Stephan, M.T. et al, Nat Med 2007, 13:1440-1449) was $AR^-/PSMA^+$. DU145 (not shown) was also $AR^-/PSMA^-$.

[0009] **Figure 2: Androgen axis activity regulates PSMA expression.** Effect of charcoal-stripped FCS (CS-FCS). With the LAPC-4 PC cell line (wild-type AR), a western blot shows PSMA was up-regulated 5.7-fold when grown in charcoal-stripped FCS medium relative to medium supplemented with FCS plus physiological levels of DHT (Figure 2A). Similarly, charcoal-stripping the growth media of LNCaP and MDA-PCa-2b (both with mutated AR) cell lines, led to 7-8-fold up-regulation of PSMA that peaked at 2 weeks (Figures 2B and 2C). Figure 2B shows that the level of PSMA up-regulation is minimal at 1 week in relation to 2 weeks. The dose-response of PSMA expression relative to media steroid concentration is shown in Figure 2D. As measured by FACS mean fluorescence intensity (MFI), PSMA expression increases approximately linearly relative to decreasing concentration of steroids in the growth medium.

[0010] **Figures 3 and 4: PSMA expression is inversely related to AR level.** Transfection of the AR gene into LNCaP to over-express AR (*i.e.*, LNCaP-AR) leads to down-regulation of PSMA by approximately 80% (Figure 3A). Conversely, silencing AR with siRNA led to a dose-dependent up-regulation of PSMA in all 4 cell lines tested (LNCaP, CWR22Rv1, MDA-Pca-2b and LAPC-4) (Figures 3B and 3C, and Figure 4A-4C). Untreated (control) and non-targeted siRNA were superimposable. The AR siRNA showed successful down-regulation of AR and up-regulation of PSMA in all cell lines: LNCaP (Figure 4A), MDA-PCa-2B (Figure 4B) and LAPC-4 (Figure 4C). Silencing AR led to a significant decrease in PSA secretion as expected (data not shown).

[0011] Figure 5: Immunohistochemical assessment of PSMA expression before and after castration. CWR22Rv1 xenografts growing in intact, androgen-replete nu/nu mice demonstrated low level expression of PSMA (Figure 5a), consistent with *in vitro* findings (*see* Figure 1, lane 5). Subsequent to surgical castration, the levels of PSMA expression rose progressively, consistent with *in vitro* findings, at 1, 2, and 4 weeks of observation (Figure 5b-d, respectively). Similarly, immunohistochemistry reveals significantly elevated PSMA expression in benign prostatic resections of patients treated with 5-α reductase therapy relative to untreated patients (data not shown).

EXAMPLES

Expression of PSMA and AR by Eight cell lines

[0012] For western blots, equal amounts of cell lysates were loaded in each lane. PSMA was detected by monoclonal antibody (mAb) J591; AR was detected by mAb anti-AR (AR441). GAPDH was used as a loading control. Six of the cell lines (MDA-PCa-2b, LNCaP, LNCaP-AR, VCAP, CWR22Rv1 and LAPC-4) expressed both AR and PSMA. MDA-PCa-2b expresses a relatively low level of AR, just barely visible at this exposure. CWR22Rv1 expresses a slightly larger AR protein (114 KD, instead of 110 KD) due to duplication of exon 3). PC3 and another cell line, DU145 (separate gel, not shown), were $AR^-/PSMA^-$. PC3-PSMA is the PC3 line stably transfected with PSMA.

[0013] Human prostate cancer cell lines, LNCaP, CWR22Rv1, MDA-PCa-2b and LAPC-4 were purchased from American Type Culture Collection (Manassas, VA). LNCaP/AR and PC3-PSMA were gifts from Charles Sawyers and Michel Sadelain, respectively (MSKCC, NY). LNCaP, LNCaP/AR, and CWR22Rv1 cells were maintained in RPMI1640 medium supplemented with 2 mM L-glutamine (Invitrogen, Carlsbad, CA), 1% penicillin-streptomycin

(Invitrogen), and 10% heat-inactivated fetal calf serum (FCS) (Invitrogen). MDA-PCa-2b cells were grown in F12K medium containing 2 mM L-glutamine, 1% penicillin-streptomycin, 20% heat-inactivated FCS, 25 ng/mL cholera toxin (Sigma-Aldrich, St. Louis, MO), 10 ng/mL epidermal growth factor (BD Biosciences, San Jose, CA), 5 μM phosphoethanolamine (Sigma-Aldrich), 100 pg/mL hydrocortisone (Sigma-Aldrich), 45 nM selenious acid (Sigma-Aldrich), and 5 μg/mL insulin (Sigma-Aldrich). LAPC-4 cells were maintained in IMDM medium supplemented with 2 mM L-glutamine, 1% penicillin-streptomycin and 5% heat-inactivated FCS. All cell lines were kept at 37°C in a 5% CO2 atmosphere. The 5α-dihydrotestosterone (DHT) was purchased from Wako Chemical USA (Richmond, VA).

[0014] MAb anti-PSMA J591 was generated (Liu *et al.*, Cancer Res 1997; 57: 3629-34). Additional antibody (Ab) reagents included: mAb anti-AR (AR441), Rabbit anti-Human AR and goat polyclonal anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA), and mAb anti-PSA (Dako, Glostrup, Denmark). Mouse mAb anti-human beta-Actin was purchased from Thermo Scientific (Rockford, IL).

[0015] In order to run Western Blots, cells were lysed with Cell Lysis Buffer (Cell Signaling Technology, Danvers, MA) containing 1mM phenylmethylsulphonyl fluoride (EMD Chemicals, Gibbstown, NJ). Equal amounts of protein were applied in each well on a 10% Tris-HCl gel (Bio-Rad Laboratories, Hercules, CA). The proteins were transferred onto Immobilon-P Membranes (Millipore, Billerica, MA), after which the filters were probed with the following reagents: murine anti-PSMA mAb J591, murine mAb anti-AR (AR441), rabbit anti-human AR, murine mAb anti-human beta-actin, and/or goat polyclonal anti-GAPDH. For quantitative western blots, the Li-cor Odyssey Infrared Imaging System (Lincoln, Nebraska) was used. With this system, two different proteins of the same molecular weight (*e.g.*, PSMA and AR) can be

detected simultaneously and quantified on the same blot using two different antibodies from two different species (mouse and rabbit) followed by detection with two IRDye labeled secondary antibodies. Anti-beta-actin is used as a loading reference. Millipore Immobilon-FL PVDF membranes were used following Licor's recommendations. MuJ591 anti-PSMA 1 ug/ml, rabbit anti-human AR 1: 500 and mouse anti-human beta-actin 1:10,000 in dry milk/PBST were combined and incubated simultaneously with the membranes for 1 hour. After washing, IRDye 800CW-goat anti-mouse secondary antibody (1:10,000) and IRDye 680LT-goat anti-rabbit secondary antibody (1:20,000) in 5% dry milk/PBST were combined and incubated simultaneously with the membranes. After washing, the membranes were scanned and the bands were quantified with the Odyssey Infrared Imaging System.

Androgen withdrawal up-regulates PSMA expression

[0016] LAPC-4, expressing wild-type AR, grown in physiological levels of DHT (10-20 nM) expresses a low level of PSMA (Lanes 1 and 2) (Figure 2A). When grown in standard 5% FCS which contains very low levels of androgens, PSMA expression increases 3.6-fold (Lane 3). When the FCS is charcoal-stripped of all steroids, PSMA level rises further, by 5.7-fold (Lane 4) that seen with physiological levels of DHT. FACS analysis demonstrates that LNCaP and MDA-PCa-2b, both with mutated AR, have elevated PSMA levels at baseline in standard FCS-supplemented medium (Figures 2B and 2C). Use of charcoal-stripped FCS further up-regulates PSMA 7-9-fold, peaking at 2 weeks. The lower cell number at 3 weeks reflects cell loss from steroid starvation. Dose response of PSMA expression by LNCaP cells grown for 2 weeks with varying levels of androgens (Figure 2D). Progressive steroid deprivation progressively leads to an increase in PSMA of 5.4-fold.

PSMA expression level is inversely related to AR level

[0017] Transfection of AR into LNCaP (LNCaP-AR) results in down-regulation of PSMA expression by approximately 80% as measured by FACS (Figure 3A). Conversely, AR-siRNA treatment silences AR and up-regulates PSMA expression in LNCaP and CWR22Rv1 at 48 hours (Figure 3B) and in MDA-PCa-2b and LAPC-4 cells at 4 days (Figure 3C).

Silencing AR up-regulates PSMA

[0018] FACS analysis of LNCaP (Figure 4A), MDA-PCa-2b, (Figure 4B) and LAPC-4 cells (Figure 4C) treated with AR-siRNA (blue line), non-targeted-siRNA (red line) and untreated control (green line) was conducted. The gray histogram is secondary antibody-only negative control. In all cases, AR-siRNA silenced AR and up-regulated PSMA; the non-targeted-siRNA control did not affect expression of either AR or PSMA.

[0019] Short interfering RNA (siRNA) duplexes specific to AR as well as non-targeting siRNA (NT-siRNA) were purchased from Dharmacon (Lafayette, CO). The AR-specific siRNA (AR-siRNA) sequence corresponds to the human AR site 5'- GACUCAGCUGCCCCAUCCA - 3'. A NT-siRNA (5'-CCUACGCCACCAAUUUCGU - 3') was used as a control for the siRNA experiments. Following overnight incubation of the suspended cells transfected with varying doses of NT-siRNA or AR-siRNA using Lipofectamine RNAiMAX Reagent (Invitrogen) according to the manufacturer's instructions, media were changed with fresh media and the cells were incubated for the time indicated.

[0020] LNCaP, MDA-PCa-2b and LAPC-4 cells were seeded in 6-well plates (1 x 105/well), grown overnight, and collected after trypsinization. Immediately after 30-min fixation with PBS containing 2% paraformaldehyde, the cells were incubated with murine anti-AR or

anti-PSMA mAb in phosphate buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 0.1% saponin (Sigma) for 1 hour, and then the cells were treated with fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse IgG (H+L, Jackson ImmunoResearch, West Grove, PA) antibody for 1 hour. After washing with PBS containing 1% BSA + 0.1% saponin, the cells were subjected to fluorescence-activated cell sorting analysis (FACS) (Becton Dickinson, Franklin Lakes, NJ).

Immunohistochemical assessment of PSMA expression before and after castration

[0021] Immunohistochemistry assessment of PSMA expression before and after castration. Baseline PSMA expression of CWR22Rv1 xenograft prior to castration (Figure 5A). PSMA expression 1 week (Figure 5B), 2 weeks (Figure 5C) and 4 weeks (Figure 5D) post-castration.

[0022] CWR22Rv1 xenografts were removed from nude mice. Tumors were pre-cooled in liquid nitrogen, snap-frozen in OCT compound (Sakura Finetek U.S.A., inc., Torrance, CA) on dry ice, and stored at -80°C. Cryostat tissue sections were fixed in cold acetone (4°C) for 10 minutes. The sections were washed in PBS. Peroxidase block (0.03%H_2O_2) was incubated for 5 minutes. After washing in PBS, humanized J591 (10 ug/ml) was incubated on the sections for 1 hour at room temperature. Antibody binding was detected using rabbit anti-human Ig-peroxidase (Dako, Carpinteria, CA) secondary antibody and diaminobenzidine (sigma-Aldrich Co., St. Louis, MO) as chromogen. The sections were counterstained with 10% Hematoxylin. The diluent (1% bovine serum albumin) was used as negative control.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0023] One aspect of the technology is a method of treating prostate cancer in a patient comprising the steps of: (a) assaying a patient's prostate-specific membrane antigen (PSMA)

expression; (b) determining, from the assay, if the patients' PSMA expression is indicative of a prostate cancer adenocarcinoma or non-adenocarcinoma; and (c) administering, to the patient, (i) an anti-androgen therapy if the PSMA expression is indicative of an adenocarcinoma or (ii) a chemotherapeutic therapy if the PSMA expression is indicative of a non-adenocarcinoma. In a related aspect, the PSMA expression is assayed by imaging. In another aspect, the imaging is conducted by employing any agent capable of specific binding to PSMA. In a related aspect, the agent is an antibody, antibody derivative, PSMA ligand, small molecule PSMA binder, PSMA enzyme inhibitor, PSMA-binding peptide, or PSMA-binding aptamer. In yet another aspect of the technology, the imaging is done by positron emission tomography (PET), PET/Computed tomography (CT), PET/Magnetic resonance (MR), planar imaging, SPECT imaging, optical imaging, or dye imaging. In one aspect of this technology, the non-adenocarcinoma comprises a prostate small cell, neuroendocrine, or sarcoma.

[0024] Another aspect of the technology is a method of treating prostate cancer in a patient comprising the steps of: (a) obtaining a first measurement of a patient's prostate-specific membrane antigen (PSMA) level prior to administering a new prostate cancer therapy with anti-androgen activity; (b) obtaining a second measurement of the patient's PSMA level after administering the new prostate cancer therapy; and (i) continuing the therapy if the second measurement is greater than the first measurement or (ii) discontinuing the therapy if the second measurement is less than or equal to the first measurement. In a related aspect, the time interval between obtaining the first and second measurements is about 2 to 4 weeks. In another aspect, the method further comprises obtaining a third measurement of the patient's PSMA level after administering the new prostate cancer therapy; and (i) continuing the therapy if the third measurement is greater than the first measurement or (ii) discontinuing the therapy if the third measurement is less than or equal to the first measurement. In a related aspect of the technology,

the first and/or second measurements of a patient's PSMA level are assayed by imaging. In one aspect, the imaging is conducted by employing any agent capable of specific binding to PSMA. In another aspect, the agent is an antibody, antibody derivative, PSMA ligand, small molecule PSMA binder, PSMA enzyme inhibitor, PSMA-binding peptide, or PSMA-binding aptamer. In one aspect of the technology, the imaging is done by positron emission tomography (PET), PET/Computed tomography (CT), PET/Magnetic resonance (MR), planar imaging, SPECT imaging, optical imaging, or dye imaging.

[0025] Yet another aspect of the technology is a method of treating prostate cancer in a patient comprising the steps of: (a) obtaining a measurement of a non-castrated patient's prostate-specific membrane antigen (PSMA) level; and (b) administering, to the patient, a prostate cancer hormonal therapy if the measurement is not elevated and is indicative of normal androgen axis function or seeking an alternative treatment for the patient if the measurement is elevated and is indicative of abnormal androgen axis function. In one aspect, the measurement of a non-castrated patient's PSMA level is assayed by imaging. In another aspect, the imaging is conducted by employing any agent capable of specific binding to PSMA. In yet another aspect, the agent is an antibody, antibody derivative, PSMA ligand, small molecule PSMA binder, PSMA enzyme inhibitor, PSMA-binding peptide, or PSMA-binding aptamer. In a one aspect, the imaging is done by positron emission tomography (PET), PET/Computed tomography (CT), PET/Magnetic resonance (MR), planar imaging, SPECT imaging, optical imaging, or dye imaging.

[0026] One other aspect of the technology is a method of treating prostate cancer in a patient comprising the steps of: (a) administering, to a patient, an anti-androgen prostate cancer therapy; and (b) administering, to the patient, an anti-PSMA prostate cancer therapy subsequent to the anti-androgen prostate cancer therapy, thereby producing a synergistic benefit as a result

of increasing PSMA density and therefore effect of PSMA-targeted agent. In a related aspect, the time interval between administering the anti-androgen prostate cancer therapy and administering the anti-PSMA prostate cancer therapy is about 2 to 4 weeks.

[0027] While the present invention has been disclosed with reference to certain embodiments, numerous modifications, alterations, and changes to the described embodiments are possible without departing from the sphere and scope of the present invention, as defined in the appended claims. Accordingly, it is intended that the present invention not be limited to the described embodiments, but that it has the full scope defined by the language of the following claims, and equivalents thereof.

What is claimed is:

1. A method of treating prostate cancer in a patient comprising the steps of:

(a) assaying a patient's prostate-specific membrane antigen (PSMA) expression;

(b) determining, from the assay, if the patients' PSMA expression is indicative of a prostate cancer adenocarcinoma or non-adenocarcinoma; and

(c) administering, to the patient,

(i) an anti-androgen therapy if the PSMA expression is indicative of an adenocarcinoma or

(ii) a chemotherapeutic therapy if the PSMA expression is indicative of a non-adenocarcinoma.

2. The method of claim 1, wherein the PSMA expression is assayed by imaging.

3. The method of claim 2, wherein the imaging is conducted by employing any agent capable of specific binding to PSMA.

4. The method of claim 3, wherein the agent is an antibody, antibody derivative, PSMA ligand, small molecule PSMA binder, PSMA enzyme inhibitor, PSMA-binding peptide, or PSMA-binding aptamer.

5. The method of claim 2, wherein the imaging is done by positron emission tomography (PET), PET/Computed tomography (CT), PET/Magnetic resonance (MR), planar imaging, SPECT imaging, optical imaging, or dye imaging.

6. The method of any one of claims 1-5, wherein the non-adenocarcinoma comprises a prostate small cell, neuroendocrine, or sarcoma.

7. A method of treating prostate cancer in a patient comprising the steps of:

(a) obtaining a first measurement of a patient's prostate-specific membrane antigen (PSMA) level prior to administering a new prostate cancer therapy with anti-androgen activity;

(b) obtaining a second measurement of the patient's PSMA level after administering the new prostate cancer therapy; and

(i) continuing the therapy if the second measurement is greater than the first measurement or

(ii) discontinuing the therapy if the second measurement is less than or equal to the first measurement.

8. The method of claim 7, wherein the time interval between obtaining the first and second measurements is about 2 to 4 weeks.

9. The method of claim 7, further comprising obtaining a third measurement of the patient's PSMA level after administering the new prostate cancer therapy; and

(i) continuing the therapy if the third measurement is greater than the first measurement or

(ii) discontinuing the therapy if the third measurement is less than or equal to the first measurement.

10. The method of any one of claims 7-9, wherein the first and/or second measurements of a patient's PSMA level are assayed by imaging.

11. The method of claim 10, wherein the imaging is conducted by employing any agent capable of specific binding to PSMA.

12. The method of claim 11, wherein the agent is an antibody, antibody derivative, PSMA ligand, small molecule PSMA binder, PSMA enzyme inhibitor, PSMA-binding peptide, or PSMA-binding aptamer.

13. The method of claim 10, wherein the imaging is done by positron emission tomography (PET), PET/Computed tomography (CT), PET/Magnetic resonance (MR), planar imaging, SPECT imaging, optical imaging, or dye imaging.

14. A method of treating prostate cancer in a patient comprising the steps of:

(a) obtaining a measurement of a non-castrated patient's prostate-specific membrane antigen (PSMA) level; and

(b) administering, to the patient, a prostate cancer hormonal therapy if the measurement is not elevated and is indicative of normal androgen axis function or seeking an alternative treatment for the patient if the measurement is elevated and is indicative of abnormal androgen axis function.

15. The method of claim 14, wherein the measurement of a non-castrated patient's PSMA level is assayed by imaging.

16. The method of claim 15, wherein the imaging is conducted by employing any agent capable of specific binding to PSMA.

17. The method of claim 16, wherein the agent is an antibody, antibody derivative, PSMA ligand, small molecule PSMA binder, PSMA enzyme inhibitor, PSMA-binding peptide, or PSMA-binding aptamer.

18. The method of claim 15, wherein the imaging is done by positron emission tomography (PET), PET/Computed tomography (CT), PET/Magnetic resonance (MR), planar imaging, SPECT imaging, optical imaging, or dye imaging.

19. A method of treating prostate cancer in a patient comprising the steps of:

(a) administering, to a patient, an anti-androgen prostate cancer therapy; and

(b) administering, to the patient, an anti-PSMA prostate cancer therapy subsequent to the anti-androgen prostate cancer therapy, thereby producing a synergistic benefit as a result of increasing PSMA density and therefore effect of PSMA-targeted agent.

20. The method of claim 19, wherein the time interval between administering the anti-androgen prostate cancer therapy and administering the anti-PSMA prostate cancer therapy is about 2 to 4 weeks.

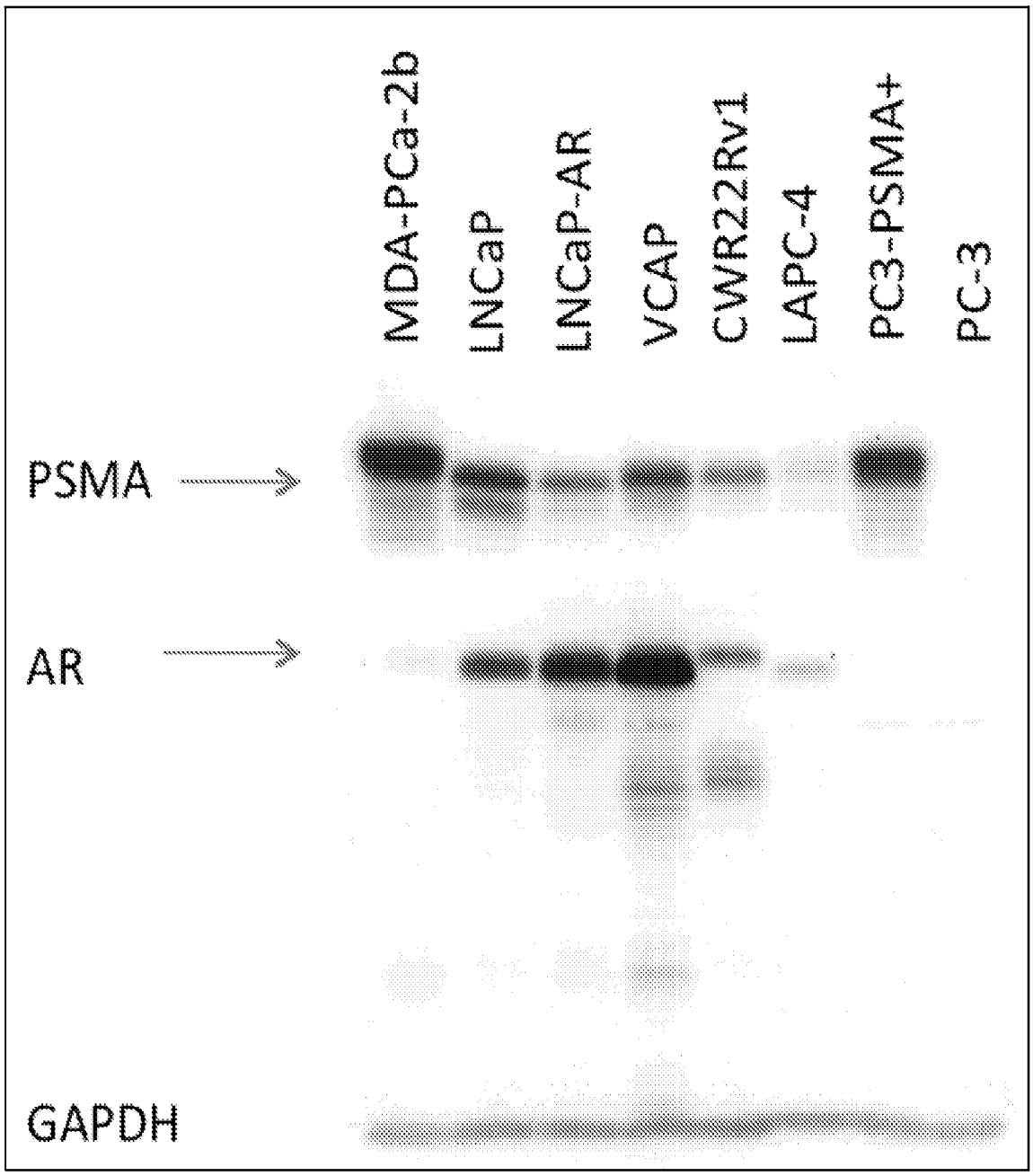

MDA-PCa-2b
LNCaP
LNCaP-AR
VCAP
CWR22Rv1
LAPC-4
PC3-PSMA+
PC-3
PSMA
AR
GAPDH

Figure 1

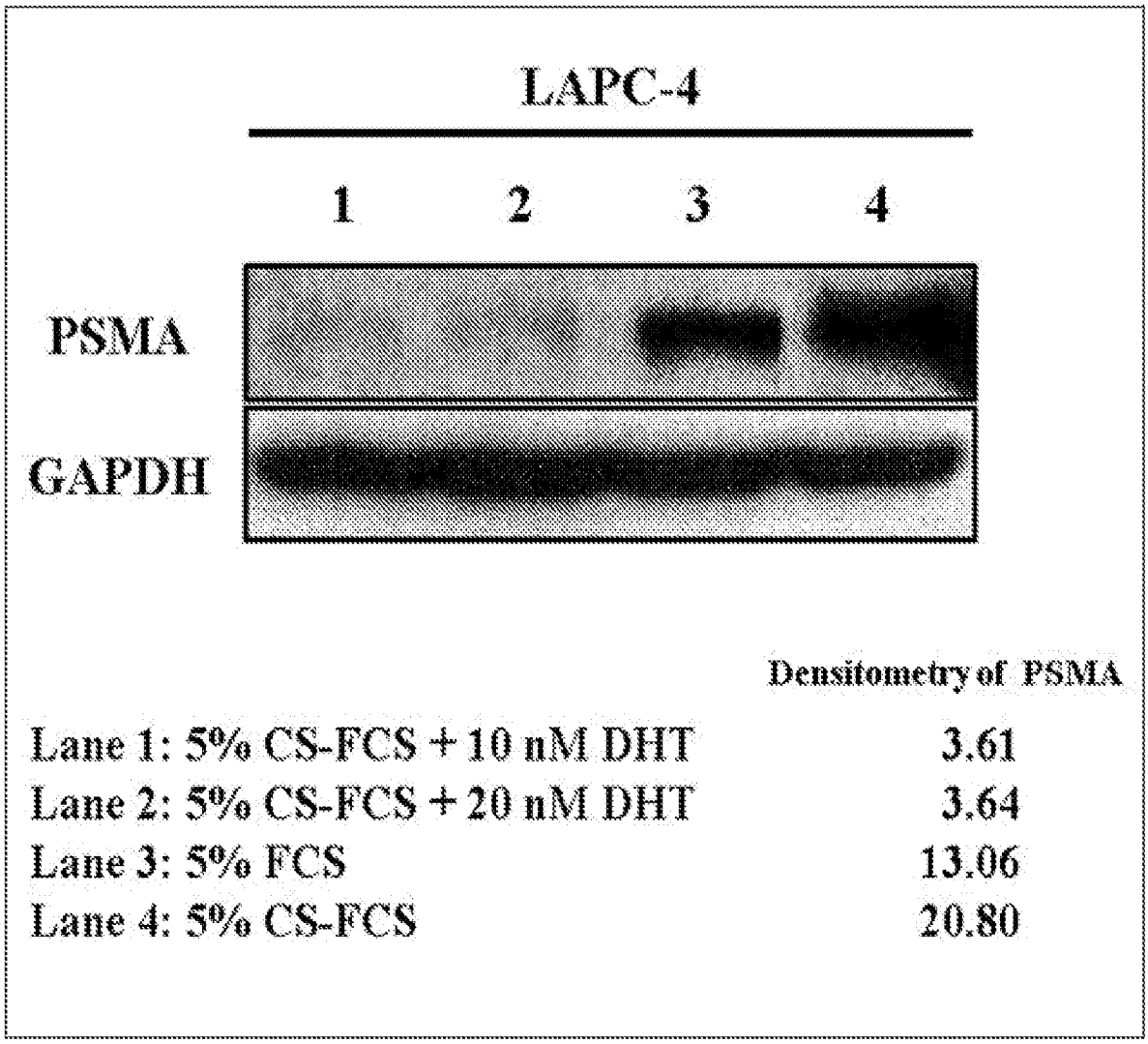

LAPC-4
1
2
3
4
PSMA
GAPDH
Densitometry of PSMA
Lane 1: 5% CS-FCS + 10 nM DHT
3.61
Lane 2: 5% CS-FCS + 20 nM DHT
3.64
Lane 3: 5% FCS
13.06
Lane 4: 5% CS-FCS
20.80

Figure 2A

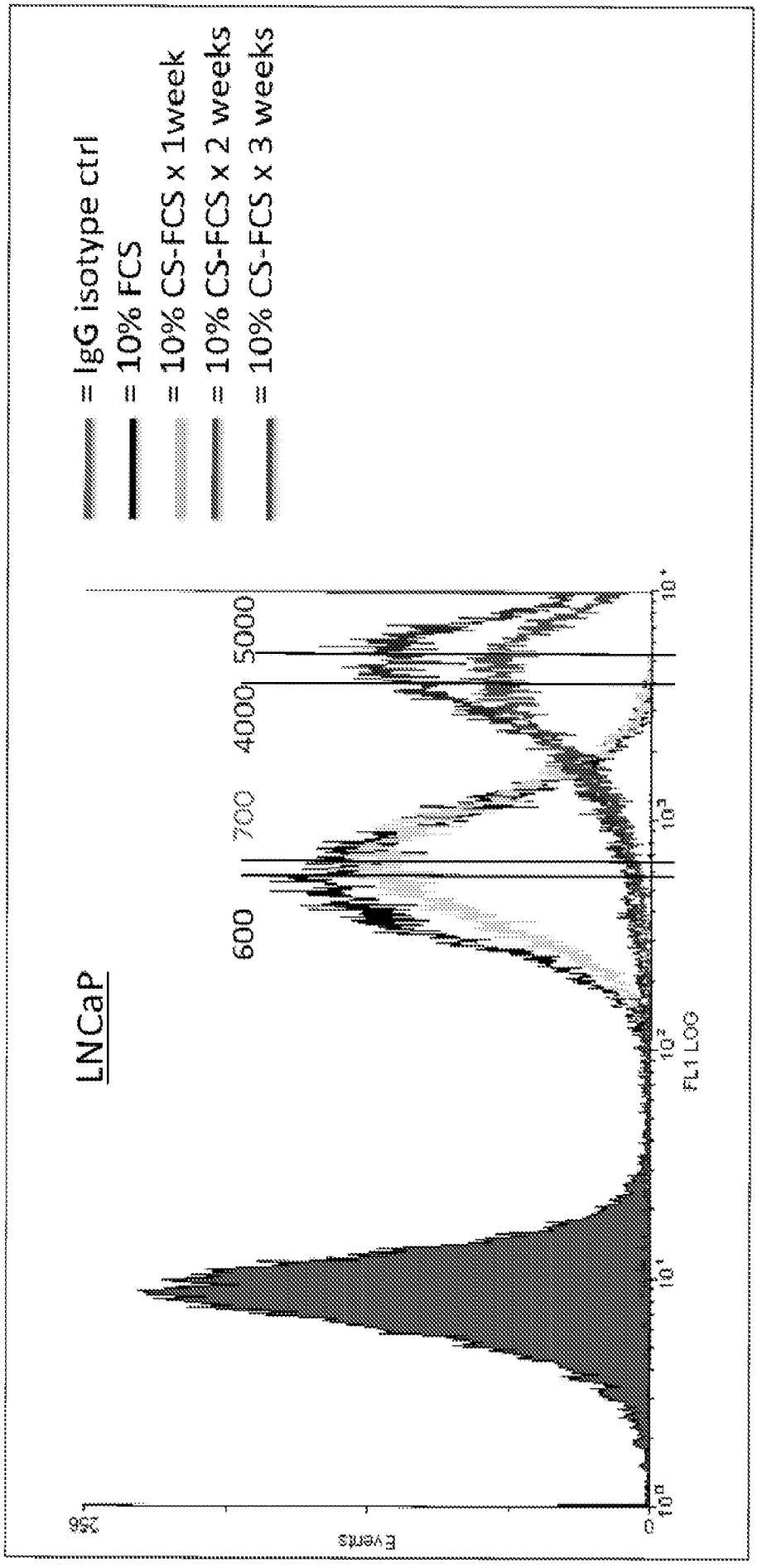

= IgG isotype ctrl
= 10% FCS
= 10% CS-FCS x 1week
= 10% CS-FCS x 2 weeks
= 10% CS-FCS x 3 weeks
LNCaP
600
700
4000
5000
Events
FL1 LOG

Figure 2B

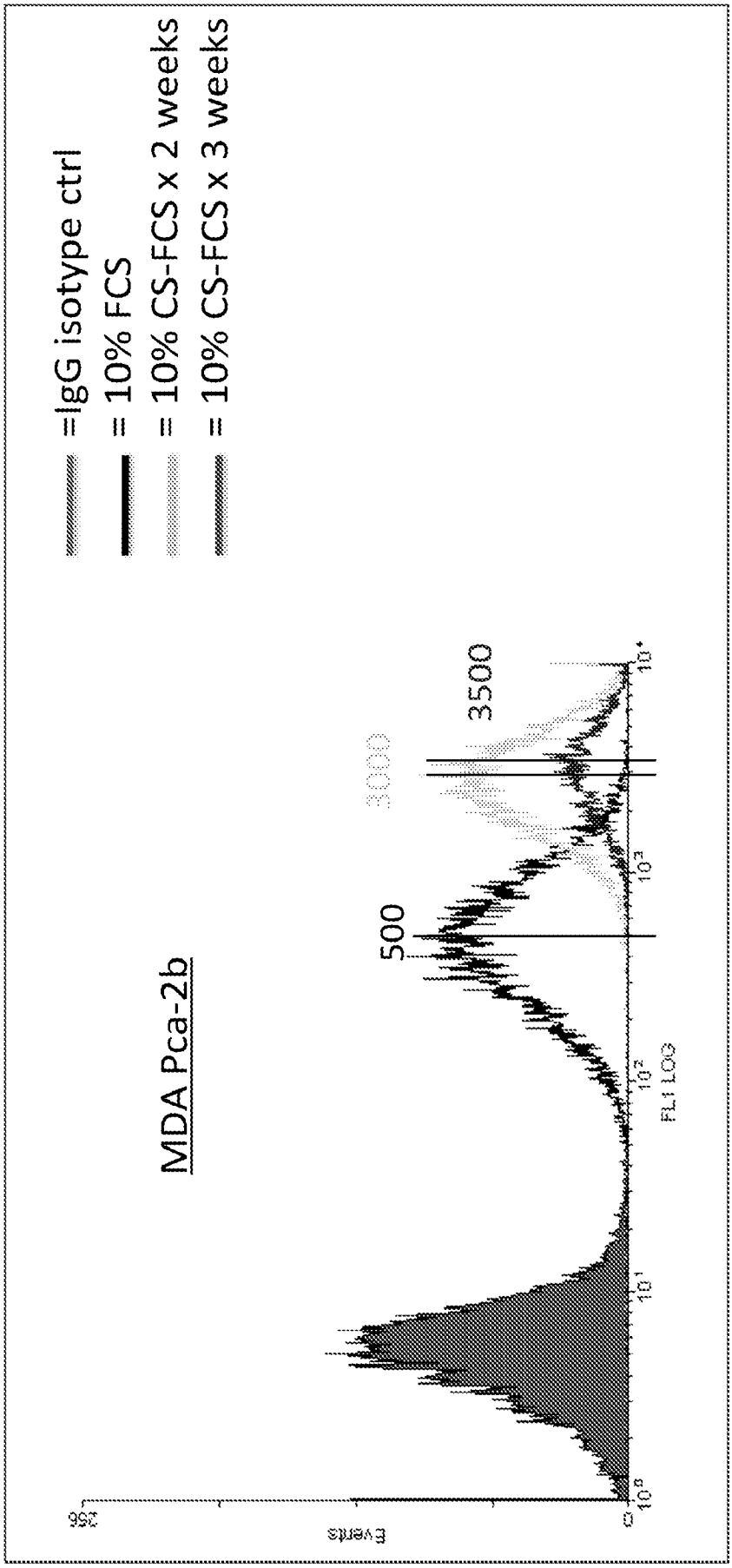
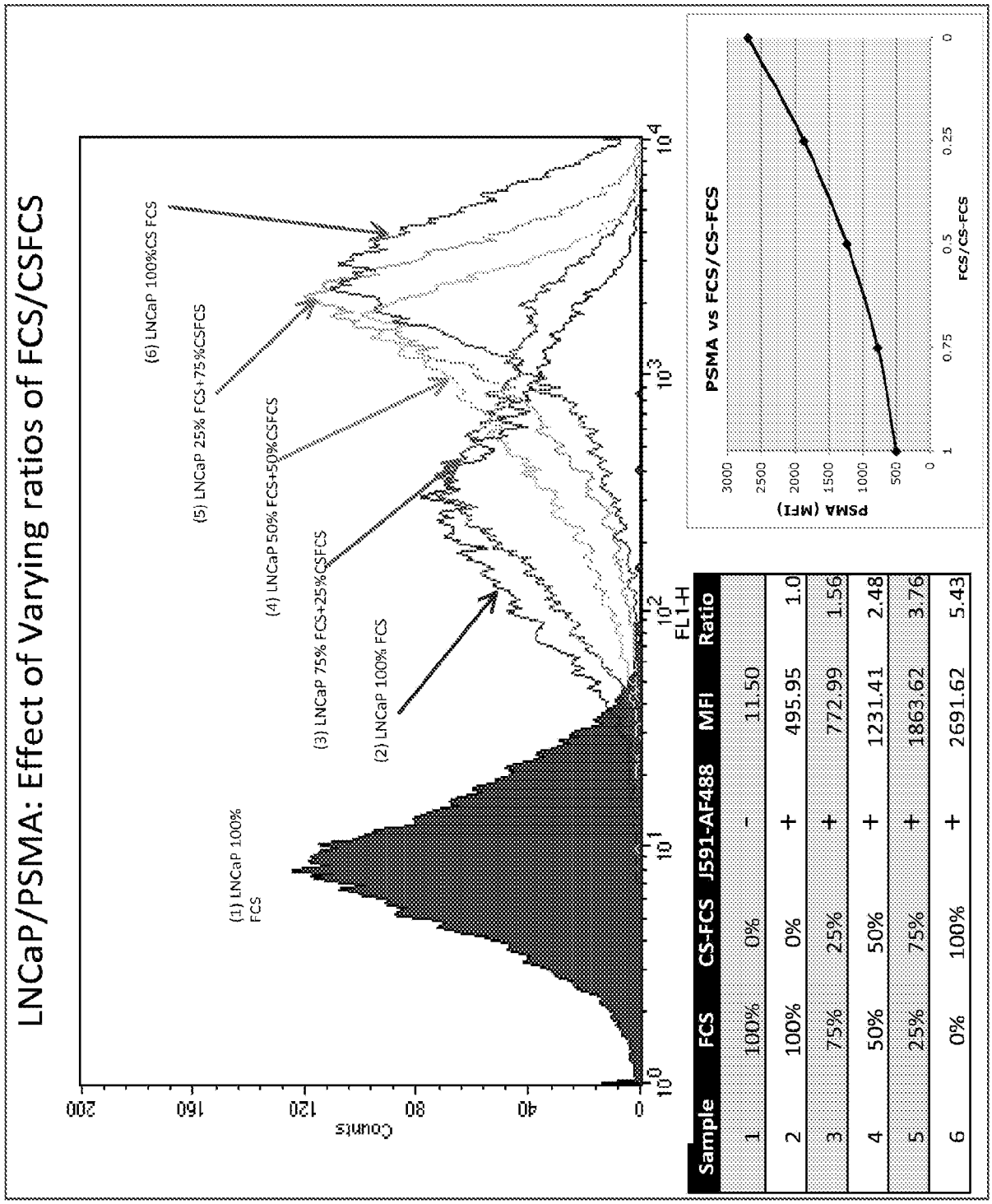

=IgG isotype ctrl
= 10% FCS
= 10% CS-FCS x 2 weeks
= 10% CS-FCS x 3 weeks
MDA Pca-2b
500
3000
3500
Events
FL1 LOG
0
256

Figure 2C



Sample	FCS	CS-FCS	J591-AF488	MFI	Ratio
1	100%	0%	-	11.50	
2	100%	0%	+	495.95	1.0
3	75%	25%	+	772.99	1.56
4	50%	50%	+	1231.41	2.48
5	25%	75%	+	1863.62	3.76
6	0%	100%	+	2691.62	5.43

Figure 2D

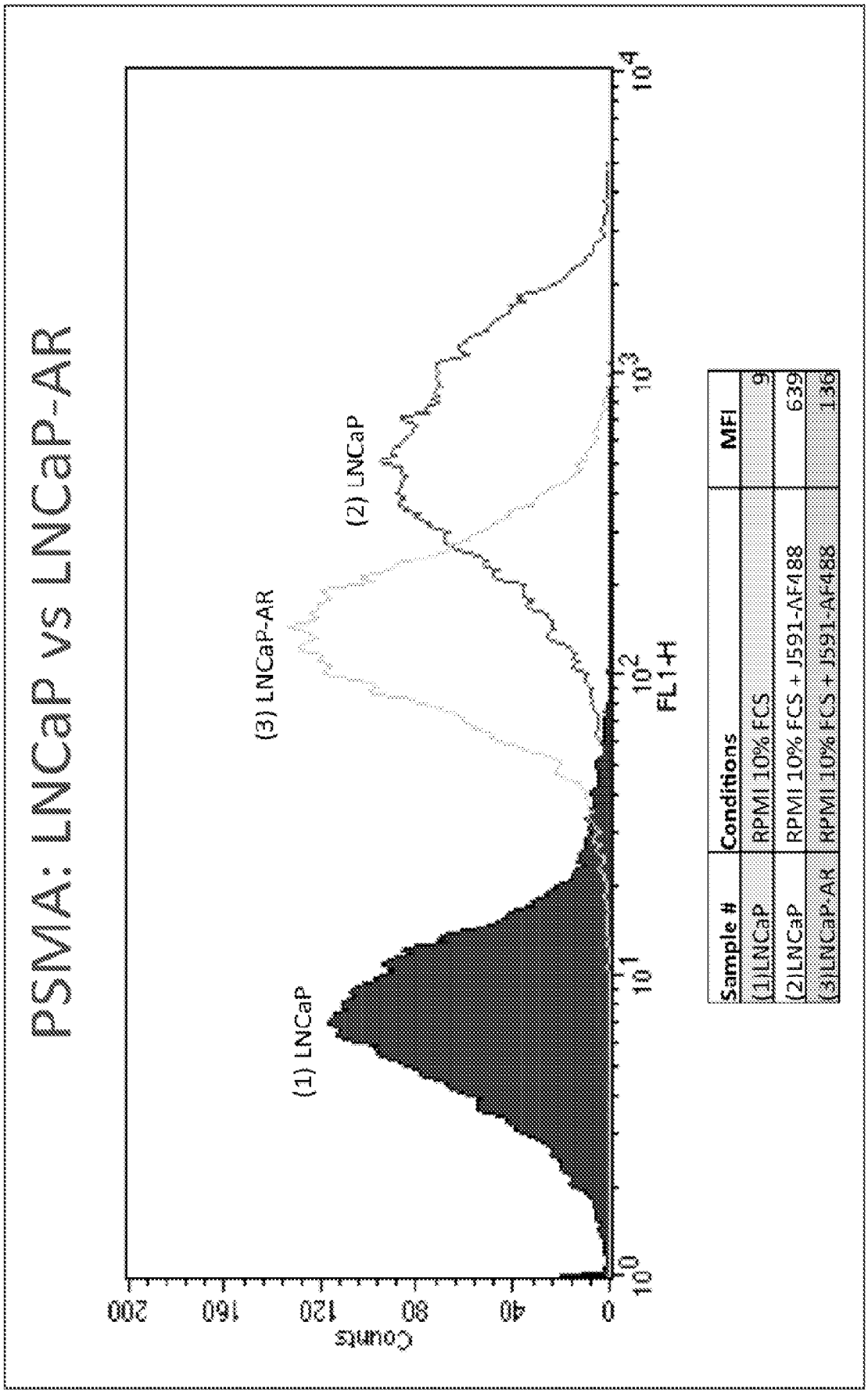

PSMA: LNCaP vs LNCaP-AR
(1) LNCaP
(2) LNCaP
(3) LNCaP-AR
Counts
FL1-H
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4
Sample # | Conditions | MFI
(1)LNCaP | RPMI 10% FCS | 9
(2)LNCaP | RPMI 10% FCS + J591-AF488 | 639
(3)LNCaP-AR | RPMI 10% FCS + J591-AF488 | 136

Figure 3A

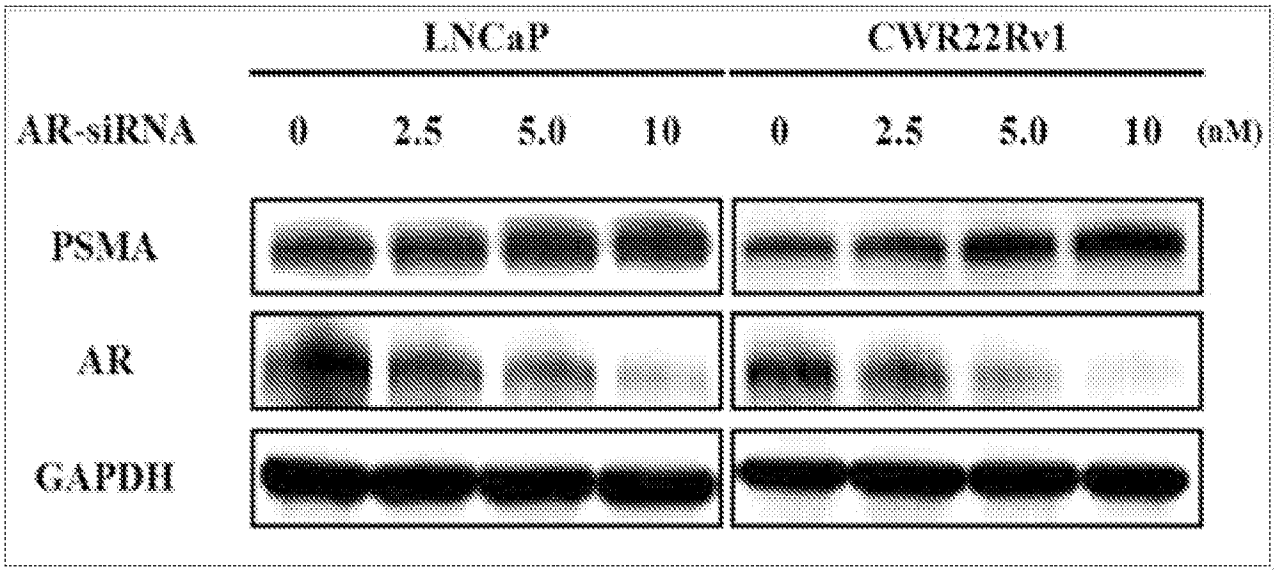


Figure 3B

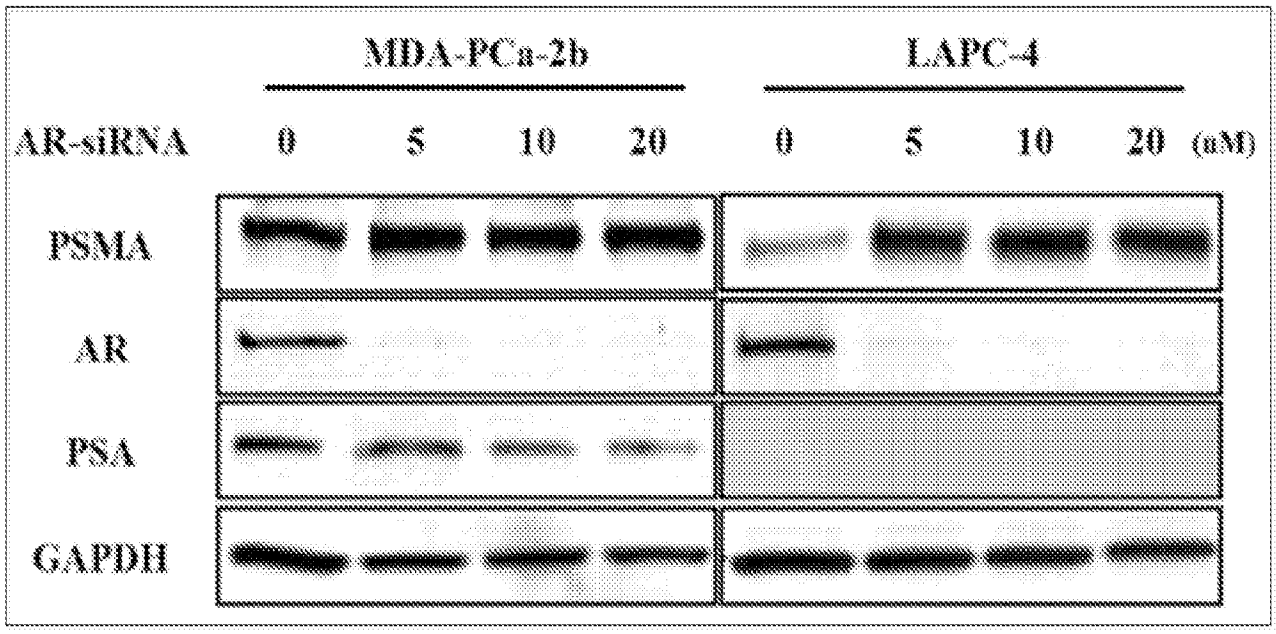
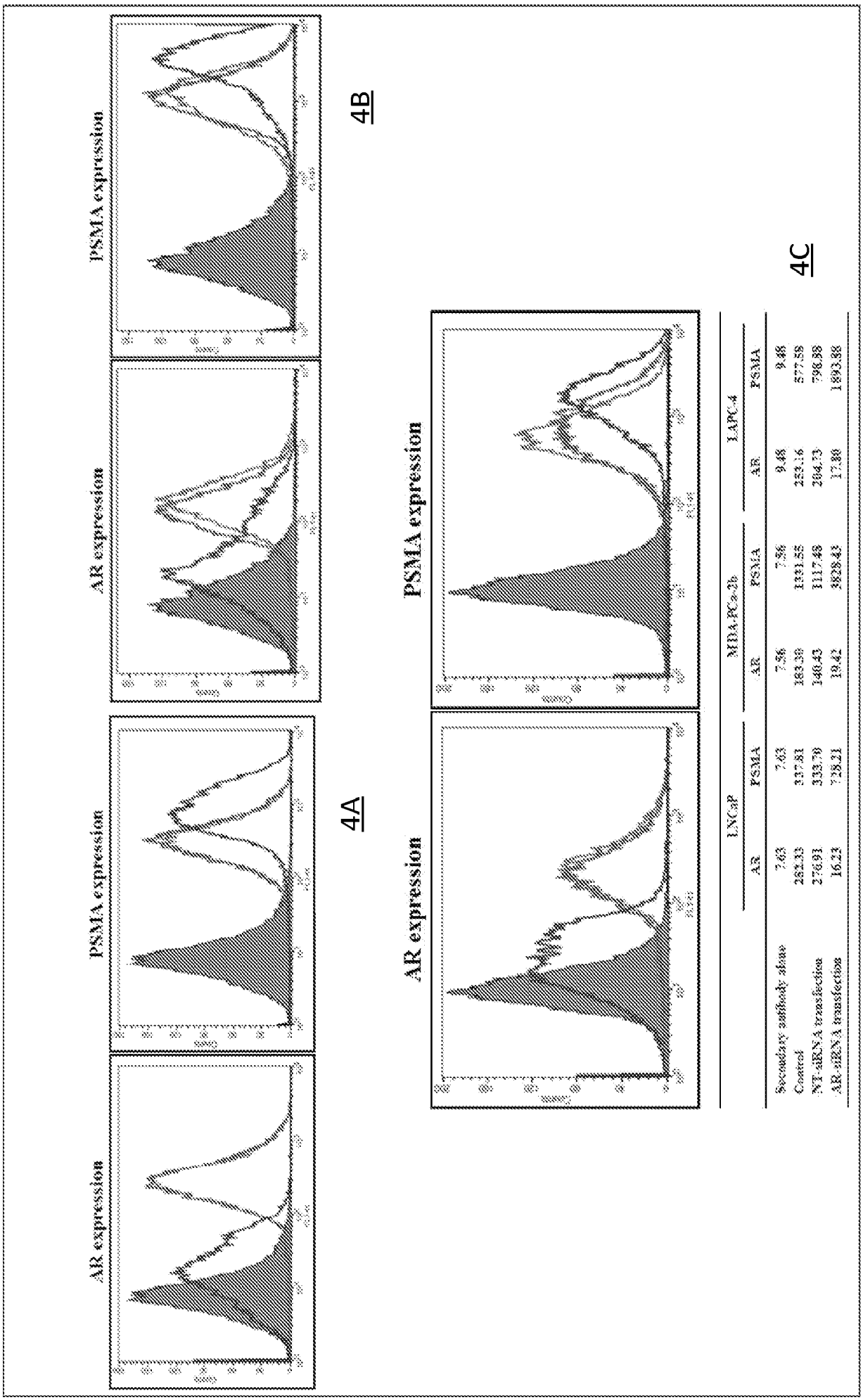


Figure 3C



	LNCaP		MDA-PCa-2b		LAPC-4	
	AR	PSMA	AR	PSMA	AR	PSMA
Secondary antibody alone	7.63	7.63	7.56	7.56	9.48	9.48
Control	282.33	337.81	183.39	1131.55	253.16	577.58
NT-siRNA transfection	276.91	333.79	140.43	1117.48	284.73	796.88
AR-siRNA transfection	16.23	728.21	19.42	3828.43	17.80	1893.88

Figure 4

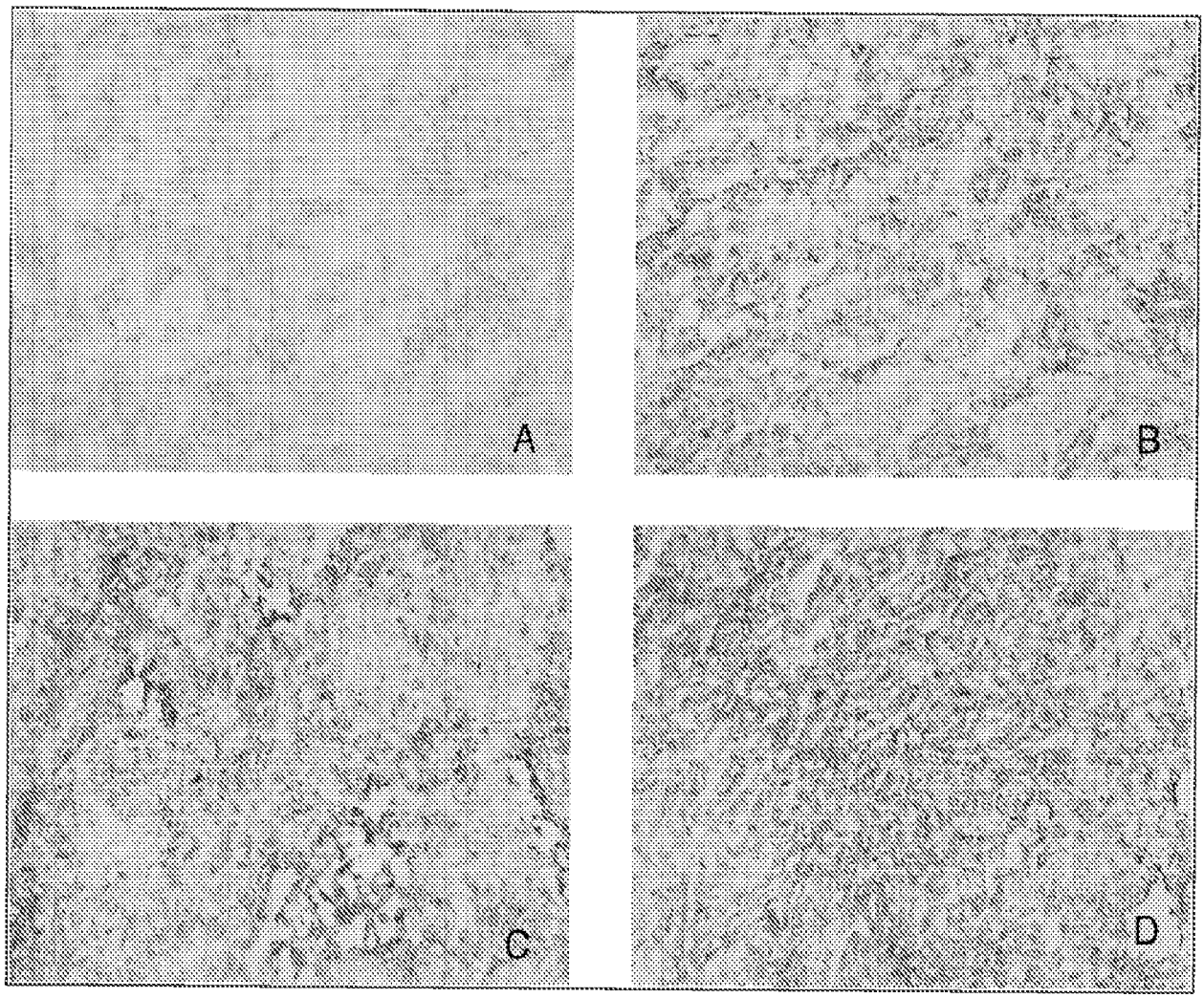


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/000051

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 49/00 (2013.01)
USPC - 424/9.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 49/00, 49/06, 49/14, 51/10; A61P 35/00; G01N 33/53 (2013.01)
USPC - 424/1.49, 9.1, 9.34, 9.4; 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 51/0482, 51/106 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patents, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0291113 A1 (BANDER) 18 November 2010 (18.11.2010) entire document	1-6, 14-20
X	US 2010/0209343 A1 (BANDER et al) 19 August 2010 (19.08.2010) entire document	7-13
A	US 2010/0047166 A1 (KANNER et al) 25 February 2010 (25.02.2010) entire document	1-20
A	US 2012/0036587 A1 (CHAUCHEREAU et al) 09 February 2012 (09.02.2012) entire document	1-20

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 May 2013	14 JUN 2013

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774