(54) Title: IDENTIFYING TAXANE SENSITIVITY IN PROSTATE CANCER PATIENTS
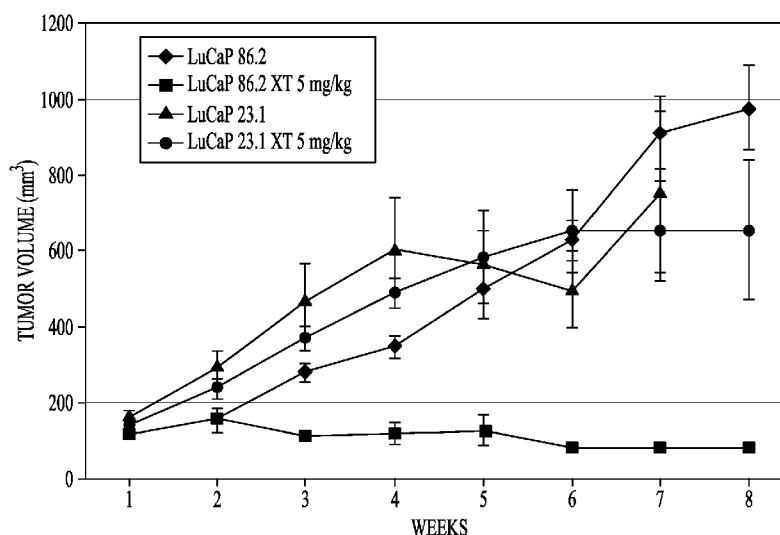

FIG. 11A

(57) Abstract: The invention relates to detection and/or treatment of a subset of prostate cancer patients who may benefit from tax-
ane treatment. The method comprises testing whether an androgen receptor (AR) splice variant is present in a test sample obtained
from the patient, wherein the androgen receptor variant can be ARv5,6,7 or ARv7, or a combination thereof.

IDENTIFYING TAXANE SENSITIVITY IN PROSTATE CANCER PATIENTS

CROSS-REFERENCE TO RELATED APPLICATION

This patent claims the benefit of priority, under 35 U.S.C. Section §119(e), to U.S. Provisional Patent Application Serial No. 61/702,983 filed on September 19, 2012, the contents of which application is incorporated by reference herein in its entirety.

GOVERNMENT SUPPORT

BACKGROUND

Androgen ablation therapy is the mainstay for prostate cancer (PC) treatment. However, it is not curative, as many patients progress to a castration-resistant stage of prostate cancer (CRPC). In fact, prostate cancer progression is dependent on continuous androgen receptor (AR) signaling and transcriptional activity. Thus, strategies designed to effectively inhibit androgen receptor transcriptional activity and signaling, are at the forefront of current research in prostate cancer. The importance of the androgen receptor in prostate cancer disease progression is highlighted by the fact that many recent therapies are designed to target the androgen axis, such as the androgen receptor antagonist enzalutamide (formerly MDV3100) (see, e.g., Hoffman-Censits & Kelly, *Clin Cancer Res* **19**, 1335 (Mar 15, 2013); Tran et al., *Science* **324**, 787 (May 8, 2009)), and the CYP17 inhibitor abiraterone (Bono et al., *N Engl J Med* **364**, 1995 (May 26, 2011)), which inhibits androgen synthesis. However, resistance to all forms of androgen deprivation therapy, including these next-generation compounds, occurs eventually and results in disease progression. Despite androgen ablation, castration-resistant prostate cancer (CRPC) remains androgen receptor driven due to several mechanisms including androgen receptor gene amplification, *in situ* androgen production and the presence of ligand-independent AR splice variants which localize to the nucleus and are constitutively active (Nelson, *J Clin Oncol* **30**, 644 (Feb 20, 2012); Locke et al., *Cancer Res* **68**, 6407 (Aug 1, 2008); Chen et al., *Nat Med* **10**, 33 (Jan, 2004); Nadiminty & Gao, *World J Urol* **30**, 287 (Jun, 2012)).

A major challenge in the clinical management of castration-resistant prostate cancer is that currently there is no biomarker that predicts clinical efficacy of chemotherapy. The taxanes represent the only class of cancer chemotherapeutics demonstrated to prolong survival in castration-resistant prostate cancer. Despite the clinical success of taxanes, treatment efficacy can be transient and the development of clinical taxane resistance is the major cause of cancer-related death. An important clinical question remains as to why patients who fail treatment with one taxane respond to another, and how clinicians can anticipate progression and proactively switch treatment.

New methods for detecting what type of therapeutic intervention is appropriate for a particular patient are needed.

SUMMARY OF THE INVENTION

As described herein, two androgen receptor splice variants ARv5,6,7 and AR-v7 can be used as markers for identifying prostate cancer patients who will, or will not, respond to taxane chemotherapy. In particular, as described herein, expression of androgen receptor splice variant v5,6,7 correlates with taxane sensitivity to patients *in vivo* while expression of androgen receptor splice variant v7 is associated with drug resistance. The variants are constitutively active in the nucleus of various prostate cancer types, rise in response to castration, and can mediate disease progression in castration-resistant prostate cancer. The choice of which drug(s) to administer to a prostate cancer patient can therefore be resolved by detection of one or the other androgen receptor slice variants in patient samples.

The invention therefore relates to a method of determining whether a prostate cancer patient can benefit from taxane drug treatment that involves testing whether an androgen receptor splice selected from the group consisting of variant v5,6,7, variant v7, or a combination thereof is present in a test sample obtained from the patient. For example, the prostate cancer patient can benefit from taxane drug treatment when more androgen receptor splice variant v5,6,7 is present in a test sample than variant v7. However, a prostate cancer patient may not benefit from taxane drug treatment when more androgen receptor splice variant v7 is present in a test sample than variant v5,6,7. The method can include testing samples such as circulating tumor cells, prostate tissue samples, blood samples, serum samples, ascites fluid samples, urine samples, semen samples, or a combination thereof. The method can also include capturing circulating tumor cells from the test sample before the testing, for example, by using a microfluidic device such as the device described herein.

Testing can involve an immunoassay, cell sorting assay, sandwich immunoassay, competition inhibition immunoassay, ELISA (Enzyme-Linked Immunosorbent Assay), immunohistochemical assay, agglutination assay, precipitation assay, radioimmunoassay or antigen-down immunoassay, immunometric assay, competitive binding assay, a direct sandwich immunoassay, an indirect sandwich assay, an immunoprecipitation assay, a nuclear immunostaining assay, an immunoblot assay, Northern blotting, nuclease protection assays, RNA fingerprinting, polymerase chain reaction, ligase chain reaction, Qbeta replicase, isothermal amplification method, strand displacement amplification, transcription based amplification systems, quantitative nucleic acid amplification assays (e.g., polymerase chain reaction assays), combined reverse transcription/nucleic acid amplification, nuclease protection (SI nuclease or RNAse protection assays), Serial Analysis Gene Expression (SAGE), next generation sequencing, gene expression microarray, in situ hybridization, nucleic acid amplification, reverse transcription, polymerase chain reaction, quantitative real time polymerase chain reaction (qRT- PCR), or a combination thereof.

In some embodiments, at least one probe or primer that specifically binds to an mRNA encoding the androgen receptor splice variant v5,6,7 can be used to detect and/or quantify mRNA expression levels of the androgen receptor splice variant v5,6,7. Alternatively, or in addition, at least one probe or primer that specifically binds to an mRNA encoding the androgen receptor splice variant v7 can be used to detect and/or quantify mRNA expression levels of the androgen receptor splice variant v7.

In other embodiments, an antibody specific for the androgen receptor splice variant v5,6,7 can be used in an immunological assay to detect whether the androgen receptor splice variant v5,6,7 is present

in the patient's test sample. Alternatively, or in addition, an antibody specific for the androgen receptor splice variant v7 can be used in an immunological assay to detect whether the androgen receptor splice variant v7 is present in the patient's test sample.

Another aspect of the invention is a method of identifying whether a prostate cancer patient will benefit from taxane drug treatment comprising determining whether an androgen receptor splice variant v5,6,7 is expressed in a test sample from the patient, and identifying a patient who will benefit from taxane drug treatment when the androgen receptor splice variant v5,6,7 is expressed in the test sample.

In some embodiments, the methods further include administering a taxane drug to a patient when the androgen receptor splice variant v5,6,7 is expressed in the test sample obtained from that patient.

Another aspect of the invention is a device for detecting androgen receptor splice variant v5,6,7 or androgen receptor splice variant v7 in a test sample, comprising a solid surface and an antibody specific for the androgen receptor splice variant (e.g., either or both of the v5,6,7 or v7 variants). The antibody can be immobilized onto the solid surface. The device can be configured for flow of a test sample through the device and binding of androgen receptor splice variant v5,6,7 to the antibody specific for the androgen receptor splice variant v5,6,7. In some embodiments, the device can be configured for flow of a test sample through the device and capture of cells that express androgen receptor splice variant v5,6,7, for example, by binding such cells to antibodies specific for the androgen receptor splice variant v5,6,7.

The test sample can be a bodily fluid sample or a tissue sample from a patient. Such a fluid sample can be blood, serum, plasma, semen, urine, lymph, or a combination thereof. The tissue sample can be a biopsy tissue sample, for example, a sample of prostate tissue.

In some embodiments, the patient is a healthy patient being tested for a susceptibility to prostate cancer. In other embodiments, the patient is a patient with prostate cancer and the method is used to evaluate the progression of the cancer and whether treatment with a taxane drug would be beneficial.

DESCRIPTION OF THE FIGURES

FIG. 1A-1D shows an exemplary GEDI microfluidic device. FIG. 1A shows schematic of the GEDI device illustrating blood flow through device. FIG. 1B shows a schematic of a device that can have a gasket, a inlet and an outlet. FIG. 1C illustrates a surface functionalization scheme that can be used to attach a binding entity such as a J591 monoclonal anti-PMSA antibody to a solid surface of the device. FIG. 1D illustrates the cell capture performance of the device as a function of shear stress and antibody concentration. Titration curves for the anti-PSMA J591 antibody in a standardized geometry indicate optimal antibody concentration for cell capture.

FIG. 2A-2F illustrate cell separation of differently sized cells by a straight-flow device versus non-linear flow pattern of the GEDI microfluidic device. FIG. 2A schematically illustrates flow through a microfluidic obstacle array with array geometric parameters. Δ = obstacle offset. Λ = obstacle spacing in the direction of bulk flow. Γ = obstacle spacing in the direction orthogonal to bulk flow. $2r$=obstacle diameter. Flow is from left to right. Path lines denote trajectories of cells of different diameters. Obstacle array spacing and orientation parameters are also defined. FIG. 2B graphically illustrates that the rate of cell-wall collisions for cells traveling through the array is a strong function of the offset parameter of the array; the GEDI design methodology indicates what offset parameter leads to size-dependent collision

rates. The results predicted for the flow through geometry illustrated in FIG. 2A are shown as a solid line in FIG. 2B. The four specific cell sizes lead to results denoted by the labeled dots in FIG. 2B. Other geometric arrangements of posts within the device lead to different results, shown at right in the dotted and dashed lines. FIG. 2C graphically illustrates the capture efficiency of straight arrays or arrays with

5   small offsets ("Straight") is lower that the capture efficiency of the GEDI device described herein. FIG. 2D illustrates that straight arrays or arrays with small offsets exhibit size independent collision rates (boxed in area to by the origin of the graph). Carefully chosen offsets such as those provided in the GEDI device exhibit size dependent collision rates (boxed in area from about offset 6 to 12 μm). Capture rates shown in FIG. 2C-2D compare GEDI (7-μm offset) and straight (no offset) geometry performance as measured

10   by LNCaP capture efficiency on J591-functionalized devices. Rates shown in the FIG. 2D describe simulated collision rates in these geometries. Both experimental results have the same surface-area-to-volume ratio. FIG. 2E schematically illustrates the offset array of the GEDI device versus a straight array. FIG. 2F graphically illustrates the average collision frequency of the GEDI device versus a device with a straight flow path. Devices with the same surface area to volume ratio give vastly different results:

15   straight arrays lead to collisions that decrease as the blood travels through the device; GEDI arrays lead to collisions that increase with travel through the device.

FIG. 3A-3D illustrate circulating tumor cell enumeration using the GEDI in comparison to capture by a CellSearch® device. FIG. 3A shows representative images of circulating tumor cells captured with the GEDI device from 1 mL of blood from prostate cancer patients. Circulating tumor cells are imaged on the

20   device and are identified following immunostaining with DAPI, PSMA, CD45, and EpCAM. Intact, nucleated cells that are PSMA$^+$/CD45$^-$ are identified as circulating tumor cells. Leucocytes are identified as DAPI$^+$/PSMA$^-$/CD45$^+$ (bottom row, arrow). Note the heterogeneity of EpCAM expression in the PSMA$^+$ cell population (top and bottom rows, EpCAM-; middle row, EpCAM$^+$). Scale bar: 10 μm. FIG. 2B graphically illustrates disease-specific GEDI capture of circulating tumor cells. Circulating tumor cell

25   enumeration (CTCs/ml) was performed using blood from healthy donors (median =3) and castration-resistant prostate cancer patients (median=54). (p<.001; Wilcoxon) FIG. 3C graphically compares the circulating tumor cells per ml as enumerated by GEDI-based- and CellSearch®-based capture devices. This comparison was performed using same-day blood draws from 25 individual castration-resistant prostate cancer patients. * indicates that CellSearch-based enumeration was performed 1 week before

30   the GEDI-based enumeration; ∉ indicates the same patient whose blood was drawn on two separate time points three months apart (blood draw no 14 occurred 3 months after blood draw no 19); # indicates the same patient whose blood was drawn on two separate time points 1 year apart (blood draw no 22 occurred 1 year before blood draw no 23). FIG. 3D graphically illustrates the effectiveness of the GEDI device versus the CellSearch® device. The graph shows a correlation between the numbers of circulating

35   tumor cells detected by the CellSearch® system vs. the GEDI system from same day blood draws. A correlation coefficient of r=0.44 (outliers were removed with Cook's distance restriction) was determined. Hashtag and asterisk denote two pairs of data each taken on the same patient at two longitudinal time points. r is not changed significantly by inclusion or rejection of these points.

FIG. 4A-4E illustrate functional characterization and detection of genetic alterations in GEDI-

40   captured cells. FIG. 4A shows that the population of captured cells is highly pure, enabling the

identification of single point mutations in the genomes of cells within the population. The T868A (ACT-GCT; Thr-Ala) androgen receptor single-point mutation is detected from RNA extracted from fifty C4-2 cells spiked into 1 ml of healthy-donor blood and captured by the GEDI device (third row, arrow, sequence CATCAGTTC**G**CTTTTGACCT, SEQ ID NO:7). Sequencing results from 1 ml blood from the same healthy donor (top row; sequence CATCAGTTC**A**CTTTTGACCT, SEQ ID NO:8) or from fifty C4-2 cells in culture (middle row; sequence CATCAGTTC**G**CTTTTGACCT, SEQ ID NO:9) are also depicted. FIG. 4B illustrates that the TMPRSS2:ERG fusion protein is detected in GEDI-captured circulating tumor cells from a castration-resistant prostate cancer patient. PSMA-captured circulating tumor cells were stained on the device with an anti-ERG antibody. Representative examples of three PSMA$^+$/CD45$^-$ circulating tumor cells are shown, two of which are positive for ERG. Scale bars: 10 microns. FIG. 4C-4E illustrate TMPRSS2:ERG detection by immunofluorescence on GEDI-captured cells. FIG. 4C illustrates ERG antibody staining of TMPRSS2:ERG fusion-positive (vCaP) and fusion-negative (C4-2) prostate cancer cell lines. Representative images acquired by confocal microscopy are displayed. Note the nuclear ERG staining in fusion-positive vCaP cells. FIG. 4D shows immunostained cells captured by the GEDI device. Two hundred vCaP cells were spiked in 1 ml of healthy-donor blood, flown through the GEDI device and processed for ERG immunofluorescence labeling. Nuclear ERG staining was detected in the GEDI-captured vCaP cells, identified as PSMA$^+$/DAPI$^+$/CD45$^-$ cells. FIG. 4E shows a representative example of ERG-negative/CD45$^+$ leucocytes identified in the blood from a castration-resistant prostate cancer patient processed by the GEDI device as in FIG. 4B.

FIG. 5A-5F illustrates on-chip assessment of effective drug-target engagement in viable GEDI-captured circulating tumor cells, and that tubulin responses to taxane treatment can be assessed in GEDI-captured cells. FIG. 5A shows images of C4-2 prostate cancer cells that were spiked (at 200 cells/ml) into a healthy-donor whole blood sample and then captured by the GEDI device. One ml of spiked blood was then passed through each of three GEDI devices. Captured cells were incubated on each device at 37°C for 24 hr with either DMSO control (upper panel) or 100 nM docetaxel (DTX; middle panel) or 1μM docetaxel (lower panel). Following drug treatment, cells were fixed and processed for immunofluorescence staining using antibodies against tubulin and CD45. DAPI was used as a DNA counterstain to evaluate nuclear integrity. Note the fine and intricate microtubule network in the DMSO control (top panel) and the distinct microtubule bundles in the docetaxel treated devices (arrows, middle and bottom panels). Apoptotic nuclei were observed at higher docetaxel concentrations (arrowhead, bottom panel). FIG. 5B shows images of GEDI-captured circulating tumor cells obtained from the blood of a castration-resistant prostate cancer patient after the cells were treated *ex-vivo* on the GEDI device with 100 nM docetaxel (top panel) or 100 nM paclitaxel (PTX; bottom panel) at 37°C for 24 hr. Following drug treatment, the PSMA-captured cells were fixed and processed for immunofluorescence staining as in FIG. 5A with the addition of cytokeratin-18 as an alternative epithelial marker. In this patient, the presence of an unperturbed microtubule network following docetaxel treatment (FIG. 5B, top panels) indicates lack of efficient drug-target engagement. In contrast, addition of paclitaxel resulted in microtubule bundling (FIG. 5B, bottom panels). FIG. 5C-5E illustrate additional examples of on-chip assessment of effective drug-target engagement from different castration-resistant prostate cancer patients: taxane-induced microtubule bundling and mitotic defects as evidence of drug-target engagement in GEDI-captured CTCs.

FIG. 5C shows GEDI-captured circulating tumor cells from the same patient as in FIG. 5B, after paclitaxel-induced prometaphase arrest of the GEDI-captured circulating tumor cells. These data provide additional evidence of effective drug-target engagement in the GEDI-captured cells. FIG. 5D shows that GEDI-captured circulating tumor cells from a patient (patient 3) following *ex-vivo* on-chip treatment with 100 nM DTX do not exhibit any evidence of a microtubule response (bundling) to drug treatment. FIG. 5E shows that GEDI-captured circulating tumor cells from a patient (patient 2) display microtubule bundling (arrow) following *ex-vivo* on-chip treatment with 100 nM or 1 uM paclitaxel (PTX). FIG. 5F shows GEDI-captured circulating tumor cells from a patient (patient 4) following *ex-vivo* on-chip treatment with 50 nM paclitaxel (PTX) exhibit a heterogeneous response to drug treatment. Note the distinct microtubule bundling in the PSMA+ cells (middle panel, barbed arrow) and no detectable microtubule network in another PSMA+ cells from the same patient (bottom panel, standard arrow). The adjacent leucocyte (PSMA−) shows clear microtubule bundling in response to paclitaxel (PTX) treatment.

FIG. 6A-6E illustrate that microtubule-binding is mediated by the C-terminal domain of the androgen receptor. FIG. 6A shows schematic diagrams of a full-length human androgen receptor, of the N-terminal domain of the human androgen receptor, and of the C-terminal domain of the human androgen receptor used in experiments described herein. The numbers (positions) of amino acids are shown below the diagrams. FIG. 6C shows schematic diagrams of a full-length human androgen receptor, and a series of C-terminal androgen receptor fragments. FIG. 6B and 6D show electrophoretically separated products of microtubule co-sedimentation assays of PC3:mCh-tub cells that transiently express GFP-AR(wt), GFP-N-terminal AR (N-ter AR) or GFP-C-terminal AR (C-ter AR) or each of the smaller deletion mutants as indicated. Following high-speed centrifugation equal volumes from each fraction (WP and WS) were resolved by SDS-PAGE and immunoblotted as indicated. WP: warm pellet containing microtubule polymers and associated proteins, WS: warm supernatant containing soluble tubulin dimers and proteins non-associated with microtubules. Boxes highlight the relative protein distribution between the WP and WS fractions from each condition. The percent protein present in the pellet fraction (%WP) was calculated using the following formula: % P = 100*WP/(WP+WS) and is presented to the right of each immunoblot. For tubulin a range of values is shown in the %WP quantitation reflecting each of the three reactions performed. FIG. 6E shows electrophoretically separated products of microtubule co-sedimentation assays of the HEK293 cell line, showing the same results as shown in FIG. 6B and 6D, namely that the C-terminal region of the androgen receptor mediates microtubule binding. The data shown in FIG. 6E was generated using HEK293 cells that were transiently transfected with full-length GFP-AR (wt), GFP-N-ter AR or GFP-C-ter AR and subjected to microtubule co-sedimentation as described earlier. Proteins from each respective fraction were resolved on SDS-PAGE and immunoblotted with the indicated antibodies. Wild-type and C-terminus-AR co-fractionated with the microtubule pellets (WP) whereas N-terminus AR remained in the WS, consistent with the results seen in the PC3:mCh-tub cells. Tubulin was detected in as microtubules in the WP in each condition.

FIG. 7A-7C illustrate that ARv5,6,7, but not ARv7, associates with the microtubule polymer. FIG. 7A is a schematic diagram of the full-length androgen receptor as well as the splice variants ARv5,6,7, encoding exons 1-4 and the first 10 amino acids of exon 8, and ARv7, encoding exons 1-3 and a cryptic exon of 16 amino acids. FIG. 7B and 7C show electrophoretically separated products of microtubule co-

sedimentation assays of whole cell lysates from PC3:mCh-tub cells transfected with GFP-AR (wt), GFP-C-ter AR, HA-ARv5,6,7 and GFP-ARv7 as carried out as described for FIG. 6. AR-wt AR and C-ter AR were used as positive controls of microtubule-binding. Tubulin was detected as microtubules in the WP in each condition. The boxes indicate the distribution of each protein between WP and WS fractions per condition. The extent of each variant's association with the microtubule polymer (% WP) was quantified by densitometry as described for FIG. 6 and is displayed to the right of each immunoblot. For tubulin a range of values is shown in the %WP quantitation reflecting each of the eight reactions performed. Actin was used as a negative control for microtubule association and is found in the supernatant fractions (WS) in all conditions.

FIGs. 8A-8D illustrate that microtubule targeting drugs inhibit ARv5,6,7 nuclear trafficking but have no effect on ARv7 nuclear accumulation. FIGs. 8A and 8B show time lapse images obtained with a spinning disk confocal microscope by acquiring an entire Z-stack at 10 min intervals for 2 hr. Maximum intensity projections are shown of representative PC3:mCherry-Tub cells expressing GFP-ARv5,6,7 (FIG. 8A) or GFP-ARv7 (FIG. 8B) at the indicated time points. The integrity of the microtubule cytoskeleton from untreated, 1 µM docetaxel (TXT) or 10 µM nocodazole (Noc) treated cells was visualized with mCherry-tagged tubulin at the beginning of the time-lapse recording and is shown in the far right panels. Arrowhead points at microtubule bundles. FIGs. 8C and 8D graphically illustrate nuclear accumulation of ARv5,6,7 (FIG. 8C) or ARv7 (FIG. 8D) over time following treatment in control versus docetaxel (TXT) pretreated cells or nocodazole (Noc) pretreated cells. Quantitative analysis of nuclear of GFP-AR was performed on each focal plane (0.5 µm Z-sections through the entire cell depth) using integrated pixel intensity values from the sum projection and the percentage of nuclear AR was calculated using the following formula: % Nuclear AR = 100*Nuclear AR/Total AR. Number of individual cells imaged per condition (n) ARv5,6,7: Control 0, 10, 20 and 30 min: n=16; 40-120 min: n=15. TXT pretreated 0-80 min: n=12; 90-120 min: n=10. Noc pretreated 0-50 min: n=11; 60-110 min: n=10; 120 min: n=3. N values for ARv7: Control 0-120 min: n=9. TXT pretreated 0-120 min: n=9. Noc pretreated 0-100 min: n=21, 110-120 min: n=7. Statistical test: One-way ANOVA with equal variances followed by multiple comparisons with Bonferroni adjustments.

FIG. 9A-9J illustrate that taxane treatment inhibits trafficking to the nucleus in M12 cells by AR-wt AR and ARv5,6,7, but not by ARv7. FIG. 9A shows M12 cells stably expressing GFP-tagged AR-wt AR. FIG. 9B shows M12 cells stably expressing GFP-tagged ARv5,6,7. FIG. 9C shows M12 cells stably expressing GFP-tagged ARv7. The various cell types were treated with 1 µM docetaxel (TXT) for 4 h either alone or followed by 10 nM R1881 for 2 h as indicated in the figure. Cells were then fixed, immunostained for tubulin, and imaged for GFP-AR and tubulin by confocal microscopy. Androgen receptor is shown as lighter staining (green in the original), while tubulin staining is darker but visible as lighter areas in the images (red in the original). Representative, high-magnification images from each condition are shown. Arrows point to cytoplasmic androgen receptor protein; arrowheads point to microtubule bundles. Scale bar: 10 µm. FIG. 9D is a bar graph depicting the ratio of cells with nuclear androgen receptor over those with cytoplasmic androgen receptor for each condition. Quantitation was performed in at least 100 GFP-positive cells. *p<0.05. FIG. 9E is a bar graph illustrating RNA expression of TMPRSS2 and FKBP51 in M12 cells expressing inducible AR-wt or AR variants after the cells were treated with 1 µM docetaxel (TXT) for 4 h either alone or followed by 10 nM R1881 overnight as indicated

in the figure. Relative mRNA expression of TMPRSS2 and FKBP51 was assessed by qPCR. Values were corrected to GAPDH and normalized to untreated M12 cells expressing control lentivirus (M12 lenti). Bar graphs represent the average of three independent experiments. * p<0.05. FIG. 9F shows images of AR-wt expressing control (non-treated) M12 cells or AR-wt expressing M12 cells treated with μM docetaxel for 4 hr either alone or followed by 10 nM R1881 for 2 hr as indicated. The control and treated cells express GFP-AR-wt that can be detected after staining with anti-androgen receptor or anti-N-terminal androgen receptor antibodies (reactive with the first 21 amino acids of the androgen receptor protein; also referred to as anti-ARN21 antibodies. FIG. 9G shows images of GFP-ARv5,6,7 expressing control (non-treated) M12 cells or GFP-ARv5,6,7 expressing M12 cells treated with μM docetaxel for 4 hr either alone or followed by 10 nM R1881 for 2 hr as indicated. Staining with anti-androgen receptor or anti-ARN-21 antibodies illuminated areas of androgen receptor and ANR-21 expression in the cells. FIG. 9H shows images of GFP-ARv7 expressing control (non-treated) M12 cells or GFP-ARv7 expressing M12 cells treated with μM docetaxel for 4 hr either alone or followed by 10 nM R1881 for 2 hr as indicated. Androgen receptor protein expression was detected by staining with anti-androgen receptor or anti-ARN-21 antibodies. FIG. 9I and 9J illustrate that R1881 of M12 cells transfected with ARv5,6,7 (FIG. 9I) and ARv7 (FIG. 9J) does not increase nuclear accumulation of androgen receptor protein. M12 cells stably expressing GFP-tagged ARv5,6,7 (A) and ARv7 (B) were treated with 1 μM docetaxel for 4 hr either alone or followed by 10 nM R1881 for 2 hr as indicated. Cells were then fixed, immunostained for tubulin, and imaged for GFP-AR and tubulin by confocal microscopy. GFP-Androgen receptor proteins are shown as lighter staining (green in the original), and tubulin expression is shown as darker staining (red in the original). Scale bar: 10 μm

FIG. 10A-10D illustrate that dynein associates with and mediates the nuclear translocation of ARv5,6,7, but not ARv7. FIG. 10A shows images of M12 cells stably expressing untagged ARv5,6,7. FIG. 10B shows images of M12 cells stably expressing untagged ARv7. The different cell types were transiently transfected with pCMVH50myc (encoding a c-Myc tagged human dynamitin). Cells were fixed, processed for double immunofluorescence labeling with anti-androgen receptor (lighter staining; green in the original) and c-Myc (darker staining; red in the original) antibodies and analyzed by confocal microscopy. Dashed arrows point to cytoplasmic androgen receptor protein and solid arrows to nuclear androgen receptor protein. FIG. 10C is a bar graph illustrating the amount of each of the variants in the nucleus. Quantification was performed using MetaMorph image analysis software. As illustrated, there is a marked decrease of nuclear ARv5,6,7 after dynein-cargo disruption. ARv7, however, remains unaffected by dynamitin overexpression. FIG. 10D shows electrophoretically separated immunoprecipitated protein from whole cell lysates of 293T cells that were transiently transfected with GFP-AR(wt), GFP-ARv5,6,7 and GFP-ARv7. The lysates were immunoprecipitated with a GFP antibody or control IgG and immunoblotted for dynein and androgen receptor.

FIG. 11A-11C shows that docetaxel treatment inhibits ARv5,6,7-mediated subcutaneous tumor growth in SCID mice. Mice with human prostate cancer xenografts LuCap 86.2, expressing predominantly ARv5,6,7, and LuCap 23.12 expressing both AR(wt) and ARv7, were treated with docetaxel 5 mg/kg weekly intraperitoneally or with vehicle control. The study was terminated when all mice in the LuCaP 23.1 5mg/kg group meet UW IACUC criteria for euthanasia. The Y axis shows tumor volumes +/- SEM, as a

function of time (x-axis). FIG. 11A graphically illustrates that LuCaP 86.2 tumor growth is markedly suppressed by a low dose of docetaxel of 5 mg/kg (P< 0.01 control versus docetaxel treated). However, LuCaP 23.1 tumor growth is unaffected by docetaxel treatment. FIG. 11B shows the tumor volume over time in mice with LuCap 86.2 xenografts after treatment with 5 mg/kg docetaxel (square symbols) or 20 mg/kg weekly docetaxel (diamond symbols) treatment. FIG. 11C shows the tumor volume over time in mice with LuCap 23.12 xenografts after treatment with 5 mg/kg docetaxel (square symbols) or 20 mg/kg weekly docetaxel (diamond symbols) treatment. LuCap 86.2 (FIG. 11B) and LuCap 23.12 (FIG. 11C) human prostate cancer xenografts were treated with docetaxel 5 mg/kg weekly intraperitoneally (square symbols) or 20 mg/kg weekly intraperitoneally (diamond symbols). The study was terminated when all mice in the 5mg/kg group meet UW IACUC criteria for euthanasia. Note that both xenografts responded to high dose docetaxel but only LuCaP 86.2 responded to low dose docetaxel. The vertical bars indicate ± SEM. Note LuCaP 86.2, which expresses predominantly ARsv5,6,7es, responds to low and high dose docetaxel but LuCaP 23.1, which expresses ARwt and ARv7 responds only to high dose docetaxel. The tumor growth curve of untreated LuCaP 86.2 xenografts is characterized by the straight line (with no symbols) in FIG. 11B. There was no difference in LuCaP 23.12 xenograft growth between untreated xenografts and those treated with a 5mg dose of docetaxel.

DESCRIPTION

As described herein, two androgen receptor splice variants, androgen receptor variant v5,6,7 (ARv5,6,7) and androgen receptor variant v7 (AR-v7) can be used as markers for identifying prostate cancer patients who will or will not respond to taxane chemotherapy.

Androgen ablation therapy induces expression of constitutively active androgen receptor splice variants, which drive prostate cancer disease progression. The experimental data described herein indicate that patients that express the androgen receptor variant v5,6,7 (ARv5,6,7) are more sensitive to taxane chemotherapy than patients that express full-length androgen receptor or other androgen receptors. Moreover, these data also show that patients who express the androgen receptor variant v7 (AR-v7) will be resistant to taxane chemotherapy.

Androgen Receptors

Androgen receptor variants ARv5,6,7 and ARv7 (also known as AR3) appear to be the two most clinically prevalent splice variants. The ARv5,6,7 variant is present in 59% of tumor specimens from castration-resistant prostate cancer patients, and its expression arises in response to androgen deprivation therapy or abiraterone treatment (Sun et al., *J Clin Invest* **120**, 2715 (Aug, 2010); Mostaghel et al., *Clin Cancer Res* **17**, 5913 (Sep 15, 2011)). The ARv7 variant is present in both benign and malignant prostate tissues but is generally enriched in metastatic disease (Gao et al., *Cancer Res* **69**, 2305 (Mar 15, 2009); Hornberg et al., *PLoS One* **6**, e19059 (2011)). Thus, the presence of androgen receptor splice variants is common in castration-resistant prostate cancer patients and is associated with resistance to current androgen deprivation therapies.

Sequences for various androgen receptors are available, for example, from the National Center for Biotechnology Information (see website at ncbi.nlm.nih.gov).

For example, a full length human androgen receptor sequence is available from the database maintained by the National Center for Biotechnology Information (see website at ncbi.nlm.nih.gov), which has accession number P10275.2 (GI:113830), and is shown below as SEQ ID NO:1.

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  1 MEVQLGLGRV YPRPPSKTYR GAFQNLFQSV REVIQNPGPR
 41 HPEAASAAPP GASLLLLQQQ QQQQQQQQQQ QQQQQQQQET
 81 SPRQQQQQQG EDGSPQAHRR GPTGYLVLDE EQQPSQPQSA
121 LECHPERGCV PEPGAAVAAS KGLPQQLPAP PDEDDSAAPS
161 TLSLLGPTFP GLSSCSADLK DILSEASTMQ LLQQQQQEAV
181 SEGSSSGRAR EASGAPTSSK DNYLGGTSTI SDNAKELCKA
241 VSVSMGLGVE ALEHLSPGEQ LRGDCMYAPL LGVPPAVRPT
281 PCAPLAECKG SLLDDSAGKS TEDTAEYSPF KGGYTKGLEG
321 ESLGCSGSAA AGSSGTLELP STLSLYKSGA LDEAAAYQSR
361 DYYNFPLALA GPPPPPPPPH PHARIKLENP LDYGSAWAAA
401 AAQCRYGDLA SLHGAGAAGP GSGSPSAAAS SSWHTLFTAE
441 EGQLYGPCGG GGGGGGGGGG GGGGGGGGGG GGEAGAVAPY
481 GYTRPPQGLA GQESDFTAPD VWYPGGMVSR VPYPSPTCVK
521 SEMGPWMDSY SGPYGDMRLE TARDHVLPID YYFPPQKTCL
561 ICGDEASGCH YGALTCGSCK VFFKRAAEGK QKYLCASRND
601 CTIDKFRRKN CPSCRLRKCY EAGMTLGARK LKKLGNLKLQ
641 EEGEASSTTS PTEETTQKLT VSHIEGYECQ PIFLNVLEAI
681 EPGVVCAGHD NNQPDSFAAL LSSLNELGER QLVHVVKWAK
721 ALPGFRNLHV DDQMAVIQYS WMGLMVFAMG WRSFTNVNSR
761 MLYFAPDLVF NEYRMHKSRM YSQCVRMRHL SQEFGWLQIT
801 PQEFLCMKAL LLFSIIPVDG LKNQKFFDEL RMNYIKELDR
841 IIACKRKNPT SCSRRFYQLT KLLDSVQPIA RELHQFTFDL
881 LIKSHMVSVD FPEMMAEIIS VQVPKILSGK VKPIYFHTQ
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The sequence of the androgen receptor can vary somewhat from one patient to another. For example, the number of the repetitive glutamine residues in androgen receptors (amino acids 58-89 of SEQ ID NO:1) can increase or decrease by any number between about 2-25 amino acids. Similarly, the number of repetitive glycine residues in androgen receptors (amino acids 446-472 of SEQ ID NO:1) can increase or decrease by any number between about 2-23 amino acids. Thus, the androgen receptor detected by the methods, reagents and devices described herein can have at least 75% sequence identity, or at least 80% sequence identity, or at least 85% sequence identity, or at least 90%, or at least 95% sequence identity sequence identity to SEQ ID NO:1.

Sequence identity can be evaluated using sequence analysis software (e.g., via the NCBI tools, or the Sequence Analysis Software Package of the Genetics Computer Group. University of Wisconsin Biotechnology Center. 1710 University Avenue. Madison, WI 53705). Such software matches similar sequences by assigning degrees of sequence identity to various substitutions, deletions, insertions, and other modifications. Conservative substitutions typically include substitutions within the following groups:

glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

Nucleotide sequences for the full-length human androgen receptor are also available from the NCBI database. For example, a cDNA sequence for the full length human androgen receptor is available as accession number M20132.1 (GI:178627), shown below as SEQ ID NO:2.

```
   1 TAATAACTCA GTTCTTATTT GCACCTACTT CAGTGGACAC
  41 TGAATTTGGA AGGTGGAGGA TTTTGTTTTT TTCTTTTAAG
  81 ATCTGGGCAT CTTTTGAATC TACCCTTCAA GTATTAAGAG
 121 ACAGACTGTG AGCCTAGCAG GGCAGATCTT GTCCACCGTG
 161 TGTCTTCTTC TGCACGAGAC TTTGAGGCTG TCAGAGCGCT
 201 TTTTGCGTGG TTGCTCCCGC AAGTTTCCTT CTCTGGAGCT
 241 TCCCGCAGGT GGGCAGCTAG CTGCAGCGAC TACCGCATCA
 281 TCACAGCCTG TTGAACTCTT CTGAGCAAGA GAAGGGGAGG
 321 CGGGGTAAGG GAAGTAGGTG GAAGATTCAG CCAAGCTCAA
 361 GGATGGAAGT GCAGTTAGGG CTGGGAAGGG TCTACCCTCG
 401 GCCGCCGTCC AAGACCTACC GAGGAGCTTT CCAGAATCTG
 441 TTCCAGAGCG TGCGCGAAGT GATCCAGAAC CCGGGCCCCA
 481 GGCACCCAGA GGCCGCGAGC GCAGCACCTC CCGGCGCCAG
 521 TTTGCTGCTG CTGCAGCAGC AGCAGCAGCA GCAGCAGCAG
 561 CAGCAGCAGC AGCAGCAGCA GCAGCAGCAG CAGCAAGAGA
 601 CTAGCCCCAG GCAGCAGCAG CAGCAGCAGG GTGAGGATGG
 641 TTCTCCCCAA GCCCATCGTA GAGGCCCCAC AGGCTACCTG
 681 GTCCTGGATG AGGAACAGCA ACCTTCACAG CCGCAGTCGG
 721 CCCTGGAGTG CCACCCCGAG AGAGGTTGCG TCCCAGAGCC
 761 TGGAGCCGCC GTGGCCGCCA GCAAGGGGCT GCCGCAGCAG
 801 CTGCCAGCAC CTCCGGACGA GGATGACTCA GCTGCCCCAT
 841 CCACGTTGTC CCTGCTGGGC CCCACTTTCC CCGGCTTAAG
 881 CAGCTGCTCC GCTGACCTTA AAGACATCCT GAGCGAGGCC
 921 AGCACCATGC AACTCCTTCA GCAACAGCAG CAGGAAGCAG
 961 TATCCGAAGG CAGCAGCAGC GGGAGAGCGA GGGAGGCCTC
1001 GGGGGCTCCC ACTTCCTCCA AGGACAATTA CTTAGGGGGC
1041 ACTTCGACCA TTTCTGACAA CGCCAAGGAG TTGTGTAAGG
1081 CAGTGTCGGT GTCCATGGGC CTGGGTGTGG AGGCGTTGGA
1121 GCATCTGAGT CCAGGGGAAC AGCTTCGGGG GGATTGCATG
1161 TACGCCCCAC TTTTGGGAGT CCACCCGCT GTGCGTCCCA
1201 CTCCTTGTGC CCCATTGGCC GAATGCAAAG GTTCTCTGCT
1241 AGACGACAGC GCAGGCAAGA GCACTGAAGA TACTGCTGAG
1281 TATTCCCCTT TCAAGGGAGG TTACACCAAA GGGCTAGAAG
1321 GCGAGAGCCT AGGCTGCTCT GGCAGCGCTG CAGCAGGGAG
1361 CTCCGGGACA CTTGAACTGC CGTCTACCCT GTCTCTCTAC
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1401 AAGTCCGGAG CACTGGACGA GGCAGCTGCG TACCAGAGTC
1441 GCGACTACTA CAACTTTCCA CTGGCTCTGG CCGGACCGCC
1481 GCCCCCTCCG CCGCCTCCCC ATCCCCACGC TCGCATCAAG
1521 CTGGAGAACC CGCTGGACTA CGGCAGCGCC TGGGCGGCTG
1561 CGGCGGCGCA GTGCCGCTAT GGGGACCTGG CGAGCCTGCA
1601 TGGCGCGGGT GCAGCGGGAC CCGGTTCTGG GTCACCCTCA
1641 GCCGCCGCTT CCTCATCCTG GCACACTCTC TTCACAGCCG
1681 AAGAAGGCCA GTTGTATGGA CCGTGTGGTG GTGGTGGGGG
1721 TGGTGGCGGC GGCGGCGGCG GCGGCGGCGG CGGCGGCGGC
1761 GGCGGCGGCG GCGGCGGCGA GGCGGGAGCT GTAGCCCCCT
1801 ACGGCTACAC TCGGCCCCCT CAGGGGCTGG CGGGCCAGGA
1841 AAGCGACTTC ACCGCACCTG ATGTGTGGTA CCCTGGCGGC
1881 ATGGTGAGCA GAGTGCCCTA TCCCAGTCCC ACTTGTGTCA
1921 AAAGCGAAAT GGGCCCCTGG ATGGATAGCT ACTCCGGACC
1961 TTACGGGGAC ATGCGTTTGG AGACTGCCAG GGACCATGTT
2001 TTGCCCATTG ACTATTACTT TCCACCCCAG AAGACCTGCC
2041 TGATCTGTGG AGATGAAGCT TCTGGGTGTC ACTATGGAGC
2081 TCTCACATGT GGAAGCTGCA AGGTCTTCTT CAAAAGAGCC
2121 GCTGAAGGGA AACAGAAGTA CCTGTGCGCC AGCAGAAATG
2161 ATTGCACTAT TGATAAATTC CGAAGGAAAA ATTGTCCATC
2201 TTGTCGTCTT CGGAAATGTT ATGAAGCAGG GATGACTCTG
2241 GGAGCCCGGA AGCTGAAGAA ACTTGGTAAT CTGAAACTAC
2281 AGGAGGAAGG AGAGGCTTCC AGCACCACCA GCCCCACTGA
2321 GGAGACAACC CAGAAGCTGA CAGTGTCACA CATTGAAGGC
2361 TATGAATGTC AGCCCATCTT TCTGAATGTC CTGGAAGCCA
2401 TTGAGCCAGG TGTAGTGTGT GCTGGACACG ACAACAACCA
2441 GCCCGACTCC TTTGCAGCCT TGCTCTCTAG CCTCAATGAA
2481 CTGGGAGAGA GACAGCTTGT ACACGTGGTC AAGTGGGCCA
2521 AGGCCTTGCC TGGCTTCCGC AACTTACACG TGGACGACCA
2561 GATGGCTGTC ATTCAGTACT CCTGGATGGG GCTCATGGTG
2601 TTTGCCATGG GCTGGCGATC CTTCACCAAT GTCAACTCCA
2641 GGATGCTCTA CTTCGCCCCT GATCTGGTTT TCAATGAGTA
2681 CCGCATGCAC AAGTCCCGGA TGTACAGCCA GTGTGTCCGA
2721 ATGAGGCACC TCTCTCAAGA GTTTGGATGG CTCCAAATCA
2761 CCCCCCAGGA ATTCCTGTGC ATGAAAGCAC TGCTACTCTT
2801 CAGCATTATT CCAGTGGATG GGCTGAAAAA TCAAAAATTC
2841 TTTGATGAAC TTCGAATGAA CTACATCAAG GAACTCGATC
2881 GTATCATTGC ATGCAAAAGA AAAAATCCCA CATCCTGCTC
2921 AAGACGCTTC TACCAGCTCA CCAAGCTCCT GGACTCCGTG
2961 CAGCCTATTG CGAGAGAGCT GCATCAGTTC ACTTTTGACC

3001 TGCTAATCAA GTCACACATG GTGAGCGTGG ACTTTCCGGA

3041 AATGATGGCA GAGATCATCT CTGTGCAAGT GCCCAAGATC

3081 CTTTCTGGGA AAGTCAAGCC CATCTATTTC CACACCCAGT

3121 GAAGCATTGG AAACCCTATT TCCCCACCCC AGCTCATGCC

3161 CCCTTTCAGA TGTCTTCTGC CTGTTATAAC TCTGCACTAC

3201 TCCTCTGCAG TGCCTTGGGG AATTTCCTCT ATTGATGTAC

3241 AGTCTGTCAT GAACATGTTC CTGAATTCTA TTTGCTGGGC

3281 TTTTTTTTTC TCTTTCTCTC CTTTCTTTTT CTTCTTCCCT

3321 CCCTATCTAA CCCTCCCATG GCACCTTCAG ACTTTGCTTC

3361 CCATTGTGGC TCCTATCTGT GTTTTGAATG GTGTTGTATG

3401 CTTTAAAATC TGTGATGATC CTCATATGGC CCAGTGTCAA

3441 GTTGTGCTTG TTTACAGCAC TACTCTGTGC CAGCCACACA

3481 AACGTTTACT TATCTTATGC CACGGGAAGT TTAGAGAGCT

3521 AAGATTATCT GGGGAAATCA AACAAAAAA CAAGCAAACA

3561 AAAAAAAAA

A sequence for androgen receptor variant 5,6,7es [Homo sapiens] is also available from the NCBI database National Center for Biotechnology Information (see website at ncbi.nlm.nih.gov). This androgen receptor variant lacks exons 5,6, and7. The NCBI database provides a sequence for a human androgen receptor variant 5,6,7es with accession number ACZ81436.1 (GI:270358642) (SEQ ID NO:3).

1 MEVQLGLGRV YPRPPSKTYR GAFQNLFQSV REVIQNPGPR

41 HPEAASAAPP GASLLLLQQQ QQQQQQQQQQ QQQQQQQQQQ

81 QETSPRQQQQ QQGEDGSPQA HRRGPTGYLV LDEEQQPSQP

121 QSALECHPER GCVPEPGAAV AASKGLPQQL PAPPDEDDSA

161 APSTLSLLGP TFPGLSSCSA DLKDILSEAS TMQLLQQQQQ

201 EAVSEGSSSG RAREASGAPT SSKDNYLGGT STISDNAKEL

241 CKAVSVSMGL GVEALEHLSP GEQLRGDCMY APLLGVPPAV

281 RPTPCAPLAE CKGSLLDDSA GKSTEDTAEY SPFKGGYTKG

321 LEGESLGCSG SAAAGSSGTL ELPSTLSLYK SGALDEAAAY

361 QSRDYYNFPL ALAGPPPPPP PPHPHARIKL ENPLDYGSAW

401 AAAAAQCRYG DLASLHGAGA AGPGSGSPSA AASSSWHTLF

441 TAEEGQLYGP CGGGGGGGGG GGGGGGGGGG GGGGGGGGEA

481 GAVAPYGYTR PPQGLAGQES DFTAPDVWYP GGMVSRVPYP

521 SPTCVKSEMG PWMDSYSGPY GDMRLETARD HVLPIDYYFP

561 PQKTCLICGD EASGCHYGAL TCGSCKVFFK RAAEGKQKYL

601 CASRNDCTID KFRRKNCPSC RLRKCYEAGM TLGARKLKKL

641 GNLKLQEEGE ASSTTSPTEE TTQKLTVSHI EGYECQPIFL

681 NVLEAIEPGV VCAGHDNNQP DSFAALLSSL NELGERQLVH

721 VVKWAKALPD CERAASVHF

The sequence of the androgen receptor splice variant v5,6,7 can vary somewhat from one patient to another. For example, the androgen receptor splice variant v5,6,7 detected by the methods, reagents and devices described herein can have at least 75% sequence identity, or at least 80% sequence identity, or at least 85% sequence identity, or at least 90%, or at least 95% sequence identity sequence identity to SEQ ID NO:3.

Nucleotide sequences for the human androgen receptor variant v5,6,7 are also available from the NCBI database. For example, a cDNA sequence for the SEQ ID NO:3 human androgen receptor variant v5,6,7 is available as accession number GU208210.1 (GI:270358641), shown below as SEQ ID NO:4.

```
   1 AGGATGGAAG TGCAGTTAGG GCTGGGAAGG GTCTACCCTC
  41 GGCCGCCGTC CAAGACCTAC CGAGGAGCTT TCCAGAATCT
  81 GTTCCAGAGC GTGCGCGAAG TGATCCAGAA CCCGGGCCCC
 121 AGGCACCCAG AGGCCGCGAG CGCAGCACCT CCCGGCGCCA
 161 GTTTGCTGCT GCTGCAGCAG CAGCAGCAGC AGCAGCAGCA
 201 GCAGCAGCAG CAGCAGCAGC AGCAGCAGCA GCAGCAGCAG
 241 CAGCAAGAGA CTAGCCCCAG GCAGCAGCAG CAGCAGCAGG
 281 GTGAGGATGG TTCTCCCCAA GCCCATCGTA GAGGCCCCAC
 321 AGGCTACCTG GTCCTGGATG AGGAACAGCA ACCTTCACAG
 361 CCGCAGTCGG CCCTGGAGTG CCACCCCGAG AGAGGTTGCG
 401 TCCCAGAGCC TGGAGCCGCC GTGGCCGCCA GCAAGGGGCT
 441 GCCGCAGCAG CTGCCAGCAC CTCCGGACGA GGATGACTCA
 481 GCTGCCCCAT CCACGTTGTC CCTGCTGGGC CCCACTTTCC
 521 CCGGCTTAAG CAGCTGCTCC GCTGACCTTA AAGACATCCT
 561 GAGCGAGGCC AGCACCATGC AACTCCTTCA GCAACAGCAG
 601 CAGGAAGCAG TATCCGAAGG CAGCAGCAGC GGGAGAGCGA
 641 GGGAGGCCTC GGGGGCTCCC ACTTCCTCCA AGGACAATTA
 681 CTTAGGGGGC ACTTCGACCA TTTCTGACAA CGCCAAGGAG
 721 TTGTGTAAGG CAGTGTCGGT GTCCATGGGC CTGGGTGTGG
 761 AGGCGTTGGA GCATCTGAGT CCAGGGGAAC AGCTTCGGGG
 801 GGATTGCATG TACGCCCCAC TTTTGGGAGT TCCACCCGCT
 841 GTGCGTCCCA CTCCTTGTGC CCCATTGGCC GAATGCAAAG
 881 GTTCTCTGCT AGACGACAGC GCAGGCAAGA GCACTGAAGA
 921 TACTGCTGAG TATTCCCCTT TCAAGGGAGG TTACACCAAA
 961 GGGCTAGAAG CGAGAGCCT AGGCTGCTCT GGCAGCGCTG
1001 CAGCAGGGAG CTCCGGGACA CTTGAACTGC CGTCTACCCT
1041 GTCTCTCTAC AAGTCCGGAG CACTGGACGA GGCAGCTGCG
1081 TACCAGAGTC GCGACTACTA CAACTTTCCA CTGGCTCTGG
1121 CCGGACCGCC GCCCCTCCG CCGCCTCCCC ATCCCCACGC
1161 TCGCATCAAG CTGGAGAACC CGCTGGACTA CGGCAGCGCC
1201 TGGGCGGCTG CGGCGGCGCA GTGCCGCTAT GGGGACCTGG
1241 CGAGCCTGCA TGGCGCGGGT GCAGCGGGAC CCGGTTCTGG
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1281 GTCACCCTCA GCCGCCGCTT CCTCATCCTG GCACACTCTC

1321 TTCACAGCCG AAGAAGGCCA GTTGTATGGA CCGTGTGGTG

1361 GTGGTGGGGG TGGTGGCGGC GGCGGCGGCG GCGGCGGCGG

1401 CGGCGGCGGC GGCGGCGGCG GCGGCGGCGG CGGCGGCGAG

1441 GCGGGAGCTG TAGCCCCCTA CGGCTACACT CGGCCCCCTC

1481 AGGGGCTGGC GGGCCAGGAA AGCGACTTCA CCGCACCTGA

1521 TGTGTGGTAC CCTGGCGGCA TGGTGAGCAG AGTGCCCTAT

1561 CCCAGTCCCA CTTGTGTCAA AAGCGAAATG GGCCCCTGGA

1601 TGGATAGCTA CTCCGGACCT TACGGGGACA TGCGTTTGGA

1641 GACTGCCAGG GACCATGTTT GCCCATTGA CTATTACTTT

1681 CCACCCCAGA AGACCTGCCT GATCTGTGGA GATGAAGCTT

1721 CTGGGTGTCA CTATGGAGCT CTCACATGTG GAAGCTGCAA

1761 GGTCTTCTTC AAAAGAGCCG CTGAAGGGAA ACAGAAGTAC

1801 CTGTGCGCCA GCAGAAATGA TTGCACTATT GATAAATTCC

1841 GAAGGAAAAA TTGTCCATCT TGTCGTCTTC GGAAATGTTA

1881 TGAAGCAGGG ATGACTCTGG GAGCCCGGAA GCTGAAGAAA

1921 CTTGGTAATC TGAAACTACA GGAGGAAGGA GAGGCTTCCA

1961 GCACCACCAG CCCCACTGAG GAGACAACCC AGAAGCTGAC

2001 AGTGTCACAC ATTGAAGGCT ATGAATGTCA GCCCATCTTT

2041 CTGAATGTCC TGGAAGCCAT TGAGCCAGGT GTAGTGTGTG

2081 CTGGACACGA CAACAACCAG CCCGACTCCT TTGCAGCCTT

2121 GCTCTCTAGC CTCAATGAAC TGGGAGAGAG ACAGCTTGTA

2161 CACGTGGTCA AGTGGGCCAA GGCCTTGCCT GATTGCGAGA

2201 GAGCTGCATC AGTTCACTTT TGACCTGCTA ATCAAGTCAC

2241 ACATGGTGAG CGTGGACTTT CCGGAAATGA TGGCAGAGAT

2281 CATCTCTGTG CAAGTGCCCA AGATCCTTTC TGGGAAAGTC

2321 AAGCCCATCT ATTTCCACAC CCAGTGAAGC ATTGGAAACC

2361 CTATTTCCCC ACCCCAGCTC ATGCCCCCTT TCAGATGTCT

2401 TCTGCCTGTT ATAACTCTGC ACTACTCCTC TGCAGTGCCT

2441 TG

A sequence for human androgen receptor variant 7 is also available from the NCBI database with accession number ACN39559.1 (GI:224181614) (SEQ ID NO:5).

1 MEVQLGLGRV YPRPPSKTYR GAFQNLFQSV REVIQNPGPR

41 HPEAASAAPP GASLLLQQQQ QQQQQQQQQQ QQQQQQQQQQ

61 QQQQQETSPR QQQQQQGEDG SPQAHRRGPT GYLVLDEEQQ

121 PSQPQSALEC HPERGCVPEP GAAVAASKGL PQQLPAPPDE

161 DDSAAPSTLS LLGPTFPGLS SCSADLKDIL SEASTMQLLQ

201 QQQQEAVSEG SSSGRAREAS GAPTSSKDNY LGGTSTISDN

241 AKELCKAVSV SMGLGVEALE HLSPGEQLRG DCMYAPLLGV

281 PPAVRPTPCA PLAECKGSLL DDSAGKSTED TAEYSPFKGG

321 YTKGLEGESL GCSGSAAAGS SGTLELPSTL SLYKSGALDE

361 AAAYQSRDYY NFPLALAGPP PPPPPHPHA RIKLENPLDY

401 GSAWAAAAAQ CRYGDLASLH GAGAAGPGSG SPSAAASSSW

441 HTLFTAEEGQ LYGPCGGGGG GGGGGGGGG GGGGEAGAVA

481 PYGYTRPPQG LAGQESDFTA PDVWYPGGMV SRVPYPSPTC

521 VKSEMGPWMD SYSGPYGDMR LETARDHVLP IDYYFPPQKT

561 CLICGDEASG CHYGALTCGS CKVFFKRAAE GKQKYLCASR

601 NDCTIDKFRR KNCPSCRLRK CYEAGMTLGE KFRVGNCKHL

641 KMTRP

The sequence of the androgen receptor splice variant v7 can vary somewhat from one patient to another. For example, the androgen receptor splice variant v7 detected by the methods, reagents and devices described herein can have at least 75% sequence identity, or at least 80% sequence identity, or at least 85% sequence identity, or at least 90%, or at least 95% sequence identity sequence identity to SEQ ID NO:5.

Nucleotide sequences for the human androgen receptor variant v7 are also available from the NCBI database. For example, a cDNA sequence for the SEQ ID NO:5 human androgen receptor variant v7 is available as accession number FJ235916.1 (GI:224181613), shown below as SEQ ID NO:6.

  1 GACACTGAAT TTGGAAGGTG GAGGATTTTG TTTTTTTCTT

 41 TTAAGATCTG GGCATCTTTT GAATCTACCC TTCAAGTATT

 81 AAGAGACAGA CTGTGAGCCT AGCAGGGCAG ATCTTGTCCA

121 CCGTGTGTCT TCTTCTGCAC GAGACTTTGA GGCTGTCAGA

161 GCGCTTTTTG CGTGGTTGCT CCCGCAAGTT TCCTTCTCTG

201 GAGCTTCCCG CAGGTGGGCA GCTAGCTGCA GCGACTACCG

241 CATCATCACA GCCTGTTGAA CTCTTCTGAG CAAGAGAAGG

281 GGAGGCGGGG TAAGGGAAGT AGGTGGAAGA TTCAGCCAAG

321 CTCAAGGATG GAAGTGCAGT TAGGGCTGGG AAGGGTCTAC

361 CCTCGGCCGC CGTCCAAGAC CTACCGAGGA GCTTTCCAGA

401 ATCTGTTCCA GAGCGTGCGC GAAGTGATCC AGAACCCGGG

441 CCCCAGGCAC CCAGAGGCCG CGAGCGCAGC ACCTCCCGGC

481 GCCAGTTTGC TGCTGCAGCA GCAGCAGCAG CAGCAGCAGC

521 AGCAGCAGCA GCAGCAGCAG CAGCAGCAGC AGCAGCAGCA

561 GCAGCAGCAG CAGCAGCAGC AAGAGACTAG CCCCAGGCAG

601 CAGCAGCAGC AGCAGGGTGA GGATGGTTCT CCCCAAGCCC

641 ATCGTAGAGG CCCCACAGGC TACCTGGTCC TGGATGAGGA

681 ACAGCAACCT TCACAGCCGC AGTCGGCCCT GGAGTGCCAC

721 CCCGAGAGAG GTTGCGTCCC AGAGCCTGGA GCCGCCGTGG

761 CCGCCAGCAA GGGGCTGCCG CAGCAGCTGC CAGCACCTCC

801 GGACGAGGAT GACTCAGCTG CCCCATCCAC GTTGTCCCTG

841 CTGGGCCCCA CTTTCCCCGG CTTAAGCAGC TGCTCCGCTG
881 ACCTTAAAGA CATCCTGAGC GAGGCCAGCA CCATGCAACT
921 CCTTCAGCAA CAGCAGCAGG AAGCAGTATC CGAAGGCAGC
961 AGCAGCGGGA GAGCGAGGGA GGCCTCGGGG GCTCCCACTT
1001 CCTCCAAGGA CAATTACTTA GGGGGCACTT CGACCATTTC
1041 TGACAACGCC AAGGAGTTGT GTAAGGCAGT GTCGGTGTCC
1081 ATGGGCCTGG GTGTGGAGGC GTTGGAGCAT CTGAGTCCAG
1121 GGGAACAGCT TCGGGGGGAT TGCATGTACG CCCCACTTTT
1161 GGGAGTTCCA CCCGCTGTGC GTCCCACTCC TTGTGCCCCA
1201 TTGGCCGAAT GCAAAGGTTC TCTGCTAGAC GACAGCGCAG
1241 GCAAGAGCAC TGAAGATACT GCTGAGTATT CCCCTTTCAA
1281 GGGAGGTTAC ACCAAAGGGC TAGAAGGCGA GAGCCTAGGC
1321 TGCTCTGGCA GCGCTGCAGC AGGGAGCTCC GGGACACTTG
1361 AACTGCCGTC TACCCTGTCT CTCTACAAGT CCGGAGCACT
1401 GGACGAGGCA GCTGCGTACC AGAGTCGCGA CTACTACAAC
1441 TTTCCACTGG CTCTGGCCGG ACCGCCGCCC CCTCCGCCGC
1481 CTCCCCATCC CCACGCTCGC ATCAAGCTGG AGAACCCGCT
1521 GGACTACGGC AGCGCCTGGG CGGCTGCGGC GGCGCAGTGC
1561 CGCTATGGGG ACCTGGCGAG CCTGCATGGC GCGGGTGCAG
1601 CGGGACCCGG TTCTGGGTCA CCCTCAGCCG CCGCTTCCTC
1641 ATCCTGGCAC ACTCTCTTCA CAGCCGAAGA AGGCCAGTTG
1681 TATGGACCGT GTGGTGGTGG TGGGGGTGGT GGCGGCGGCG
1721 GCGGCGGCGG CGGCGGCGGC GGCGGCGGCG AGGCGGGAGC
1761 TGTAGCCCCC TACGGCTACA CTCGGCCCCC TCAGGGGCTG
1801 GCGGGCCAGG AAAGCGACTT CACCGCACCT GATGTGTGGT
1841 ACCCTGGCGG CATGGTGAGC AGAGTGCCCT ATCCCAGTCC
1881 CACTTGTGTC AAAAGCGAAA TGGGCCCCTG GATGGATAGC
1921 TACTCCGGAC CTTACGGGGA CATGCGTTTG GAGACTGCCA
1961 GGGACCATGT TTTGCCCATT GACTATTACT TTCCACCCCA
2001 GAAGACCTGC CTGATCTGTG GAGATGAAGC TTCTGGGTGT
2041 CACTATGGAG CTCTCACATG TGGAAGCTGC AAGGTCTTCT
2081 TCAAAAGAGC CGCTGAAGGG AAACAGAAGT ACCTGTGCGC
2121 CAGCAGAAAT GATTGCACTA TTGATAAATT CCGAAGGAAA
2161 AATTGTCCAT CTTGTCGTCT TCGGAAATGT TATGAAGCAG
2201 GGATGACTCT GGGAGAAAAA TTCCGGGTTG GCAATTGCAA
2241 GCATCTCAAA ATGACCAGAC CCTGAAGAAA GGCTGACTTG
2281 CCTCATTCAA AATGAGGGCT CTAGAGGGCT CTAGTGGATA
2321 GTCTGGAGAA ACCTGGCGTC TGAGGCTTAG GAGCTTAGGT
2361 TTTTGCTCCT CAACACAGAC TTTGACGTTG GGGTTGGGGG
2401 CTACTCTCTT GATTGCTGAC TCCCTCCAGC GGGACCAATA

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2441 GTGTTTTCCT ACCTCACAGG GATGTTGTGA GGACGGGCTG

2481 TAGAAGTAAT AGTGGTTACC ACTCATGTAG TTGTGAGTAT

2521 CATGATTATT GTTTCCTGTA ATGTGGCTTG GCATTGGCAA

2561 AGTGCTTTTT GATTGTTCTT GATCACATAT GATGGGGGCC

2601 AGGCACTGAC TCAGGCGGAT GCAGTGAAGC TCTGGCTCAG

2641 TCGCTTGCTT TTCGTGGTGT GCTGCCAGGA AGAAACTTTG

2681 CTGATGGGAC TCAAGGTGTC ACCTTGGACA AGAAGCAACT

2721 GTGTCTGTCT GAGGTTCCTG TGGCCATCTT TATTTGTGTA

2761 TTAGGCAATT CGTATTTCCC CCTTAGGTTC TAGCCTTCTG

2801 GATCCCAGCC AGTGACCTAG ATCTTAGCCT CAGGCCCTGT

2841 CACTGAGCTG AAGGTAGTAG CTGATCCACA GAAGTTCAGT

2881 AAACAAGGAC CAGATTTCTG CTTCTCCAGG AGAAGAAGCC

2921 AGCCAACCCC TCTCTTCAAA CACACTGAGA GACTACAGTC

2961 CGACTTTCCC TCTTACATCT AGCCTTACTG TAGCCACACT

3001 CCTTGATTGC TCTCTCACAT CACATGCTTC TCTTCATCAG

3041 TTGTAAGCCT CTCATTCTTC TCCCAAGCCA GACTCAAATA

3081 TTGTATTGAT GTCAAAGAAG AATCACTTAG AGTTTGGAAT

3121 ATCTTGTTCT CTCTCTGCTC CATAGCTTCC ATATTGACAC

3161 CAGTTTCTTT CTAGTGGAGA AGTGGAGTCT GTGAAGCCAG

3201 GGAAACACAC ATGTGAGAGT CAGAAGGACT CTCCCTGACT

3241 TGCCTGGGGC CTGTCTTTCC CACCTTCTCC AGTCTGTCTA

3281 AACACACACA CACACACACA CACACACACA CACACACACA

3321 CACACGCTCT CTCTCTCTCT CCCCCCCCAA CACACACACA

3361 CTCTCTCTCT CACACACACA CACATACACA CACACTTCTT

3401 TCTCTTTCCC CTGACTCAGC AACATTCTGG AGAAAAGCCA

3441 AGGAAGGACT TCAGGAGGGG AGTTTCCCCC TTCTCAGGGC

3481 AGAATTTTAA TCTCCAGACC AACAAGAAGT TCCCTAATGT

3521 GGATTGAAAG GCTAATGAGG TTTATTTTTA ACTACTTTCT

3561 ATTTGTTTGA ATGTTGCATA TTTCTACTAG TGAAATTTTC

3601 CCTTAATAAA GCCATTAATA CACCCAAAAA AAAAAAAAAA

3641 A

The inventors have recently determined that the androgen receptor (AR) binds cellular microtubules (MTs) and utilizes them as tracks for nuclear translocation, with the aid of the microtubule-associated motor protein dynein (Darshan et al., Taxane-Induced Blockade to Nuclear Accumulation of the Androgen Receptor Predicts Clinical Responses in Metastatic Prostate Cancer. Cancer research. (2011)). In this application, the inventors extend these observations to the clinical setting by analyzing the subcellular androgen receptor localization in circulating tumor cells (CTCs) isolated from castration-resistant prostate cancer patients (e.g., about 30 patients) receiving taxane chemotherapy. These analyses revealed a significant relationship between androgen receptor cytoplasmic sequestration and

clinical response to taxane treatment using PSAWG2 or Prostate Cancer Working Group 2 criteria. The Prostate Cancer Working Group 2 criteria are generally described, for example, by Bubley et al. (J. Clin. Oncol. 17:3461-67 (1999)); and Scher et al. (J. Clin. Oncol. 26(7): 1148-59 (2008)), which are both specifically incorporated herein by reference in their entireties. Taken together, the results challenge the existing paradigm in which the clinical activity of the taxanes is largely attributed to their antimitotic effects.

As described herein, the ARv5,6,7 and AR-v7 androgen receptors were evaluated for their ability to bind microtubules, translocate to the nucleus with dynein and respond to taxane treatment. Data described herein shows that the two variants behave differently in all aspects. Microtubule co-sedimentation revealed that ARv5,6,7 is associated with microtubules, in contrast to the significantly reduced association displayed by AR-v7. Dynamitin overexpression inhibited the nuclear accumulation of full length androgen receptor and ARv5,6,7 but had no effect on AR-V7, indicating that AR-v7's nuclear translocation is independent of dynein-based microtubules transport.

To examine the impact of taxane treatment on variant activity we microinjected GFP-tagged full length androgen receptor, or variants into the nucleus of PC3 prostate cancer cells and monitored the dynamics of nuclear translocation of androgen receptor proteins using live-cell confocal microscopy and androgen receptor transcriptional activity using a luciferase reporter assay. The data described herein shows that taxanes significantly inhibited the nuclear accumulation and activity of full length androgen receptor and ARv5,6,7 but not that of AR-v7. Moreover, the tumor volume of human xenografts models expressing the ARv5,6,7 were significantly reduced in response to taxane treatment as compared with xenografts expressing either full length androgen receptor or other variants. Taken together these data reveal that functional microtubules are required for ARv5,6,7 nuclear transport, but this not so for AR-v7, which is not under microtubule control and thus, remains insensitive to taxane treatment.

Clinically, the taxanes (e.g., paclitaxel, docetaxel and cabazitaxel), represent the only class of chemotherapy drugs that improve survival in castration-resistant prostate cancer patients. However, not all patients respond and the clinical efficacy of taxanes can be transient. Currently, there are two clinical questions that remain to be answered:

1) how can we predict which patients will benefit the most from taxane-based chemotherapy and can we identify patients more likely to respond to paclitaxel, docetaxel or cabazitaxel

2) what are the mechanisms contributing to the development of clinical taxane resistance in metastatic prostate cancer.

As described herein, the presence of distinct androgen receptor splice variants that were identified in castration-resistant prostate cancer patients can be used as a predictive biomarker of taxane sensitivity. The new data show that the commonly expressed ARv5,6,7 (which lacks exons 5, 6 and 7 harboring the ligand-binding domain) binds microtubules and is sensitive to taxane-induced inhibition of its nuclear accumulation; while the ARv7 variant does not bind microtubules and its nuclear accumulation is not affected by taxane treatment.

Further work has demonstrated that xenografts with tumors expressing the ARv5,6,7 variant are much more sensitive to docetaxel treatment compared with xenografts whose tumors express full-length wild-type androgen receptor.

Detection Methods

A variety of methods can be used for identifying patients for treatment with taxanes, and for avoiding taxane treatment when it will likely have no beneficial effects.

Prostate cancer patients who can benefit from administration of one or more taxanes can be identified by observation that the androgen variant v5,6,7 is expressed by cells within samples taken from the patient(s). Such taxane responsive patients can be identified through detection of the ARv5,6,7 variant in RNA molecules or via the encoded ARv5,6,7 variant protein in a biological sample comprising tumor cells obtained from the patient.

A patient population of individuals who are likely to be resistant or non-respondent to taxane treatment can be identified by observation that the androgen variant v7 is expressed by cells within samples taken from the individual(s). Such non-taxane responsive patients can be identified through detection of the ARv7 variant in RNA molecules or via the encoded ARv7 variant protein in a biological sample comprising tumor cells obtained from the patient.

The biological sample can, for example, be circulating tumor cells, or prostate tissue sample. The biological sample can also, for example, be a fresh or frozen or archived paraffin-embedded and fixed (e.g. formalin-fixed) tissue sample, routinely prepared and preserved in everyday clinical practice. The biological sample can also be a different sample obtained from the patient, such as a biological fluid, including, without limitation, blood, urine, saliva, ascites fluid, or derivatives such as blood serum and blood plasma, and the like.

Various methods for determining expression of mRNA or protein include, but are not limited to, gene expression profiling, polymerase chain reaction (PCR) including quantitative real time PCR (qRT-PCR), microarray analysis that can be performed by commercially available equipment, following manufacturer's protocols, such as by using the Affymetrix GenChip technology, serial analysis of gene expression (SAGE) (Velculescu et al, Science 270:484-487 (1995); and Velculescu el al, Cell 88:243-51 (1997)), MassARRAY, Gene Expression Analysis by Massively Parallel Signature Sequencing (MPSS) (Brenner et al, Nature Biotechnology 18:630-634 (2000)), proteomics, immunohistochemistry (IHC), etc. For example, methods for detecting and/or quantifying expression of mRNA can include Northern blotting, nuclease protection assays, RNA fingerprinting, polymerase chain reaction, ligase chain reaction, Qbeta replicase, isothermal amplification method, strand displacement amplification, transcription based amplification systems, quantitative nucleic acid amplification assays (e.g., polymerase chain reaction assays), combined reverse transcription/nucleic acid amplification, nuclease protection (SI nuclease or RNAse protection assays), Serial Analysis Gene Expression (SAGE), next generation sequencing, gene expression microarray, and combinations thereof.

The mRNA levels encoding the androgen receptor variant can be quantified. Such mRNA analysis is can be performed using the technique of polymerase chain reaction (PCR), or by microarray analysis. Quantitative real time PCR (qRT- PCR) can be used to quantify the mRNA levels.

Polynucleotide microarrays can be used to simultaneously measure whether or not any of several microRNAs are expressed. Generally, microarrays include probes for a plurality of microRNAs informative for benign / malignancy determination, for a particular disease or condition, and, in particular, for

individuals having specific combinations of genotypic or phenotypic characteristics of the disease or condition (i.e., that are prognosis-informative for a particular patient subset).

A standard Northern blot assay can be used to ascertain an RNA transcript size, and the relative amounts of mRNA in a sample, in accordance with conventional Northern hybridization techniques known to those persons of ordinary skill in the art. In Northern blots, RNA samples are first separated by size via electrophoresis in an agarose gel under denaturing conditions. The RNA is then transferred to a membrane, crosslinked and hybridized with a labeled probe. Nonisotopic or high specific activity radiolabeled probes can be used including random-primed, nick-translated, or PCR-generated DNA probes, in vitro transcribed RNA probes, and oligonucleotides. Additionally, sequences with only partial homology (e.g., a microRNA from a different species or genomic DNA fragments that might contain an exon) may be used as probes. The labeled probe can be a labeled cDNA; a full-length, single stranded labeled RNA or DNA, or a labeled fragment of that RNA or DNA sequence.

Such a RNA or DNA (or fragments therefore) may serve as a probe, for example, when it is at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or at least 16 consecutive nucleotides in length. In some embodiments, the probe is about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21 or about 22 consecutive nucleotides in length. In further embodiments, the probe may be at least 20, at least 30, at least 50, or at least 70 consecutive nucleotides in length. The primers and/or probes can be less than about 80, less than about 70, less than about 60, less than about 50, less than about 45, less than about 40, less than about 39, less than about 38, less than about 37, less than about 36, less than about 35, less than about 34, less than about 33, less than about 32, less than about 31, or less than about 30 consecutive nucleotides in length.

The probe can be labeled by any of the many different methods known to those skilled in this art. The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals that fluoresce when exposed to ultraviolet light, and others. A number of fluorescent materials are known and can be utilized as labels. These include, but are not limited to, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow.

Methods for profiling gene expression using fixed, paraffin-embedded tissues as the RNA source can also be employed. Such methods can include mRNA isolation, purification, primer extension and amplification are given in various published journal articles (for example: Godfrey et al J. Molec. Diagnostics 2: 84-91 (2000); Specht et al, Am. J. Pathol 158: 419-29 (2001)). Briefly, a representative process starts with cutting about 10 microgram thick sections of paraffin-embedded tumor tissue samples. The mRNA is then extracted, and protein and DNA are removed. General methods for mRNA extraction are well known in the art and are disclosed in standard textbooks of molecular biology, including Aiisubel et al, Current Protocols of Molecular Biology, John Wiley and Sons (1997). Methods for RNA extraction from paraffin embedded tissues are disclosed, for example, in Rupp and Locker, Lab Invest. 56:A67 (1987), and De Andres et al, BioTechniques 18:42044 (1995). In particular, RNA isolation can be performed using purification kit, buffer set and protease from commercial manufacturers, such as Qiagen, according to the manufacturer's instructions. For example, total RNA from cells in culture can be isolated using Qiagen RNeasy mini-columns. Other commercially available RNA isolation kits include

MasterPure™ Complete DNA and RNA Purification Kit (EPICENTRE®, Madison, WI), and Paraffin Block RNA Isolation Kit (Ambion, Inc.). Total RNA from tissue samples can be isolated using RNA Stat-60 (Tel-Test). RNA prepared from tumor can be isolated, for example, by cesium chloride density gradient centrifugation. After analysis of the RNA concentration, RNA repair and/or amplification steps may be

5   included, if necessary, and RNA is reverse transcribed using gene specific promoters followed by PCR. Nuclease protection assays such as ribonuclease protection assays and S1 nuclease assays can be used to detect and quantify specific microRNAs. In nuclease protection assays, an antisense probe (labeled with, e.g., radiolabeled or nonisotopic) hybridizes in solution to an RNA sample. Following hybridization, single-stranded, unhybridized probe and RNA are degraded by nucleases. An acrylamide gel is used to

10   separate the remaining protected fragments. Typically, solution hybridization is more efficient than membrane-based hybridization, and it can accommodate up to 100 μg of sample RNA, compared with the 20-30 μg maximum of blot hybridizations.

       A ribonuclease protection assay employs RNA probes. Oligonucleotides and other single-stranded DNA probes can only be used in assays containing S1 nuclease. The single-stranded, antisense

15   probe must typically be completely homologous to target RNA to prevent cleavage of the probe:target hybrid by nuclease.

       Serial Analysis Gene Expression (SAGE), which is described in e.g., Velculescu et al., 1995, *Science* 270:484-7; Carulli, et al., 1998, *Journal of Cellular Biochemistry Supplements* 30/31:286-96, can also be used to determine RNA abundances in a cell sample.

20   Quantitative reverse transcriptase PCR (qRT-PCR) can also be used to determine the expression profiles of microRNA genes (see, e.g., U.S. Patent Application Publication No. 2005/0048542A1). The first step in gene expression profiling by RT-PCR is the reverse transcription of the RNA template into cDNA, followed by its exponential amplification in a PCR reaction. The two most commonly used reverse transcriptases are avilo myeloblastosis virus reverse transcriptase (AMV-RT) and Moloney murine

25   leukemia virus reverse transcriptase (MLV-RT). The reverse transcription step is typically primed using specific primers, random hexamers, or oligo-dT primers, depending on the circumstances and the goal of expression profiling. For example, extracted RNA can be reverse-transcribed using a GeneAmp RNA PCR kit (Perkin Elmer, Calif., USA), following the manufacturer's instructions. The derived cDNA can then be used as a template in the subsequent PCR reaction.

30   An assay involving PCR can use a variety of thermostable DNA-dependent DNA polymerases. Commonly employed polymerases include the Taq DNA polymerase, which has a 5'-3' nuclease activity but lacks a 3'-5' proofreading endonuclease activity. TaqMan® PCR typically utilizes the 5'-nuclease activity of Taq or Tth polymerase to hydrolyze a hybridization probe bound to its target amplicon, but any enzyme with similar or equivalent 5' nuclease activity can be used. Two oligonucleotide primers are used

35   to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA

40   polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments

disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

TaqMan® RT-PCR can be performed using commercially available equipment, such as, for example, ABI PRISM 7700™. Sequence Detection System™ (Perkin-Elmer-Applied Biosystems, Foster City, Calif., USA), or Lightcycler (Roche Molecular Biochemicals, Mannheim, Germany). In one embodiment, the 5′ nuclease procedure is run on a real-time quantitative PCR device such as the ABI PRISM 7700™ Sequence Detection System™. The system consists of a thermocycler, laser, charge-coupled device (CCD), camera and computer. The system includes software for running the instrument and for analyzing the data.

For example, the following primers and/or probes can be used to detect the v5,6,7 variant of the human androgen receptor:

Probe: 5'-CCTTGCCTGATTGCGAGA-3' (SEQ ID NO:10).

Forward primer: 5'-CCTTGCTCTCTAGCCTCAATGAA-3' (SEQ ID NO:11).

Reverse primer: 5'-CTTGATTAGCAGGTCAAAAGTGAACT-3' (SEQ ID NO:12).

To minimize errors and the effect of sample-to-sample variation, RT-PCR is often performed using an internal standard. The ideal internal standard is expressed at a constant level among different tissues, and is unaffected by the experimental treatment. RNAs most frequently used to normalize patterns of gene expression are 18S rRNA or mRNAs for the housekeeping genes such as glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) or β-actin.

Real time PCR can be used in the methods, which is compatible both with quantitative competitive PCR, where internal competitor for each target sequence is used for normalization, and with quantitative comparative PCR using a normalization gene contained within the sample, or a housekeeping gene for RT-PCR. For further details see, e.g. "PCR: The Polymerase Chain Reaction", Miillis et al., eds., 1994; and Held et al, Genome Research 6:986-994 (1996); Held et al., Genome Research 6:986-994 (1996). Finally, the data are analyzed to identify the best treatment option(s) available to the patient on the basis of the characteristic gene expression pattern identified in the tumor sample examined.

In some embodiments, the quantitative RT-PCR assay data are presented as Ct values, also referred to as ΔCt thresholds. The ΔCt (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross a detectable threshold. The ΔCt is a measure of when the amount of RNA expressed exceeds background levels. Ct threshold levels are inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the Ct threshold the greater the amount of target nucleic acid in the sample). Fluorescence values are recorded during every cycle and represent the amount of product amplified to that point in the amplification reaction. The point when the fluorescent signal is first recorded as statistically significant is the threshold cycle (ΔCt).

Thus, many of the RNA detection and quantification methods involve hybridization to a probe (e.g., probe(s) or primer(s) selected from a segment of a nucleic acid encoding androgen variant v5,6,7 or androgen variant v7). Nucleic acid hybridization involves contacting a probe and target nucleic acid under conditions where the probe and its complementary target can form stable hybrid duplexes through complementary base pairing (see Lockhart et al., 1999, WO 99/32660, for example). The target of

hybridization is an androgen receptor mRNA, such as a wild type, androgen receptor mRNA, an androgen variant v5,6,7 mRNA, an androgen variant v7, or a combination thereof. The nucleic acids that do not form hybrid duplexes during hybridization are washed away during a washing step, leaving the hybridized nucleic acids to be detected, for example, through detection of a label that intercalates into the hybrid or that is attached to the probe or primer. It is generally recognized that nucleic acids are denatured by increasing the temperature and/or decreasing the salt concentration of the buffer containing the nucleic acids.

Under low stringency conditions (e.g., low temperature and/or high salt) hybrid duplexes (e.g., DNA-DNA, RNA-RNA or RNA-DNA) will form even where the annealed sequences are not perfectly complementary. Thus specificity of hybridization is reduced at lower stringency. Conversely, at higher stringency (e.g., higher temperature or lower salt) successful hybridization occurs with fewer mismatches.

One of skill in the art will appreciate that hybridization conditions may be selected to provide any degree of stringency. Stringency can also be increased by addition of agents such as formamide. Hybridization specificity may be evaluated by comparison of hybridization to the test probes with hybridization to the various controls that can be present (e.g., expression level control, normalization control, mismatch controls, etc.). For example, one control can be a normalization control such as a housekeeping gene. Examples of controls for normalization of expression include expression of 18S rRNA, actin, or GAPDH.

In general, there is a tradeoff between hybridization specificity (stringency) and signal intensity. Thus, in some embodiments, the wash is performed at the highest stringency that produces consistent results and that provides a signal intensity greater than approximately 10% of the background intensity. To better distinguish between the signal and the background, the hybridized sequences (e.g., on a microarray) may be washed at successively higher stringency solutions and read between each wash. Analysis of the data sets thus produced will reveal a wash stringency above which the hybridization pattern is not appreciably altered and which provides adequate signal for the particular oligonucleotide probes of interest.

As used herein, the terms "hybridize" and "hybridization" refer to the annealing of a complementary sequence to the target nucleic acid, i.e., the ability of two polymers of nucleic acid (polynucleotides) containing complementary sequences to anneal through base pairing. The terms "annealed" and "hybridized" are used interchangeably throughout, and are intended to encompass any specific and reproducible interaction between a complementary sequence and a target nucleic acid, including binding of regions having only partial complementarity. Certain bases not commonly found in natural nucleic acids may be included in the nucleic acids of the present invention and include, for example, inosine and 7-deazaguanine. Those skilled in the art of nucleic acid technology can determine duplex stability empirically considering a number of variables including, for example, the length of the complementary sequence, base composition and sequence of the oligonucleotide, ionic strength and incidence of mismatched base pairs. The stability of a nucleic acid duplex is measured by the melting temperature, or "T_m". The T_m of a particular nucleic acid duplex under specified conditions is the temperature at which on average half of the base pairs have disassociated.

The term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds, under which nucleic acid hybridizations are conducted. With "high stringency" conditions, nucleic acid base pairing will occur only between nucleic acid fragments that have a high frequency of complementary base sequences. Thus, conditions of "medium" or "low" stringency are often required when it is desired that nucleic acids which are not completely complementary to one another be hybridized or annealed together. The art knows well that numerous equivalent conditions can be employed to comprise medium or low stringency conditions. The choice of hybridization conditions is generally evident to one skilled in the art and is usually guided by the purpose of the hybridization, the type of hybridization (DNA-DNA or DNA-RNA), and the level of desired relatedness between the sequences (e.g., Sambrook et al. (1989); Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington D.C. 1985, for a general discussion of the methods).

The stability of nucleic acid duplexes is known to decrease with an increased number of mismatched bases, and further to be decreased to a greater or lesser degree depending on the relative positions of mismatches in the hybrid duplexes. Thus, the stringency of hybridization can be used to maximize or minimize stability of such duplexes. Hybridization stringency can be altered by: adjusting the temperature of hybridization; adjusting the percentage of helix destabilizing agents, such as formamide, in the hybridization mix; and adjusting the temperature and/or salt concentration of the wash solutions. For filter hybridizations, the final stringency of hybridizations often is determined by the salt concentration and/or temperature used for the post-hybridization washes.

"High stringency conditions" when used in reference to nucleic acid hybridization include conditions equivalent to binding or hybridization at 42 °C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed. In general, the stringency of hybridization is determined by the wash step. Hence, a wash step involving 0.1X SSPE, 1.0% SDS at a temperature of at least 42°C can yield a high stringency hybridization product. In some instances the high stringency hybridization conditions include a wash in 1X SSPE, 1.0% SDS at a temperature of at least 50ºC, or at about 65ºC.

"Medium stringency conditions" when used in reference to nucleic acid hybridization include conditions equivalent to binding or hybridization at 42☐ºC in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0X SSPE, 1.0% SDS at 42ºC when a probe of about 500 nucleotides in length is employed. Hence, a wash step involving 1.0X SSPE, 1.0% SDS at a temperature of 42ºC can yield a medium stringency hybridization product.

"Low stringency conditions" include conditions equivalent to binding or hybridization at 42ºC in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5X Denhardt's reagent [50X Denhardt's contains per 500 ml: 5 g Ficoll (Type 400, Pharmacia), 5 g BSA (Fraction V; Sigma)] and 100 g/ml denatured salmon sperm DNA followed by washing in a solution comprising 5X SSPE, 0.1% SDS at 42ºC when a probe of about 500 nucleotides in

length is employed. Hence, a wash step involving 5X SSPE, 1.0% SDS at a temperature of 42ºC can yield low stringency hybridization product.

Expression levels can also be determined at the protein level, for example, using various types of immunoassays or proteomics techniques.

In immunoassays, the target diagnostic protein marker (e.g., ARv5,6,7 and/or ARv7) is detected by using an binding entity or antibody that specifically binds to the marker(s). The antibody can be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories. A variety of immunoassay procedures can be employed such as a cell sorting assay (where cells are labeled with a selected binding entity or antibody), sandwich immunoassay, competition inhibition immunoassay, ELISA (Enzyme-Linked Immunosorbent Assay), agglutination assay, precipitation assay, radioimmunoassay or antigen-down immunoassay or immunometric assay.

Fluorescent labels such as rare earth chelates (europium chelates) or fluorescein and its derivatives, rhodamine and its derivatives, dansyl. Lissamine, phycoerythrin and Texas Red are available. The fluorescent labels can be conjugated to the antibody using the techniques disclosed in Current Protocols in Immunology, supra, for example. Fluorescence can be quantified using a fluorimeter.

The antibody can be labeled with the radioisotope such as ^{35}S, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, using the techniques described in Current Protocols in Immunology, Volumes 1 and 2, Coligen et al. (1991) Ed. Wiley-Interscience, New York, New York, Pubs, for example, and radioactivity can be measured using scintillation counting.

Various enzyme-substrate labels are available and U.S. Patent No. 4,275,149 provides a review of some of these. The enzyme generally catalyzes a chemical alteration of the chromogenic substrate which can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are available in the art. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor.

Examples of enzymatic labels include luciferases (e.g., firefly Luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases {e.g., glucose oxidase, galactose oxidase, and gliicose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan et al. (1981) Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in Methods in Enzymol. (eds. J. Langone & H. Van Vunakis), Academic press, New York 73: 147-166.

Examples of enzyme-substrate combinations include, for example: horseradish peroxidase (HRPO) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (e.g., orthophenylene diamine (OPD) or 3, 3', 5,5'- tetramethylbenzidine hydrochloride (TMB)); alkaline phosphatase (AP) with para-nitrophenyl phosphate as chromogenic substrate; and β-D-

galactosidase (β-D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl-β-D- galactosidase) or fluorogenic substrate 4-methylumbelliferyl-β-D-galactosidase.

Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review of these, see U.S. Patent Nos. 4,275,149 and 4,318,980.

Sometimes, the label is indirectly conjugated with the antibody. The skilled artisan will be aware of various techniques for achieving this. For example, the antibody can be conjugated with biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin, or vice versa. Biotin binds selectively to avidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody is conjugated with a small hapten (e.g., digoxin) and one of the different types of labels mentioned above is conjugated with an anti-hapten antibody (e.g., anti-digoxin antibody). Thus, indirect conjugation of the label with the antibody can be achieved.

In other versions of immunoassay techniques, the antibody need not be labeled, and the presence thereof can be detected using a labeled antibody which binds to the antibody. Thus, the diagnostic immunoassays herein may be in any assay format, including, for example, competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, Monoclonal Antibodies: A Manual of Techniques, pp.147- 158 (CRC Press, Inc. 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyze for binding with a limited amount of antibody. The amount of antigen in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and analyze that are bound to the antibodies may conveniently be separated from the standard and analyze which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyze is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyze, thus forming an insoluble three-part complex. See, e.g., U.S. Pat No. 4,376,1 10. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

Protein levels can also be detected using proteomics techniques. The term '"proteome" refers to the totality of the proteins present in a sample (e.g. tissue, organism, or cell culture) at a certain point of time. Proteomics includes, among other things, study of the global changes of protein expression in a sample (also referred to as "expression proteomics"). Proteomics typically includes the following steps: (1) separation of individual proteins in a sample by 2-dimensional gel electrophoresis (e.g., 2-D PAGE); (2) identification of the individual proteins within or recovered from the gel, e.g. immunoblotting, by mass spectrometry, or N-terminal sequencing, and (3) analysis of the data using bioinformatics. Proteomics methods arc valuable alternatives or supplements to other methods of gene expression profiling, and can

be used, alone or in combination with other methods, to detect the products of the tumor resistance markers of the present invention.

Measurement of biomarker expression levels can be performed by using a software program executed by a suitable processor. Suitable software and processors are well known in the art and are

5   commercially available. The program may be embodied in software stored on a tangible medium such as CD-ROM, a floppy disk, a hard drive, a DVD, or a memory associated with the processor, but persons of ordinary skill in the art will readily appreciate that the entire program or parts thereof could alternatively be executed by a device other than a processor, and/or embodied in firmware and/or dedicated hardware by available procedures.

10   Following the measurement of the expression levels of the genes identified herein, or their expression products, and the determination that a subject is likely or not likely to respond to treatment with taxane, the assay results, findings, diagnoses, predictions and/or treatment recommendations are typically recorded and communicated to technicians, physicians and/or patients, for example. In certain embodiments, computers will be used to communicate such information to interested parties, such as,

15   patients and/or the attending physicians. In some embodiments, the assays will be performed or the assay results analyzed in a country or jurisdiction which differs from the country or jurisdiction to which the results or diagnoses are communicated.

A diagnosis, prediction and/or treatment recommendation based on the expression of a particular marker (e.g., ARv5,6,7 or ARv7), or upon the level of a marker in a test subject is communicated to the

20   subject as soon as possible after the assay is completed and the diagnosis and/or prediction is generated. The results and/or related information may be communicated to the subject by the subject's treating physician. Alternatively, the results may be communicated directly to a test subject by any means of communication, including writing, electronic forms of communication, such as email, or telephone.

In certain embodiments, the communication containing results of a diagnostic test and/or

25   conclusions drawn from and/or treatment recommendations based on the test, may be generated and delivered automatically to the subject using a combination of computer hardware and software which will be familiar to artisans skilled in telecommunications. One example of a healthcare-oriented communications system is described in U.S. Pat. No. 6,283,761; however, the present invention is not limited to methods which utilize this particular communications system.

30   The taxane sensitivity of a patient, and the expression of an androgen receptor variant, can also be detected by whether genes normally regulated by wild type androgen receptor are normally expressed. For example, the following genes normally interact with androgen receptors: AKT1, BAG1, Beta-catenin, BRCA1, C-jun, Calmodulin 1, Caveolin 1, CDK9, COX5B, CREB-binding protein, Cyclin D1, Cyclin-dependent kinase 7, Death associated protein 6, Deleted in Colorectal Cancer, EFCAB6, Epidermal

35   growth factor receptor, FOXO1, GAPDH, Gelsolin, GNB2L1, GSK3B, HDAC1, HSP90AA1, HTATIP, MAGEA11, MED1, MYST2, NCOA1, NCOA2, NCOA3, NCOA4, NCOA6, NCOR2, NONO, PA2G4, PAK6, PATZ1,PIAS2, PRPF6, PTEN, RAD9A, RANBP9, RCHY1, Retinoblastoma protein, RNF14, RNF4, SART3, SMAD3, Small heterodimer partner, Src, SRY, STAT3, SVIL, Testicular receptor 2, Testicular receptor 4, TGFB1I1, TMF1, TRIM68, UBE2I, UXT, ZMIZ1, and any combination thereof. These genes

40   are referred to herein as "androgen receptor regulated genes." When an androgen receptor variant (e.g.,

ARv5,6,7 or Arv7) is expressed instead of, or in addition to, the wild type androgen receptor, the expression of these androgen receptor regulated genes is altered. Thus, the expression of an androgen receptor variant (e.g., ARv5,6,7 or Arv7) can be detected by detecting alternations in the expression of one or more androgen receptor-related genes in a test sample relative to expression levels of these

5   androgen receptor-related genes in a wild type, healthy sample of cells.

Capture and Detection of Markers in Circulating Tumor Cells

The development of metastases in patients with solid tumor malignancies can result from tumor cells entering the circulatory system and migrating to distant organs, where they extravasate and multiply.

10   Circulating tumor cells (CTCs) are rare—as few as one cell per 100 million blood cells.

A variety of technologies has been developed to improve the detection and capture of circulating tumor cells from the peripheral blood. These include density gradient centrifugation, immunomagnetic bead separation using monoclonal antibodies targeting epithelial cell-surface antigens, cell sorting using flow cytometry, filtration based size separation and microfluidic devices. Although advances in circulating

15   tumor cell capture have been made, the low frequency of circulating tumor cells in cancer patients, their heterogeneity, the lack of organ-specific capture approaches, and the plasticity of the circulating tumor cell population has limited the ability to capture and track all circulating tumor cells. Currently, the epithelial cell-adhesion molecule (EpCAM), represents an antigen of choice for the majority of microfluidic devices that have been developed to capture circulating tumor cells.

20   However, accumulating evidence indicates that the expression of EpCAM during cancer progression and, in particular, during epithelial-to-mesenchymal transition has not been well characterized, raising concerns about the universality of this antigen for immunocapture systems (Mani et al., Cell 133: 704-715 (2008); Polyak & Weinberg, Nature reviews Cancer 9: 265-273 (2009)). EpCAM has been reported to have oncogenic potential and its presence can correlate with proliferation in cell

25   lines. However, it is down-regulated during epithelial-to-mesenchymal transition, and epithelial-to-mesenchymal transition markers have been shown to be more important than epithelial markers (e.g., cytokeratin) in predicting cancer progression (Gradilone et al., J. Cell. Molec. Med. 15: 1066-1070 (2011)). Thus, while EpCAM is clearly useful in identifying circulating tumor cell populations in many cancers, the biases associated with EpCAM enrichment are currently unknown.

30   In addition to the uncertainties regarding surface antigens, the specificity of immunocapture from the blood is confounded by the non-specific adhesive properties of leukocytes on most antibody surfaces. Because of the presence of numerous leukocytes in blood at an approximately 10^4-10^5:1 ratio with respect to the circulating tumor cells, immunospecific surfaces enrich circulating tumor cells but cannot isolate them from contaminating leukocytes entirely. Identifying circulating tumor cells requires additional steps

35   and often involves staining with DAPI to ensure the presence of an intact nucleus and immunostaining to identify the presence of epithelial markers (i.e., cytokeratin) and the lack of the leukocytic marker CD45. Such immunostaining has identified a family of criteria that correlate circulating tumor cell number with patient prognosis (Coumans et al., J. Eur. Soc. Med. Oncol. 21: 1851-57 (2010), but these criteria are based upon fixation and staining procedures for circulating tumor cell identification, as in the commercial

40   CellSearch® system (Danila et al., Clin. Cancer Res. 13:7053-58 (2007; Scher et al.., The Lancet Oncol.

10: 233-239 (2009)). Although enumeration of circulating tumor cells from patients with advanced prostate cancer receiving chemotherapy using the commercially available circulating tumor cell capture system by CellSearch® can be useful as a prognostic indicator of patient survival, the presence of contaminating leukocytes impede the downstream utility of circulating tumor cell capture devices, in that assays based on RNA or protein quantification are obfuscated by the need for fixation and material of leukocytic origin.

To facilitate high-efficiency capture of prostate circulating tumor cells, a microfluidic device is described herein that employs an approach termed 'geometrically enhanced differential immunocapture' (GEDI). This device combines a geometry that reduces capture of contaminating leukocytes by generating size-dependent cell-wall collisions. This geometric approach is combined with a prostate-specific immunocapture surface using the J591 monoclonal antibody that recognizes the extracellular domain of prostate-specific membrane antigen (PSMA) (Gleghorn et al., Lab on a Chip 10: 27-29 (2010)).

The microfluidic device includes a solid support with a length and width, and with rows of posts configured for flow of a cell sample through the length of the device from a cell sample application area, to an outlet; wherein each row is perpendicular to the length of the device; and where the posts of one row do not align with the posts of adjacent rows. The microfluidic device can have a cover sheet on top of the posts, so that each post is linked to the solid support and the cover sheet. Thus, when sample is introduced through the cell sample application area, the sample flow is constrained to channels between the posts, and does not flow on top or below the posts.

The posts of one row do not align with the posts of one or two adjacent rows because the posts of one row are offset across the width of the device, relative to posts in the one or two adjacent rows by 0.1 to 100 microns. The term "offset" when used to describe the position of posts in a row relative to the one or two adjacent rows, means that a line drawn from each post in the row to its nearest neighbor in the one or two adjacent rows is not parallel to the length of the device. Instead, each post in a row is shifted (offset) along the width of the device, relative to its nearest neighbor in the one or two adjacent rows. The offset can be about 0.1 to 100 microns, or about 0.2 to 75 microns, or about 0.5 to 50 microns, or about 0.75 to 10 microns, or about 1 microns to about 25 microns.

The solid support and/or the cover sheet can include silicon, silica, glass, polydimethylsiloxane (PDMA), cellulose, ethylcellulose, methylcellulose, nitrocellulose, hydroxypropylcellulose, hydroxypropyl methylcellulose, polystyrene, polyethylene, nylon, rayon, cotton, Teflon, mica, sephadex, sepharose, polyacrylonitrile, glass, glass-fiber paper, gold, metal, paper, and combinations thereof. In some embodiments, the solid support is silicon, glass, metal (e.g., gold or gold plated), or a combination thereof. The cover sheet can, for example, be polydimethylsiloxane (PDMA), polystyrene, polyethylene, nylon, rayon, Teflon, mica, glass

The device can include one or more types of binding entities immobilized to at least one section of the solid support and/or the cover sheet of the device. The binding entities can be specific for the cell type of interest. For example, the binding entities can selectively bind to circulating tumor cells. In some embodiments, the binding entities can be an anti-PMSA antibody. For example, the binding entity can be the monoclonal antibody J591 developed at Weill Cornell Medical College. PSMA is a cell surface peptidase highly expressed by malignant prostate epithelial cells. PSMA is an attractive target for

prostate cancer circulating tumor cell capture, as it is expressed on virtually all prostate cancer cells and expression increases following castration.

The Examples further describe the utility of the GEDI device, including a comparison of circulating tumor cell enumeration with CellSearch®, the detection of a specific androgen receptor mutation from blood samples spiked with only 50 cells; the identification of the TMPRSS2-ERG fusion by immunostaining, and the ex-vivo assessment of circulating tumor cell sensitivity to taxane-treatment using microtubule bundling as a marker of drug-target engagement.

Circulating tumor cells are typically large, nucleated, $PSMA^+/CD45^-$ cells. Enumeration and analysis of circulating tumor cells optimally focuses on intact cells that fulfill such circulating tumor cell criteria while ignoring cell fragments, and non-nucleated cells that might qualify as circulating tumor cells (despite a recent report suggesting that enumeration of such events can impact the prognostic value of the circulating tumor cell assay (Coumans et al., Annals of Oncology : official journal of the European Society for Medical Oncology / ESMO 21: 1851-1857 (2010)). A microfluidic device such as the device described herein can be used to provide substantially pure, intact circulating tumor cells for determining whether an androgen receptor splice variant (e.g., one with SEQ ID NO:3 or 5) is expressed in patient test samples that contain circulating tumor cells.

When circulating tumor cells are captured by the device, the cells can be evaluated by any of the methods and assays described herein, either while the captured cells are within the device or after removal of the captured cells from the device.

Binding Entities

As used herein, "binding entities" include any molecule that can specifically bind to an androgen receptor or an androgen receptor splice variant. Each binding entity binds a its target androgen receptor or splice variant with specificity. Binding entities are typically binding regions of affinity molecules available in the biological sciences including, but not limited to, antibodies, antibody fragments, leucine zippers, histones, complementary determining regions (CDRs), single chain variable fragments (scFv's), receptors, ligands, aptamers, lectins, nucleic acid probes and the like. Binding entities can include binding regions that are generated, for example, of full sized versions of an affinity molecule, fragments of an affinity molecule, or the smallest portion of the affinity molecule providing binding that is useful in the detection of a target of interest (an androgen receptor or an androgen receptor splice variant).

In a one embodiment, the devices include binding entities which are members of the immunoglobulin family of proteins, or derivatives thereof. For example, the binding entity can be a complete immunoglobulin or antibody, a fragment, a single chain variable fragment (scFv), a heavy or light chain variable region, a CDR peptide sequence, and/or the like.

As used herein, "antibody" refers to an immunoglobulin molecule, and fragments thereof, which are immunologically reactive with a particular antigen. The term "antibodies" refers to a plurality of such molecules and is not limited to homogeneous populations of a single type of antibody. The term "antibody" also includes genetically engineered forms such as chimeric antibodies, heteroconjugate antibodies (e.g., bispecific antibodies), and recombinant single chain Fv fragments (scFv), and disulfide stabilized (dsFv) Fv fragments (see, for example U.S. Pat. No. 5,747,654). The term "antibody" also includes antigen

binding forms of antibodies (e.g., Fab', F(ab')2, Fab, Fv and IgG. See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.). The term "antibody," includes immunologically-active fragment of an immunoglobulin molecule such as the Fab or F(ab')2 fragment generated by, for example, cleavage of the antibody with an enzyme such as pepsin or co-expression of an antibody light chain and

5   an antibody heavy chain in bacteria, yeast, insect cell or mammalian cell. The antibody can also be an IgG, IgD, IgA, IgE or IgM antibody.

Antibodies for use in the methods and devices described herein can be obtained commercially or can be generated by available methods. Methods of making antibody fragments are available in the art (see for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory,

10   New York, (1988), specifically incorporated herein by reference in its entirety). For example, antibodies suitable for use the devices can be obtained by immunizing an animal such as a rabbit, goat, sheep, horse, or guinea pig. Such antibodies are present in the blood (e.g., serum) of immunized animals.

Antibody fragments can be prepared by proteolytic hydrolysis of the antibody or by expression of nucleic acids encoding the antibody fragment in a suitable host. Antibody fragments can be obtained by

15   pepsin or papain digestion of whole antibodies conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment described as $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally using a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, enzymatic cleavage using pepsin produces two monovalent Fab'

20   fragments and an Fc fragment directly. These methods are described, for example, in U.S. Patents No. 4,036,945 and No. 4,331,647, and references contained therein. These patents are hereby incorporated herein by reference in their entireties.

A number of proteins can serve as protein scaffolds to which binding domains can be attached and thereby form a suitable binding entity. The binding domains bind or interact with androgen receptors

25   while the protein scaffold merely holds and stabilizes the binding domains so that they can bind. A number of protein scaffolds can be used. For example, phage capsid proteins can be used. See Review in Clackson & Wells, Trends Biotechnol. 12:173-184 (1994). Phage capsid proteins have been used as scaffolds for displaying random peptide sequences, including bovine pancreatic trypsin inhibitor (Roberts et al., PNAS 89:2429-2433 (1992)), human growth hormone (Lowman et al., Biochemistry 30:10832-

30   10838 (1991)), Venturini et al., Protein Peptide Letters 1:70-75 (1994)), and the IgG binding domain of Streptococcus (O'Neil et al., Techniques in Protein Chemistry V (Crabb, L,. ed.) pp. 517-524, Academic Press, San Diego (1994)). These scaffolds have displayed a single randomized loop or region that can be modified to include binding domains for androgen receptor or an androgen receptor splice variants.

Fibronectin type III domain has also been used as a protein scaffold to serve as a binding entity

35   platform. Fibronectin type III is part of a large subfamily (Fn3 family or s-type Ig family) of the immunoglobulin superfamily. Sequences, vectors and cloning procedures for using such a fibronectin type III domain as a protein scaffold portion of a binding entity (e.g. that includes CDR peptides) are provided, for example, in U.S. Patent Application Publication 20020019517. See also, Bork, P. & Doolittle, R. F. (1992) Proposed acquisition of an animal protein domain by bacteria. Proc. Natl. Acad. Sci.

40   USA 89, 8990-8994; Jones, E. Y. (1993) The immunoglobulin superfamily Curr. Opinion Struct. Biol. 3,

846-852; Bork, P., Hom, L. & Sander, C. (1994) The immunoglobulin fold. Structural classification, sequence patterns and common core. J. Mol. Biol. 242, 309-320; Campbell, I. D. & Spitzfaden, C. (1994) Building proteins with fibronectin type III modules Structure 2, 233-337; Harpez, Y. & Chothia, C. (1994).

It can be useful to employ a binding entity that binds to a selected androgen receptor or androgen receptor splice variant with specificity. For example, the binding entity can have an affinity for an androgen receptor or an androgen receptor splice variant of about 1×10^7 M^{-1} to about 1×10^{10} M^{-1}, or about 1×10^8 M^{-1} to about 1×10^9 M^{-1}. For example, the affinity of a binding entity can be measured by detecting and quantifying the formation of a binding entity-androgen receptor complex, generally referred to as an antigen-antibody complex [Ag-Ab]. The formation of such an antigen-antibody complex [Ag-Ab] is illustrated by the following reaction.
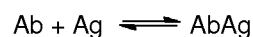
$$Ab + Ag \rightleftharpoons AbAg$$

The formation of such an Ag-Ab complex is therefore at equilibrium with its dissociation, and the equilibrium association constant (K_A) of the complex can be calculated as follows:

$$K_A=1/k_d=[Ag\text{-}Ab]/[Ag][Ab]$$

As used herein, the term "binds specifically" or "specifically binds," in reference to a binding entity or antibody interaction with an androgen receptor or androgen receptor variant protein, means that the binding entity or antibody binds with a particular antigen (e.g., androgen receptor splice variant v5,6,7, such as one with SEQ ID NO:3, or androgen receptor splice variant v7, such as one with SEQ ID NO:5) without substantially binding to a full-length androgen receptor or other androgen receptor isoform.

For example, in some embodiments, selected binding entities can bind with greater affinity or selectivity to androgen receptor splice variant v5,6,7 (such as one with SEQ ID NO:3) than to full-length androgen receptor, or the androgen receptor splice variant v7. Thus, for example, a binding entity binds to androgen receptor splice variant v5,6,7 (such as one with SEQ ID NO:3) with at least 50 % or greater affinity (or selectivity), or 60% greater affinity (or selectivity), or 70% greater affinity (or selectivity), or 80% greater affinity (or selectivity), or 85% greater affinity (or selectivity), or 90% greater affinity (or selectivity), or 95% greater affinity (or selectivity) for androgen receptor splice variant v5,6,7 (such as one with SEQ ID NO:3) than to full-length androgen receptor or to other androgen receptor isoforms. Similarly, for example, a binding entity binds to androgen receptor splice variant v7 (such as one with SEQ ID NO:5) with at least 50 % or greater affinity (or selectivity), or 60% greater affinity (or selectivity), or 70% greater affinity (or selectivity), or 80% greater affinity (or selectivity), or 85% greater affinity (or selectivity), or 90% greater affinity (or selectivity), or 95% greater affinity (or selectivity) for androgen receptor splice variant v7 (such as one with SEQ ID NO:5) than to full-length androgen receptor or to other androgen receptor isoforms.

Binding entities can be separated from impurities before incorporation into the devices. For example, the binding entities can be purified or isolated using purification methods such as electrophoretic, molecular, immunological and chromatographic techniques, including ion exchange, hydrophobic, affinity, and reverse-phase HPLC chromatography, and chromatofocusing, and the like. The degree of purification necessary will vary depending on the contaminants present with the binding entities. In some instances no purification will be necessary (e.g., when binding entities are commercially available and provided in purified form).

Antibodies directed against androgen receptor splice variants (such as ARv5,6,7, e.g., with SEQ ID NO:3; or ARv7, e.g., with SEQ ID NO:5) are generally monoclonal antibodies.

A monoclonal antibody is a population of molecules having a common antigen binding site that binds specifically with a particular antigenic epitope. A monoclonal antibody can be obtained by selecting an antibody-producing cell from a mammal that has been immunized with a selected androgen receptor splice variant (such as ARv5,6,7, e.g., with SEQ ID NO:3; or ARv7, e.g., with SEQ ID NO:5) and fusing the antibody-producing cell, e.g. a B cell, with a myeloma to generate an antibody-producing hybridoma. A monoclonal antibody can also be obtained by screening a recombinant combinatorial library such as an antibody phage display library. See, for example, PHAGE DISPLAY – A LABORATORY MANUAL, Barbas, et al., eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001; and Kontermann & Dübel, ANTIBODY ENGINEERING, Heidelberg: Springer-Verlag. Berlin, 2001. Techniques for preparing monoclonal antibody-secreting hybridoma cells are also described, for example, by Kohler and Milstein, Nature 256:495-97 (1975) and Kozbor et al. Immunol Today 4: 72 (1983).

A monoclonal antibody against androgen receptor splice variant v5,6,7 or androgen receptor splice variant v7 can also be prepared using other methods available in the art. For example, the antibodies can be obtained by screening of a recombinant combinatorial immunoglobulin library using a selected androgen receptor splice variant protein (such as ARv5,6,7, e.g., with SEQ ID NO:3; or ARv7, e.g., with SEQ ID NO:5). Immunoglobulins that selectively bind to a selected androgen receptor protein can be produced by recombinant expression from cells encoding the immunoglobulin of interest.

The antibodies can be evaluated for affinity to a selected androgen receptor splice variant (such as ARv5,6,7, e.g., with SEQ ID NO:3; or ARv7, e.g., with SEQ ID NO:5) using standard procedures including, for example, enzyme linked immunosorbent assay (ELISA) to determine antibody titer and protein A chromatography to obtain the antibody-containing an IgG fraction.

Another method for generating antibodies involves a Selected Lymphocyte Antibody Method (SLAM). The SLAM technology permits the generation, isolation and manipulation of monoclonal antibodies without needing to generate a hybridoma. The methodology principally involves the growth of antibody forming cells, the physical selection of specifically selected antibody forming cells, the isolation of the genes encoding the antibody and the subsequent cloning and expression of those genes.

The nucleic acids encoding the antibodies can be mutated to optimize the affinity, selectivity, binding strength or other desirable property of an antibody. A mutant antibody refers to an amino acid sequence variant of an antibody. In general, one or more of the amino acid residues in the mutant antibody is different from what is present in the reference antibody. Such mutant antibodies necessarily have less than 100% sequence identity or similarity with the reference amino acid sequence. In general, mutant antibodies have at least 75% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the reference antibody. Preferably, mutant antibodies have at least 80%, more preferably at least 85%, even more preferably at least 90%, and most preferably at least 95% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the reference antibody.

Labels

A variety of different labels can be used in the methods, kits, and devices described herein. Labels can be covalently attached to any of the binding entities, primers or probes described herein. Alternatively, the labels can non-covalently associate with a hybridized probe or primer that is specifically bound to a target nucleic acid (e.g., an mRNA encoding an androgen receptor or androgen receptor variant protein). Similarly, a label can be non-covalently or indirectly bound to a binding entity. For example, the label can be an enzyme substrate that is transformed by an enzyme bound to a binding entity into a colored signal.

So called "direct labels" are detectable labels that are directly attached to or incorporated into a binding entity that then can bind to an androgen receptor or androgen receptor variant. In contrast, so-called "indirect labels" are joined to a complex formed between an androgen receptor or androgen receptor variant, and a binding entity after complex formation. For example, an indirect label can be attached to a secondary antibody that binds to a different epitope on an androgen receptor or androgen receptor variant, than does a primary antibody.

Examples of labels include, but not limited to, fluorophores, chromophores, radiophores, enzymatic tags, antibodies, chemiluminescence, electroluminescence, and affinity labels. One of skill in the art will recognize that these and other labels can be used with success in this invention. Examples of enzyme labels include enzymes such as urease, alkaline phosphatase or peroxidase to mention a few. Colorimetric indicator substrates can be employed to provide a detection means visible to the human eye or spectrophotometrically. Examples of fluorophores include, but are not limited to, Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy2, Cy3, Cy5, 6-FAM, Fluorescein, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, ROX, TAMRA, TET, Tetramethylrhodamine, and Texas Red.

Means of detecting such labels are well known to those of skill in the art. For example, fluorescent markers may be detected using a microscope, photodetector or fluorimeter to detect emitted light. In still further examples, enzymatic labels can be detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label or reaction product; or by use of spectrometer.

Taxanes

A variety of taxanes can be used in the methods, devices and compositions described herein.

Taxanes refer to a class of compounds having a core ring system of three rings, A, B and C, as shown below.
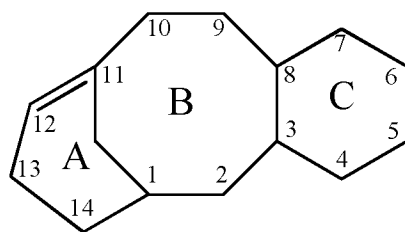
Examples of taxanes that can be used in the methods, devices and compositions described herein include paclitaxel, docetaxel, cabazitaxel, baccatin III, 10-deacetylbaccatin, hongdoushan A, hongdoushan B, hongdoushan C, and combinations thereof. In some instances, the taxane is paclitaxel, docetaxel, or a combination thereof.

Kits

Kits are also described here for use in the diagnostic and therapeutic applications described or suggested above. The kits can include a carrier means for the devices and reagents as well as other components of the kits. Such a carrier can be a box, a bag, a satchel, plastic carton (such as molded plastic or other clear packaging), wrapper (such as, a sealed or sealable plastic, paper, or metallic wrapper), or other container. In some examples, kit components will be enclosed in a single packaging unit, such as a box or other container, which packaging unit may have compartments into which one or more components of the kit can be placed. In other examples, a kit includes one or more containers, for instance vials, tubes, and the like that can separately contain, for example, one or nucleic acid probes, one or more binding entities, one or more devices, as well as positive and/or negative control samples or solutions.

For example, at least one of the containers can include at least one binding entity that binds with specificity or selectivity to an androgen receptor or androgen receptor variant protein. In another embodiment, one of the containers can include a nucleic acid probe or primer that selectively hybridizes to an androgen receptor or androgen receptor variant mRNA. The binding entities, probes and primers are or can be detectably labeled. For example, the binding entities, probes and primers can be packaged separately from the labels, and the label can be added to the binding entities, probes and primers during or after performance of an assay for an androgen receptor or androgen receptor variant.

Kits can also contain vials, needles, syringes, finger-prick devices, alcohol swabs, gauze squares, cotton balls, bandages, latex gloves, incubation trays with variable numbers of troughs, adhesive plate sealers, data reporting sheets, which may be useful for handling, collecting and/or processing biological samples. Kits may also optionally contain implements useful for introducing samples into an assay chamber or a cell capturing device, including, for example, droppers, Dispo-pipettes, capillary tubes, rubber bulbs (e.g., for capillary tubes), and the like. Other components can also be present in the kits such as disposal means for discarding used devices and/or other items used with the device (such as patient samples, etc.). Such disposal means can include, without limitation, containers that are capable of containing leakage from discarded materials, such as plastic, metal or other impermeable bags, boxes or containers.

The kits can include instructions for use of a cell capturing device, or for performing an assay such as an immunoassay, cell sorting assay, Northern blotting, nuclease protection assays, RNA fingerprinting, polymerase chain reaction, ligase chain reaction, Qbeta replicase, isothermal amplification method, strand displacement amplification, transcription based amplification systems, quantitative nucleic acid amplification assays (e.g., polymerase chain reaction assays), combined reverse transcription/nucleic acid amplification, nuclease protection (SI nuclease or RNAse protection assays), Serial Analysis Gene Expression (SAGE), next generation sequencing, gene expression microarray, in situ hybridization,

nucleic acid amplification, reverse transcription, polymerase chain reaction, quantitative real time polymerase chain reaction (qRT- PCR), and the like.

The following non-limiting Examples illustrate some of the experimental work involved in developing the invention.

5

EXAMPLE 1: Materials and Methods

This Example describes some of the materials and methods employed in the development of the invention.

10   **Device Fabrication**

All device fabrication was carried out at the Cornell NanoScale Science and Technology Facility (Ithaca, NY). Standard photolithography techniques were used to define array geometries on silicon wafers. The wafers were etched with an oxygen plasma deep reactive ion etcher (Uniaxis SLR770) to a depth of 100 μm, and cleaned using sulfuric acid and hydrogen peroxide prior to antibody surface

15   functionalization. The J591 monoclonal antibody (manufactured by Lonza plc (Slough, England) for BZL Biologics, Inc.) was immobilized on the device surfaces using MPTMS-GMBS-NeutrAvidin-biotin chemistry (Gleghorn et al., Lab on a chip 10: 27-29 (2010); see FIG. 1C). Polydimethylsiloxane (PDMS) sheets (5:1 base:curing agent), approximately 3 mm thick, were polymerized for 18 hours at 60°C and trimmed to form covers for the GEDI device. A PDMS sheet was clamped to the top of the device with a

20   custom jig to create closed channels populated with post arrays. Inlet and outlet holes were created with a biopsy punch, and 23-gauge metal tubes were inserted into the PDMS to connect inlet and outlets to external tubing. Devices were primed with a 50/50 isopropanol/water mixture, and then flushed with DI water and PBS before experiments.

25   **Sample Collection and Microfluidic Capture**

Peripheral blood samples were collected in tubes containing sodium citrate anticoagulant (Becton-Dickinson) from healthy volunteers and patients with metastatic castrate-resistant prostate cancer under a clinical protocol entitled "Analysis of circulating tumor cells in prostate cancer. Predicting response to taxanes: a pilot study" which was approved by the Institutional Review Board (IRB) of Weill

30   Cornell Medical College of Cornell University. Blood was obtained from patients or healthy donors following written informed consent, which was also approved by the IRB committee of Weill Cornell Medical College of Cornell University. As previously described by Gleghorn et al. (Lab on a chip 10: 27-29 (2010)), 1ml of blood from each specimen was processed through the GEDI chip within 24 h of blood draw by pushing the blood through the device at a volumetric flow rate of 1ml/hr (Chemyx syringe pump).

35

Cell staining and analysis

The cell lines used in these experiments as controls for staining or in spiked experiments are: the human leukemia cell line U937, and the human prostate cancer cell lines PC-3, LNCaP and C4-2. All cell lines were purchased from ATCC. Post-capture, cells were fixed on-chip with PHEMO fixative at 37°C

40   (PHEMO buffer: PIPES acid, HEPES acid, EGTA disodium salt, Mg-Cl2-6H20, 10%DMSO),

glutaraldehyde, and 3.7%formaldehyde. Cells were then blocked (10% Normal Goat Serum – Jackson
Immuno Research) and immunostained with FITC-conjugated humanized mAb J591 to detect PSMA
expression. Monoclonal mouse anti-CD-45 (BD Biosciences) followed by AlexaFluor568 labeled goat
anti-mouse secondary (Invitrogen) and mouse anti-EpCAM directly conjugated to AlexaFluor647

5   (Biolegend). For the detection of intracellular antigens, cells were permeabilized with 0.1% Triton X-100
(Sigma-Aldrich) in PBS and stained by use of rat anti-alpha tubulin (YL1/2, Millipore) and rabbit anti-ERG
monoclonal antibody (clone EPR 3864; Epitomics, Burlingame, CA). The anti-ERG antibody was a
generous gift from Dr. Mark Rubin (Weill Cornell Medical College, New York, NY). All primary antibodies
were incubated for 1 hour at room temperature; secondary antibodies were stained at room temperature

10 for 30 minutes. DAPI was used for DNA counterstaining. GEDI devices were mounted to coverslips with
Mowiol and stored at -20°C before analysis.

Circulating tumor cells enumeration

Blinded circulating tumor cell enumeration following antibody labeling was performed by use of a

15 Zeiss LSM-700 point scanning confocal microscope, equipped with 405-, 488-, 555-, and 632-nm laser
lines. All $PSMA^+/CD45^-$ nucleated cells were identified as circulating tumor cells. Initial validation of
circulating tumor cell enumeration was accomplished by two independent, blinded testers. Positive and
negative controls for antibody performance and staining were included in each experiment: U937 human
leukemia cells ($CD45^+/PSMA^-/EpCAM^-$), and the human prostate cancer cell lines (C4-2 and LNCaP:

20 $PSMA^+/CD45-/EpCAM^+$ and PC-3 (PSMA-/Cd45-/EpCAM dim). Individual *z*-stacks were acquired using
100X/NA 1.46 and 63X/NA 1.3 Plan-Apo Zeiss objectives controlled by Zen software (Zeiss) and
presented as maximum intensity projections.

RNA extraction

25 Following cell capture, the GEDI device was rinsed by flowing PBS for 30 min at a rate of 1ml/hr.
Cells were lysed with 700 µl of RLT Lysis buffer supplemented with 1% β-mercaptoethanol at a flow rate
of 15 ml/hr. The lysate was collected, and RNA was extracted using the QiagenRNEasy Micro Plus Kit
(Qiagen Inc, Valencia, CA) according to the manufacturer's instructions.

30 **Ex-vivo Drug treatments and Analysis**

For *ex-vivo* drug treatment experiments, the blood sample from each patient was divided and 1 ml
was flown to each of three GEDI devices simultaneously. After circulating tumor cell capture and
subsequent PBS wash, each GEDI microdevice was gently placed in a culture dish with RPMI-1640
media containing 2% serum and supplemented with either 0.1 % DMSO control or paclitaxel at

35 concentrations of 100 nM or 1 µM and incubated at 37°C for 24hr. At the end of treatment, the GEDI-
captured cells were fixed with PHEMO buffer and processed for multiplex confocal microscopy following
immunostaining with different cell surface and cytoplasmic antibodies as indicated. All circulating tumor
cells ($PSMA^+/CD45-/DAPI^+$) were assessed for the presence of microtubule bundles as evidence of
effective drug-target engagement. The percent of circulating tumor cells with evidence of microtubule

40 bundling was calculated. In all samples analyzed, bundling was clearly apparent by the distinct shape,

width, orientation and increased fluorescence intensity of microtubule bundles as compared with microtubules from untreated cells. In addition, DAPI counterstain was used to assess the presence of mitotic or apoptotic nuclei following drug treatment.

5   **Statistical Analysis**

Statistical analysis was performed to compare the mean circulating tumor cell counts obtained from castration-resistant prostate cancer patients and healthy donors. We used a non-parametric (Wilcoxon signed-rank) analysis, as circulating tumor cell counts did not exhibit normal distribution. Statistical significance was defined with $\alpha = 0.05$.

10

EXAMPLE 2: Cell Capturing Device

The cell capture rates of PSMA-positive cancer cells were determined to evaluate the performance of the device depicted in FIG. 1A. Cell capture was determined as a function of varying mAb J591 concentrations (1.5-20 ug/ml) using shear stress magnitudes representative of those experienced by

15   the functionalized surfaces of the device (0.08-0.24 Pa). These experiments revealed a dose-dependent increase in cell capture up to mAb concentration of 10 ug/ml, which was used for all subsequent experiments (FIG. 1D).

Although the J591 antibody is specific for PSMA-expressing cells, non-specific leukocyte adhesion has been a major problem for all blood-based immunocapture techniques. To minimize

20   leukocyte adhesion, a parametric study was conducted to characterize collision rate (CpR; collision per row) as a function of cell size and obstacle offset. Collision rates from a subset of these offsets exhibit a sharp cutoff according to cell size, as shown in FIG. 2B. Hence an obstacle offset (7 μm) was selected that generates a sharp cutoff at the cell diameter of 14 μm. The physics describing this cutoff is illustrated by the size-dependent cell path lines (FIG. 2A), which show how large cells experience repeated collision

25   whereas small cells separate from the obstacles and escape capture.

This hypothesis was then tested by measuring capture of LNCaP prostate cancer cells (FIG. 2C). In this experiment, spiked LNCaP cells were flown into J591-functionalized devices that had a 7 μm offset (GEDI) *versus* those that had no offset (straight). Although these two devices have the same surface-area-to-volume ratio, the GEDI geometry greatly increased cell capture efficiency, as measured by

30   captured and enumerated cells normalized by input cell counts.

Because of the dependence of cell trajectory on cell diameter, collision rates are a complicated function of both cell diameter and array parameters such as row offsets. The collision rate per row (CpR) is a strong function of the row offset, exhibiting discontinuities, size dependence, and startup effects related to the finite array size (FIG. 2E). The dramatic difference between the performance of different

35   designs is caused by the deflection of particles—in poorly-chosen geometries, the deflection causes cells to deflect onto streamlines that do not come into proximity with later obstacles, whereas in well-chosen geometries, the deflection causes cells to deflect onto streamlines that do come into proximity with later obstacles. Thus the collision rate increases as the cells proceed through the device for the GEDI design, and decreases for poorly chosen designs such as straight arrays (FIG. 2E). This cutoff allows the user to

identify a cutoff between hematocytes (<14 µm) and the cell population that will experience maximum collisions (>15 µm).

EXAMPLE 3: Cell capture, imaging, and enumeration of circulating tumor cells from metastatic prostate cancer patients.

This Example describes use of the GEDI device to capture and characterize circulating tumor cells (CTCs) from the blood of patients with metastatic castration-resistant prostate cancer.

One ml of peripheral blood was passed through the device, captured cells were fixed and immunostained for PSMA, CD45, EpCAM and DAPI, and the captured cells were analyzed by confocal microscopy. Circulating tumor cells were defined as intact, nucleated, PSMA$^+$/CD45$^-$ cells. Different cell lines were used as controls for antibody staining for PSMA, CD45, and EpCAM, as follows: the C4-2 prostate cancer cells are PSMA$^+$/EpCAM$^+$/CD45$^-$ while the PC3 prostate cancer cells are PSMA$^-$/EpCAM$^-$/CD45$^-$. The U937 leukemic cell line was used as a positive control for the leukocyte marker CD45. DAPI was used to stain the DNA.

Representative examples of circulating tumor cells and leucocytes are shown in FIG. 3A. Interestingly, PSMA$^+$ cells had variable EpCAM staining, ranging from highly-positive to weak to negative in terms of EpCAM fluorescent intensity. In a subset of patients, the percent of PSMA-captured circulating tumor cells was quantitated that were EpCAM positive. About 40-70% of GEDI-captured circulating tumor cells were positive for both markers, with the median being 60% (data not shown). Controls for antibody performance were included with every experiment using two prostate cancer cell lines expressing different levels of PSMA and EpCAM (C4-2:PSMA$^+$/EpCAM$^+$/CD45$^-$ and PC3: PSMA$^-$/EpCAM$^-$/CD45$^-$) and the CD45$^+$ leukemia cell line U937.

Blood samples obtained from 10 healthy donors (controls) and 30 patients with metastatic castration-resistant prostate cancer were processed using the GEDI device. The median number of circulating tumor cells/ml detected was 3 (range 0 to 22) and 54 (range 0 to 1200), respectively (p<0.001; FIG. 3B).

A direct comparison of circulating tumor cell capture and enumeration was then performed by comparing the GEDI microdevice with the FDA-approved EpCAM-based CellSearch$^®$ CTC Test on same-day blood draws from 25 castration-resistant prostate cancer patients (FIG. 3C). A 2 to 400-fold increase was detected in the number of circulating tumor cells/ml reported with the GEDI microdevice relative to the CellSearch$^®$ CTC Test (FIG. 3C; *p*<.0001, calculated with Wilcoxon test). A weak correlation (*r*=0.44; outliers removed with Cook's distance restriction) between GEDI circulating tumor cell counts and CellSearch® (FIG. 3D).

EXAMPLE 4: Markers for Circulating Tumor Cells

This Example provides a comparison of cell capture by the GEDI device and the CellSearch$^®$ device.

The results presented in FIG. 3 illustrate both that the EpCAM expression level of captured PSMA$^+$/DAPI$^+$/CD45$^-$ cells is variable and that the correlation between GEDI PSMA$^+$ capture and CellSearch$^®$ EpCAM$^+$ capture is only weak. The two capture methodologies both correlate with the

disease state, but the population captured by the GEDI device is different from that captured by the currently available CellSearch® device, presumably owing to different expression levels of EpCAM and PSMA in the circulating tumor cell population. The data described herein shows detection of a significantly higher circulating tumor cell population using the GEDI microfluidic device compared with CellSearch®. Thus the GEDI microdevice has enhanced sensitivity for detecting circulating tumor cells in samples obtained from prostate cancer patients. Because circulating tumor cells expressing low EpCAM levels are frequently missed using CellSearch® (Scher et al., Journal of clinical oncology : official journal of the American Society of Clinical Oncology 29: 2191-2198 (2011)), it is likely that anti-PSMA capture maybe be a more consistent methodology for circulating tumor cell isolation in metastatic prostate cancer patients.

To further investigate the distribution of the two antigens in castration-resistant prostate cancer isolated circulating tumor cells, in an unbiased manner (i.e. no antibody-specific circulating tumor cell capture) circulating tumor cells were isolated from castration-resistant prostate cancer patient sample by performing immuno-magnetic CD45 depletion of Ficoll-isolated peripheral blood mononuclear cells. These immuno-depleted cells were subsequently labeled with antibodies against PSMA (J591), EpCAM and CD45. In this assay, circulating tumor cells were classified as nucleated CD45- cells that were positive for PSMA, EpCAM, or both PSMA and EpCAM. A total of eleven patient samples were analyzed and circulating tumor cells were identified in seven patients. Over 80% of dual $PSMA^+/EpCAM^+$ circulating tumor cells in six of the seven patients (Table 1).

Table 1: Circulating Tumor Cell Counts

Patient	PSMA	EpCAM	PSMA + EpCaM	Total
1	176	172	169 (94.4%)	179
2	125	130	119 (87.5%)	136
3	104	103	96 (86.4%)	111
4	1	0	0	1
5	2	1	1 (50%)	2
6	1	1	1 (100%)	1
7	6	6	6 (100%)	6
8	0	0	NA	0
9	0	0	NA	0
10	0	0	NA	0
11	0	0	NA	0

Interestingly, using this technique, the EpCAM staining intensity was variable which is consistent with the data obtained using the GEDI-microdevice (FIG. 3).

Taken together these data indicate that an important contributing factor to the enhanced sensitivity of the GEDI is that the GEDI microdevice is designed to optimize circulating tumor cell capture and minimize leukocyte capture; it does this by inducing a fluid flow that generates size-dependent trajectories of cells that lead to size-dependent collision rates. Spatial separation of cells based on size

alone would be of limited use in circulating tumor cell capture from blood—although circulating tumor cells tend to be larger on average that hematological cells, the sizes of cells and cell fragments of epithelial origin has a broad distribution, and size is much less specific to the circulating tumor cell phenotype than surface markers such as EpCAM, PSMA, or EGFR. However, when a surface antibody is present, size-dependent particle trajectories enable the captured cell population to be biased to reject nonspecific leukocyte adhesion. The surface collision rate and capture rate (FIG. 2) can be made size-specific, enhancing the receiver-operator characteristic of the rare cell capture. By capturing circulating tumor cells at high efficiency and purity, functional and molecular assays, exemplified by the ERG and SNP measurements in FIG. 4 and the tubulin measurements in FIG. 5 can be applicable in a clinical setting.

EXAMPLE 5: Molecular characterization of captured cells:
Detection of a single point mutation in the androgen receptor and expression of the TMPRSS2-ERG fusion in spiked cells and circulating tumor cells

This Example describes proof of principle experiments performed to assess the GEDI device.

To molecularly characterize the GEDI-captured circulating tumor cells, proof-of-principle experiments were performed in which prostate cancer cells were spiked into 1 ml of blood from a healthy donor, captured by the device and analyzed for the presence of androgen receptor (AR) point mutations or expression of a TMPRSS2:ERG gene fusion protein. To detect the T868A (ACT-GCT) single point mutation in the AR ligand-binding domain, fifty C4-2 cells were added into 1 ml of healthy donor blood that had passed through the GEDI device. RNA was extracted from the captured cells by direct lysis on the device followed by cDNA sequencing. In parallel, sequencing was performed on RNA extracted from 1 ml of the same healthy donor blood processed similarly (negative control) and on RNA extracted from fifty C4-2 cells (positive control).

The sequencing results are illustrated in FIG 4A. As expected, the T868A mutation was clearly detected in the C4-2 cells (FIG. 4A, top and middle panel) but absent from the negative control. The point mutation was also detected in the spiked blood sample, with the mutant peak (A) accounting for 70% of the nucleotide present at this position and the wild-type (T) for 30%. This result is consistent with the previously reported cell capture purity rate of 68% obtained with fluorescently labeled prostate cancer cells spiked into 1 ml peripheral blood from a healthy donor and flown through the GEDI microdevice (Gleghorn et al., Lab on a chip 10: 27-29 (2010).

In addition, the presence of the TMPRSS2:ERG fusion protein could be detected by immunostaining with the rabbit monoclonal anti-ERG antibody (Park et al., Neoplasia 12: 590-598 (2010)) in fusion-positive vCaP prostate cancer cells captured by the device and analyzed by multiplex confocal microscopy (FIG. 4C-4D). Similar analysis on GEDI-captured circulating tumor cells from a castration-resistant prostate cancer patient revealed the presence of both ERG positive and ERG negative cells, while CD45^{+} leucocytes were negative for ERG protein (FIG. 4E), indicating the specificity of ERG staining for prostate cancer-derived cells.

EXAMPLE 6: Tubulin bundling upon exposure to taxanes

This Example describes experiments showing that taxanes chemosensitivity can be detected in cells captured on the GEDI microdevice.

GEDI-captured circulating tumor cells are highly pure and viable, which is desirable for detection of drug resistance and/or drug sensitivity. The GEDI-captured circulating tumor cells were tested *in situ* (i.e., while in the GEDI device) to evaluate their chemosensitivity to taxanes, and predict a patient's clinical response to therapy. Taxanes (paclitaxel, docetaxel and cabazitaxel), which are commonly used to treat castration-resistant prostate cancer patients, act by stabilizing microtubule polymers and inducing the formation of microtubule bundles. Microtubule bundling is readily detectable by immunofluorescence staining, due to their increased fluorescence intensity, distinct shape and cytoplasmic organization when viewed in maximum signal projection (Marcus et al., J. Biol. Chem. 280: 11569-11577 (2005)). Microtubule bundling is the first event induced by taxane treatment, resulting in downstream mitotic arrest and apoptotic cell death, and is therefore an appropriate marker of efficient taxane drug-target engagement.

To first determine the optimal concentration and duration of *ex-vivo* on-chip treatment of patient-derived circulating tumor cells, 200 C4-2 cells were spiked into 1 ml of blood from a healthy donor, processed the sample in the GEDI device, and then incubated the captured cells on the device for 24 h in media containing 100 nM or 1μM of docetaxel (DTX) at 37°C. Docetaxel treatment with 100 nM resulted in distinct microtubule bundles in captured C4-2 cells (FIG. 5A, middle panel arrows), in contrast to the fine and intricate microtubule network of the untreated GEDI-captured cells (FIG. 5A, upper panel). Treatment with 1 μM docetaxel resulted in robust microtubule bundling and induction of apoptotic events (FIG. 5A, lower panel arrowheads). Apoptotic events were determined by the presence of bright and fragmented nuclei accompanied by loss of tubulin staining. Similar results were obtained with 48 hour, on-chip, *ex-vivo* treatment (data not shown). These results, together with the fact that the AUC of serum docetaxel concentration for patients administered 55-100 mg/m^2 of docetaxel is approximately equal to 1.5-5 hours mg/l, indicated that 24 hour treatment with 100 nM of a taxane (1.92 hours mg/l) can be used to evaluate the *ex-vivo* response of patient-derived circulating tumor cells.

A number of assays were performed to show the potential for functional assay in the described device. In these cases cells captured in the device were treated *ex vivo* with docetaxel and/or paclitaxel for 24 hr (FIG. 5B and 4E). These highlight the ability to perform functional assays on chip and assay drug-target engagement in patients in the context of their clinical response. Non-response, as indicated by a lack of evidence of microtubule bundling or apoptotic nuclei following *ex-vivo* docetaxel treatment, could be observed in some patients. FIG. 5E shows a patient that was non-responsive by the microtubule assay, consistent with this patient's lack of a clinical response using established RECIST and PSAWG2 criteria. This response was often heterogeneous within the captured cell population; FIG. 5F shows images of cells from a patient with heterogeneous response within the PSMA$^+$ circulating tumor cell population, with only 45% of circulating tumor cells displaying clear evidence of microtubule bundling (whereas nearly 100% of the leucocytes present on the chip showed microtubule bundling). Interestingly, cell response was often specific to the taxane used. As an example of this, FIG. 5B (top panel) shows a sample in which *ex vivo* docetaxel had no effect on the microtubule cytoskeleton of captured circulating tumor cells, whereas isolated circulating tumor cells from the same patient (docetaxel progressor) treated

with paclitaxel demonstrated clear evidence of microtubule bundling (FIG. 5B, bottom panel) as well as signs of aberrant mitotic arrest (FIG. 5C). FIG. 5D shows exemplary results of a patient (docetaxel progressor on cabazitaxel) whose circulating tumor cells exhibited apoptotic nuclei with paclitaxel but not with docetaxel.

EXAMPLE 7: Additional Methods
for Evaluation of Androgen Receptor Splice Variants

This Example describes additional materials and methods that have been used for evaluation of androgen receptor splice variants.

Cell lines and reagents:

PC3 and HEK293T cells were obtained from ATCC (Manassas, VA) and a PC3 stable cell line expressing mCherry-tubulin (PC3:mCherry-tub) was generated and maintained as previously described by Darshan et al. (Cancer Res. 71: 6019 (2011)).

M12 stable cell lines expressing untagged wild-type androgen receptor (AR-wt), ARv5,6,7es and ARv7 or expressing Cumate-inducible FLAG-tagged AR-wt or variants were generated in Dr. Plymate's laboratory (University of Washington School of Medicine, Seattle, WA) and maintained in RPMI 1640 supplemented with 5% FBS, 0.01 µM dexamethasone (Sigma Aldrich, St Louis, MO), 10 ng/ml epidermal growth factor (Invitrogen), 10 ml/L insulin-transferrin-selenium (Cellgro, Manassas, VA), 100 I.U./ml penicillin plus 100 µg/µl streptomycin and 200 µg/µl G418 (Cellgro, Manassas, VA) at 37°C with 5% CO_2.

In addition, M12 cells stably expressing GFP-tagged AR-wt, AR or variants were generated in Dr Giannakakou's laboratory. GFP^+ cells from all three androgen receptor cell lines were sorted using fluorescence activated cell sorting (FACS) on a BD FACSAria II cell sorter (BD Biosciences, Franklin Lakes, NJ, USA) and subsequently expanded in media containing G418 (400 ng/mL).

Unless otherwise stated, all reagents used were from Sigma Aldrich (St. Louis, MO). For immunofluorescence the following primary and secondary antibodies were used: rat monoclonal anti α-tubulin (Novus Biologicals, Littleton, CO), mouse monoclonal anti-c-Myc (Merck, Darmstadt, Germany) and species-matching Alexa Fluor 488 and Alexa Fluor 568 conjugated antibodies from Invitrogen (Carlsbad, CA). For immunoprecipitation rat monoclonal anti α-tubulin, rabbit polyclonal anti-GFP (Novus Biologicals) and mouse anti-dynein (Covance, Emeryville, CA) antibodies were used. For the immunoblot assays mouse monoclonal anti-androgen receptor 441 (Novus Biologicals); rabbit monoclonal anti-androgen receptor (EP670Y from Abcam, Cambridge, MA) specific for the C-terminus; rat monoclonal anti α-tubulin; rabbit polyclonal anti-GFP (Abcam) and rabbit polyclonal anti-actin (Sigma Aldrich) antibodies were used. Alexa Fluor 680 (Invitrogen) and IRDye 800 (Rockland) conjugated antibodies were used as secondary antibodies. Protease inhibitor cocktail tablets (including serine and cysteine proteases) were purchased from Roche (Indianapolis, IN). NLP-005 Methyltrienolone (R1881) was purchased from Perkin Elmer (Boston, MA). Nonidet P-40 was purchased from US Biological (Swampscott, MA). Paclitaxel was obtained from Sigma Aldrich and docetaxel from Sanofi Aventis (Kansas City, MO). Generation of androgen receptor truncated mutants:

The full-length GFP-AR plasmid (pEGFP-C1-AR-Q22) was generously provided by Dr. Michael Mancini (Baylor College of Medicine, Houston, TX) and used as the template to generate all androgen receptor-truncated mutant constructs. All PCR-generated androgen receptor -truncated mutant constructs were sub-cloned into the expression vector pEGFP-C1. All cloning was performed using AccuPrime Taq DNA Polymerase High Fidelity (Invitrogen), with 10 µM forward and reverse cloning primers (IDT, San Diego, CA). All primers were designed using the human androgen receptor mRNA reference sequence (GenBank NM_000044).

AR 540-724 and AR 725-919 were subcloned into the p3xFLAG-CMV-14 expression vector (Sigma) with the following specific primers:

AR 540-724 forward:

    5'-CGCACGATATCGCCACCATGTTGGAGACTGCCAGGGACC-3' (SEQ ID NO:14);

AR 540-724 reverse:

    5'-CGCACGGATCCAGGCAAGGCCTTGGCCCAC-3' (SEQ ID NO:15); and

AR 725-919 forward

    5'-CGCACGATATCGCCACCATGGGCTTCCGCAACTTACACGTG-3'(SEQ ID NO:16);

AR 725-919 reverse:

    5'-CGCACGGATCCCTGGGTGTGGAAATAGATGGG-3' (SEQ ID NO:17).

Amplification products were analyzed by Sanger sequencing to confirm the integrity of all constructs.

Immunoprecipitation and Western Blotting:

HEK293T cells transiently transfected with GFP-tagged AR-wt AR, ARv5,6,7 or ARv7 were lysed in TNES buffer and subjected to immunoprecipitation as previously described by Darshan *et al.* (*Cancer Res* **71**, 6019 (Sep 15, 2011)).

Microtubule co-sedimentation assay:

PC3-mCherry-tubulin and HEK293T cells were transiently transfected with GFP-tagged androgen receptor, AR-wt or GFP-ARv7 or HA-tagged ARv5,6,7 and subjected to microtubule co-sedimentation assay as previously described by Giannakakou *et al.* (*Nat Cell Biol* **2**, 709 (Oct, 2000)). Briefly, 1 mg of total cell lysate was first pre-cleared by high-speed centrifugation, the pellet (HSP) was discarded while the supernatant (HSS) was supplemented exogenous purified with bovine brain tubulin (Cytoskeleton, Denver, CO) reconstituted at a final concentration of 10 µM in the presence of 1 mM GTP, and 20 µM paclitaxel (PTX) and subjected to a cycle of polymerization for 30 min at 37°C. Samples were centrifuged at 100,000 x g for 30 min at room temperature and the warm supernatant (WS) was separated from the warm pellet (WP), which was resuspended in an equal volume of PEM buffer. Equal volumes from each respective fraction were loaded onto a SDS-PAGE and transferred and immunoblotted with antibodies against androgen receptor, α-tubulin and actin. Densitometry for each respective protein was performed using ImageJ (National Institutes of Health) software and the percentage of the protein present in the pellet fraction was calculated using the following formula: % P = 100*WP/(WP+WS).

Quantitative real time PCR:

M12-cumate inducible AR-wt or variant cells were treated with cumate (Cu) for 48h and then starved for 24h in CSS media. Cells were treated with 1 µM docetaxel (TXT) for 4 h either alone or followed by 10 nM R1881 overnight. QPCR for TMPRSS2, FKBP51 and GAPDH was performed as previously described by Chan et al. (*J Biol Chem* **287**, 19736 (Jun 1, 2012)).

Dynamitin overexpression, immunofluorescence and confocal microscopy:

M12 cells stably expressing the untagged androgen receptor constructs were transiently transfected with c-Myc-tagged pCMVH50m plasmid containing dynamitin (gifted by R. Vallee, Columbia University, New York, NY) using FuGENE 6, according to the manufacturer's instructions. Twenty four hours post-transfection, the cells were treated with the indicated drugs. Images were acquired by confocal microscopy and image analysis was performed as previously described by Darshan *et al.* (*Cancer Res* **71**, 6019 (Sep 15, 2011)).

Live cell imaging:

PC3-mCherry-tubulin cells were plated on MatTek (Ashland, MA) 5 mm, Poly-d-lysine coated glass bottom dishes and cells were pressure-microinjected intranuclearly with plasmids encoding the different GFP-AR cDNAs as previously described by Darshan *et al.* (*Cancer Res* **71**, 6019 (Sep 15, 2011)). Time-lapse images were taken using a Spinning Disk microscopy system consisting of a Zeiss Axiovert 200 system fitted with a Yokogawa CSU-X1 spinning disk head (Tokio, Japan). Time lapse microscopy and image analyses were performed as previously described by Darshan *et al.* (*Cancer Res* **71**, 6019 (Sep 15, 2011)).

Xenograft tumors

LuCaP human prostate cancer xenografts were grown as previously described by Mostaghel *et al.* (*Clin Cancer Res* **17**, 5913 (Sep 15, 2011)) and Wu et al. (*Clin Cancer Res* **12**, 6153 (Oct 15, 2006)) in non-castrated SCID mice. Briefly, when the tumor volume reached an estimated size of 200mm^3 (lxw^2/2) mice were treated with either vehicle or docetaxel at 5 or 20-mgm/kg intraperitoneally. weekly until tumors in the control group reached a tumor volume of 1000 mm^3. At this time all animals in a group were sacrificed.

Immunohistochemistry

Androgen receptor immunostaining of explanted LuCaP tumors was conducted as previously described by Zhang et al. (*PLoS One* **6**, e27970 (2011)).

Statistical analysis:

The p-values for androgen receptor nuclear localization in control, taxane and nocodazole treated cells were calculated using one-way analysis of variance followed by multiple comparisons with Bonferroni adjustments using Stata Statistical Software: release 10. The p-value for androgen receptor nuclear *versus* cytoplasmic localization in M12 cells were calculated using two-tailed unpaired T-Test.

EXAMPLE 8: Microtubule binding

is mediated by the C-terminal domain of the androgen receptor

This Example describes experimental results showing that the C-terminal domain of androgen receptors bind to microtubules.

The inventors have previously shown that wild type androgen receptor (AR-wt) associates with the microtubule cytoskeleton and that this association is important for androgen receptor cytoplasmic to nuclear translocation and transcriptional activity in castration-resistant prostate cancer (Darshan *et al., Cancer Res* **71**, 6019 (Sep 15, 2011); Thadani-Mulero et al., *Cancer Res* **72**, 4611 (Sep 15, 2012)). However, the androgen receptor protein domain required for microtubule association has previously not been identified.

To determine where the microtubule binding domain is within the androgen receptor protein a microtubule co-sedimentation assay was performed using cells expressing overlapping truncated androgen receptor mutants, which were generated by a serial mutagenesis approach. Initially, two large androgen receptor truncations were generated and sub-cloned into a pEGFP-C1 vector: the N-terminal domain (N-ter, amino acids 1-539) encompassing exon 1 of the androgen receptor and the C-terminal domain (C-ter, amino acids 540-919) encompassing the DNA binding domain, the hinge region and the ligand binding domain of AR (FIG. 6A). Each deletion mutant was individually expressed in either PC3:mCherry-tub or HEK293T cells by transient transfection and cells were subjected to microtubule co-sedimentation in order to assess each proteins' association with microtubule polymers. In this assay cell lysates from each condition (HSS) were supplemented with exogenous purified tubulin and subjected to a cycle of microtubule polymerization at 37 ºC and in the presence of GTP and Taxol. Under these conditions purified tubulin along with endogenous cellular tubulin is robustly polymerized enabling microtubule interactions with cellular proteins. Following the polymerization reaction, high-speed centrifugation separated microtubule polymers along with any cellular proteins with affinity for them into the warm pellet (WP) fraction, while soluble tubulin and other proteins that did not have affinity for microtubules segregated with the warm supernatant (WS). The distribution of androgen receptor proteins between the WP and WS fractions indicates its ability to associate with the microtubule polymers, as in all conditions the majority of tubulin was identified in the WP fraction indicating efficient polymerization. GFP-tagged AR-wt androgen receptor was used as a positive control for microtubule association.

As shown in FIGs. 6B and 6E, the C-terminal domain of the androgen receptor associated preferentially with microtubule polymers in both cell lines. In particular, 86% of the C-terminal androgen receptor co-fractionated with microtubules in the WP fraction, in a manner similar to the AR-wt. In contrast, the N-terminal androgen receptor showed minimal association (13%) with polymerized tubulin (FIGs. 6B and 6E). Tubulin was efficiently polymerized in all conditions as shown by the majority of tubulin (over 80%) found in respective WP fractions.

In order to further narrow down the minimum AR-microtubule binding domain, seven additional androgen receptor truncation mutants were generated within the C-terminal region (FIG. 6C). The truncations were designed to correspond to androgen receptor functional domains as well as to cover the entire C-terminal region. In particular, the following truncations were generated:

47

the DNA binding domain (amino acids 559-624),

the hinge region (amino acids 625-663),

the DNA binding domain plus the hinge region (amino acids 559-663) and

the 540-724 amino acid fragment,

5   the 540-558 amino acid fragment,

the 664-724 amino acid fragment, and

the 725-919 amino acid fragment.

These deletion mutants were subcloned into a pEGFP-C1 vector, with the exception of the
fragment containing amino acids 540-724 and the fragment containing amino acids 725-919 mutants were
10   subcloned into a p3XFLAG-CMV vector, and with the GFP-tag gave rise to a protein of approximately 50
kDa, which is also the size of tubulin, whose excess made the detection of the truncated proteins very
challenging.

Of the seven truncation mutants, the androgen receptor amino acid 559-663 fragment,
corresponding to the DNA binding domain plus the hinge region, showed the most extensive association
15   with the microtubule polymers with 35% protein seen in the WP (FIG. 6D). Surprisingly, none of these
androgen receptor deletion mutants showed as extensive association with the microtubule polymer
fraction as the original C-terminal androgen receptor fragment (FIG. 6B), indicating a likelihood that
different parts of the C-terminal region of the androgen receptor contribute to effective tubulin association.

20   **EXAMPLE 9: ARv5,6,7 associates**
                          more extensively with microtubules than ARv7

This Example describes experiments showing that the ARv5,6,7 variant co-fractionated almost
exclusively (70%) with microtubule polymers, while the ARv7, only partially co-fractionated with
microtubules.
25   Experiments were performed to investigate whether any of the two most clinically prevalent AR
splice variants with truncations in their C-terminus, namely ARv5,6,7 (Sun et al., *J Clin Invest* **120**, 2715
(Aug, 2010)) and ARv7 (Guo et al., *Cancer Res* **69**, 2305 (Mar 15, 2009); Hu et al., *Cancer Res* **69**, 16
(Jan 1, 2009)), would associate with microtubules similar to the AR-wt. Microtubule co-sedimentation
revealed that the ARv5,6,7 variant co-fractionated almost exclusively (70%) with microtubule polymers in
30   the WP fraction while ARv7, only partially co-fractionated with microtubules at 42% (FIG. 7B).

                          EXAMPLE 10: Nuclear translocation of ARv5,6,7, but not of ARv7,
                                    is impaired by microtubule targeting drugs

This Example describes experiments demonstrating that drugs that target microtubules impair the
35   nuclear translocation of the Arv5,6,7 variant, which affects the transcription of genes normally regulated
by the androgen receptor.

The distinct pattern of microtubule association exhibited by the two AR variants suggested
potentially distinct mechanisms of nuclear translocation. To test this hypothesis, the microtubule network
was perturbed by 2 hr treatment with drugs that either stabilize (docetaxel) or depolymerize (nocodazole)
40   microtubules. The nuclear accumulation of the androgen receptor variants was then assessed in cells

microinjected with GFP-tagged ARv5,6,7 or ARv7. Live cell confocal microscopy was then used to image the dynamics of androgen receptor variant nuclear accumulation by obtaining z-stack images every 10 minutes for a total of 120 min.

Representative images from each condition are shown in FIG. 8. As shown in FIG. 8, the nuclear accumulation of ARv5,6,7 was significantly impaired following microtubule perturbation with either of the docetaxel or nocodazole drugs. In contrast, the ARv7 remained largely unaffected and remained substantially localized within the nucleus.

The two variants were found predominantly in the nucleus of untreated cells as soon as the GFP-tagged protein was expressed following microinjection (time 0). Despite their initial nuclear localization at baseline, the two variants exhibited entirely distinct responses to drug-induced microtubule disruption. The extent of nuclear localized ARv5,6,7 was quantified, revealing a significant decrease in its nuclear localization following microtubule perturbation at all time points observed (FIG. 8C and Tables 2 and 3; $p < 0.01$ at baseline and $p < 0.001$ at all other time points).

Table 2: P-values for the live cell imaging experiments
with PC3:mCh-tub-ARv5,6,7, shown in FIG. 7C-7D

Time (min)	P value (Control vs. TXT)	P value (Control vs. Noc)
0	0.02	0.00
10	0.02	0.00
20	0.01	0.00
30	0.00	0.00
40	0.00	0.00
50	0.00	0.00
60	0.00	0.00
70	0.00	0.00
80	0.00	0.00
90	0.00	0.00
100	0.00	0.00
110	0.00	0.00
120	0.001	0.00

Table 3: P-values for the live cell imaging experiments
with PC3:mCh-tub-ARv7 displayed in FIG. 7C and 7D

Time (min)	P value (Control vs. TXT)	P value (Control vs. Noc)
0	1.000	1.000
10	1.000	1.000
20	1.000	0.561
30	1.000	0.382
40	1.000	0.621
50	1.000	0.249
60	1.000	0.482
70	1.000	0.491
80	1.000	0.646
90	1.000	0.545
100	1.000	0.922
110	1.000	0.750
120	1.000	0.787

The integrity of the microtubule cytoskeleton was assessed in each condition prior to time lapse image acquisition and is shown in the right panels of FIG. 8A and 8B, indicating effective drug-target engagement for each of the conditions. Microtubule bundling with docetaxel is shown in FIG. 8A-8B (middle row, arrowhead). Depolymerized tubulin upon nocodazole treatment is also shown in FIG. 8A-8B (third row).

In contrast and despite effective drug-target engagement, drug treatment had no effect on the other clinically relevant and constitutively active androgen receptor splice variant, ARv7. Treatment of the ARv7-microinjected cells with microtubule targeting drugs did not impact this variant's nuclear localization at any time point (FIG. 8B and 8D and Tables 2 and 3).

Taken together, these data indicate that the ARv5,6,7 variant, but not the ARv7, is dependent on microtubules for effective nuclear accumulation.

These studies were also performed on the M12 prostate cancer cell line, a tumorigenic cell line representative of the metastatic stage of prostate cancer. Isogenic cell lines were engineered to stably express GFP-tagged AR-wt AR, ARv5,6,7 or ARv7. The effects of docetaxel (TXT) treatment on the nuclear localization of each of the GFP-tagged receptors were then investigated in these M12 prostate cancer cells. As seen by the GFP fluorescence exhibited in FIG. 9A-9B), or antibody-based detection (FIG. 9F-9H), docetaxel inhibited ligand-induced AR-wt nuclear accumulation downstream of microtubule stabilization (FIG. 9A, arrowhead for microtubule bundles, arrows for cytoplasmic AR). Docetaxel treatment also inhibited the nuclear localization of the ligand-independent ARv5,6,7 variant (FIG. 9B).

Similar results were obtained in the presence of R1881, which did not induce any further nuclear accumulation of ARv5,6,7 (FIG. 9I). However, as shown in FIG. 9C, docetaxel treatment failed to alter the nuclear localization of ARv7 variant in the absence or presence of R1881 (see also, FIG. 9J).

The extent of AR nuclear localization for each variant and condition was quantified by counting the number of cells with androgen receptor in the nucleus versus number of cells with cytoplasmic androgen receptor. FIG. 9D shows the ratio of cells in the nucleus : cytoplasm for cells having the wild type or variant androgen receptors under various drug exposure conditions. This analysis revealed that docetaxel treatment resulted in a significant decrease of nuclear localization in AR-wt and ARv5,6,7, but had no effect on ARv7. These data confirm and corroborate the live cell imaging results showing that ARv7 does not depend on microtubules for its nuclear trafficking.

To assess the effect of docetaxel treatment on AR transcriptional activity quantitative real-time PCR was performed using transcriptional targets previously shown to be differentially induced by AR-wt and AR variants. Specifically TMPRSS2 was identified as a target specific for AR-wt while FKBP51 was transcriptionally activated by the AR variants. In this assay M12 cells expressing inducible AR-wt or variants were used. As seen in FIG. 9E, in M12 cells the AR-wt increased TMPRSS2 expression and docetaxel treatment significantly inhibited TMPRSS2 expression, consistent with the drug's effects on androgen receptor cytoplasmic sequestration. In the case of the AR variants, TMPRSS2 expression was no longer regulated while FKBP51 transcription was induced by both ARv7 and ARv5,6,7. Thus, docetaxel treatment significantly inhibited ARv5,6,7 mediated induction of FKBP51 but had no effect on ARv7 transcriptional activity, in agreement with the differential effects of docetaxel on each variant's nuclear localization (FIG. 9B-9C). These data confirm that the AR variants have a distinct transcriptome compared to AR-wt but that nuclear localization is necessary for AR-V and AR-wt activity.

EXAMPLE 11: Dynamitin overexpression
impairs ARv5,6,7 nuclear translocation, but has no effect on ARv7.

This Example describes experiments demonstrating that Dynamitin adversely affect Arv5,6,7 nuclear translocation but does not affect Arv7 nuclear translocation.

The data described in previous Examples indicate that a functional microtubule network is required for effective nuclear translocation of ARv5,6,7 variant, and for the AR-wt. In contrast, variant ARv7 behaves in a microtubule independent manner in all assays and cell lines tested.

To further dissect the mechanism regulating the cytoplasmic to nuclear translocation of each variant the involvement of the minus-end directed microtubule motor protein dynein was investigated, because the inventors have shown that dynein mediates nuclear translocation (Darshan et al., *Cancer Res* **71**, 6019 (Sep 15, 2011)). Dynein works in concert with several accessory proteins to drive subcellular motile functions including dynactin, which is an adapter that mediates the binding of dynein to cargo structures enhancing dynein's motor function. Overexpression of the dynactin associated protein, dynamitin, disrupts dynein-cargo interactions (Burkhardt et al., *J Cell Biol* **139**, 469 (Oct 20, 1997)). Such dynamitin overexpression was used in experiments to dissect the involvement of dynein in the transport of the androgen receptor splice variants to the nucleus.

M12 cells stably expressing untagged ARv5,6,7 (M12-ARv5,6,7) or ARv7 (M12-ARv7) were transiently transfected with a c-Myc-tagged p50-dynamitin vector and processed for double-labeling immunofluorescence with anti-androgen receptor and anti-c-Myc antibodies. Overexpression of dynamitin impaired nuclear accumulation of ARv5,6,7 (FIG. 10A, dashed arrows point to cytoplasmic androgen receptor). However, overexpression of dynamitin had no effect on the nuclear accumulation of ARv7 (FIG. 10B arrows point to nuclear androgen receptor). Quantification of the extent of nuclear accumulation of androgen receptor proteins revealed a significant decrease of ARv5,6,7 in the nucleus of dynamitin expressing cells as compared with dynamitin-overexpressing M12-ARv7 cells (FIG. 10C). However, no such effect was observed on the nuclear accumulation of ARv7 (FIG. 10C).

To further investigate the role of the dynein microtubule-based motor protein on androgen receptor variant trafficking, a co-immunoprecipitation (co-IP) experiment was performed in HEK293T cells transiently transfected with GFP-tagged AR-wt AR, ARv5,6,7 or ARv7. Co-precipitation using an antibody against GFP revealed that both the AR-wt AR and ARv5,6,7, but not ARv7, associated with dynein (FIG. 10D).

Taken together these data support a model whereby AR-wt AR and ARv5,6,7 utilize microtubules and dynein-dependent transport for their nuclear accumulation and subsequent activity. On the other hand, ARv7 does not utilize this mechanism of transport and hence remains insensitive to taxane treatment.

EXAMPLE 12: Docetaxel treatment inhibits ARv5,6,7-mediated subcutaneous tumor growth in SCID mice

This Example shows that Docetaxel is an effective anti-tumor agent for prostate cancer cells that express Arv5,6,7.

Recent work and the current data suggest that inhibition of the nuclear accumulation and transcriptional activity of androgen receptor is related, as least in part, to the clinical activity of taxanes. In order to determine the impact of androgen receptor variant expression on taxane sensitivity *in vivo*, the effects of docetaxel on the growth of two LuCaP xenograft tumors grown subcutaneously in SCID mice was evaluated (see Example 7 for a description of the methods employed).

The tumors were LuCaP 86.2, a human xenograft tumor in which the majority of the androgen receptor is ARv5,6,7, and LuCaP 23.1, a human xenograft expressing both AR-wt and ARv7 (Mostaghel et al., *Clin Cancer Res* **17**, 5913 (Sep 15, 2011)). All xenografts were grown in non-castrate SCID mice. There were 15 mice in each group. LuCaP 86.2 tumor cells are resistant to castration. FIG. 11 indicates that LuCaP 86.2 tumor cells are driven by ARv5,6,7, because the growth of these cells is markedly suppressed by a low dose of docetaxel of 5 mg/kg (P< 0.01 control vs. docetaxel treated). The inventors have previously shown that the 5 mg/kg dose of docetaxel is ineffective on the growth of LuCaP 35 xenograft expressing primarily AR-wt (Wu et al., *Clin Cancer Res* **12**, 6153 (Oct 15, 2006)). In contrast, there was no effect of docetaxel on the growth of LuCaP23.1 tumors (p = NS control vs. docetaxel treatment).

The effect of docetaxel treatment on androgen receptor nuclear accumulation was further assessed by immunohistochemistry on explanted LuCaP tumors, as previously described by Zhang et al.

(*PLoS One* **6**, e27970 (2011)). Quantitation of nuclear accumulation of androgen receptor protein in tumors from untreated *versus* treated animals revealed that docetaxel treatment resulted in a statistically significant reduction of nuclear androgen receptor in LuCaP86.2 tumors, while it had a minimal effect on LuCaP 23.1 tumors (FIGs. 10 - 11).

These data indicate that drug-induced inhibition of androgen receptor nuclear accumulation underlies taxane antitumor activity. These data also indicated that androgen receptor variant expression can determine whether a prostate cancer patient will respond to treatment with taxanes.

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All patents and publications referenced or mentioned herein (as well as those listed in Appendix A) are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "an antibody" includes a plurality (for example, a solution of antibodies or a series of antibody preparations) of such antibodies, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The following statements summarize and describe aspects of the invention.

Statements:

1.   A method of determining whether a prostate cancer patient can benefit from taxane drug treatment comprising testing whether an androgen receptor splice selected from the group consisting of variant v5,6,7, variant v7, or a combination thereof is present in a test sample obtained from the patient.

2.   The method of statement 1, wherein the prostate cancer patient can benefit from taxane drug treatment when androgen receptor splice variant v5,6,7 is present in a test sample than variant v7.

3.   The method of statement 1 or 2, wherein the prostate cancer patient may not benefit from taxane drug treatment when androgen receptor splice variant v7 is present in a test sample than variant v5,6,7.

4.   The method of any of statements 1-3, wherein the test sample comprises circulating tumor cells, a prostate tissue sample, a blood sample, a serum sample, ascites fluid, a urine sample, semen sample, or a combination thereof.

5.   The method of any of statements 1-4, further comprising capturing circulating tumor cells from the test sample before the testing.

6.   The method of any of statements 1-5, further comprising capturing circulating tumor cells from the test sample in a microfluidic device.

7.   The method of any of statements 1-6, further comprising capturing circulating tumor cells from the test sample in a microfluidic device that comprises a solid support with a length and width, a cover sheet, and rows of posts between the solid support and the covers sheet, the device configured for flow of a cell sample through the length of the device from a cell sample application area, to an outlet; wherein each row is perpendicular to the length of the device; and where the posts of one row do not align with the posts of adjacent rows.

8.   The method of any of statements 1-7, further comprising capturing circulating tumor cells from the test sample in a microfluidic device comprising rows posts, each row is perpendicular to the length of the device, wherein the posts in each row do not align with the posts of one or two adjacent rows.

9.   The method of any of statements 1-8, further comprising capturing circulating tumor cells from the test sample in a microfluidic device comprising rows posts, each row is perpendicular to the length of the device, wherein the posts in each row are offset from the posts in the one or two adjacent rows by 0.1 to 100 microns, or about 0.2 to 75 microns, or about 0.5 to 50 microns, or about 0.75 to 10 microns, or about 1 microns to about 25 microns.

10.   The method of any of statements 1-9, further comprising capturing circulating tumor cells from the test sample in a microfluidic device comprising a solid support and/or the cover sheet can comprises include silicon, silica, glass, polydimethylsiloxane (PDMA), cellulose, ethylcellulose, methylcellulose, nitrocellulose, hydroxypropylcellulose, hydroxypropyl methylcellulose, polystyrene, polyethylene, nylon, rayon, cotton, teflon, mica, sephadex, sepharose, polyacrylonitrile, glass, glass-fiber paper, gold, metal, paper, and combinations thereof.

11.   The method of any of statements 6-10, wherein a binding entity is immobilized on at least a section of the solid support, the cover sheet, or a combination thereof.

12.   The method of statement 11, wherein the binding entity specifically binds to a selected cell type.

13.   The method of statement 11 or 12, wherein the binding entity is an anti-PMSA antibody.

14.   The method of any of statements 1-13, further comprising capturing circulating tumor cells from the test sample in a microfluidic device, and testing captured circulating tumor cells in the microfluidic device.

15.   The method of any of statements 1-14, further comprising capturing circulating tumor cells from the test sample in a microfluidic device, removing captured circulating tumor cells from the device and testing captured circulating tumor cells.

16.   The method of any of statements 1-15, wherein testing comprises detection of one or more androgen receptor variant proteins.

17.   The method of any of statements 1-16, wherein testing comprises quantification of one or more androgen receptor variant proteins to generate quantified level of androgen receptor variant expression for each androgen receptor variant protein.

18.   The method of any of statements 1-17, wherein testing comprises quantification of one or more androgen receptor variant proteins to generate quantified level of androgen receptor variant expression for each androgen receptor variant protein, and comparison of one or more quantified level of androgen receptor variant expression to a control expression level of the same androgen receptor variant.

19.   The method of any of statements 1-18, wherein the androgen receptor splice variant v5,6,7 protein has at least 75% sequence identity, or at least 80% sequence identity, or at least 85% sequence identity, or at least 90%, or at least 95% sequence identity to SEQ ID NO:3.

20.   The method of any of statements 1-19, wherein the androgen receptor splice variant v7 protein has at least 75% sequence identity, or at least 80% sequence identity, or at least 85% sequence identity, or at least 90%, or at least 95% sequence identity to SEQ ID NO:5.

21.   The method of any of statements 1-20, wherein testing comprises an assay of the test sample with an antibody specific for the androgen receptor variant protein.

22.   The method of any of statements 1-21, wherein testing comprises an assay comprising contacting the test sample with an antibody having at least 50 % or greater selectivity, or 60% greater selectivity, or

70% greater selectivity, or 80% greater selectivity, or 85% greater selectivity, or 90% greater selectivity, or 95% greater selectivity for the androgen receptor splice variant.

23.   The method of any of statements 1-22, wherein testing comprises an assay comprising contacting the test sample with an antibody and detecting a signal from a conjugate of the antibody and the androgen receptor variant protein, if binding between the antibody and the androgen receptor variant protein occurs.

24.   The method of any of statements 1-23, wherein testing comprises an assay comprising contacting the test sample with an antibody and detecting a signal from a conjugate of the antibody and the androgen receptor variant protein within cells from the test sample, if binding between the antibody and the androgen receptor variant protein occurs.

25.   The method of any of statements 1-24, wherein testing comprises an assay comprising contacting the test sample with an antibody and detecting a signal from a conjugate of the antibody and the androgen receptor variant protein within nuclei of cells from the test sample, if binding between the antibody and the androgen receptor variant protein occurs.

26.   The method of any of statements 1-25, wherein testing comprises an assay comprising contacting the test sample with an antibody and detecting a signal from a conjugate of the antibody, the androgen receptor variant protein, and cellular microtubules from the test sample cells, if binding between the antibody and the androgen receptor variant protein occurs.

27.   The method of any of statements 1-26, wherein testing comprises an assay comprising contacting the test sample with an antibody and detecting a signal from a conjugate of the antibody and the androgen receptor variant protein within cytoplasm of cells from the test sample, if binding between the antibody and the androgen receptor variant protein occurs.

28.   The method of any of statements 1-27, wherein testing comprises an assay comprising contacting the test sample with an antibody and a taxane, and detecting a signal from a conjugate of the antibody and the androgen receptor variant protein within cells from the test sample, if binding between the antibody and the androgen receptor variant protein occurs.

29.   The method of any of statements 1-28, wherein testing comprises an assay comprising contacting the test sample with an antibody and a taxane, and detecting whether a signal from a conjugate of the antibody and the androgen receptor variant protein is localized within cell nuclei of the test sample.

30.   The method of any of statements 1-29, wherein testing comprises an assay comprising contacting the test sample with a directly or indirectly labeled antibody.

31.   The method of any of statements 1-30, wherein testing comprises an immunoassay selected from the group consisting of an immunoassay, cell sorting assay, sandwich immunoassay, competition inhibition immunoassay, ELISA (Enzyme-Linked Immunosorbent Assay), immunohistochemical assay, agglutination assay, precipitation assay, radioimmunoassay or antigen-down immunoassay, immunometric assay, competitive binding assay, a direct sandwich immunoassay, an indirect sandwich assay, an immunoprecipitation assay, a nuclear immunostaining assay, an immunoblot assay, or a combination thereof.

32.   The method of any of statements 1-15, wherein testing comprises detection of the androgen receptor variant transcript.

33.   The method of any of statements 1-15 or 32, wherein testing comprises Northern blotting, nuclease protection assays, RNA fingerprinting, polymerase chain reaction, ligase chain reaction, Qbeta replicase, isothermal amplification method, strand displacement amplification, transcription based amplification systems, quantitative nucleic acid amplification assays (e.g., polymerase chain reaction assays), combined reverse transcription/nucleic acid amplification, nuclease protection (SI nuclease or RNAse protection assays), Serial Analysis Gene Expression (SAGE), next generation sequencing, gene expression microarray, in situ hybridization, nucleic acid amplification, reverse transcription, polymerase chain reaction, quantitative real time polymerase chain reaction (qRT- PCR), or a combination thereof.

34.   The method of any of statements 1-15, 32 or 33, wherein testing comprises an assay comprising contacting the test sample with at least one probe or primer that selectively hybridizes to the androgen receptor splice variant mRNA.

35.   The method of any of statements 1-15, 32-34, wherein testing comprises an assay comprising contacting the test sample with at least one probe or primer that selectively hybridizes to the androgen receptor splice variant mRNA, wherein the probe or primer is covalently attached to a label that can emit a detectable signal.

36.   The method of any of statements 1-15, 32-35, wherein testing comprises an assay comprising contacting the test sample with at least one probe or primer that selectively hybridizes to the androgen receptor splice variant mRNA, wherein hybridization of the probe or the primer to the androgen receptor splice variant mRNA emits a signal.

37.   The method of any of statements 1-15, 32-36, wherein testing comprises an assay comprising contacting the test sample with at least one probe or primer that selectively hybridizes to the androgen receptor splice variant mRNA, wherein primer extension of the androgen receptor splice variant mRNA emits a signal.

38.   The method of any of statements 1-15, 32-37, wherein testing comprises an assay comprising contacting the test sample with at least one probe or primer that can hybridize to the androgen receptor splice variant mRNA under high stringency hybridization conditions.

39.   The method of any of statements 1-15, 32-38, wherein testing comprises an assay comprising contacting the test sample with at least one probe or primer that can hybridize to the androgen receptor splice variant mRNA under high stringency hybridization conditions comprising a wash in 0.1X SSPE, 1.0% SDS at a temperature of at least 42ºC.

40.   The method of any of statements 1-15, 32-39, wherein testing comprises quantification of one or more androgen receptor variant mRNAs to generate quantified level of androgen receptor variant expression for each androgen receptor variant mRNA.

41.   The method of any of statements 1-15, 32-40, wherein testing comprises quantification of one or more androgen receptor variant mRNAs to generate a quantified level of androgen receptor variant expression for each androgen receptor variant mRNA, and comparison of one or more quantified level of androgen receptor variant expression to a control expression level of the same androgen receptor variant.

42.   The method of statement 41, wherein control expression level of the same androgen receptor variant is the expression level of the same androgen receptor variant in non-tumor prostate cells.

43.   The method of any of statements 1-15, 32-42 wherein testing comprises quantification of androgen receptor variant v5,6,7 mRNA to generate a quantified level of androgen receptor variant v5,6,7 expression level, and comparison of the quantified level of androgen receptor variant v5,6,7 expression level to a quantified level of androgen receptor variant v7.

44.   The method of any of statements 1-15, 32-43 wherein testing comprises quantification of androgen receptor variant v7 mRNA to generate a quantified level of androgen receptor variant v7 expression level, and comparison of the quantified level of androgen receptor variant v7 expression level to a quantified level of androgen receptor variant v5,6,7.

45.   The method of any of statements 1-44, wherein a patient is responsive to taxane administration when a quantified level of androgen receptor variant v5,6,7 expression in the patient's test sample is greater than a quantified level of androgen receptor variant v7 expression in the patient's test sample.

46.   The method of any of statements 1-45, wherein a patient is non-responsive to taxane administration when a quantified level of androgen receptor variant v7 expression in the patient's test sample is greater than a quantified level of androgen receptor variant v5,6,7 expression in the patient's test sample.

47.   The method of any of statements 1-15, wherein testing comprises detection of a gene product or a gene transcript regulated by the androgen receptor splice variant in the test sample.

48.   The method of any of statements 1-15 or 47, wherein the gene product or the gene transcript regulated by the androgen receptor splice variant is from AKT1, BAG1, Beta-catenin, BRCA1, C-jun, Calmodulin 1, Caveolin 1, CDK9, COX5B, CREB-binding protein, Cyclin D1, Cyclin-dependent kinase 7, Death associated protein 6, Deleted in Colorectal Cancer, EFCAB6, Epidermal growth factor receptor, FOXO1, GAPDH, Gelsolin, GNB2L1, GSK3B, HDAC1, HSP90AA1, HTATIP, MAGEA11, MED1, MYST2, NCOA1, NCOA2, NCOA3, NCOA4, NCOA6, NCOR2, NONO, PA2G4, PAK6, PATZ1,PIAS2, PRPF6, PTEN, RAD9A, RANBP9, RCHY1, Retinoblastoma protein, RNF15, RNF4, SART3, SMAD3, Small heterodimer partner, Src, SRY, STAT3, SVIL, Testicular receptor 2, Testicular receptor 4, TGFB1I1, TMF1, TRIM68, UBE2I, UXT, ZMIZ1, and any combination thereof.

49.   The method of any of statements 1-15, 47 or 48, wherein testing comprises quantification of a selected gene product or a selected gene transcript regulated by the androgen receptor splice variant in the test sample to generate a quantified regulated selected gene expression level, and comparison of the quantified regulated selected gene expression level to a control expression level of the selected regulated gene.

50.   The method of statement 49, wherein control expression level of the selected regulated gene is the expression level of the selected gene in non-tumor prostate cells.

51.   The method of any of statements 1-50, further comprising administering a taxane to a patient when more androgen receptor splice variant v5,6,7 is present in the patient's test sample than variant v7.

52.   The method of statement 51, wherein the taxane is paclitaxel, docetaxel, cabazitaxel, baccatin III, 10-deacetylbaccatin, hongdoushan A, hongdoushan B, hongdoushan C, or any combination thereof.

53.   A microfluidic device for capturing circulating tumor cells, comprising a solid support with a length and width, a cover sheet, and rows of posts between the solid support and the cover sheet, the device configured for flow of a cell sample through the length of the device from a cell sample application area, to an outlet; wherein each row is perpendicular to the length of the device; and where the posts of one row do not align with the posts of adjacent rows.

54.   The device of statement 53, wherein each row is perpendicular to the length of the device, and wherein the posts in each row do not align with the posts of one or two adjacent rows.

55.   The device of statement 53 or 54, wherein each row is perpendicular to the length of the device, and wherein the posts in each row are offset from the posts in the one or two adjacent rows by 0.1 to 100

microns, or about 0.2 to 75 microns, or about 0.5 to 50 microns, or about 0.75 to 10 microns, or about 1 microns to about 25 microns.

56.   The device of any of statements 53-55, wherein the solid support and/or the cover sheet can comprise include silicon, silica, glass, polydimethylsiloxane (PDMA), cellulose, ethylcellulose, methylcellulose, nitrocellulose, hydroxypropylcellulose, hydroxypropyl methylcellulose, polystyrene, polyethylene, nylon, rayon, cotton, teflon, mica, sephadex, sepharose, polyacrylonitrile, glass, glass-fiber paper, gold, metal, paper, and combinations thereof.

57.   The device of any of statements 53-56, wherein a binding entity is immobilized on at least a section of the solid support, the cover sheet, or a combination thereof.

58.   The device of statement 57, wherein the binding entity specifically binds to a selected cell type.

59.   The device of statement 58, wherein the binding entity is an anti-PMSA antibody.

60.   A kit comprising instructions for use of the kit components, and any of the following separately packaged components:
(a)   at least one probe or primer that specifically binds to and androgen receptor variant v5,6,7, or v7;
(b)   a binding entity that specifically binds to and androgen receptor variant v5,6,7, or v7;
(c)   the device of any of statements 50-57; or
(d)   any combination thereof.

61.   The kit of statement 60, wherein the probe or primer specifically binds to and androgen receptor variant v5,6,7 under high stringency conditions.

62.   The kit of statement 60 or 61, wherein the probe or primer specifically binds to and androgen receptor variant v7 under high stringency conditions.

63.   The kit of any of statements 60-62, further comprising at least one probe, primer or binding entity that specifically binds to full length androgen receptor.

64.   The kit of any of statements 60-63, wherein the instructions describe use of the kit components for isolation of circulating tumor cells, immunoassay, cell sorting assay, sandwich immunoassay, competition inhibition immunoassay, ELISA (Enzyme-Linked Immunosorbent Assay), immunohistochemical assay, agglutination assay, precipitation assay, radioimmunoassay or antigen-down immunoassay, immunometric assay, competitive binding assay, a direct sandwich immunoassay, an indirect sandwich assay, an immunoprecipitation assay, a nuclear immunostaining assay, an immunoblot assay, Northern blotting, nuclease protection assays, RNA fingerprinting, polymerase chain reaction, ligase chain reaction, Qbeta replicase, isothermal amplification method, strand displacement amplification,

transcription based amplification systems, quantitative nucleic acid amplification assays (e.g., polymerase chain reaction assays), combined reverse transcription/nucleic acid amplification, nuclease protection (SI nuclease or RNAse protection assays), Serial Analysis Gene Expression (SAGE), next generation sequencing, gene expression microarray, in situ hybridization, nucleic acid amplification, reverse transcription, polymerase chain reaction, quantitative real time polymerase chain reaction (qRT- PCR), or a combination thereof.

65.   The kit of any of statements 60-64, further comprising at least one vial, needle, syringe, finger-prick device, alcohol swab, gauze square, cotton ball, bandage, latex glove, incubation tray, adhesive label, or data reporting sheet.

66.   The kit of any of statements 60-65, further comprising a container comprising a taxane composition.

67.   The kit of statement 66, wherein the taxane is paclitaxel, docetaxel, cabazitaxel, baccatin III, 10-deacetylbaccatin, hongdoushan A, hongdoushan B, hongdoushan C, or any combination thereof.

68.   A method of determining whether a prostate cancer patient can benefit from taxane drug treatment comprising testing whether an androgen receptor splice variant v5,6,7 is present in a test sample obtained from the patient.

69.   The method of statement 68, wherein the androgen receptor splice variant v5,6,7 has SEQ ID NO:3.

70.   The method of statement 68 or 69, wherein the test sample is a bodily fluid (e.g. blood) or a tissue sample from the patient.

71.   The method of any of statements 68-70, wherein the testing comprises an assay that comprises contacting an antibody specific for the androgen receptor splice variant v5,6,7 with the test sample to form a test mixture, and detecting whether androgen receptor splice variant v5,6,7 binds to the antibody.

72.   The method of any of statements 68-71, wherein the testing comprises contacting the test sample with a device comprising an antibody specific for the androgen receptor splice variant v5,6,7 and detecting whether androgen receptor splice variant v5,6,7 binds to the antibody.

73.   The method of statement 71 or 72, further comprising separating unbound material from the antibody.

74.   The method of any of statements 71-73, further comprising contacting the test mixture or the device with a labeling agent capable of binding to a complex formed between the antibody and androgen receptor splice variant v5,6,7.

75.   The method of statement 74, wherein the agent does not detectably bind to the antibody when androgen receptor splice variant v5,6,7 is not bound to the antibody.

76.   The method of any of statements 68-75, further comprising administering a taxanes drug to the patient when androgen receptor splice variant v5,6,7 is present in a test sample obtained from the patient.

77.   A device comprising a solid surface and an antibody immobilized thereon, wherein the antibody is specific for androgen receptor splice variant v5,6,7, or for androgen receptor splice variant v7.

78.   The device of statement 77, wherein the device is configured for flow of a test sample through the device and binding of a cell expressing androgen receptor splice variant v5,6,7 or for androgen receptor splice variant v7 to the antibody.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

WHAT IS CLAIMED:

1.   A method of determining whether a prostate cancer patient can benefit from taxane drug treatment comprising testing whether an androgen receptor splice selected from the group consisting of variant v5,6,7, variant v7, or a combination thereof is present in a test sample obtained from the patient.

2.   The method of claim 1, wherein the prostate cancer patient can benefit from taxane drug treatment when more androgen receptor splice variant v5,6,7 is present in a test sample than variant v7.

3.   The method of claim 1 or 2, wherein the prostate cancer patient may not benefit from taxane drug treatment when more androgen receptor splice variant v7 is present in a test sample than variant v5,6,7.

4.   The method of any of claims 1-3, wherein the test sample comprises circulating tumor cells, a prostate tissue sample, a blood sample, a serum sample, ascites fluid, a urine sample, semen sample, or a combination thereof.

5.   The method of any of claims 1-4, further comprising capturing circulating tumor cells from the test sample before the testing.

6.   The method of any of claims 1-5, further comprising capturing circulating tumor cells from the test sample in a microfluidic device.

7.   The method of any of claims 1-6, wherein the androgen receptor splice variant v5,6,7 protein has at least 75% sequence identity to SEQ ID NO:3.

8.   The method of any of claims 1-7, wherein the androgen receptor splice variant v7 protein has at least 75% sequence identity to SEQ ID NO:5.

9.   The method of any of claims 1-8, wherein testing comprises an immunoassay, cell sorting assay, sandwich immunoassay, competition inhibition immunoassay, ELISA (Enzyme-Linked Immunosorbent Assay), immunohistochemical assay, agglutination assay, precipitation assay, radioimmunoassay or antigen-down immunoassay, immunometric assay, competitive binding assay, a direct sandwich immunoassay, an indirect sandwich assay, an immunoprecipitation assay, a nuclear immunostaining assay, an immunoblot assay, Northern blotting, nuclease protection assays, RNA fingerprinting, polymerase chain reaction, ligase chain reaction, Qbeta replicase, isothermal amplification method, strand displacement amplification, transcription based amplification systems, quantitative nucleic acid amplification assays (e.g., polymerase chain reaction assays), combined reverse transcription/nucleic acid amplification, nuclease protection (SI nuclease or RNAse protection assays), Serial Analysis Gene Expression (SAGE), next generation sequencing, gene expression microarray, in situ hybridization,

nucleic acid amplification, reverse transcription, polymerase chain reaction, quantitative real time polymerase chain reaction (qRT- PCR), or a combination thereof.

10.   The method of any of claims 1-9, further comprising administering a taxane to a patient when more androgen receptor splice variant v5,6,7 is present in the patient's test sample than variant v7.

11.   The method of claim 10, wherein the taxane is paclitaxel, docetaxel, cabazitaxel, baccatin III, 10-deacetylbaccatin, hongdoushan A, hongdoushan B, hongdoushan C, or any combination thereof.

12.   A kit comprising instructions for use of the kit components, and any of the following separately packaged components:
        a.   at least one probe or primer that specifically binds to and androgen receptor variant v5,6,7, androgen receptor variant v7, or a combination thereof;
        b.   a binding entity that specifically binds to and androgen receptor variant v5,6,7, or v7;
        c.   a microfluidic device; or
        d.   any combination thereof.

13.   The kit of claim 12, wherein the microfluidic device comprises a solid support with a length and width, a cover sheet, and rows of posts between the solid support and the cover sheet; the device configured for flow of a cell sample through the length of the device from a cell sample application area, to an outlet; wherein each row is perpendicular to the length of the device; and where the posts of one row do not align with the posts of adjacent rows.

14.   The kit of claim 12 or 13, wherein each row is perpendicular to the length of the device, and wherein the posts in each row are offset from the posts in the one or two adjacent rows by 0.1 to 100 microns.

15.   The kit of any of claims 13 or 14, wherein the solid support and/or the cover sheet can comprise include silicon, silica, glass, polydimethylsiloxane (PDMA), cellulose, ethylcellulose, methylcellulose, nitrocellulose, hydroxypropylcellulose, hydroxypropyl methylcellulose, polystyrene, polyethylene, nylon, rayon, cotton, teflon, mica, sephadex, sepharose, polyacrylonitrile, glass, glass-fiber paper, gold, metal, paper, and combinations thereof.

16.   The kit of any of claims 13-15, wherein a binding entity is immobilized on at least a section of the solid support, the cover sheet, or a combination thereof.

17.   The kit of any of claims 13-16, wherein the binding entity specifically binds to a selected cell type.

18.   The kit of any of claims 13-17, wherein the binding entity is an anti-PMSA antibody.

19.   The kit of any of claims 13-18, further comprising a taxane.
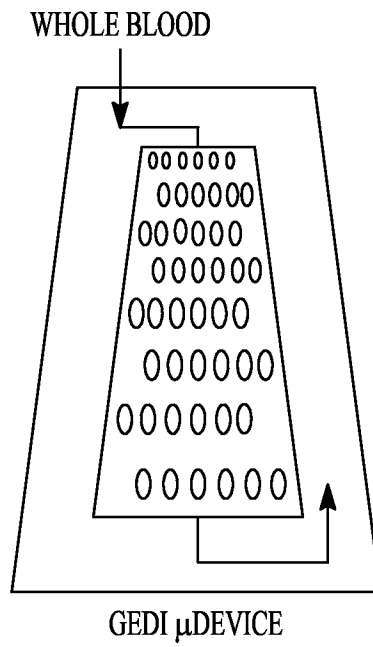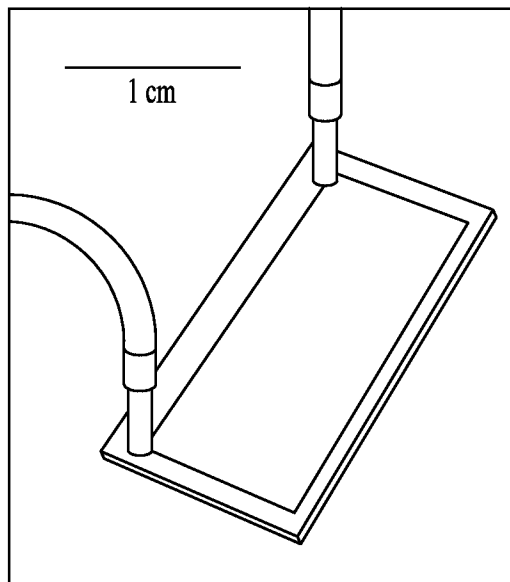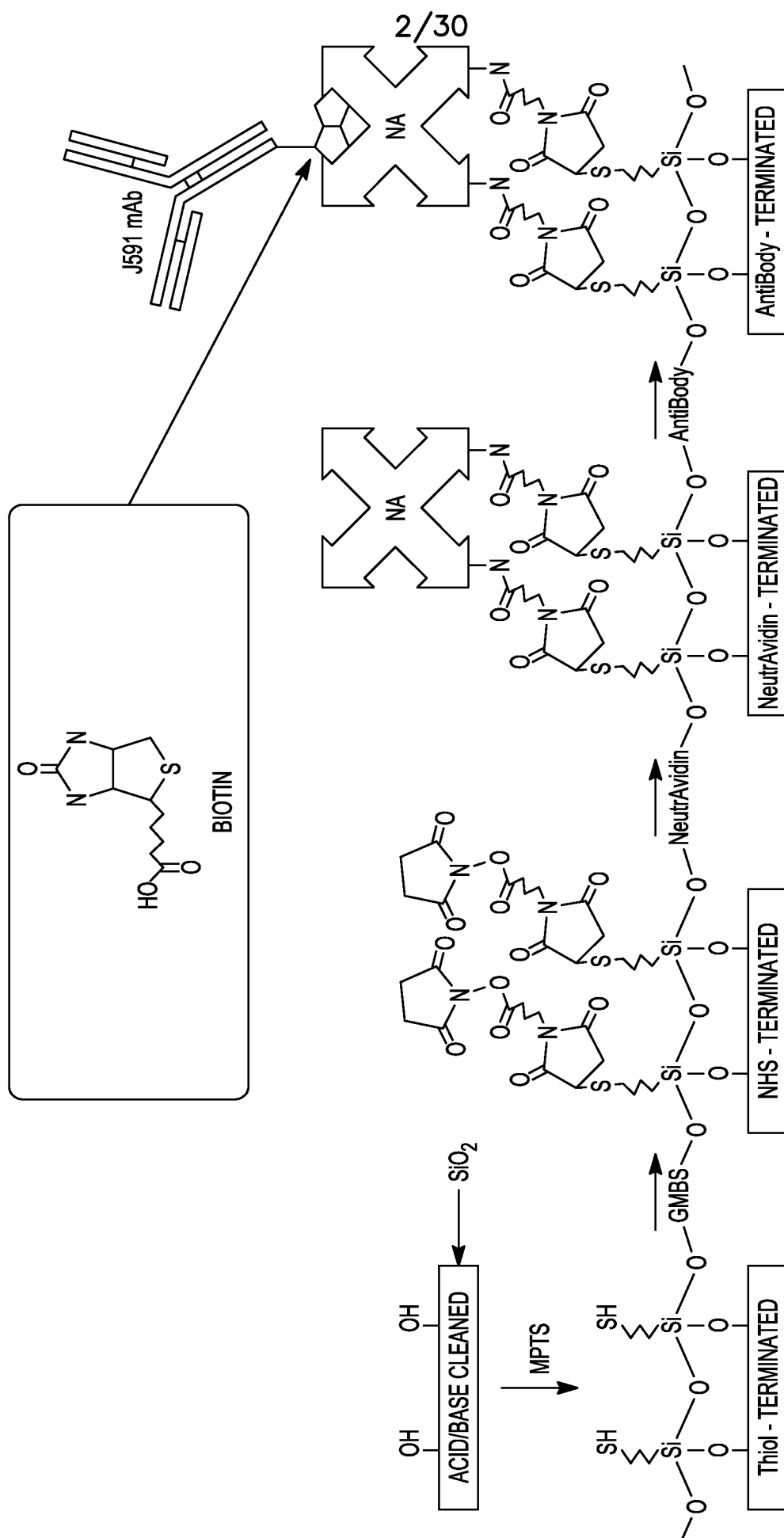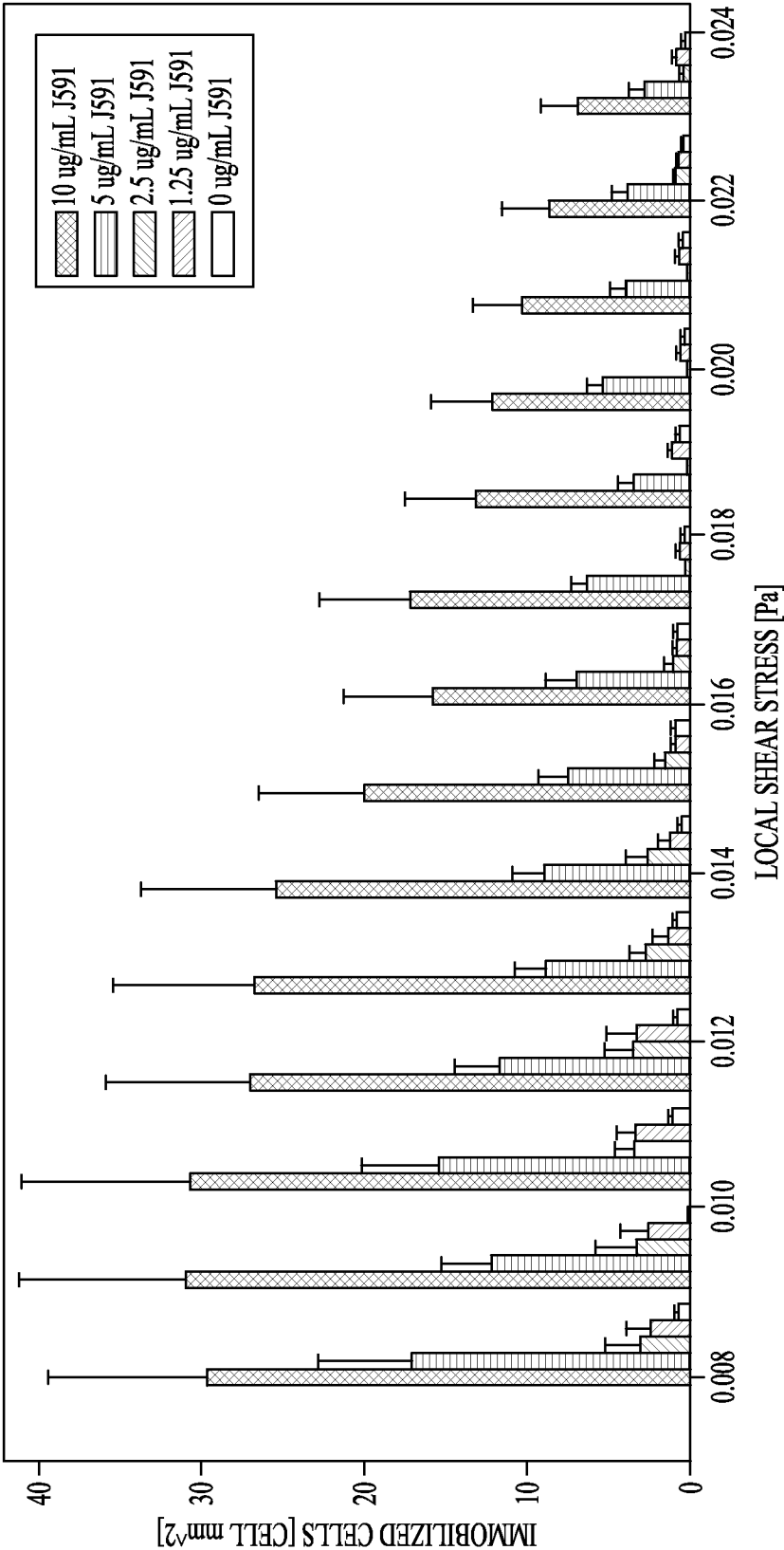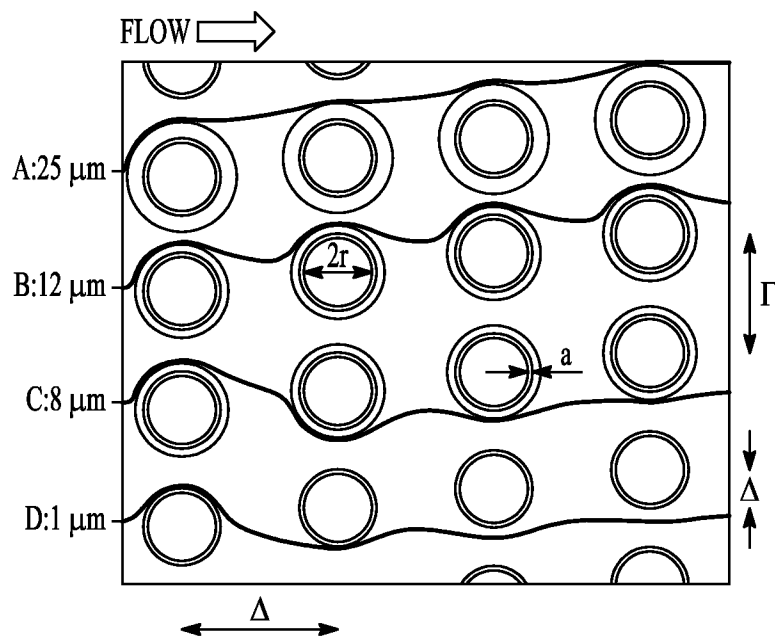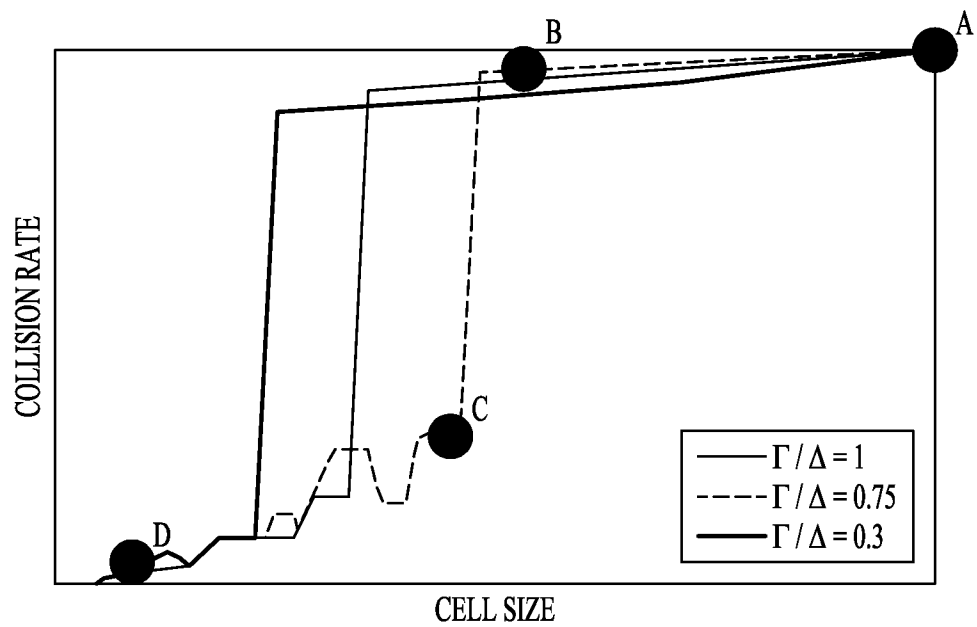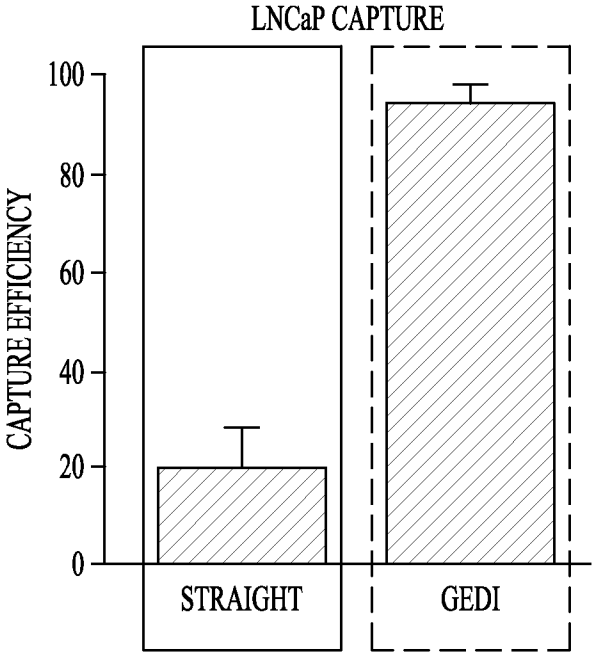
WHOLE BLOOD

GEDI μDEVICE

FIG. 1A

1 cm

FIG. 1B

2/30

FIG. 1C

FIG. 1D

FIG. 2A

FIG. 2B

FIG. 2C

FIG. 2D

FIG. 2E

FIG. 2F

FIG. 3A

FIG. 3B

FIG. 3C

FIG. 3D

CATCAGTTCACTTTTGACCT

CATCAGTTCGCTTTTGACCT

CATCAGTTCGCTTTTGACCT
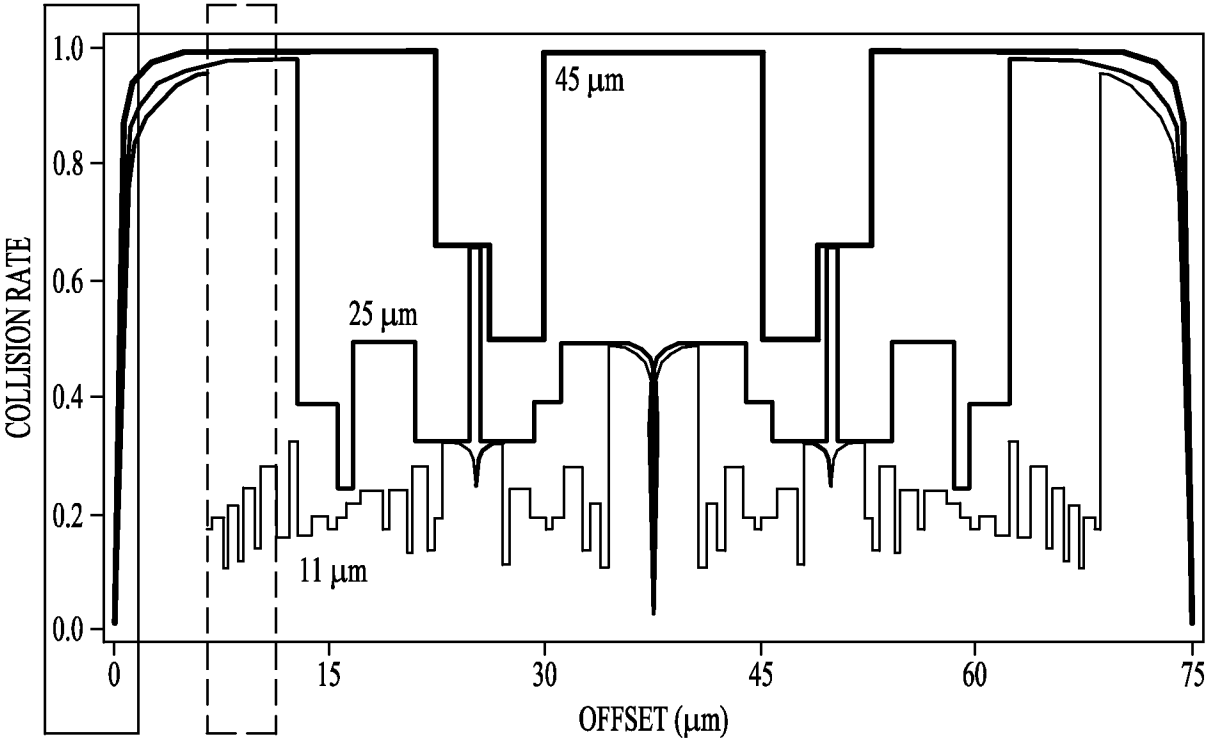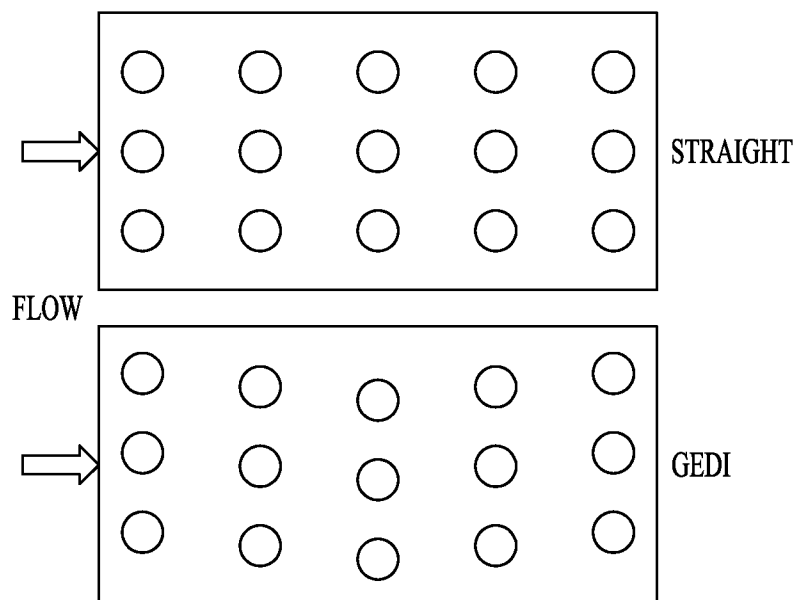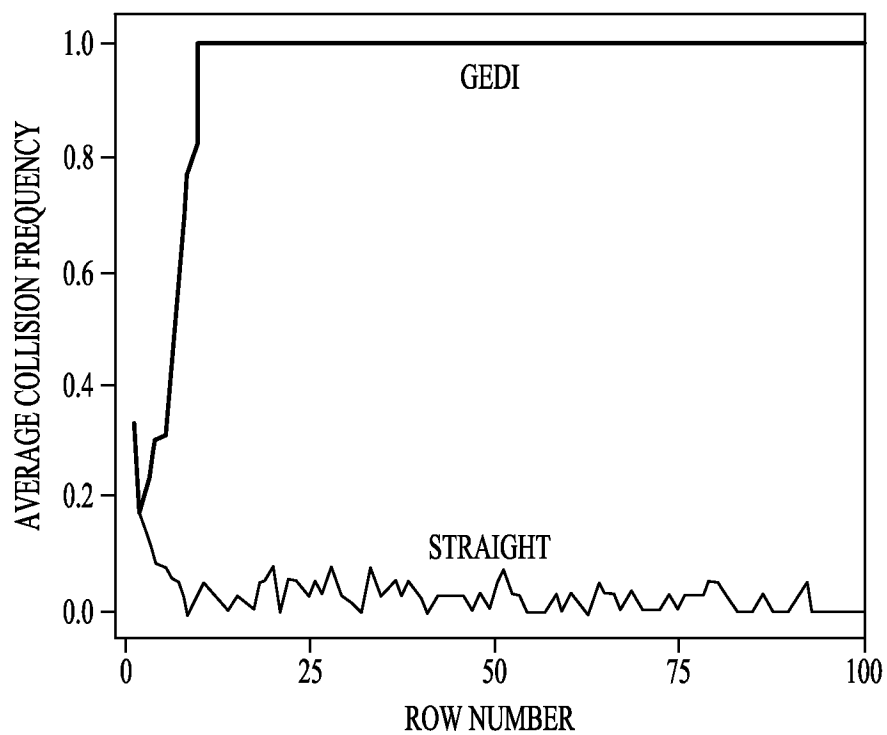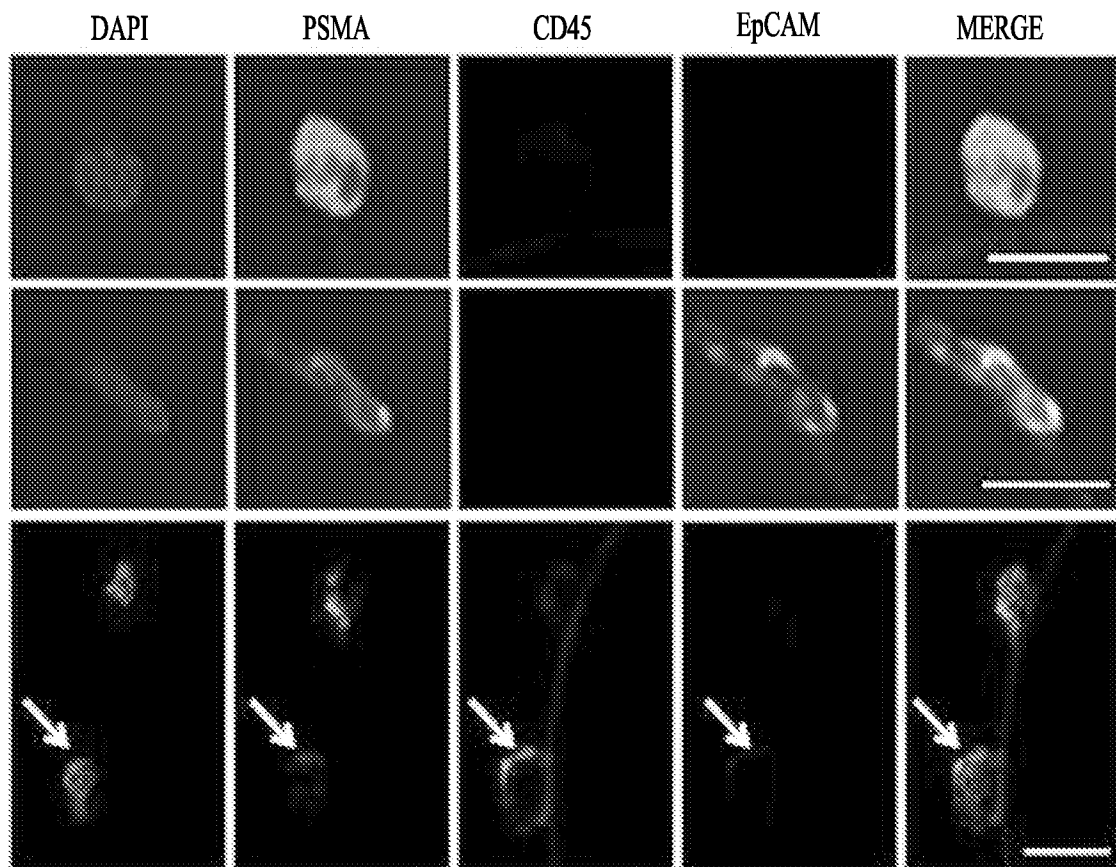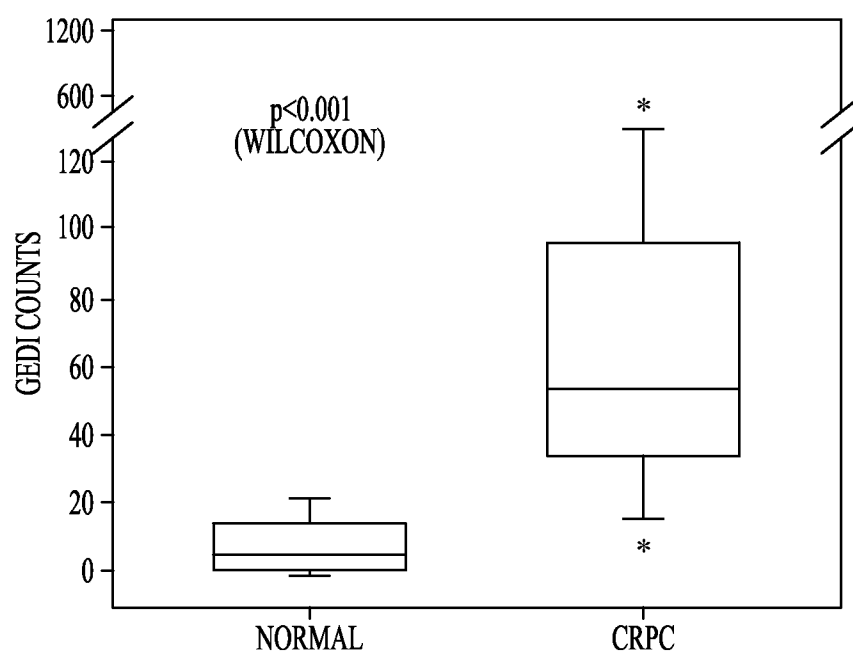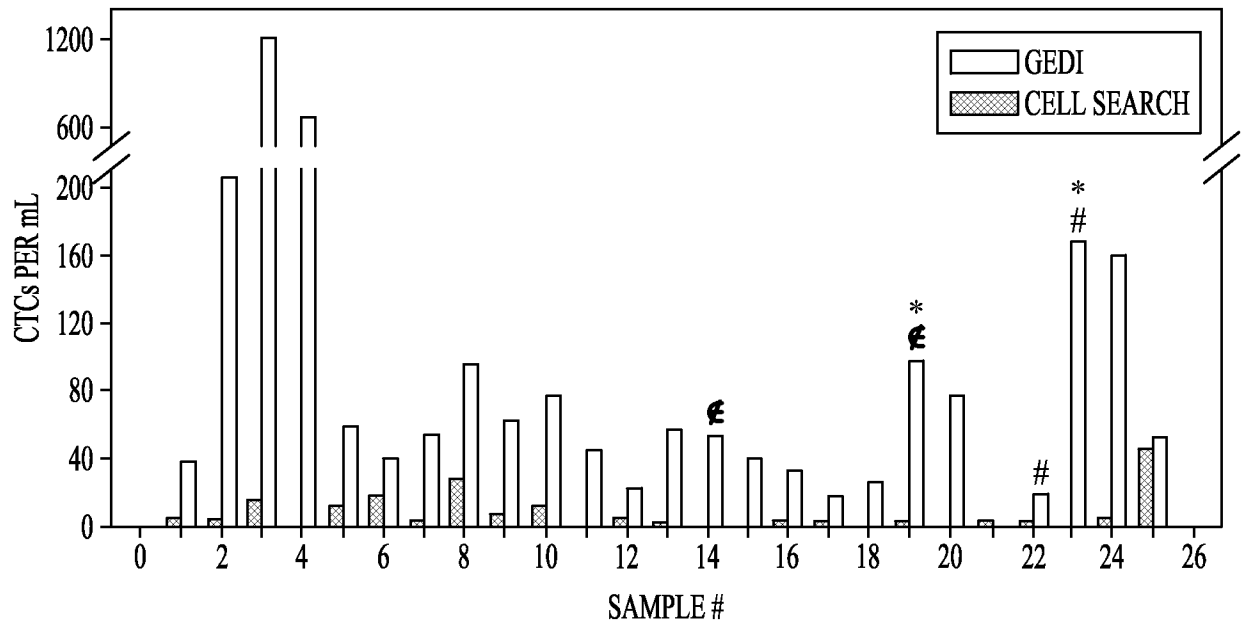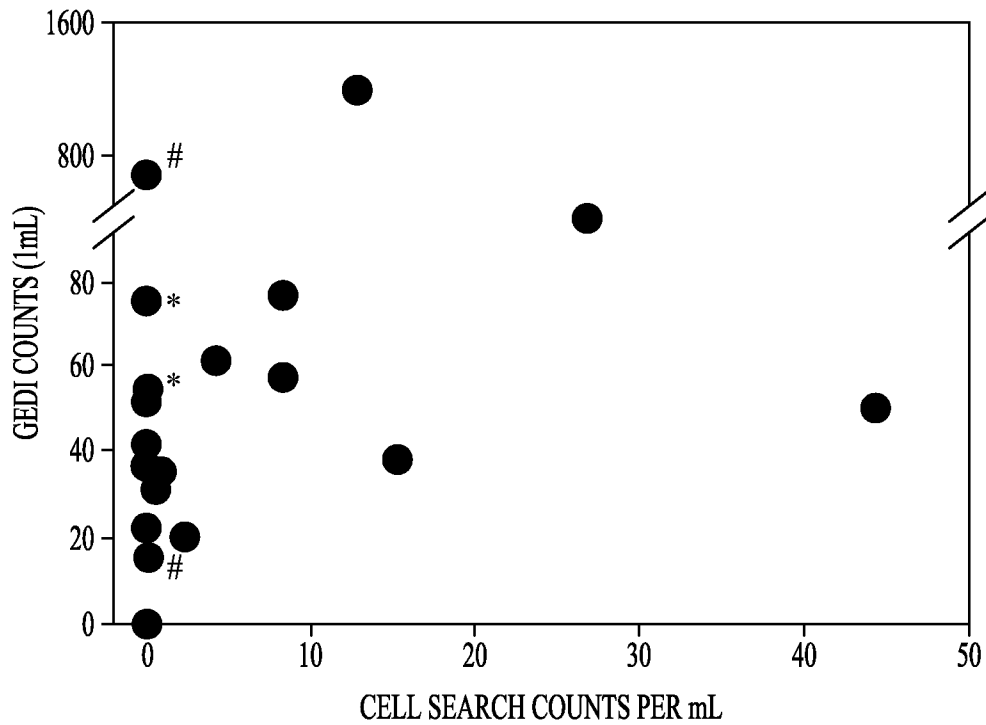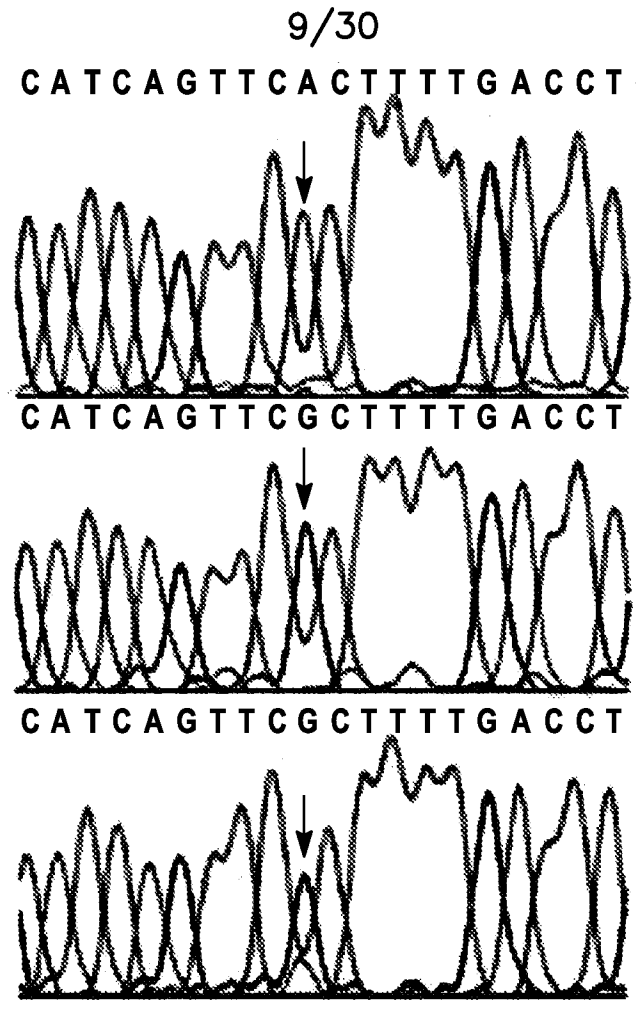
FIG. 4A
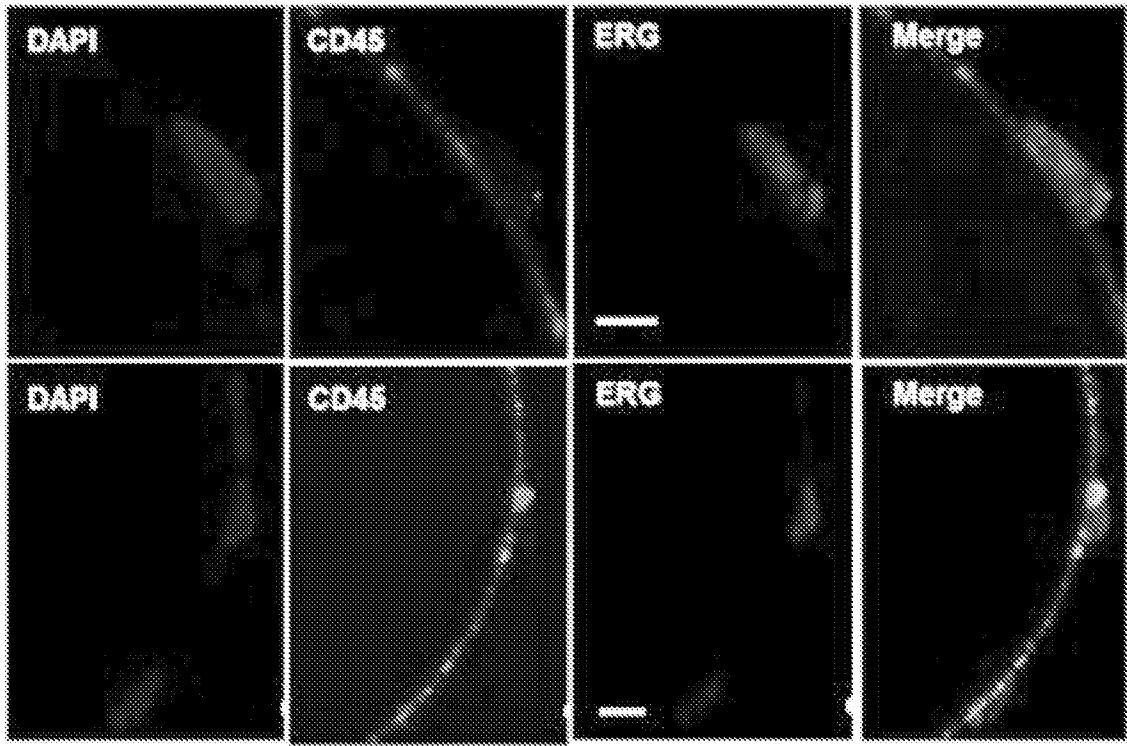
FIG. 4B
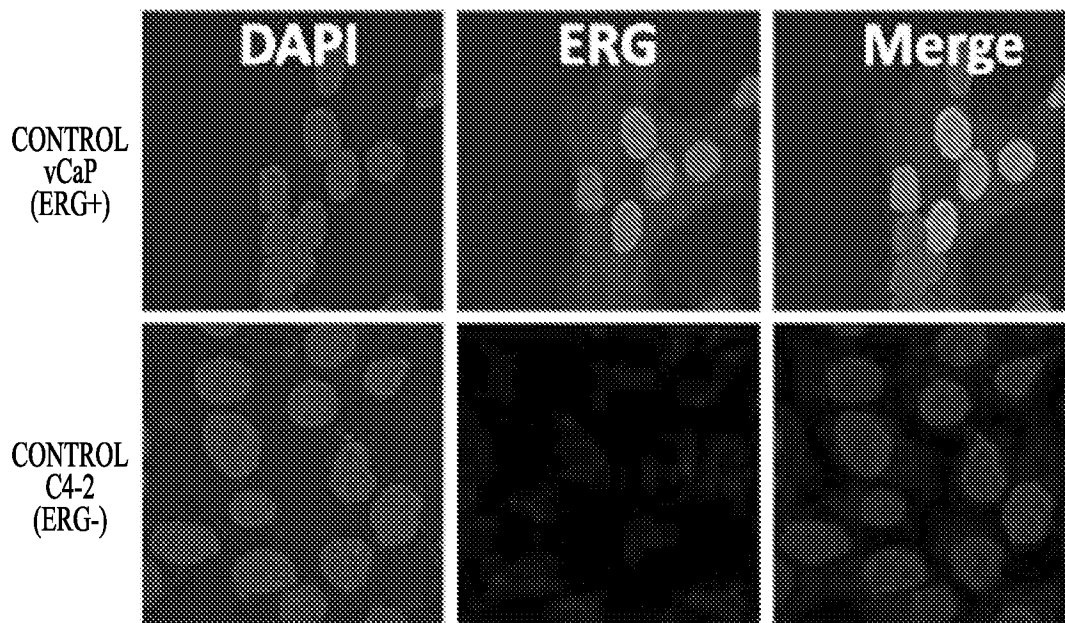
FIG. 4C
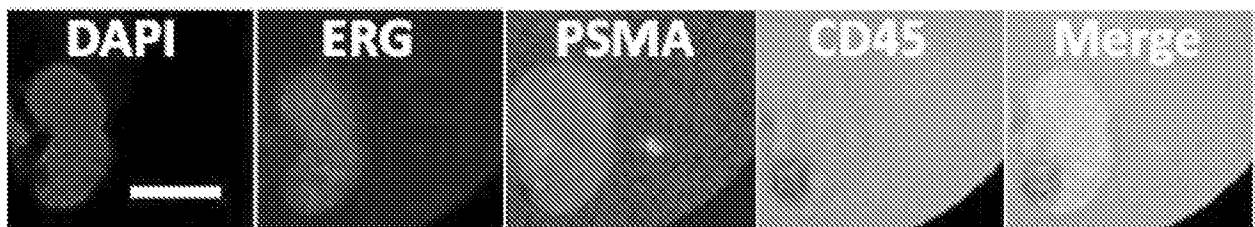
FIG. 4D
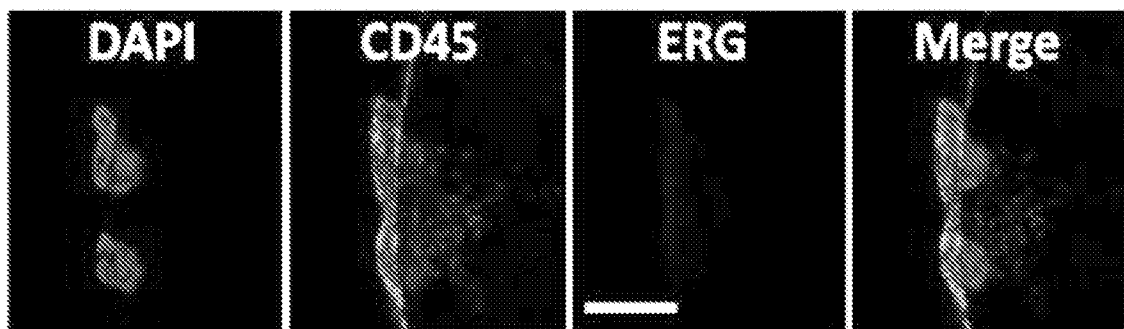
FIG. 4E
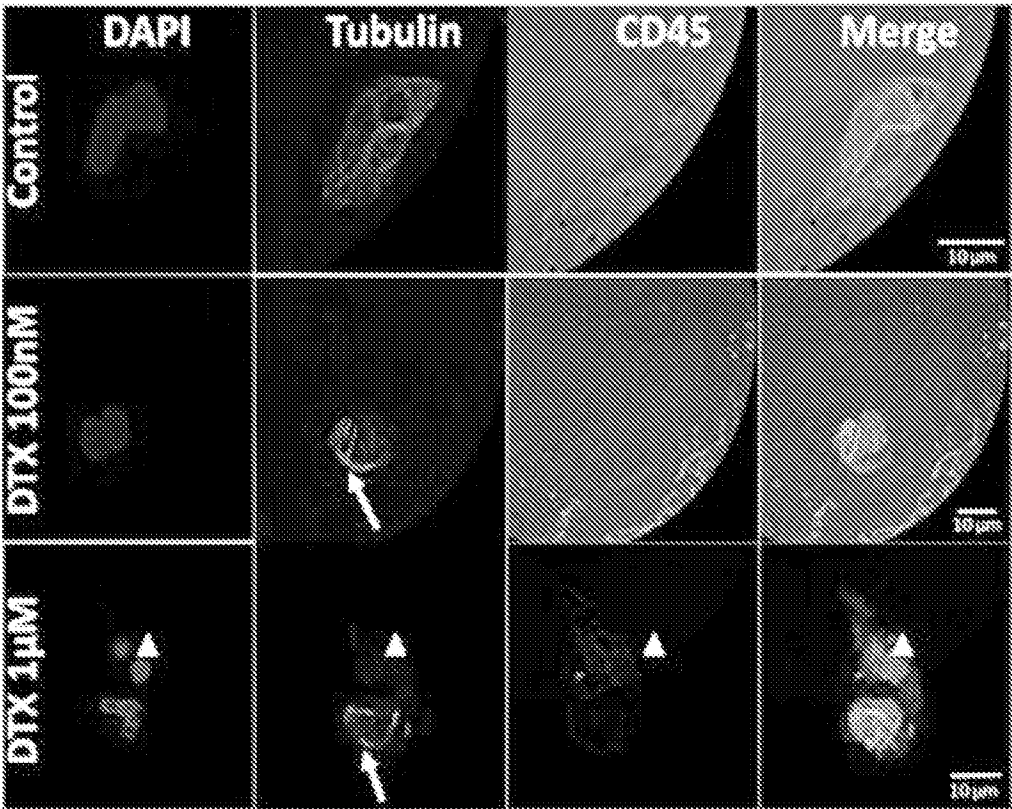
FIG. 5A
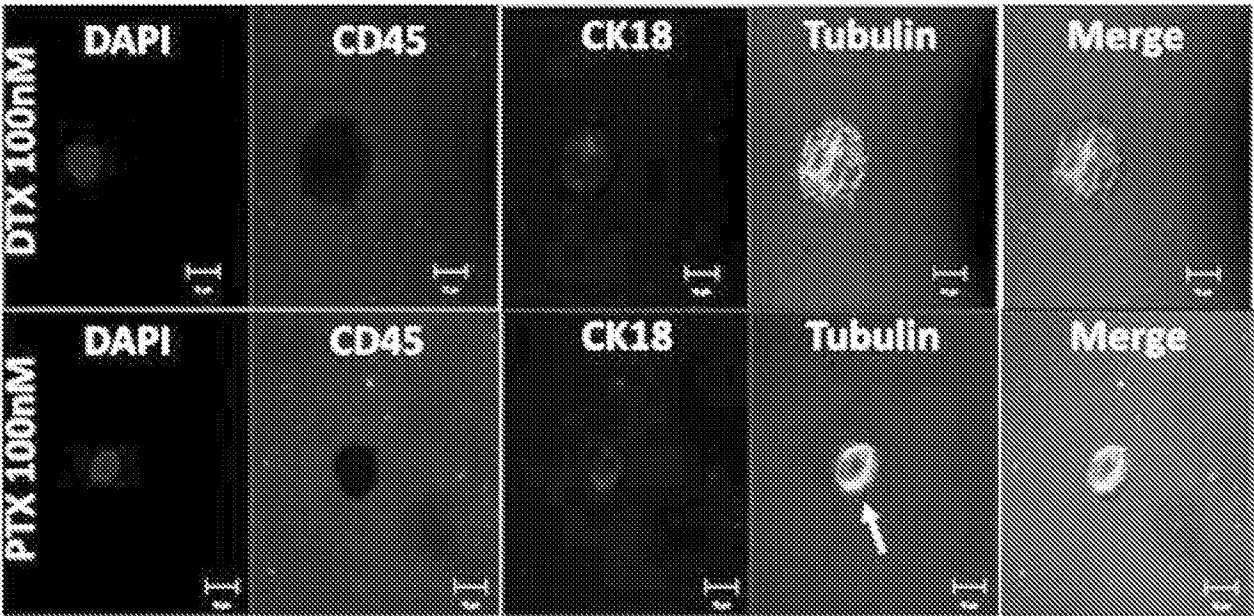
FIG. 5B
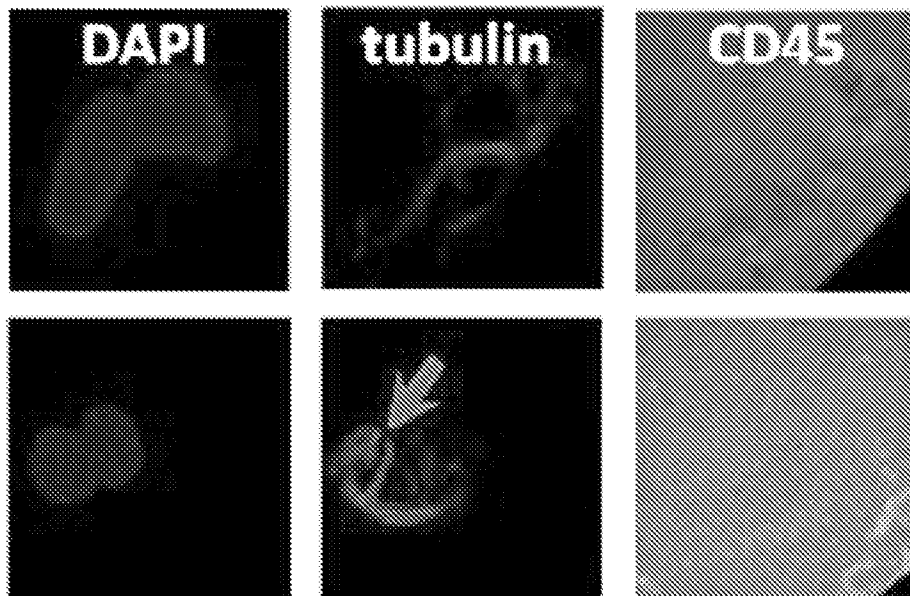
FIG. 5C
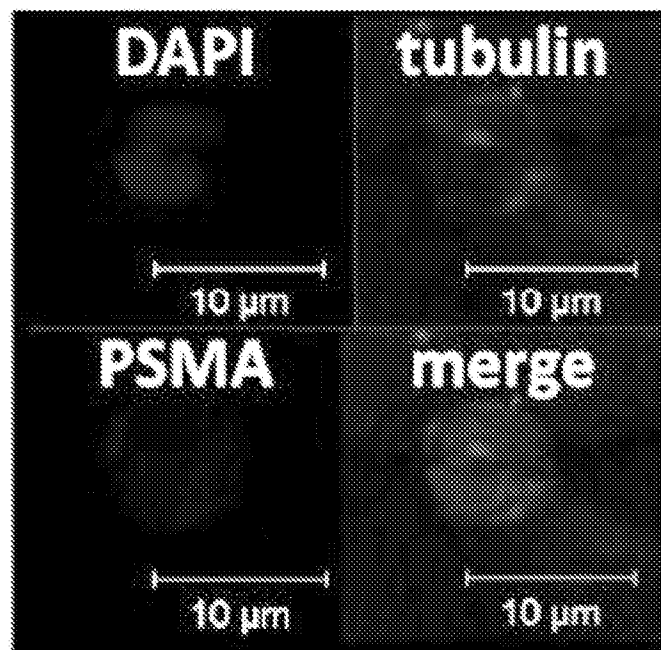
FIG. 5D
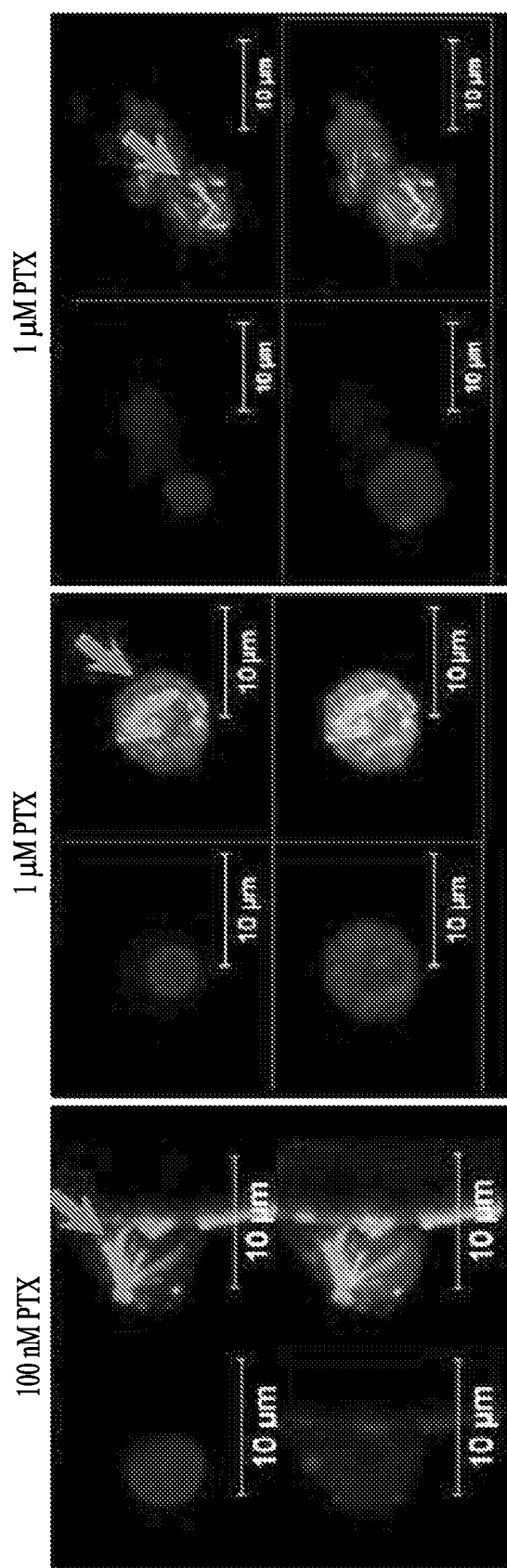
FIG. 5E
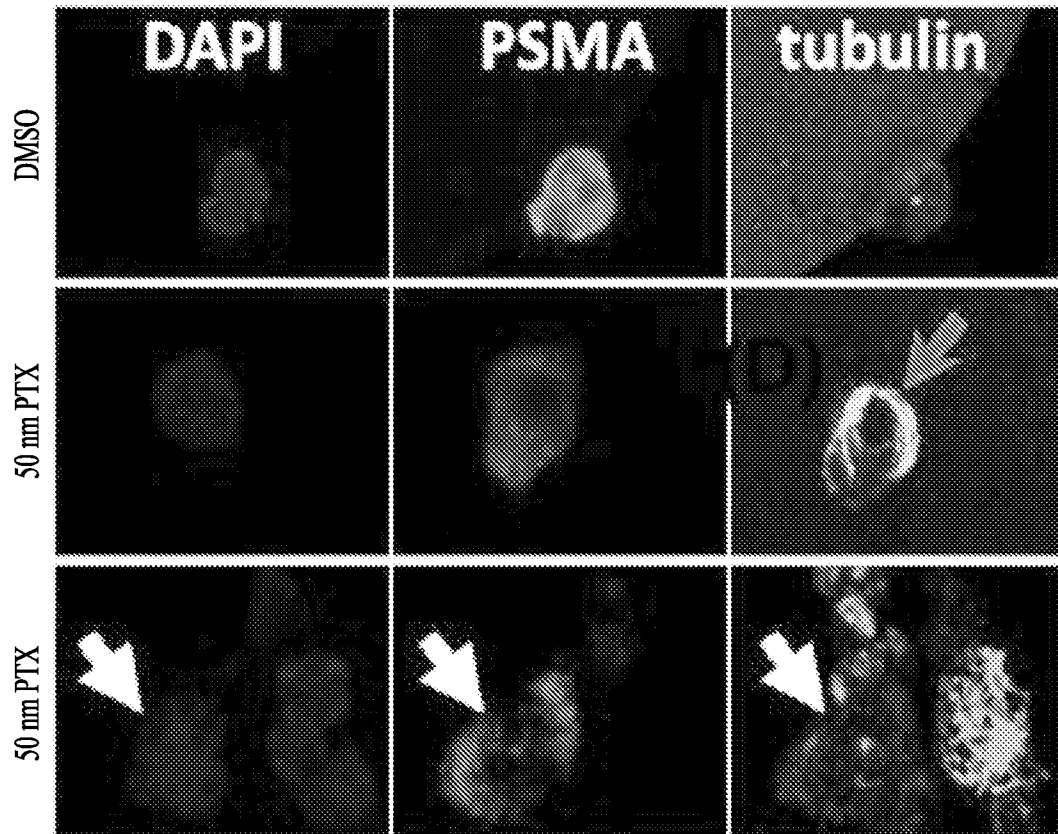
FIG. 5F

FIG. 6A
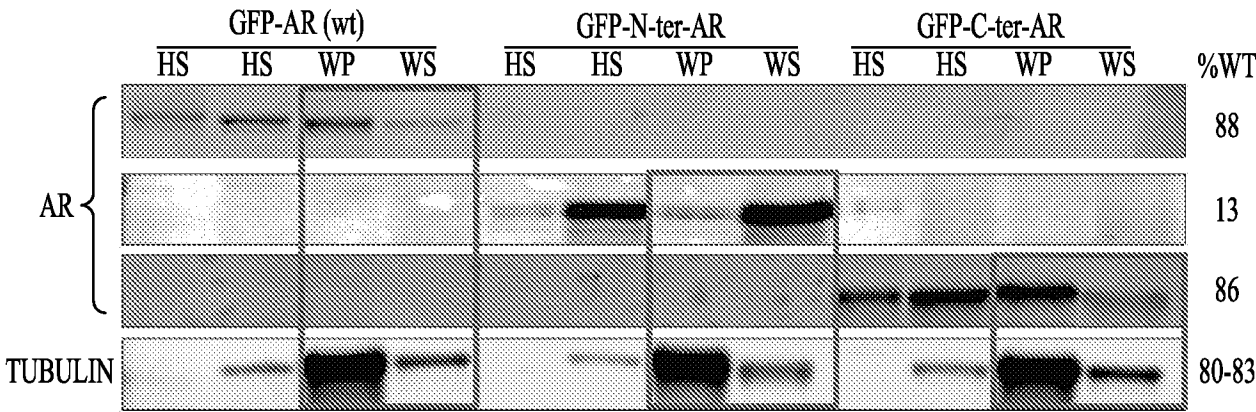
FIG. 6B
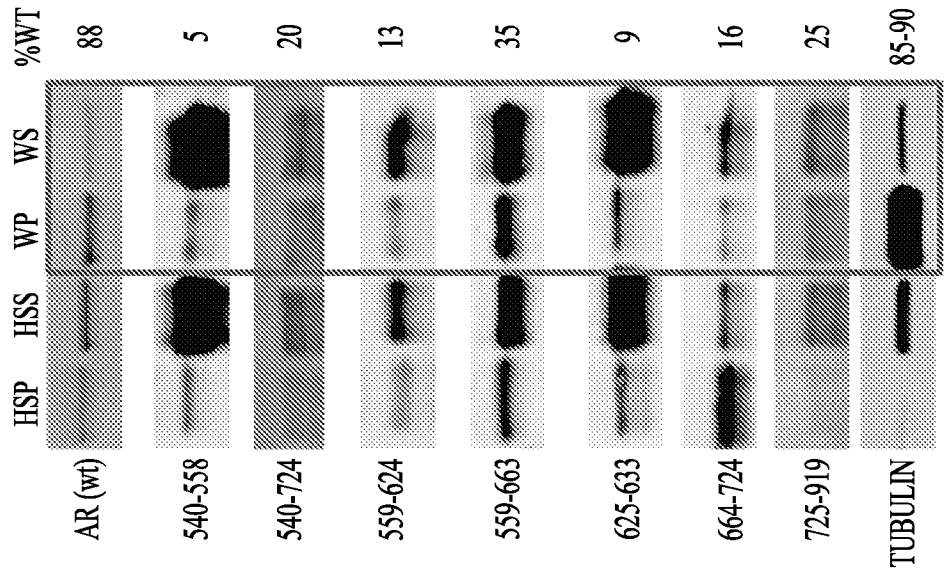
FIG. 6D
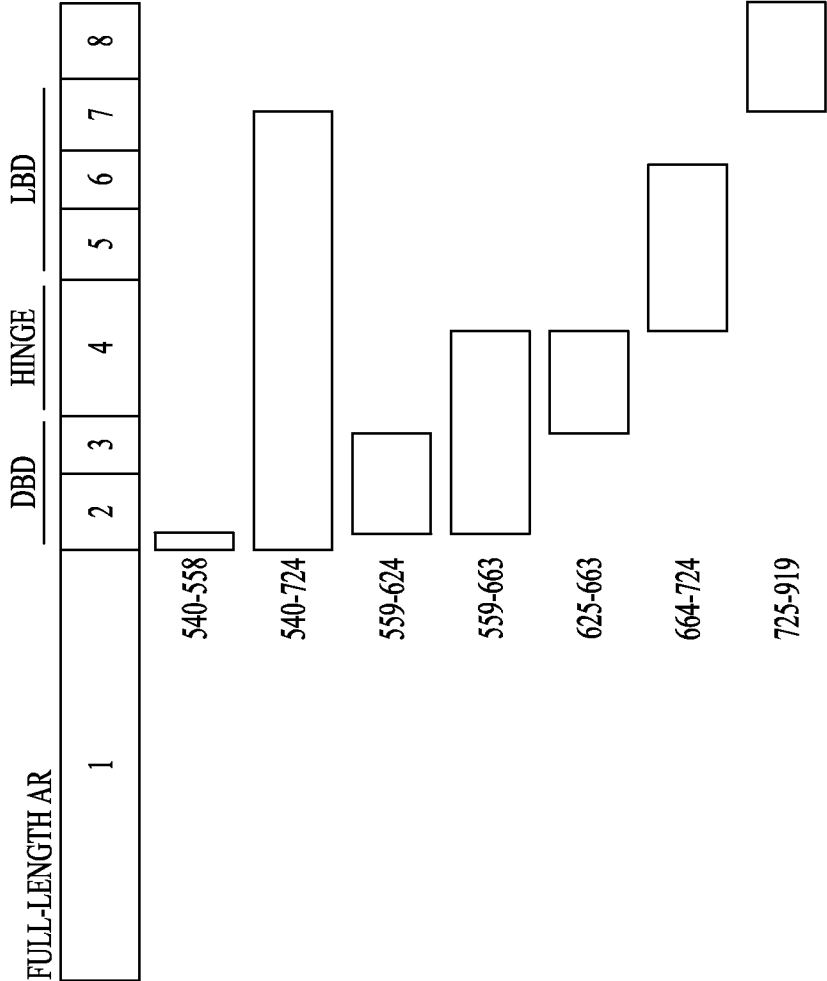
FIG. 6C
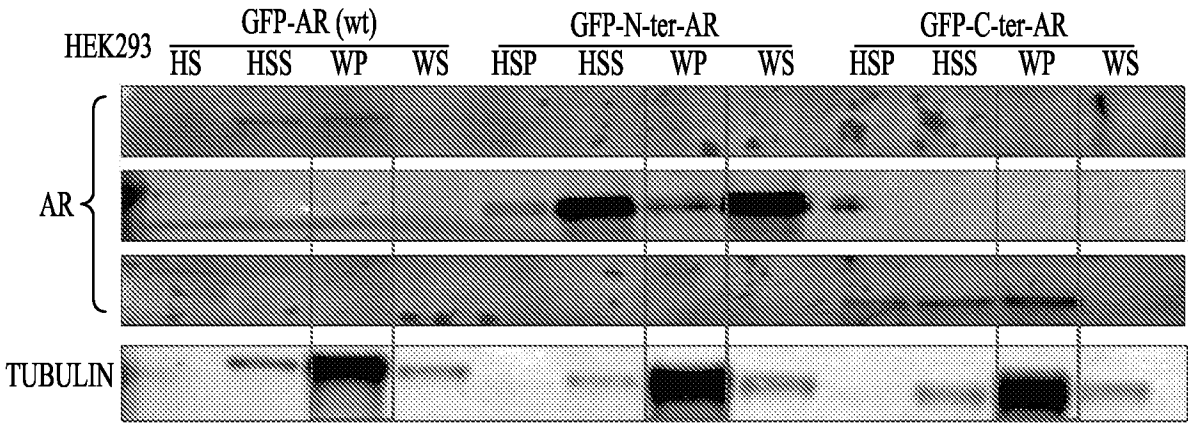
FIG. 6E
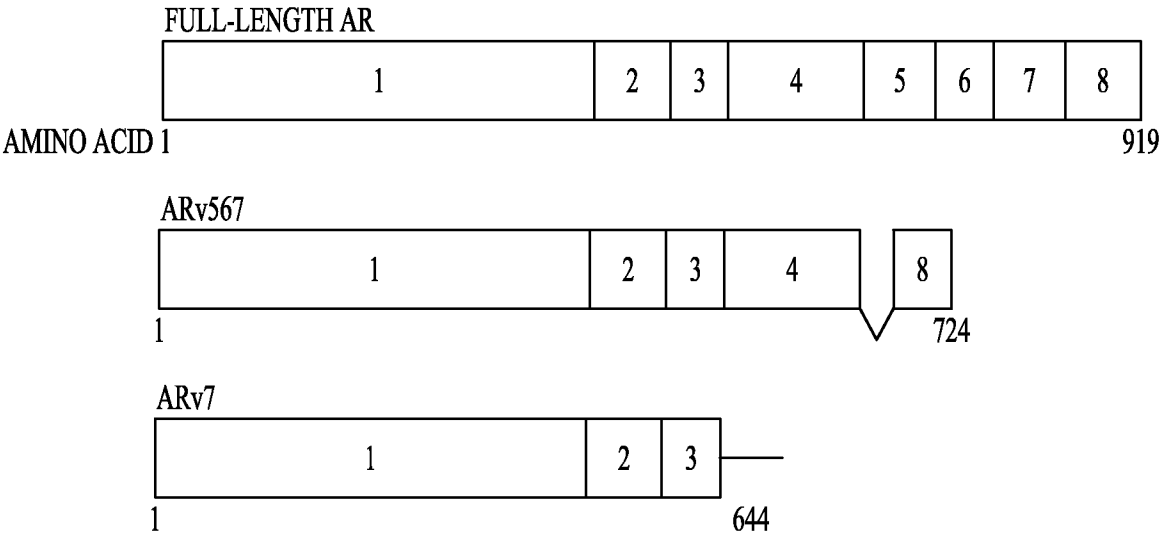
FIG. 7A
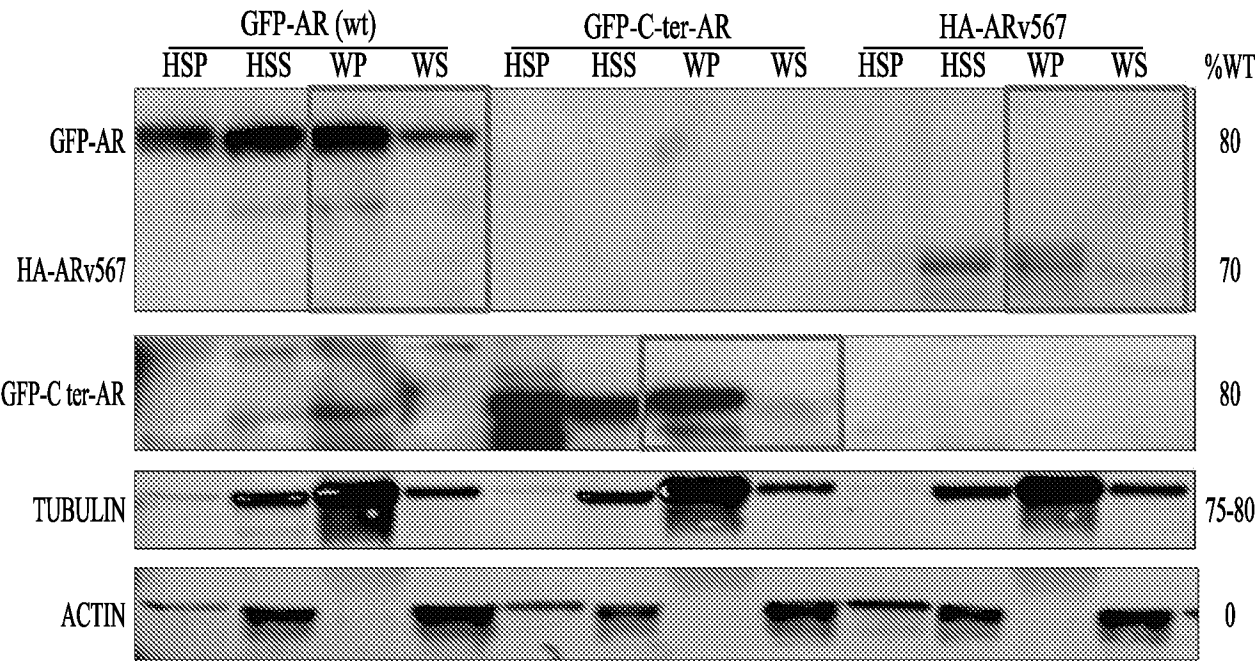
FIG. 7B
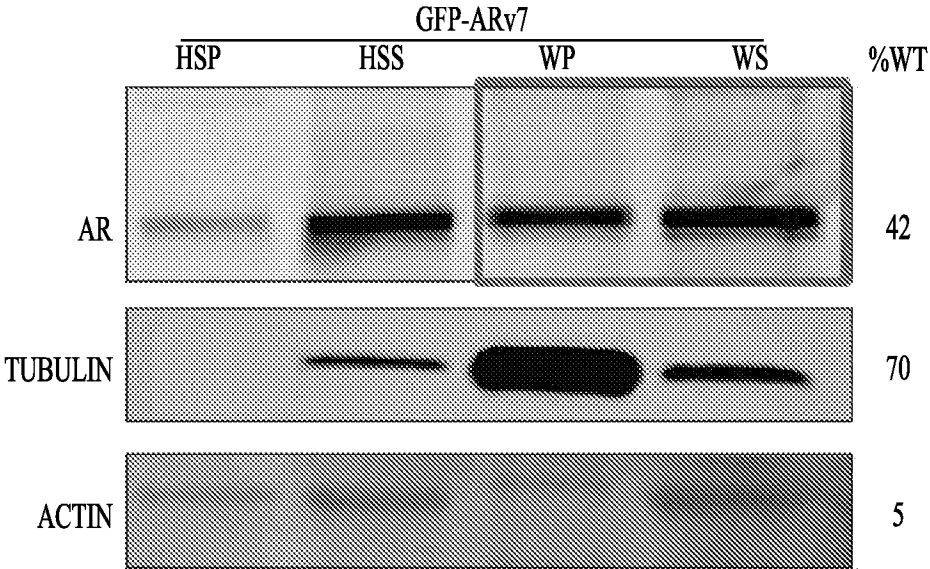
FIG. 7C
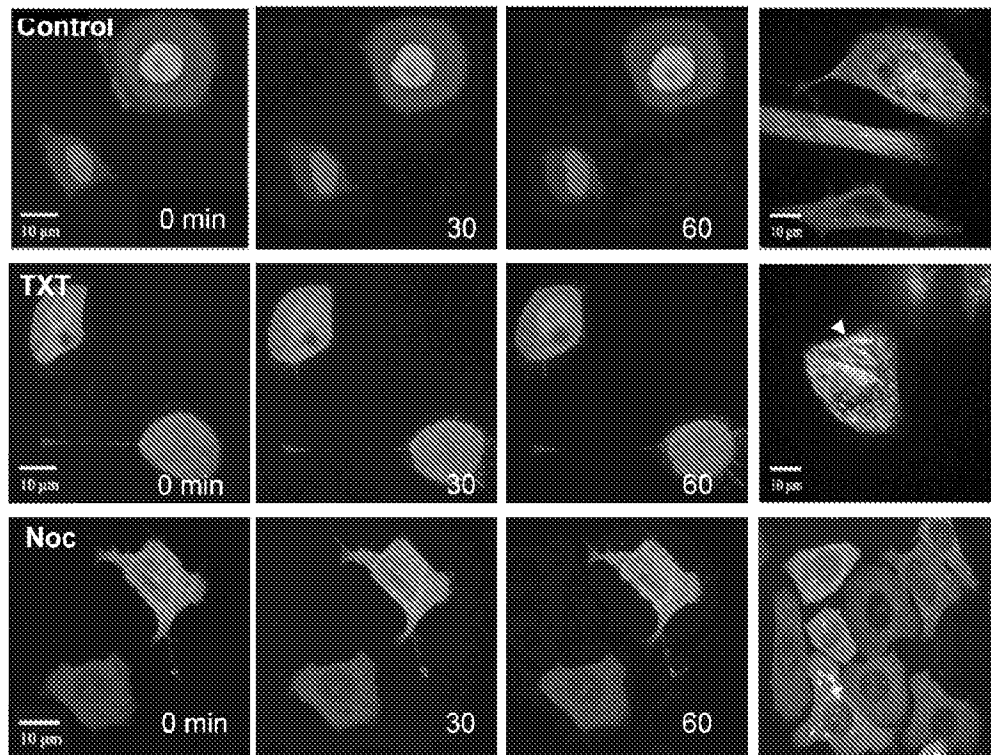
FIG. 8A
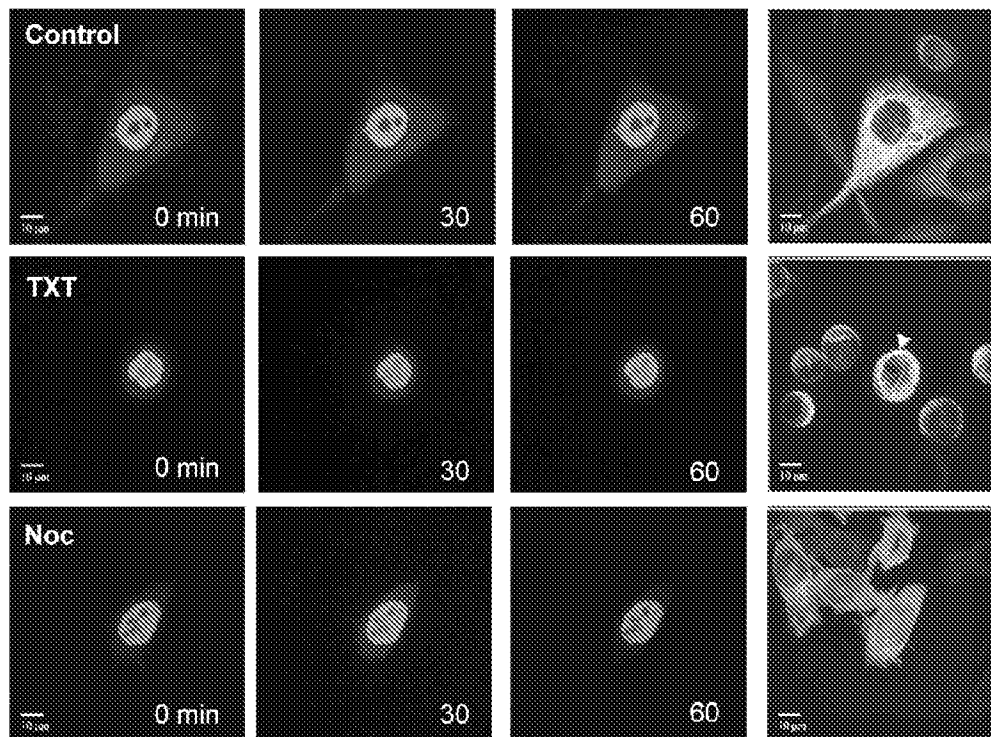
FIG. 8B
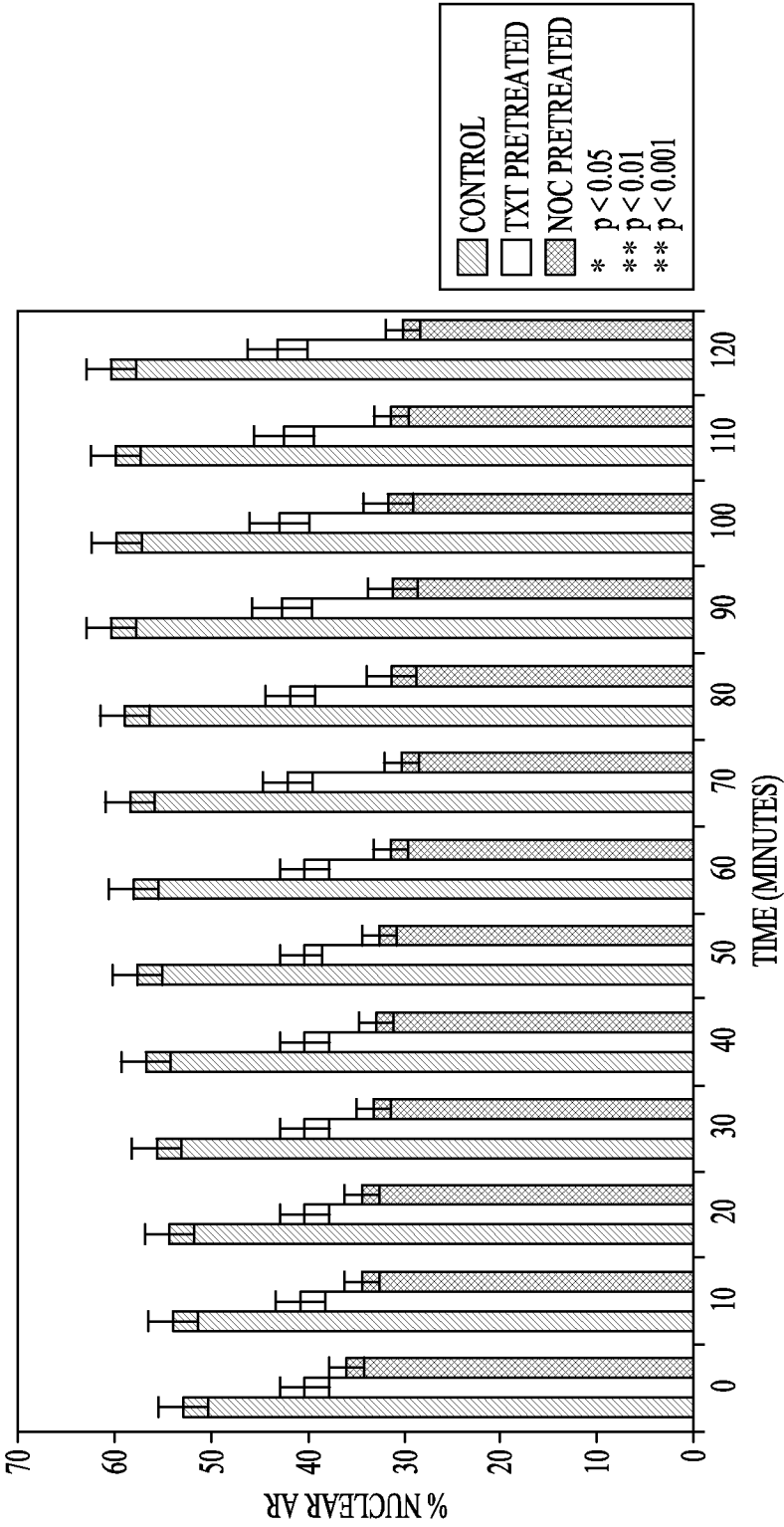
FIG. 8C
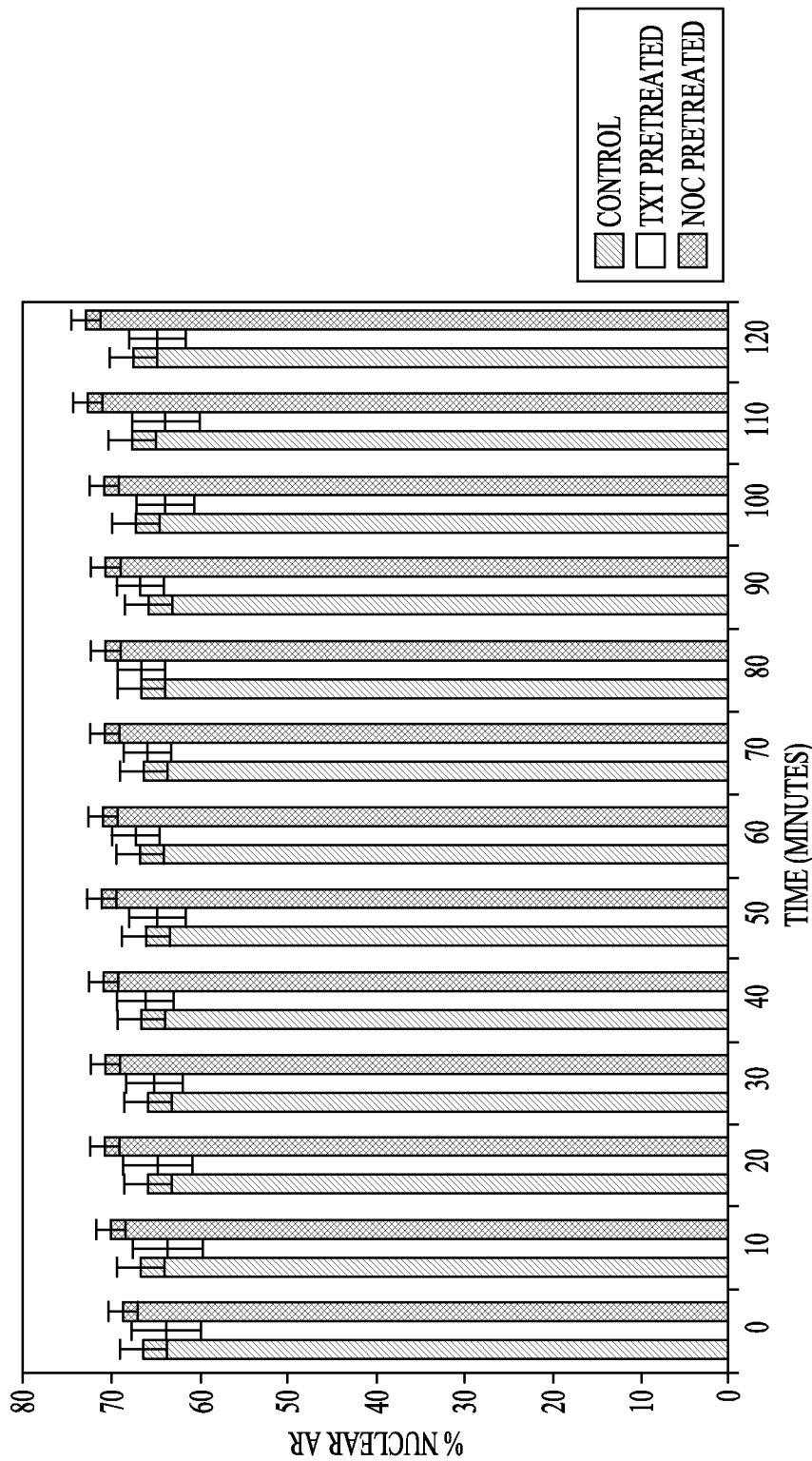
FIG. 8D
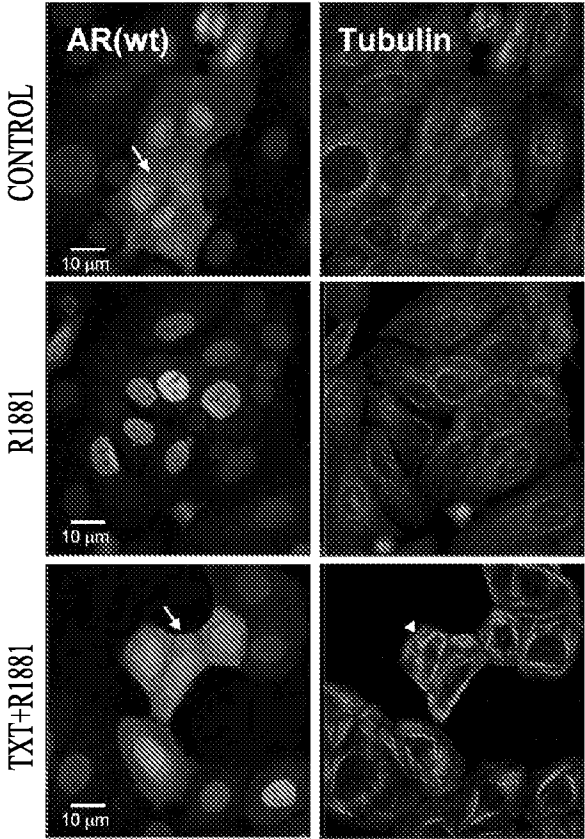
FIG. 9A
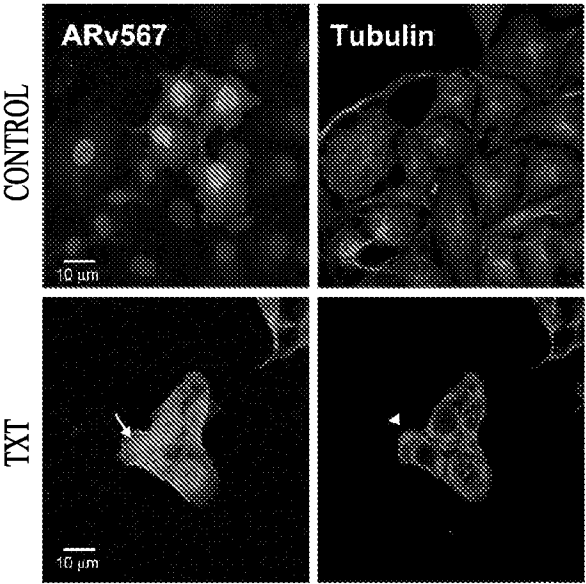
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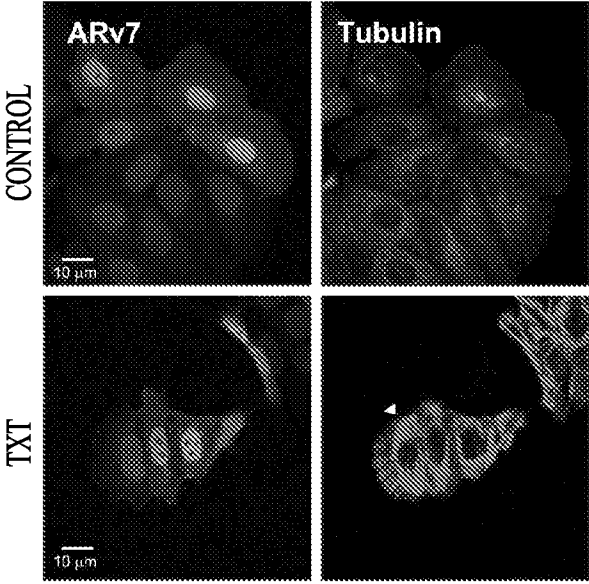
FIG. 9C

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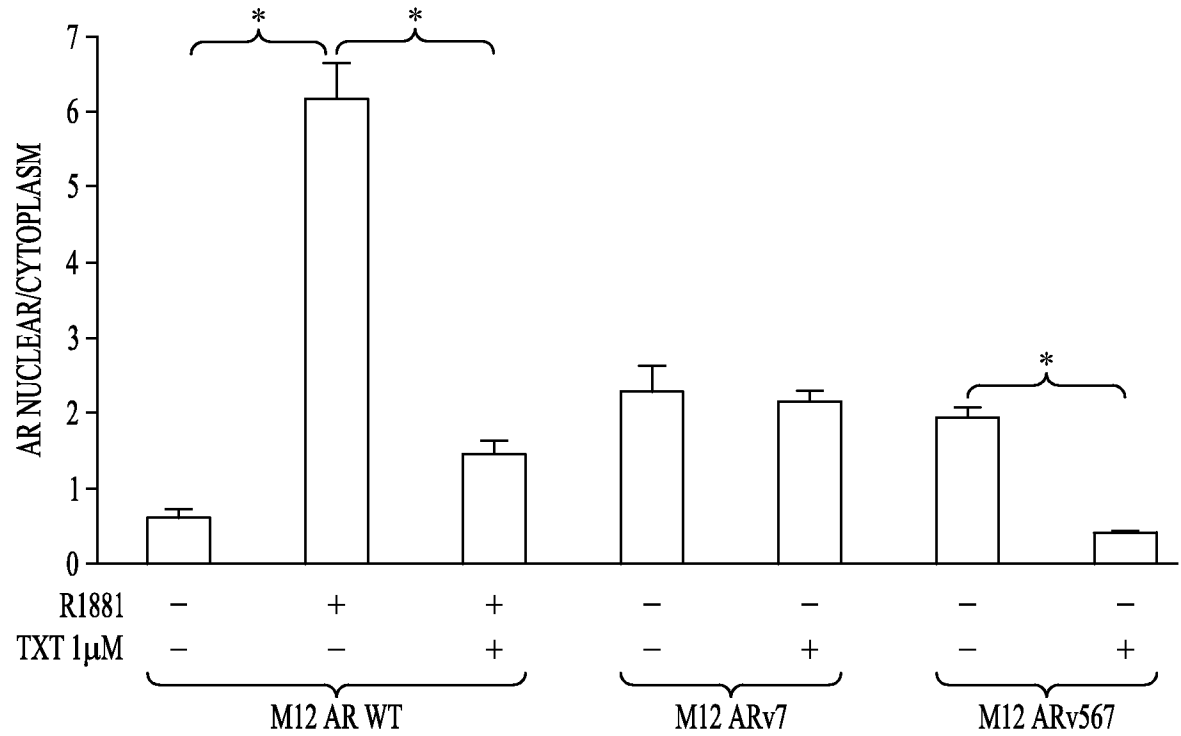
FIG. 9D
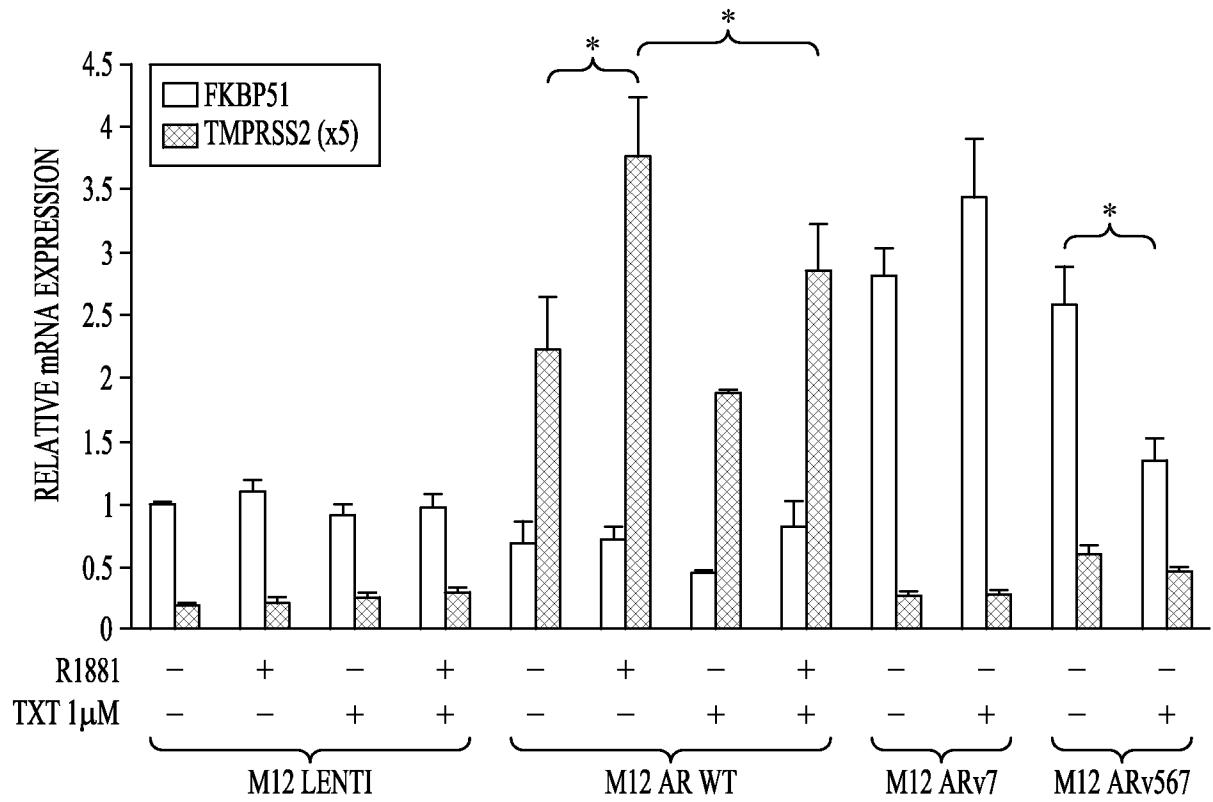
FIG. 9E
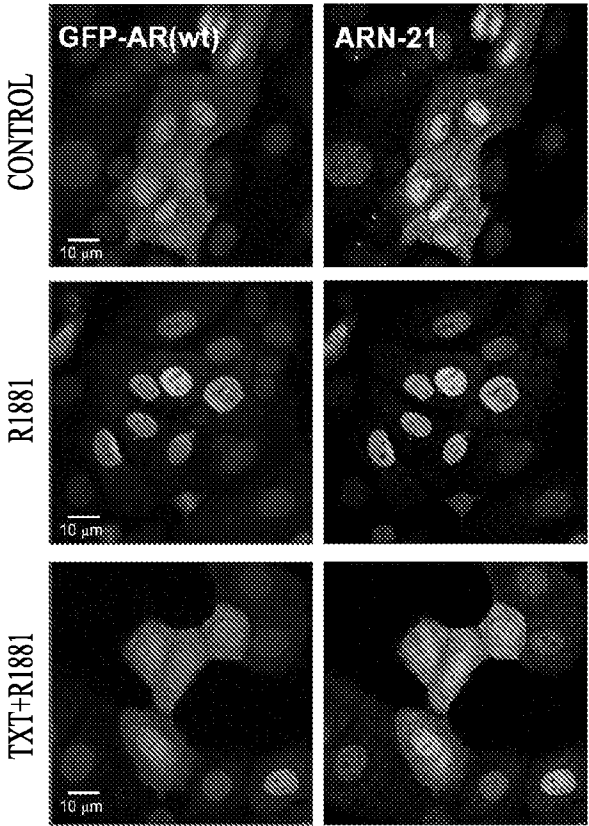
FIG. 9F
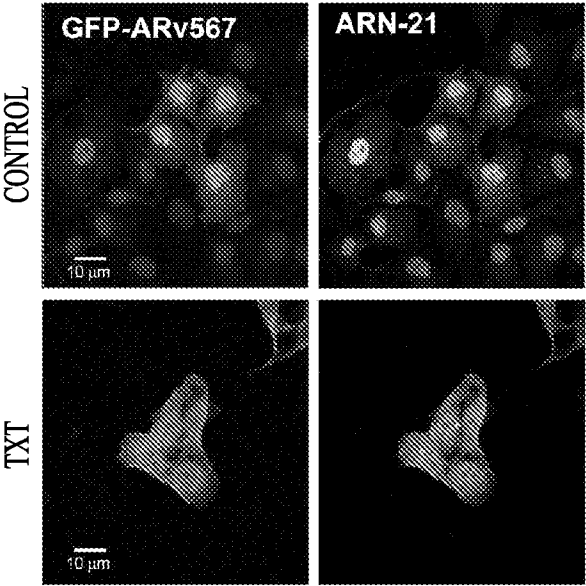
FIG. 9G
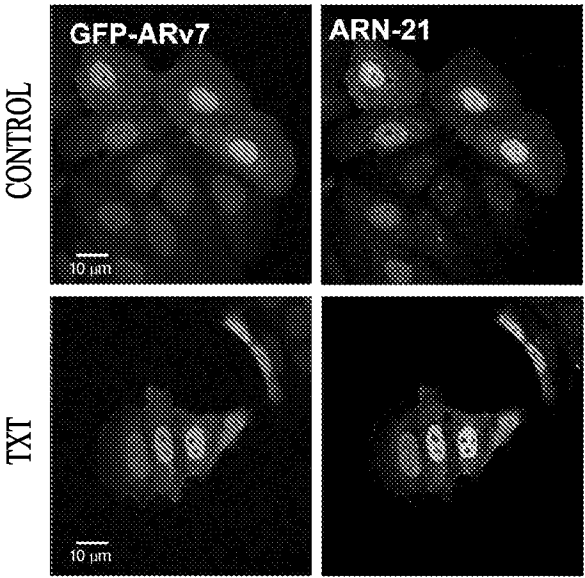
FIG. 9H
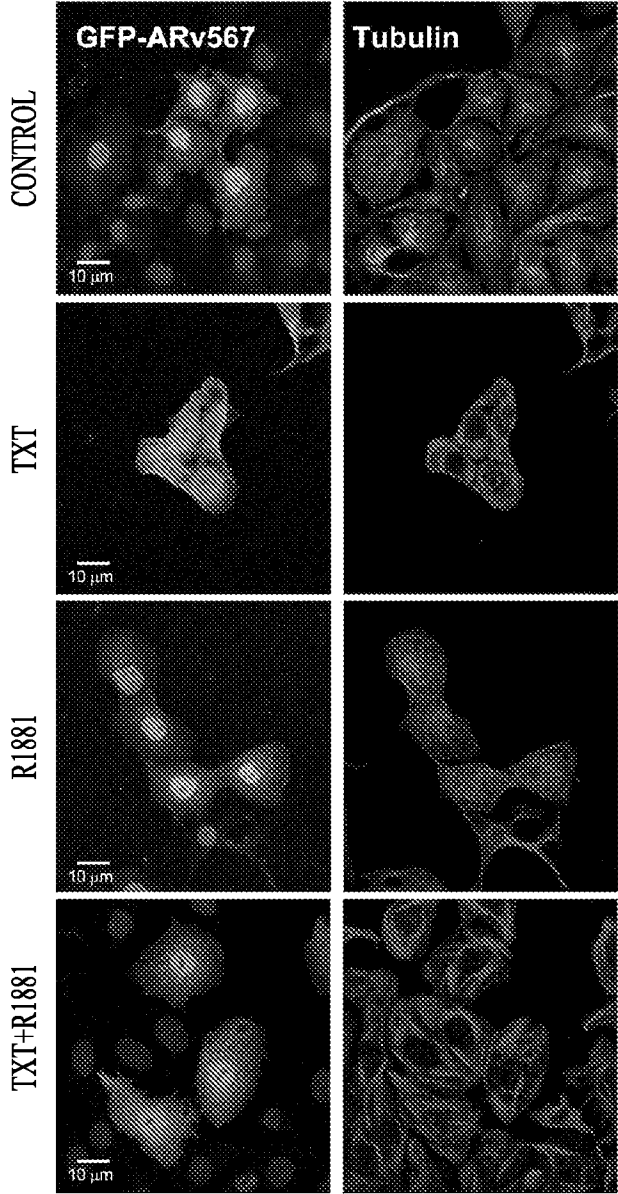
FIG. 9I
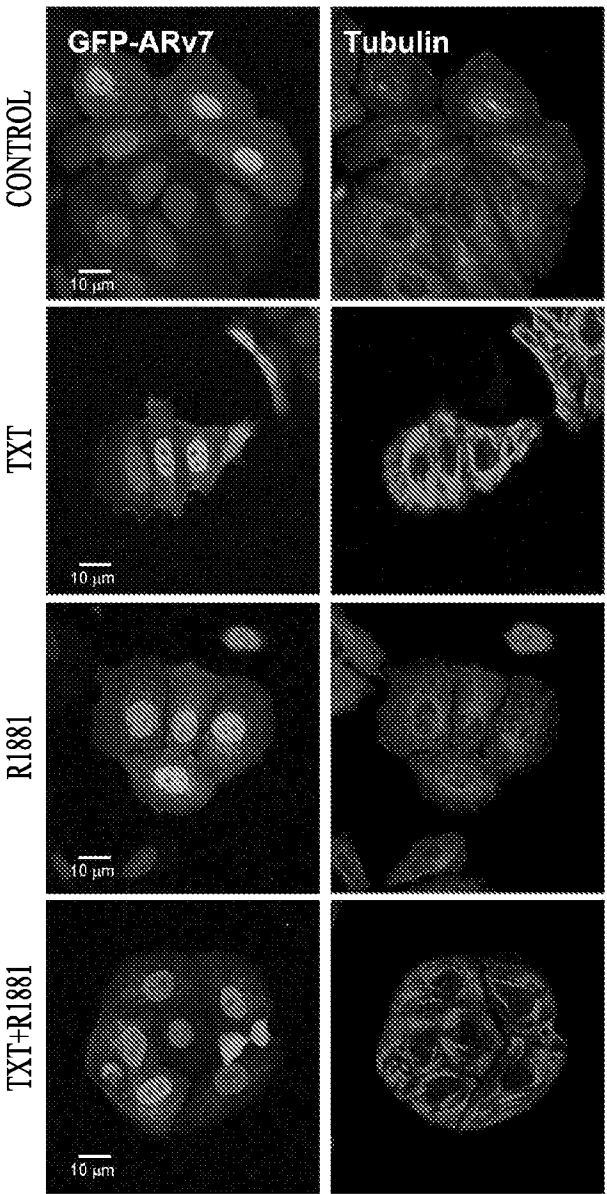
FIG. 9J
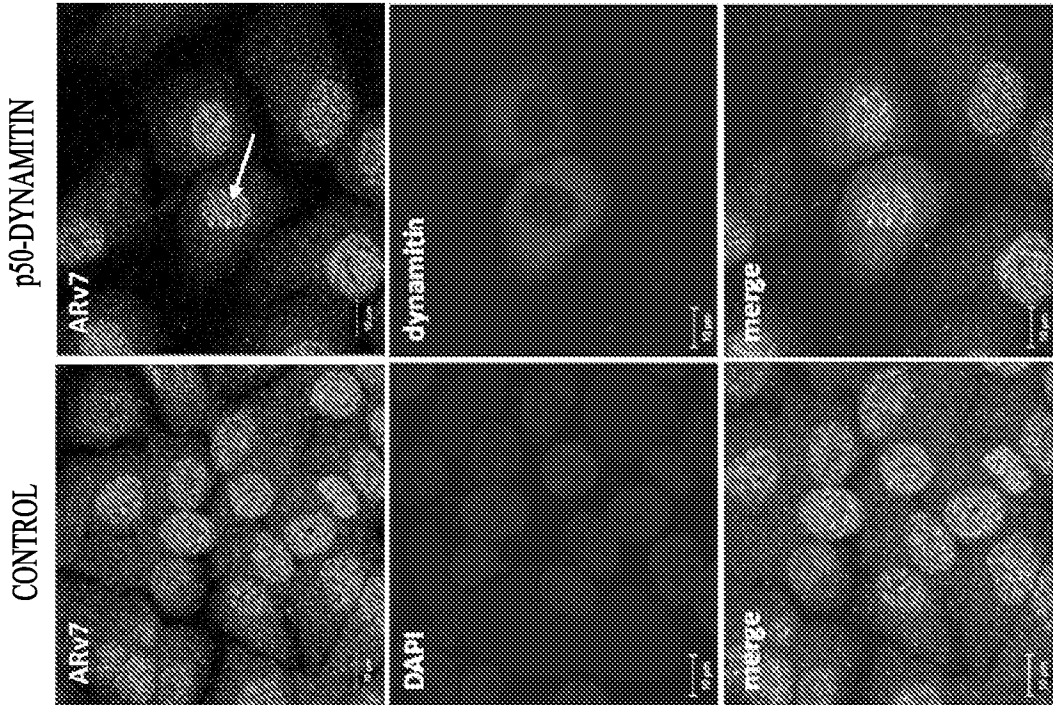
FIG. 10B
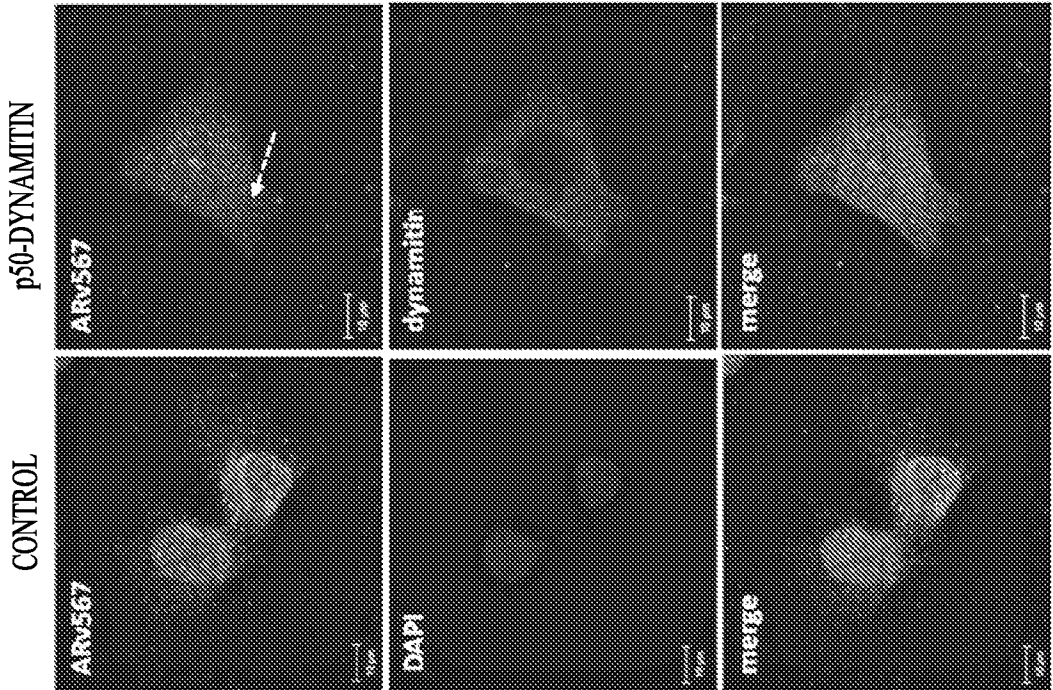
FIG. 10A
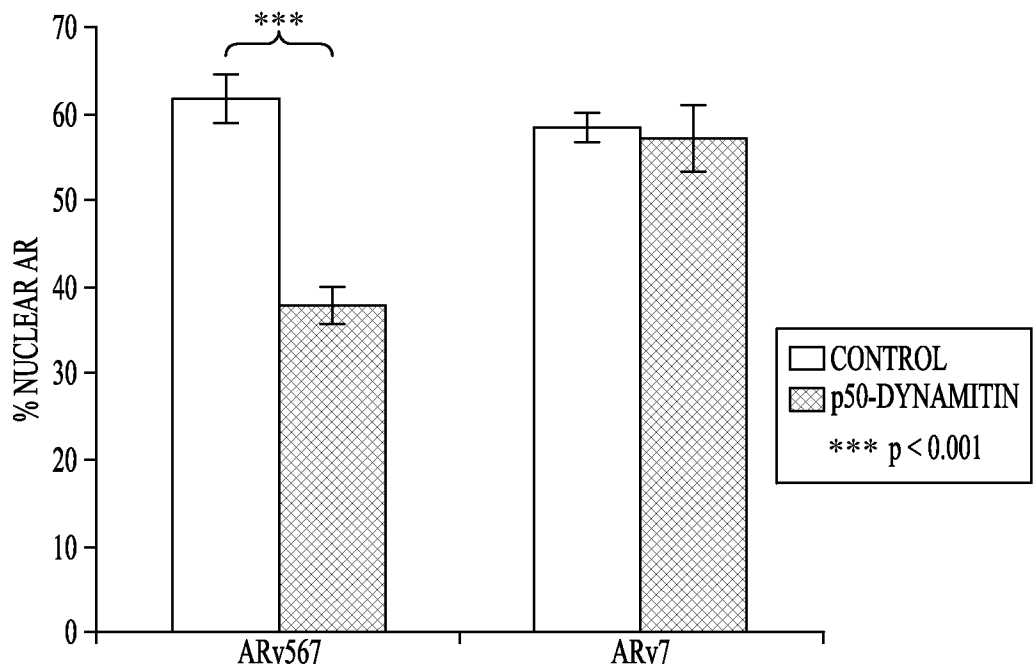
FIG. 10C
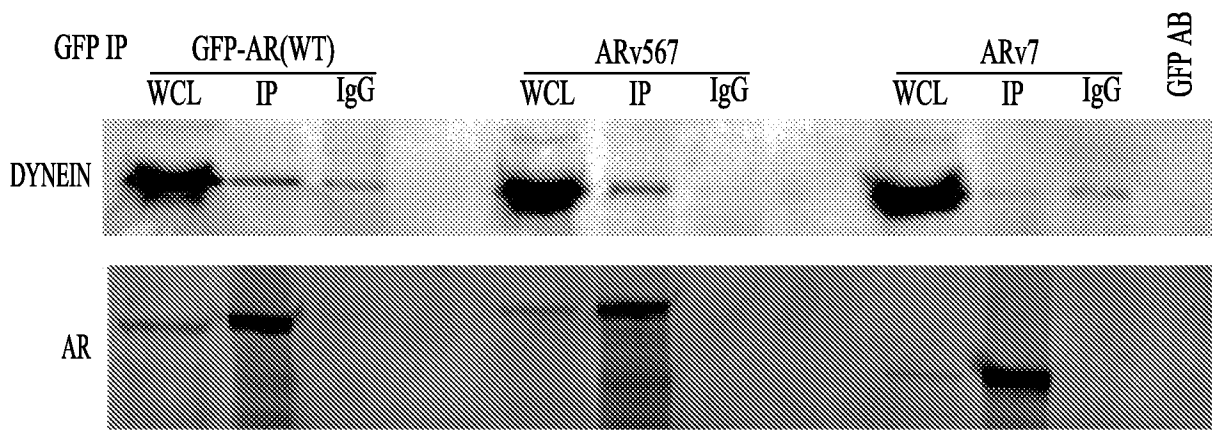
FIG. 10D
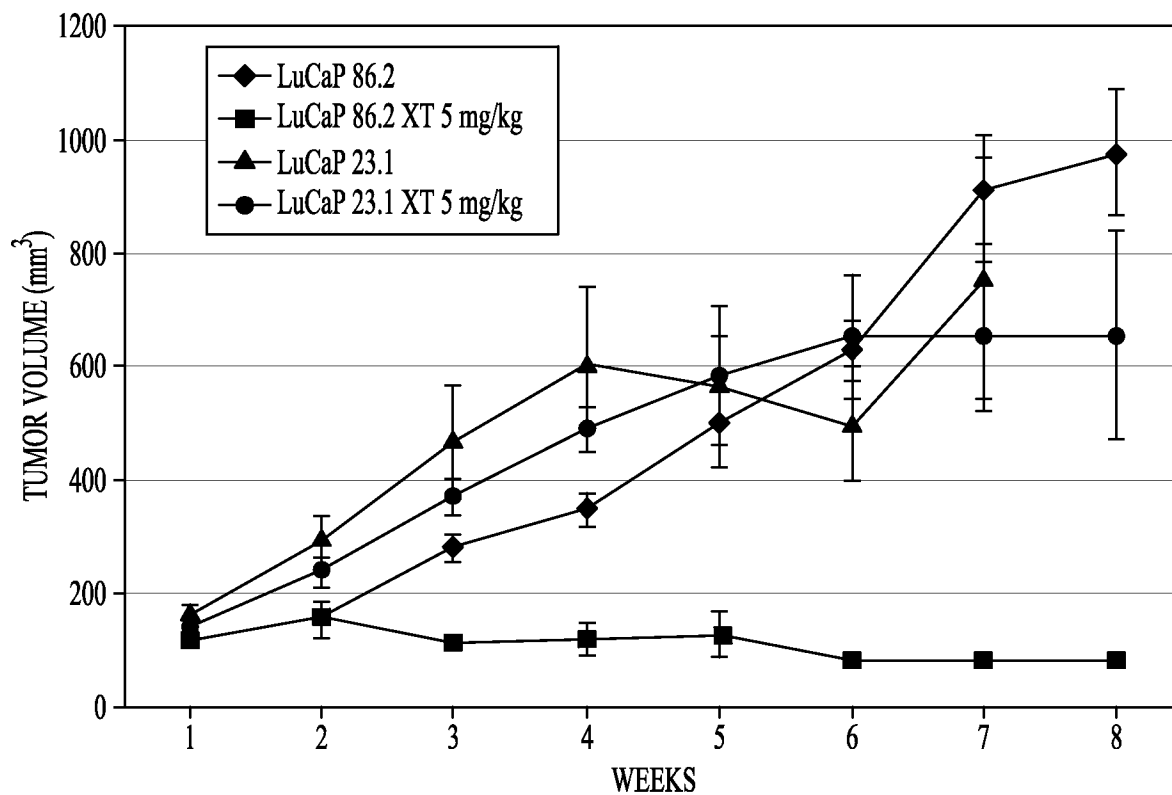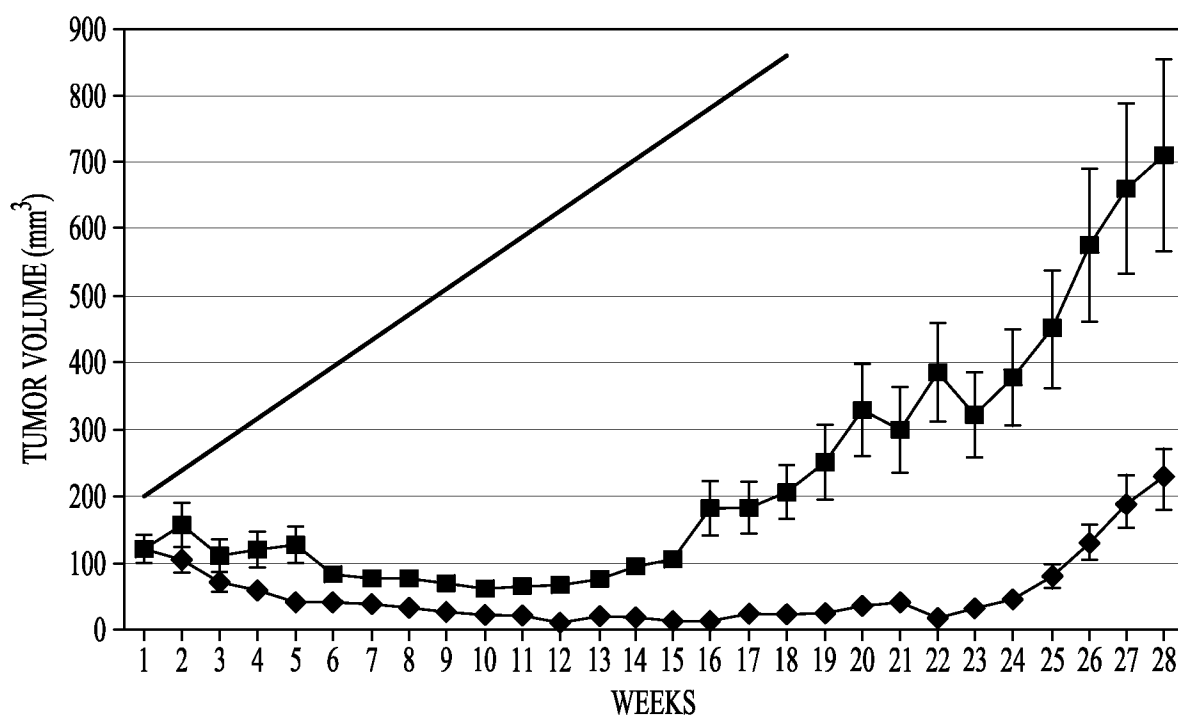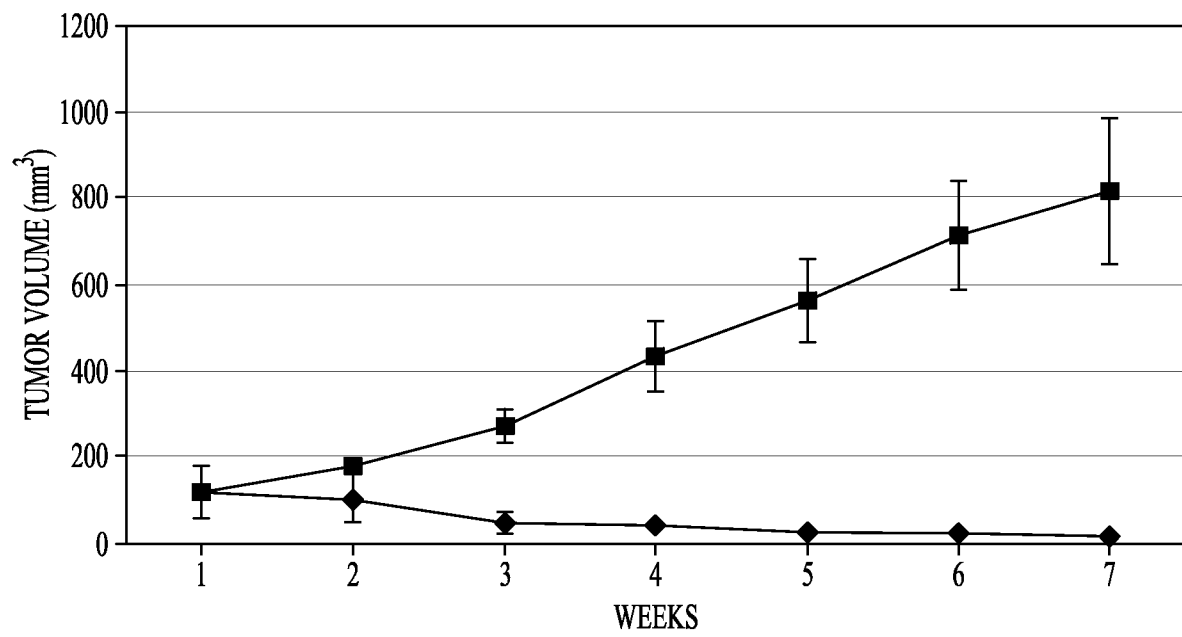
FIG. 11A

FIG. 11B

FIG. 11C

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/50   G01N33/574   A61K31/337   C07K14/72
ADD.

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)

G01N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/128936 A2 (UNIV JOHNS HOPKINS [US]; LUO JUN [US]; BOVA GEORGE STEVEN [US]; ISAACS) 22 October 2009 (2009-10-22) abstract; claims 1,2,21,22, 26,27 paragraph bridging page 65-66; page 66 last paragraph; page 33 (SEQ ID 1); page 75 sequence 1 ----- -/--	1,4-6, 8-19

[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

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"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

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 December 2013	11/12/2013

Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Mulder, Lonneke

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	JASON P. GLEGHORN ET AL: "Capture of circulating tumor cells from whole blood of prostate cancer patients using geometrically enhanced differential immunocapture (GEDI) and a prostate-specific antibody", LAB ON A CHIP, vol. 10, no. 1, 1 January 2010 (2010-01-01), page 27, XP055091201, ISSN: 1473-0197, DOI: 10.1039/b917959c abstract title -----	6,12-19
A	US 2010/068802 A1 (QIU YUN [US] ET AL) 18 March 2010 (2010-03-18) abstract; claims 1,2,4 -----	1-11
A	NAGALAKSHMI NADIMINTY ET AL: "Mechanisms of persistent activation of the androgen receptor in CRPC: recent advances and future perspectives", WORLD JOURNAL OF UROLOGY, SPRINGER, BERLIN, DE, vol. 30, no. 3, 19 October 2011 (2011-10-19), pages 287-295, XP035062123, ISSN: 1433-8726, DOI: 10.1007/S00345-011-0771-3 abstract; table 1 -----	1-11
A	S. M. DEHM ET AL: "Alternatively spliced androgen receptor variants", ENDOCRINE RELATED CANCER, vol. 18, no. 5, 21 July 2011 (2011-07-21), pages R183-R196, XP055091089, ISSN: 1351-0088, DOI: 10.1530/ERC-11-0141 abstract; figure 4 -----	1-11
A	WO 2005/020794 A2 (UNIV TEXAS [US]; SYSMEX CORP [JP]; UENO NAOTO T [US]; ISHIHARA HIDEKI) 10 March 2005 (2005-03-10) abstract; claims 1,21,22,23 -----	1-11

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	Gang Liu ET AL: "AR Variant AR v567es Induces Carcinogenesis in a Novel Transgenic Mouse Model of Prostate Cancer 1,2", Neoplasia, 1 January 2013 (2013-01-01), pages 1009-1017, XP055091100, DOI: 10.1593/neo.13784 Retrieved from the Internet: URL:http://www.neoplasia.com/pdf/manuscript/v15i09/neo13784.pdf [retrieved on 2013-12-02] abstract -----	1-11

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Information on patent family members

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