



(51) International Patent Classification:

C12N 15/113 (2010.01) G01N 33/574 (2006.01)
C12Q 1/68 (2006.01) G06F 19/10 (2011.01)
G01N 33/50 (2006.01) G06F 19/18 (2011.01)

(21) International Application Number:

PCT/US2017/038803

(22) International Filing Date:

22 June 2017 (22.06.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/353,331 22 June 2016 (22.06.2016) US
62/362,964 15 July 2016 (15.07.2016) US

(71) Applicant: **THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK** [US/US]; 412 Low Memorial Library, 535 West 116th Street, New York, NY 10027 (US).

(72) Inventors: **ABATE-SHEN, Corinne**; 1130 St. Nicholas Avenue, New York, NY 10031 (US). **SHEN, Michael M.**; 1130 St. Nicholas Avenue, New York, NY 10031 (US).

(74) Agent: **DAVITZ, Michael, A.** et al.; Leason Ellis LLP, One Barker Avenue, Fifth Floor, White Plains, NY 10601 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: TRANSDIFFERENTIATION AS A MECHANISM OF TREATMENT RESISTANCE FOR CASTRATION-RESISTANT PROSTATE CANCER

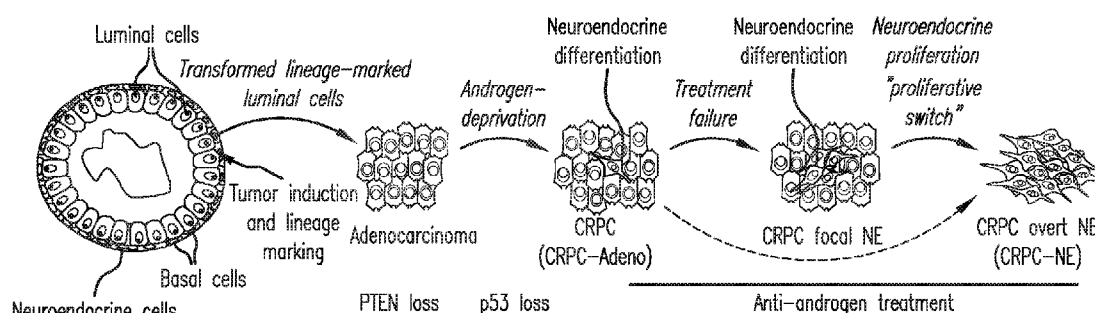


FIG.6

(57) Abstract: Provided herein are methods and markers for determining the development of castration resistant prostate cancer and related treatments. In certain embodiments, the present invention relates to a method of detecting castration-resistant prostate cancer (CRPC) and/or super resistant prostate tumors in a subject diagnosed with prostate cancer, comprising assaying gene expression levels of one or more genes, or corresponding protein levels, in a sample from the subject to obtain a test expression profile, comparing the test expression profile of the genes or proteins with a reference expression profile for the genes or proteins, wherein differential expression or protein levels is indicative of CRPC and/or the development of super-resistant prostate tumors. The present invention relates a kit comprising one or more probes for detecting expression levels of one or more genes.



Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

TRANSDIFFERENTIATION AS A MECHANISM OF TREATMENT RESISTANCE FOR CASTRATION-RESISTANT PROSTATE CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority to provisional application U.S. Patent
5 Application No. 62/353,331, filed June 22, 2016, and provisional application U.S.
Application No. 62/362,964 filed July 15, 2016, each of which is incorporated by reference
in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been filed
10 electronically in ASCII format and is hereby incorporated by reference in its entirety. Said
ASCII copy, created on June 15, 2017, is named 01001-005247-WO0_SL.txt and is 4,015
bytes in size.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under CA196662, DK076602 and
15 CA173481 awarded by the National Institutes of Health. The government has certain rights in
the invention.

FIELD OF THE INVENTION

Provided herein are markers for determining the development of castration resistant
prostate cancer and related treatments and methods.

BACKGROUND

For many decades, androgen-deprivation therapy (ADT) has been a standard
treatment for prostate adenocarcinoma, due to the requirement of androgen receptor (AR)
signaling at all stages of prostate cancer progression. ADT initially results in tumor
regression, but often results in relapse with a more aggressive disease called castration-
25 resistant prostate cancer (CRPC), which remains dependent on AR signaling despite
depletion of androgens (1). Treatments for CRPC have focused on inhibition of AR signaling,
using anti-androgen agents such as enzalutamide or abiraterone (2). While treatment with
these agents improve survival, these gains are often transient as most patients ultimately fail
treatment. Notably, in a significant proportion of cases, treatment failure is associated with
30 the emergence of a highly aggressive variant (CRPC-NE) with neuroendocrine differentiation

and histopathological features of small cell carcinomas, often mixed together with adenocarcinoma (3, 4).

Thus, there is an urgent need for elucidation of the mechanisms producing aggressive variants, related diagnostic predictors of such variants, and for improved treatments for prostate cancer conditions.

SUMMARY OF THE INVENTION

In certain embodiments, the present invention relates to a method of detecting castration-resistant prostate cancer (CRPC) and/or super resistant prostate tumors in a subject diagnosed with prostate cancer, comprising:

- 10 a. assaying gene expression levels of one or more genes listed in the gene signatures of Table S4 or Table S8, or corresponding protein levels, in a sample from the subject to obtain a test expression profile,
- b. comparing the test expression profile of the genes or proteins with a reference expression profile for the genes or proteins, wherein the reference expression profile
15 comprises the expression level of the same genes or proteins in a control;
- c. detecting gene expression levels of one or more genes listed in Table S4 or Table S8 or corresponding protein levels, in the test expression profile compared to the gene expression levels of one or more genes in the reference expression profile, or corresponding protein levels, wherein differential expression or protein levels is
20 indicative of CRPC and/or the development of super-resistant prostate tumors as a prognosis and wherein anti-androgen treatment is counter-indicated under these conditions.

In certain embodiments, the control is selected from the group consisting of a healthy control, an untreated subject, and a composite reference value for human prostate tumors.

- 25 In additional embodiments, when CRPC and/or the development of super-resistant prostate tumors is prognosed, the method further comprises treating the subject with one or more alternatives to anti-androgen treatment, comprising administering to the subject a therapeutically effective amount of one or any combination of treatments selected from the group consisting of an autologous active cellular immunotherapy (e.g. Sipuleucel-T), a
30 poxvirus based vaccine, a T-cell checkpoint inhibiting monoclonal antibody (anti-CTL4 or anti-PD-1 or anti-PDL1), and cabazitaxel, and optionally further comprising monitoring the subject at least monthly.

In additional embodiments, the method further comprises administering to the subject one or more of the following treatments: chemotherapy, radiation therapy, surgery, or any combination thereof, based on the prognosis.

In additional embodiments, the method further comprises treating the subject with one or more alternatives to anti-androgen treatment when CRPC and/or the development of super-resistant prostate tumors is prognosed, wherein the method further comprises administering to the subject an effective amount of one or any combination of the following: androgen synthesis inhibitors (TAK700), androgen receptor (AR) inhibitors (ARN-509, ODM-201, and EZN-4176), AR DNA binding domain inhibitors (EPI-001), selective AR downregulators or SARDs (AZD-3514), including agents that inhibit both androgen synthesis and receptor binding (TOK-001/galeterone).

In certain embodiments, the present invention relates to a method of monitoring the treatment of a subject diagnosed with prostate cancer, comprising:

- a. assaying gene expression of one or more genes from Table S4 or Table S8 or corresponding protein levels, in a sample from the subject to obtain a test expression profile,
- b. comparing the test expression profile of the genes or corresponding proteins with a reference expression profile, wherein the reference expression profile comprises the expression level of the same genes or corresponding proteins obtained from the subject prior to treatment;
- c. detecting gene expression levels of one or more genes from Table S4 or Table S8, or corresponding proteins in the test expression profile that are differential compared to the gene expression of the same one or more genes in the reference expression profile or corresponding proteins, wherein the differential expression or protein levels is indicative of CRPC and/or the development of super-resistant prostate tumors and wherein anti-androgen treatment is counter-indicated under these conditions.

In additional embodiments, the method further comprises treating the subject with one or more alternatives to anti-androgen treatment when CRPC and/or the development of super-resistant prostate tumors is prognosed, comprising administering to the subject a therapeutically effective amount of one or any combination of treatments selected from the group consisting of an autologous active cellular immunotherapy (e.g. Sipuleucel-T), a poxvirus based vaccine, a T-cell checkpoint inhibiting monoclonal antibody (anti-CTL4 or

anti-PD-1 or anti-PDL1), and cabazitaxel, and further comprising monitoring the subject at least monthly.

In additional embodiments, the method further comprises detecting one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and 1-DOPA Decarboxylase (DDC), or any combination thereof, wherein the presence any one or more neuroendocrine markers indicates a likelihood of CRPC and/or the development of super-resistant prostate tumors.

In additional embodiments, assaying the gene expression levels is performed using a method comprising at least one of: PCR, RNA sequencing, and microarray.

In additional embodiments, assaying the protein levels is performed using a method comprising at least one of the following methods: quantitative Western blot, immunoblot, quantitative mass spectrometry, enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIA), immunoradiometric assays (IRMA), and immunoenzymatic assays (IEMA) and sandwich assays using monoclonal and polyclonal antibodies.

In certain embodiments, a combination of gene expression and protein expression is performed to arrive at a differential profile, wherein the differential profile indicates CRPC and/or the development of super-resistant prostate tumors, and/or castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE) or CRPC-NE likelihood.

In certain embodiments, the present invention relates to a method for detecting castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE) or CRPC-NE likelihood in a patient sample comprising obtaining a sample comprising prostate cancer tumor tissue from the patient and detecting one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and 1-DOPA Decarboxylase (DDC), or any combination thereof, in the sample and diagnosing the patient with CRPC-NE or prognosing the patient as having an increased likelihood of prostate cancer progression (CRPC-NE likelihood) when one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and 1-DOPA Decarboxylase (DDC), or any combination thereof is detected in the sample.

In additional embodiments, anti-androgen treatment is counter-indicated when the patient is diagnosed with CRPC-NE or CRPC-NE likelihood.

In additional embodiments, the method further comprises administering to the subject one or more of chemotherapy, radiation therapy or surgery based on the prognosis.

In additional embodiments, the method further comprises administering to the subject one or more of chemotherapy alternatives to anti-androgen treatment, radiation, surgery, or any combination thereof, based on the prognosis.

In additional embodiments, the sample is selected from the group consisting of blood, 5 bronchial lavage fluid, sputum, saliva, urine, amniotic fluid, lymph fluid, tissue or fine needle biopsy samples, peritoneal fluid, and cerebrospinal fluid.

In additional embodiments, the sample also exhibits a decrease in expression or loss of *RBI*, *TP53*, *PTEN*, or a combination thereof; and optionally further exhibits an increase in expression of *SOX11*, or any combination thereof.

10 In certain embodiments, the present invention relates to a method of screening or identifying a test agent for the prevention and/or treatment of prostate cancer comprising:

- a. contacting or incubating a test agent with a cell comprising a gene listed in Table S4 or Table S8, or corresponding proteins;
- b. detecting differential expression of the gene or corresponding protein in the 15 host cell;
- c. contacting the host cell with the test agent or compound; and
- d. detecting restored differential expression of the gene or corresponding protein in the host cell after contact with the test agent or compound, wherein restoration to a reference or normal level of the differential expression of the gene or corresponding protein 20 in the host cell after contact with the test agent would identify the test agent as a therapeutic or preventative for prostate cancer.

In additional embodiments, the test agent is therapeutic or preventative for castration-resistant prostate cancer (CRPC) and/or super resistant prostate tumors.

In certain embodiments, the present invention relates a kit comprising one or more 25 probes for detecting expression levels of one or more genes listed in the gene signatures of Table S4 or Table S8 or corresponding proteins, and optionally, any one or more of *TP53*, *PTEN*, *SOX11* and/or *RBI*, and further optionally, wherein any one or more probes are detectably labeled.

In certain embodiments, the kit further comprises at least one sealed container which 30 contains the one or more probes, and optionally, the kit further comprising printed material providing instructions.

In certain embodiments, the kit further comprises any one or combination of antibodies for detecting one or more neuroendocrine markers including Synaptophysin (Syn),

chromogranin A (CgA), Neuron-specific enolase (NSE), and l-DOPA Decarboxylase (DDC), and any combination thereof.

In certain embodiments, the present invention relates a kit comprising one or more antibodies for detecting one or more neuroendocrine markers including Synaptophysin (Syn),
 5 chromogranin A (CgA), neuron-specific enolase (NSE), and l-DOPA Decarboxylase (DDC), and any combination thereof, and optionally, any one or more of *TP53*, *PTEN*, *SOX11* and/or *RBI*, and further optionally, wherein any one or more of the antibodies or probes are detectably labeled.

In certain embodiments, the kit further comprises at least one sealed container which
 10 contains the one or more probes or antibodies, and optionally, the kit further comprising printed material providing instructions.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-F are images, graphs and plots showing that a refined GEM model of
 15 **CRPC is conserved with human CRPC.** Figs. 1A-B show details and percentages of *PTEN* and *TP53* gene alterations in human prostate cancer. Fig. 1A is an oncoprint depicting alterations of *PTEN* and *TP53* in metastatic castration-resistant prostate cancer (CRPC) as reported by the SU2C consortium. Fig. 1B is a graph showing the prevalence of alterations in primary prostate tumors (from TCGA (16)) and metastatic CRPC (from SU2C (17)). Fig. 1C
 20 are histopathological analyses to determine phenotypic characterization of GEM models. Histopathological analyses of androgen-intact prostate tumors or CRPC from *NP* and *NPp53* mice, as indicated. Shown are representative H&E images and representative immunostaining for the indicated antibodies. Scale bars represent 50 microns. Figs. 1D-F are graphs showing cross-species gene set enrichment analyses (GSEA). Fig. 1D is a comparison of reference
 25 mouse master regulator (MR) signatures from *NP* CRPC (left) or *NPp53* CRPC (right) versus control mouse prostate (*N*) with a query human MR signature compares treatment-naïve androgen-independent versus androgen-dependent primary prostate tumors (PCa) (n=10/group) from Best *et al* (28). Fig. 1E is a comparison of reference mouse MR signature from *NPp53* CRPC versus *NP* CRPC with a query human MR signature comparing
 30 metastatic CRPC (n=35) versus primary tumors (PCa) (n=59) from Grasso *et al* (15). Fig. 1F is a comparison of reference mouse MR signature from *NPp53* CRPC versus *NP* CRPC with a query human MR signature comparing metastatic CRPC having low *PTEN*/low *TP53* versus low *PTEN*/high *TP53* (n=5/group from SU2C). In Figs. 1D-F, “NES” stands for

normalized enrichment score; GSEA p-values were calculated using 1000 gene permutations. *See also* additional related analyses in Figure 7, Table S1 for description of human datasets, and Dataset 1 for complete list of differentially expressed genes in the mouse tumors, which can be accessed through the Gene Expression Omnibus (GEO) database (GSE92721).

5 **Figures 2A-G are images, graphs, and plots of preclinical analyses of abiraterone showing acceleration of the tumor phenotype in *NPp53* CRPC.** Fig. 2A is a schematic showing preclinical trial design for the GEM model. Tumors were induced in cohorts of *NP* or *NPp53* mice by delivery of tamoxifen, and mice were subsequently castrated to induce CRPC. Mice were randomly assigned to the treatment or vehicle groups, and treated with
10 abiraterone-acetate (200 mg/kg) or vehicle 5 times weekly for 1 month. MRI was performed immediately prior to the first treatment and immediately after the last treatment. At the conclusion of the study, mice were sacrificed and tissues were collected for analysis, as indicated. Fig. 2B are stains showing the histological phenotype of *NP* and *NPp53* CRPC treated with vehicle or abiraterone. Shown are representative H&E images and
15 immunohistochemical staining for the indicated antibodies. Abiraterone-treated *NPp53* CRPC show representative examples of adenocarcinoma and alternative histopathologies, as in Table S3. Scale bars represent 50 microns. Fig. 2C are graphs showing the quantification of cellular proliferation by analysis of Ki67 immunostaining. Data represent the average from 5-8 independent images from 3 independent mice. p-values indicate the difference between
20 bracketed groups and were calculated by a t-test. Note that cellular proliferation for the abiraterone-treated *NPp53* CRPC was determined separately for regions of adenocarcinoma and alternative histopathologies. Fig. 2D-E are images and graphs reflecting MRI analysis. The abiraterone-treated *NPp53* CRPC show examples of Group 1 and Group 2 tumor, as described in the text. Fig. 2D are representative images from 2-D MRI performed
25 immediately prior to the first treatment (pre-treatment) and immediately after the last treatment (post-treatment). Tumor volumes are indicated. Fig. 2E are representative waterfall plots showing the change in tumor volume after treatment; each bar represents a single mouse. Fig. 2F is a plot of principal component analysis (PCA) based on comparison of RNA-sequencing gene expression profiles from *NPp53* CRPC treated with vehicle, or
30 abiraterone (Group 1 and Group 2). Fig. 2G is a heatmap of single-sample GSEA (ssGSEA). Single sample gene signatures were defined by comparing each individual abiraterone-treated *NPp53* CRPC sample to the average of expression levels in the pooled group of corresponding vehicle-treated samples. Cross-species GSEA was performed to compare the

single-sample signatures to a human gene signature of treatment-naïve androgen-independent versus androgen-dependent tumors of Best *et al.* The comparison is shown as a heatmap, wherein upper and lower boxes correspond to NESs from the GSEA between the single-sample reference signature and the top 200 over-expressed (top) and under-expressed (bottom) genes from the Best *et al.* signature (28). *See also Figures 8-13* for additional phenotypic and molecular analyses, Tables S2 for data summary, Table S3 for quantitative analysis of the histopathology phenotypes, and Dataset 1 for list of differentially expressed genes.

Figures 3A-D are graphs and plots showing cross-species computational analyses of adverse treatment response. Fig. 3A is a schematic diagram showing the computational strategy used to predict molecular drivers of adverse treatment response. Illustrated are three possible outcomes: In scenario 1, the “responder” group, drug treatment is predicted to reverse the direction of MR signatures (*i.e.*, activated MRs are repressed (as indicated) and vice versa). In scenario 2, the “non-responder” group, drug treatment is predicted to have minimal effect on MR signatures. In scenario 3, the “exceptional non-responder group, drug treatment is predicted to enhance the activation or repression of MR signatures (*i.e.*, activated red MRs (as indicated) are further activated (cross hatching) and repressed MRs (indicated) are further repressed (vertical hatching)). Figs. 3B-C are plots showing cross-species GSEA. Fig. 3B shows the reference signature, a human MR signature from Balk *et al.*(34) comparing CRPC bone metastasis (n=29) versus primary hormone-naïve prostate tumors (PCa) (n=22), was compared with three independent mouse MR query signatures from abiraterone- versus vehicle-treated “responders” (*NP* CRPC), “non-responders” (*NPp53* CRPC, Group 1), or “exceptional non-responders” (*NPp53* CRPC, Group 2). Fig. 3C is the reference signature, a human MR signature from Beltran *et al.* (5) comparing CRPC-NE (n=15) with CRPC-Adeno (n=34), was compared with a mouse MR query signature comparing abiraterone-treated “exceptional non-responders” (*NPp53* CRPC, Group 2) versus abiraterone-treated “responders” (*NP* CRPC). In Fig. 3B and Fig. 3C, “NES” and p-value were calculated using 1000 gene permutations. Fig. 3D is a Kaplan-Meier survival analysis estimated based on the activity levels of adverse treatment response MRs (as in panel Fig. 3C and Table S4) based on Sboner *et al.* (35) using prostate cancer-specific survival as the endpoint (n=281 patients). The p-value was estimated using a log-rank test to determine the difference in outcomes between patients with higher activity levels of adverse treatment response MR (thin, lower line) versus those with lower/no MR activity (darker, upper line). *See also Figures 12* for

additional analyses, Table S1 for description of human datasets, and Table S4 for summary of MRs identified in Panel Fig. 3C.

Figures 4A-E are graphs and plots showing that neuroendocrine differentiation in *TP53*-deficient CRPC is mediated in part by *SOX11*. Fig. 4A is a heat map depicting relative expression levels of genes associated with neuroendocrine differentiation in vehicle- or abiraterone-treated mouse CRPC (6). The top right label indicates results for abiraterone (e) which are “exceptional non-responders”. Fig. 4B are graphs showing relative expression level of *SOX11* in human prostate cancer showing primary tumors segregated by Gleason grade (from TCGA) or CRPC-Adeno versus CRPC-NE (5). Fig. 4C is GSEA showing the enrichment of *SOX11* target genes, predicted from analyses of a human prostate cancer interactome, in a human signature comparing CRPC-Adeno or CRPC-NE (5). NES and p-value were calculated using 1000 gene permutations. Fig. 4D is a graph of Quantitative real-time PCR results showing expression levels of *Sox11* and neuron-specific enolase (NSE) in two independent mouse prostate epithelial cell lines from *NP* or *NPp53* tumors (see Fig. 10). Fig. 4E are graphs showing expression levels of *Sox11*, *NSE* and *Synaptophysin* in mouse *NPp53* cell lines following knock-down using two independent shRNA for *Sox11* (shSox11#1 and shSox11#1). shNT is the non-targeting control vector. In Figs. 4B, D, and E, p-values were calculated using a t-test.

Figures 5A-C are graphs showing focal and overt neuroendocrine differentiation arise through transdifferentiation of luminal cells. Fig. 5A shows immunostaining results for Synaptophysin in intact or castrated *NP* and *NPp53* tumors treated with vehicle or abiraterone, as indicated. Shown are regions of focal neuroendocrine differentiation. Scale bars represent 50 microns. Fig. 5B are graphs showing quantification of the percentage of Synaptophysin positive (Syn+) cells in regions of focal and overt neuroendocrine differentiation. Data quantification are provided in Table S5. p-values were calculated by one-way ANOVA. Fig. 5C are images showing histological phenotypes of lineage-marked Synaptophysin+ cells in regions of focal and overt *NPp53* CRPC. Shown is representative immunostaining for the indicated antibodies, with arrows indicating key staining. Data quantification are provided in Table S5. Scale bars represent 50 microns.

Figure 6 is a schematic depicting the molecular and phenotypic events associated with progression to CRPC including treatment-failure and transdifferentiation to CRPC-NE.

Figures 7A-B are graphs showing additional analysis of NP and NPp53 GEM models. Fig. 7A are graphs showing Levels of steroid hormones, as determined by mass spectrometry, in serum or dorsolateral prostate from intact or castrated NP or NPp53 mice, as indicated. p-values represent comparisons between bracketed groups and were calculated using a t-test. Boxes without error bars were at or below level of detection. The number of samples analyzed were: 1) Serum samples: NP intact n=10, NP castrated n=10, NPp53 intact n=7, and NPp53 castrated n=10; 2) Prostate tissue samples: NP intact n=16, NP castrated n=13, NPp53 intact n=7, NPp53 castrated n=10. Note that although the NP and NPp53 intact mice have differing levels of androgens, which may be due to differing levels of androgen synthesis enzymes as reported previously (1), following castration, the levels of androgens in the NP and NPp53 were reduced to similar levels. Fig. 7B shows cross species gene set enrichment analyses (GSEA). Comparison of a reference mouse MR signature from NPp53 CRPC versus NP CRPC with a query human MR signature comparing primary tumors having low PTEN/low TP53 versus low PTEN/high TP53 based on the TCGA dataset (low versus high TP53 (TCGA)). “NES” stands for normalized enrichment score; GSEA p-values were calculated using 1000 gene permutations.

Figures 8A-D are graphs showing tolerance and efficacy of abiraterone in mouse prostate. Figs. 8A-C are graphs showing optimization of dose and scheduling for abiraterone-acetate treatment performed using non-tumor bearing strain-matched littermates; abiraterone-acetate was delivered by oral gavage. Fig. 8A shows tolerance for abiraterone-acetate as a function of body weight over a period of 20 days. Mice (n=5/group) were treated once daily for 20 days at the indicated concentration. Note that treatments resulted in no appreciable loss of total body weight over the 20-day study period. Fig. 8B shows efficacy of abiraterone-acetate after 20 days of treatment (as in Fig. 8A) as assessed by measurement of the wet weight of androgen-dependent (i.e., prostate and the seminal vesicle) and androgen independent (i.e., kidney) tissues. p-values represent comparisons between treatment and vehicle groups (n = 5/group), and were calculated using a t-test. Note that the levels of abiraterone-acetate used are similar to published studies in other mouse strains, including studies that have evaluated human xenografts (2, 3). Fig. 8C are graphs confirming uptake of abiraterone-acetate in the mouse prostate and its metabolism to the active form, as well as to determine the temporal kinetics, mice (n = 3/group) were given a single dose of abiraterone-acetate (200 mg/kg) at time zero and sacrificed at the indicated time-points. Levels of abiraterone were measured in serum and prostate tissue using mass spectrometry. Fig. 8D are

graphs showing levels of steroid hormones in serum and prostate tumors following treatment of NPp53 CRPC with abiraterone-acetate (200 mg/kg) for 1 month. Serum and prostate tumors were collected within 24 hours of the last treatment and snap-frozen until processed for mass spectrometry analysis, which was performed in the Steroid Analysis Core of the Fred Hutchinson Cancer Research Center. Shown are the levels of abiraterone and metabolites of steroid biosynthesis, some of which are below the level of detection for the mass spectrometry assay. These data confirm uptake and processing of abiraterone-acetate in mouse CRPC, in addition to the nontumor bearing mice evaluated in Fig. 8A-C, and show that steroids downstream of abiraterone-acetate, namely testosterone and androsterone, are significantly affected by the treatment. The number of samples analyzed were: 1) Serum samples: vehicle treated n=3, abiraterone treated n=4; 2) Tissue samples: vehicle treated n=3, abiraterone treated n=4. p-values represent comparison between abiraterone- and vehicle-treated group and were calculated by a t-test. Boxes without error bars were at or below level of detection.

Figures 9A-C are graphs showing additional analyses of tumor volumes in GEM models before and after treatment. Fig. 9A are images with anterior views from a 3-dimensional volumetric analysis of NP or NPp53 CRPC following treatment with abiraterone. Shown are images collected immediately prior to treatment (pre-treatment) and directly after the last treatment (post-treatment) with vehicle or abiraterone, as indicated. Shown are the positions of the tumor, as well as the bladder and kidneys as a reference. Fig. 9B are growth curve graphs showing the relative tumor volume for individual NPp53 mice after castration and subsequent treatment with abiraterone or vehicle, as indicated. Fig. 9C shows the comparison of tumor volume for individual NPp53 CRPC cases prior to or immediately following treatment with vehicle or abiraterone, as indicated. Mice were randomly assigned to the vehicle or abiraterone treatment groups. Note that there was no significant difference in the tumor volumes of between these groups at the time of enrollment.

Figures 10A-D are blots and graphs showing analyses of preclinical treatment of cell lines from NP and NPp53 tumors. Epithelial cell lines from treatment-naïve independent NPp53 or NP tumors (n=2 lines each) were generated as described previously (6). Fig. 10A shows western blot analysis using total cell extracts (10 µg) to detect the indicated proteins. Fig. 10B are graphs using cell culture assays, as follows. To measure the consequences of abiraterone for cell growth, NP or NPp53 cell lines, as indicated, were grown in charcoal-

stripped serum in the absence or presence of DHT (20 nM) followed by treatment with abiraterone (10 μ M) or vehicle for 0 to 72 hours. Samples, in triplicate, were analyzed by MTT assays. p-values represent comparisons between bracketed groups and were calculated by a t-test. The conditions used here for these mouse cell lines were similar to those reported previously in human cell lines (4). Note that inhibition of cell growth by abiraterone in the NP cell lines occurs in the absence of DHT, suggestive of anti-AR mechanism as has been reported for human cells (4). Figs. 10C-D are graphs showing *in vivo* tumor growth under different conditions. NP or NPp53 cells (1×10^6 cells) were implanted into the flank of nude mice, and tumor growth was monitored by caliper measurement until they reached a size of 100-300mm³. The mice were then randomly enrolled for treatment with abiraterone (200 mg/kg/day) or vehicle 5 times per week for 4 weeks. The number of samples analyzed were: 1) NP allografts: vehicle n=6, abiraterone n=4; 2) NPp53 allografts: vehicle n=6, abiraterone n=4. Fig. 10C shows graphs of tumor growth measurements. * and ** indicate p-values < 0.05 or 0.01, respectively, calculated by a t-test. Fig. 10D is a graph showing tumor weight at the conclusion of the study. p-values represent comparisons between bracketed groups and were calculated by a t-test.

Figure 11 shows images of alternative histopathologies of NPp53 CRPC. Representative H&E images of alternative histopathologies of NPp53 CRPC as indicated.

Figures 12A-B are additional cross-species computational analyses. Fig. 12A is principal component analysis (PCA) of gene expression profiles from NP CRPC that were treated with vehicle (veh) or abiraterone (abi), as indicated. Fig. 12B shows cross-species GSEA. The reference signature, a human MR signature from Best et al. comparing treatment-naïve androgen independent versus androgen-dependent prostate tumors, was compared with three independent mouse MR query signatures comparing abiraterone- versus vehicle-treated samples from the “responder”, “non-responder”, or “exceptional non-responder” groups. “NES” and p-value were calculated using 1000 gene permutations.

Figures 13A-B are blots and graphs showing analysis of expression of SOX11 and other NE genes. Fig. 13A are graphs of quantitative real-time PCR showing relative expression levels of Sox11, Rb1, and Sox2 in individual mouse CRPC tumors treated with vehicle or abiraterone as indicated. The individual mouse ID numbers correspond to the cases shown in Table S3. Gapdh was used as a reference; p values were calculated using a t-test to compare the indicated groups (shown as bracketed) consisting of multiple cases. Data show

the mean $\pm$ S.D. for triplicates done for each individual tumor. Fig. 13B are additional graphs of quantitative real-time PCR showing relative expression levels of Sox2 and Sox7, using Gapdh as a reference, in the mouse NPp53 cell line 1 (see Fig. 10).

DETAILED DESCRIPTION

5 The progression of prostate cancer is also marked by profound changes in its mutational landscape, as revealed by recent genomic analyses (5-10). Genomic alterations that occur in early localized disease include deletion of 8p21, resulting in haploinsufficiency for the *NKX3.1* homeobox gene, as well as the *TMPRSS2-ERG* chromosomal rearrangement (6). In contrast, several other common genomic alterations, such as those affecting the *TP53*
10 tumor suppressor and the *PTEN* tumor suppressor, occur at low frequencies in primary tumors, but are more prevalent in advanced disease (10).

 In particular, the most common somatic alterations in human castration-resistant prostate cancer (CRPC) affect *TP53* and *PTEN*. Current treatments for castration-resistant prostate cancer (CPRC) target androgen receptor (AR) signaling and initially improve patient
15 survival, yet often ultimately fail. To investigate treatment failure in aggressive CRPC, we have generated a genetically engineered mouse (GEM) model based on combinatorial loss of function of p53 and Pten, which are frequently co-mutated in human CRPC. In response to treatment with the antiandrogen abiraterone, these *NPp53* mice frequently display an accelerated aggressive tumor phenotype that shares phenotypic and molecular features with
20 treatment-induced neuroendocrine prostate cancer. Notably, lineage-tracing *in vivo* demonstrates that neuroendocrine-like cells arise through transdifferentiation of luminal prostate adenocarcinoma cells. These findings demonstrate principal roles for p53 and Pten in modulating cellular plasticity, and implicate transdifferentiation as a key mechanism of drug resistance in cancer.

25 In certain embodiments, the present invention relates to human prostate cancer in which one or a combination of PTEN and TP53 gene alterations are present and indicate a potential to develop non-adenocarcinoma histopathology, which is augmented by abiraterone treatment, and for which abiraterone treatment is counter-indicated, or should be stopped. Consequently, CRPC comprising PTEN and/or TP53 gene alterations may benefit from
30 alternative androgen pathway inhibitors. Novel androgen pathway inhibitors that may be suitable alternatives to abiraterone include androgen synthesis inhibitors (TAK700), androgen receptor inhibitors (ARN-509, ODM-201, and EZN-4176), AR DNA binding domain inhibitors (EPI-001), selective AR downregulators or SARDs (AZD-3514), and agents that

inhibit both androgen synthesis and receptor binding (TOK-001/galeterone) (reviewed by Agarwal et al. *Ann Oncol.* 2014 Sep;25(9):1700-9; and Leibowitz-Amit R and Joshua AM, *Curr Oncol.* 2012 Dec; 19(Suppl 3): S22–S31).

In certain embodiments, the effects of abiraterone treatment on human prostate cancer in which one or a combination of PTEN and TP53 gene alterations are present, may be due to complete lack of dependence on AR signaling in the tumors. Thus, in these scenarios it is likely that most AR-targeted therapies would exhibit negative effects comparable to those of abiraterone, as shown in the present data. Accordingly, early detection of MR associated with CRPC-NE would allow for identification of patients that would not benefit from AR-targeted therapies. Such patients could be selected for alternative treatment regimen, such as chemotherapy, immunotherapy, or co-targeting therapeutic approaches. For example, Sipuleucel-T, an autologous active cellular immunotherapy, has shown evidence of efficacy in prolonged overall survival among men with metastatic castration-resistant prostate cancer (Kantoff et al. *N Engl J Med.* 2010;363(5):411-22.). Additional promising immunotherapeutic agents include poxvirus based vaccines, T-cell checkpoint inhibiting monoclonal antibodies, as well as antibody conjugates. Furthermore, cabazitaxel, a new therapeutic option for patients with mCRPC resistant to docetaxel was recently approved by the FDA (Matthew D, *Nature Reviews Drug Discovery* 2010, 9, 677-678). Eribulin mesylate, a nontaxane halichondrin B analog microtubule inhibitor, has also demonstrated activity in CRPC (Agarwal et al. *Ann Oncol.* 2014 Sep;25(9):1700-9).

Molecular biology

In accordance with the present invention, there may be numerous tools and techniques within the skill of the art, such as those commonly used in molecular immunology, cellular immunology, pharmacology, and microbiology. See, e.g., Sambrook et al. (2001) *Molecular Cloning: A Laboratory Manual*. 3rd ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y.; Ausubel et al. eds. (2005) *Current Protocols in Molecular Biology*. John Wiley and Sons, Inc.: Hoboken, N.J.; Bonifacino et al. eds. (2005) *Current Protocols in Cell Biology*. John Wiley and Sons, Inc.: Hoboken, N.J.; Coligan et al. eds. (2005) *Current Protocols in Immunology*, John Wiley and Sons, Inc.: Hoboken, N.J.; Coico et al. eds. (2005) *Current Protocols in Microbiology*, John Wiley and Sons, Inc.: Hoboken, N.J.; Coligan et al. eds. (2005) *Current Protocols in Protein Science*, John Wiley and Sons, Inc.: Hoboken, N.J.;

and Enna et al. eds. (2005) Current Protocols in Pharmacology, John Wiley and Sons, Inc.: Hoboken, N.J.

The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and the specific context where each term is used. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the methods of the invention and how to use them. Moreover, it will be appreciated that the same thing can be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein, nor is any special significance to be placed upon whether or not a term is elaborated or discussed herein. Synonyms for certain terms are provided. A recital of one or more synonyms does not exclude the use of the other synonyms. The use of examples anywhere in the specification, including examples of any terms discussed herein, is illustrative only, and in no way limits the scope and meaning of the invention or any exemplified term. Likewise, the invention is not limited to its preferred embodiments.

“Treating” or “treatment” of a state, disorder or condition includes:

- (1) preventing or delaying the appearance of clinical symptoms of the state, disorder, or condition developing in a person who may be afflicted with or predisposed to the state, disorder or condition but does not yet experience or display clinical symptoms of the state, disorder or condition; or
- (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof (in case of maintenance treatment) or at least one clinical symptom, sign, or test, thereof; or
- (3) relieving the disease, i.e., causing regression of the state, disorder or condition or at least one of its clinical or sub-clinical symptoms or signs.

The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Acceptable excipients, diluents, and carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington: The Science and Practice

of Pharmacy. Lippincott Williams & Wilkins (A. R. Gennaro edit. 2005). The choice of pharmaceutical excipient, diluent, and carrier can be selected with regard to the intended route of administration and standard pharmaceutical practice.

5 An “immune response” refers to the development in the host of a cellular and/or antibody-mediated immune response to a composition or vaccine of interest. Such a response usually consists of the subject producing antibodies, B cells, helper T cells, suppressor T cells, regulatory T cells, and/or cytotoxic T cells directed specifically to an antigen or antigens included in the composition or vaccine of interest.

10 A “therapeutically effective amount” means the amount of a compound that, when administered to an animal for treating a state, disorder or condition, is sufficient to effect such treatment. The “therapeutically effective amount” will vary depending on the compound, the disease and its severity and the age, weight, physical condition and responsiveness of the animal to be treated.

15 The compositions of the invention may include a “therapeutically effective amount” or a “prophylactically effective amount” of a compound described herein. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of an antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired
20 response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the compound are outweighed by the therapeutically beneficial effects. A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the
25 prophylactically effective amount will be less than the therapeutically effective amount.

While it is possible to use a composition provided by the present invention for therapy as is, it may be preferable to administer it in a pharmaceutical formulation, e.g., in admixture with a suitable pharmaceutical excipient, diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice. Accordingly, in one
30 aspect, the present invention provides a pharmaceutical composition or formulation comprising at least one active composition, or a pharmaceutically acceptable derivative thereof, in association with a pharmaceutically acceptable excipient, diluent and/or carrier.

The excipient, diluent and/or carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The compositions of the invention can be formulated for administration in any convenient way for use in human or veterinary medicine. The invention therefore includes
5 within its scope pharmaceutical compositions comprising a product of the present invention that is adapted for use in human or veterinary medicine.

In a preferred embodiment, the pharmaceutical composition is conveniently administered as an oral formulation. Oral dosage forms are well known in the art and include tablets, caplets, gelcaps, capsules, and medical foods. Tablets, for example, can be made by
10 well-known compression techniques using wet, dry, or fluidized bed granulation methods.

Such oral formulations may be presented for use in a conventional manner with the aid of one or more suitable excipients, diluents, and carriers. Pharmaceutically acceptable excipients assist or make possible the formation of a dosage form for a bioactive material and include diluents, binding agents, lubricants, glidants, disintegrants, coloring agents, and other
15 ingredients. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, ascorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used. An excipient is pharmaceutically acceptable if, in addition to performing its desired function, it is non-toxic, well tolerated upon ingestion, and does not interfere with absorption
20 of bioactive materials.

Acceptable excipients, diluents, and carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington: The Science and Practice of Pharmacy. Lippincott Williams & Wilkins (A. R. Gennaro edit. 2005). The choice of pharmaceutical excipient, diluent, and carrier can be selected with regard to the intended
25 route of administration and standard pharmaceutical practice.

As used herein, the phrase “pharmaceutically acceptable” refers to molecular entities and compositions that are “generally regarded as safe”, e.g., that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Preferably, as used herein, the term
30 “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopeias for use in animals, and more particularly in humans.

“Patient” or “subject” refers to mammals and includes human and veterinary subjects.

The dosage of the therapeutic formulation will vary widely, depending upon the nature of the disease, the patient's medical history, the frequency of administration, the manner of administration, the clearance of the agent from the host, and the like. The initial
5 dose may be larger, followed by smaller maintenance doses. The dose may be administered as infrequently as weekly or biweekly, or fractionated into smaller doses and administered daily, semi-weekly, etc., to maintain an effective dosage level. In some cases, oral administration will require a higher dose than if administered intravenously. In some cases, topical administration will include application several times a day, as needed, for a number of
10 days or weeks in order to provide an effective topical dose.

The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, olive oil, sesame oil and the like. Water or aqueous solution
15 saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Alternatively, the carrier can be a solid dosage form carrier, including but not limited to one or more of a binder (for compressed pills), a glidant, an encapsulating agent, a flavorant, and a colorant. Suitable pharmaceutical carriers are described in “Remington's Pharmaceutical Sciences” by E. W. Martin.

20 The term “subject” as used in this application means an animal with an immune system such as avians and mammals. Mammals include canines, felines, rodents, bovine, equines, porcines, ovines, and primates. Avians include, but are not limited to, fowls, songbirds, and raptors. Thus, the invention can be used in veterinary medicine, *e.g.*, to treat companion animals, farm animals, laboratory animals in zoological parks, and animals in the
25 wild. The invention is particularly desirable for human medical applications.

The term “patient” as used in this application means a human subject. In some embodiments of the present invention, the “patient” is diagnosed with prostate cancer.

The terms “screen” and “screening” and the like as used herein means to test a subject or patient to determine if they have a particular illness or disease, or a particular
30 manifestation of an illness or disease. The term also means to test an agent to determine if it has a particular action or efficacy.

The terms “identification”, “identify”, “identifying” and the like as used herein means to recognize a disease state or a clinical manifestation or severity of a disease state in a

subject or patient. The term also is used in relation to test agents and their ability to have a particular action or efficacy.

The terms “prediction”, “predict”, “predicting” and the like as used herein means to tell in advance based upon special knowledge.

5 The term “reference value” as used herein means an amount or a quantity of a particular protein or nucleic acid in a sample from a healthy control or healthy donor.

The terms “healthy control”, “healthy donor” and “HD” are used interchangeably in this application and are a human subject who is not suffering from prostate cancer or any other cancer-related condition.

10 The terms “treat”, “treatment”, and the like refer to a means to slow down, relieve, ameliorate or alleviate at least one of the symptoms of the disease, or reverse the disease after its onset.

The terms “prevent”, “prevention”, and the like refer to acting prior to overt disease onset, to prevent the disease from developing or minimize the extent of the disease or slow its course of development.

15 The term “agent” as used herein means a substance that produces or is capable of producing an effect and would include, but is not limited to, chemicals, pharmaceuticals, biologics, small organic molecules, antibodies, nucleic acids, peptides, and proteins.

The phrase “therapeutically effective amount” is used herein to mean an amount sufficient to cause an improvement in a clinically significant condition in the subject, or delays or minimizes or mitigates one or more symptoms associated with the disease, or results in a desired beneficial change of physiology in the subject.

As used herein, the term “isolated” and the like means that the referenced material is free of components found in the natural environment in which the material is normally found.

25 In particular, isolated biological material is free of cellular components. In the case of nucleic acid molecules, an isolated nucleic acid includes a PCR product, an isolated mRNA, a cDNA, an isolated genomic DNA, or a restriction fragment. In another embodiment, an isolated nucleic acid is preferably excised from the chromosome in which it may be found. Isolated nucleic acid molecules can be inserted into plasmids, cosmids, artificial

30 chromosomes, and the like. Thus, in a specific embodiment, a recombinant nucleic acid is an isolated nucleic acid. An isolated protein may be associated with other proteins or nucleic acids, or both, with which it associates in the cell, or with cellular membranes if it is a membrane-associated protein. An isolated material may be, but need not be, purified.

The term “purified” and the like as used herein refers to material that has been isolated under conditions that reduce or eliminate unrelated materials, *i.e.*, contaminants. For example, a purified protein is preferably substantially free of other proteins or nucleic acids with which it is associated in a cell; a purified nucleic acid molecule is preferably substantially free of proteins or other unrelated nucleic acid molecules with which it can be found within a cell. As used herein, the term “substantially free” is used operationally, in the context of analytical testing of the material. Preferably, purified material substantially free of contaminants is at least 50% pure; more preferably, at least 90% pure, and more preferably still at least 99% pure. Purity can be evaluated by chromatography, gel electrophoresis, immunoassay, composition analysis, biological assay, and other methods known in the art.

The terms “expression profile” or “gene expression profile” refers to any description or measurement of one or more of the genes that are expressed by a cell, tissue, or organism under or in response to a particular condition. Expression profiles can identify genes that are up-regulated, down-regulated, or unaffected under particular conditions. Gene expression can be detected at the nucleic acid level or at the protein level. The expression profiling at the nucleic acid level can be accomplished using any available technology to measure gene transcript levels. For example, the method could employ *in situ* hybridization, Northern hybridization or hybridization to a nucleic acid microarray, such as an oligonucleotide microarray, or a cDNA microarray. Alternatively, the method could employ reverse transcriptase-polymerase chain reaction (RT-PCR) such as fluorescent dye-based quantitative real time PCR (TaqMan® PCR). In the Examples section provided below, nucleic acid expression profiles were obtained using Affymetrix GeneChip® oligonucleotide microarrays. The expression profiling at the protein level can be accomplished using any available technology to measure protein levels, *e.g.*, using peptide-specific capture agent arrays.

The terms “gene signature” and “signature genes” will be used interchangeably herein and mean the particular transcripts that have been found to be differentially expressed in some prostate cancer patients, as described herein. For example, those differentially expressed genes listed in Table S4 and/or Table S8. It is noted that differential levels of the corresponding proteins, will also be useful as a marker or signature for prognosing or diagnosing patients as described herein.

The terms “gene”, “gene transcript”, and “transcript” are used somewhat interchangeable in the application. The term “gene”, also called a “structural gene” means a DNA sequence that codes for or corresponds to a particular sequence of amino acids which comprise all or part of one or more proteins or enzymes, and may or may not include

regulatory DNA sequences, such as promoter sequences, which determine for example the conditions under which the gene is expressed. Some genes, which are not structural genes, may be transcribed from DNA to RNA, but are not translated into an amino acid sequence. Other genes may function as regulators of structural genes or as regulators of DNA transcription. "Transcript" or "gene transcript" is a sequence of RNA produced by transcription of a particular gene. Thus, the expression of the gene can be measured via the transcript.

The term "antisense DNA" is the non-coding strand complementary to the coding strand in double-stranded DNA.

10 The term "genomic DNA" as used herein means all DNA from a subject including coding and non-coding DNA, and DNA contained in introns and exons.

The term "nucleic acid hybridization" refers to anti-parallel hydrogen bonding between two single-stranded nucleic acids, in which A pairs with T (or U if an RNA nucleic acid) and C pairs with G. Nucleic acid molecules are "hybridizable" to each other when at least one strand of one nucleic acid molecule can form hydrogen bonds with the complementary bases of another nucleic acid molecule under defined stringency conditions. Stringency of hybridization is determined, *e.g.*, by (i) the temperature at which hybridization and/or washing is performed, and (ii) the ionic strength and (iii) concentration of denaturants such as formamide of the hybridization and washing solutions, as well as other parameters. Hybridization requires that the two strands contain substantially complementary sequences. Depending on the stringency of hybridization, however, some degree of mismatches may be tolerated. Under "low stringency" conditions, a greater percentage of mismatches are tolerable (*i.e.*, will not prevent formation of an anti-parallel hybrid).

25 The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (*e.g.* a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (*e.g.* transcription and translation) of the introduced sequence. Vectors include, but are not limited to, plasmids, phages, and viruses.

Vectors typically comprise the DNA of a transmissible agent, into which foreign DNA is inserted. A common way to insert one segment of DNA into another segment of DNA involves the use of enzymes called restriction enzymes that cleave DNA at specific sites (specific groups of nucleotides) called restriction sites. A "cassette" refers to a DNA coding sequence or segment of DNA which codes for an expression product that can be inserted into a vector at defined restriction sites. The cassette restriction sites are designed to ensure insertion of the cassette in the proper reading frame. Generally, foreign DNA is

inserted at one or more restriction sites of the vector DNA, and then is carried by the vector into a host cell along with the transmissible vector DNA. A segment or sequence of DNA having inserted or added DNA, such as an expression vector, can also be called a "DNA construct" or "gene construct." A common type of vector is a "plasmid", which generally is a self-contained molecule of double-stranded DNA, usually of bacterial origin, that can readily accept additional (foreign) DNA and which can readily introduced into a suitable host cell. A plasmid vector often contains coding DNA and promoter DNA and has one or more restriction sites suitable for inserting foreign DNA. Coding DNA is a DNA sequence that encodes a particular amino acid sequence for a particular protein or enzyme. Promoter DNA is a DNA sequence which initiates, regulates, or otherwise mediates or controls the expression of the coding DNA. Promoter DNA and coding DNA may be from the same gene or from different genes, and may be from the same or different organisms. A large number of vectors, including plasmid and fungal vectors, have been described for replication and/or expression in a variety of eukaryotic and prokaryotic hosts. Non-limiting examples include pKK plasmids (Clontech), pUC plasmids, pET plasmids (Novagen, Inc., Madison, WI), pRSET or pREP plasmids (Invitrogen, San Diego, CA), or pMAL plasmids (New England Biolabs, Beverly, MA), and many appropriate host cells, using methods disclosed or cited herein or otherwise known to those skilled in the relevant art. Recombinant cloning vectors will often include one or more replication systems for cloning or expression, one or more markers for selection in the host, *e.g.* antibiotic resistance, and one or more expression cassettes.

The term "host cell" means any cell of any organism that is selected, modified, transformed, grown, used or manipulated in any way, for the production of a substance by the cell, for example, the expression by the cell of a gene, a DNA or RNA sequence, a protein or an enzyme. Host cells can further be used for screening or other assays, as described herein.

A "polynucleotide" or "nucleotide sequence" is a series of nucleotide bases (also called "nucleotides") in a nucleic acid, such as DNA and RNA, and means any chain of two or more nucleotides. A nucleotide sequence typically carries genetic information, including the information used by cellular machinery to make proteins and enzymes. These terms include double or single stranded genomic and cDNA, RNA, any synthetic and genetically manipulated polynucleotide, and both sense and anti-sense polynucleotide. This includes single- and double-stranded molecules, *i.e.*, DNA-DNA, DNA-RNA and RNA-RNA hybrids, as well as "protein nucleic acids" (PNA) formed by conjugating bases to an amino acid

backbone. This also includes nucleic acids containing modified bases, for example thio-uracil, thio-guanine and fluoro-uracil.

“Nucleic acid” refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The nucleic acids herein may be flanked by natural regulatory (expression control) sequences, or may be associated with heterologous sequences, including promoters, internal ribosome entry sites (IRES) and other ribosome binding site sequences, enhancers, response elements, suppressors, signal sequences, polyadenylation sequences, introns, 5'- and 3'- non-coding regions, and the like. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. The nucleic acids may also be modified by many means known in the art. Non-limiting examples of such modifications include methylation, “caps”, substitution of one or more of the naturally occurring nucleotides with an analog, and internucleotide modifications such as, for example, those with uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoroamidates, and carbamates) and with charged linkages (*e.g.*, phosphorothioates, and phosphorodithioates). Polynucleotides may contain one or more additional covalently linked moieties, such as, for example, proteins (*e.g.*, nucleases, toxins, antibodies, signal peptides, and poly-L-lysine), intercalators (*e.g.*, acridine, and psoralen), chelators (*e.g.*, metals, radioactive metals, iron, and oxidative metals), and alkylators. The polynucleotides may be derivatized by formation of a methyl or ethyl phosphotriester or an alkyl phosphoramidate linkage. Modifications of the ribose-phosphate backbone may be done to facilitate the addition of labels, or to increase the stability and half-life of such molecules in physiological environments. Nucleic acid analogs can find use in the methods of the invention as well as mixtures of naturally occurring nucleic acids and analogs. Furthermore, the polynucleotides herein may also be modified with a label capable of providing a detectable signal, either directly or indirectly. Exemplary labels include radioisotopes, fluorescent molecules, and biotin.

The term “polypeptide” as used herein means a compound of two or more amino acids linked by a peptide bond. “Polypeptide” is used herein interchangeably with the term “protein.”

The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system, *i.e.*,

the degree of precision required for a particular purpose, such as a pharmaceutical formulation. For example, “about” can mean within 1 or more than 1 standard deviations, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value.

- 5 Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated, the term “about” meaning within an acceptable error range for the particular value should be assumed.

10 **Methods of Treatment and Monitoring and Targeting Treatment**

In certain embodiments, the present invention relates to a method of detecting castration-resistant prostate cancer (CRPC) and/or super resistant prostate tumors in a subject diagnosed with prostate cancer, comprising:

- 15 a. assaying gene expression levels of one or more genes listed in the gene signatures of Table S4 or Table S8, or corresponding protein levels, in a sample from the subject to obtain a test expression profile,
- b. comparing the test expression profile of the genes or proteins with a reference expression profile for the genes or proteins, wherein the reference expression profile comprises the expression level of the same genes or proteins in a control;
- 20 c. detecting gene expression levels of one or more genes listed in Table S4 or Table S8 or corresponding protein levels, in the test expression profile compared to the gene expression levels of one or more genes in the reference expression profile, or corresponding protein levels, wherein differential expression or protein levels is indicative of CRPC and/or the development of super-resistant prostate tumors as a
- 25 prognosis and wherein anti-androgen treatment is counter-indicated under these conditions.

In certain embodiments, the control is selected from the group consisting of a healthy control, an untreated subject, and a composite reference value for human prostate tumors.

- 30 In certain embodiments, the present invention relates to a method of monitoring the treatment of a subject diagnosed with prostate cancer, comprising:

- a. assaying gene expression of one or more genes from Table S4 or Table S8 or corresponding protein levels, in a sample from the subject to obtain a test expression profile,

b. comparing the test expression profile of the genes or corresponding proteins with a reference expression profile, wherein the reference expression profile comprises the expression level of the same genes or corresponding proteins obtained from the subject prior to treatment;

5 c. detecting gene expression levels of one or more genes from Table S4 or Table S8, or corresponding proteins in the test expression profile that are differential compared to the gene expression of the same one or more genes in the reference expression profile or corresponding proteins, wherein the differential expression or protein levels is indicative of CRPC and/or the development of super-resistant prostate tumors and wherein anti-androgen treatment is counter-indicated under these
10 conditions.

In additional embodiments, the method further comprises treating the subject with one or more alternatives to anti-androgen treatment when CRPC and/or the development of super-resistant prostate tumors is prognosed, comprising administering to the subject a
15 therapeutically effective amount of one or any combination of treatments selected from the group consisting of an autologous active cellular immunotherapy (e.g. Sipuleucel-T), a poxvirus based vaccine, a T-cell checkpoint inhibiting monoclonal antibody (anti-CTL4 or anti-PD-1 or anti-PDL1), and cabazitaxel, and further comprising monitoring the subject at least monthly.

20 In additional embodiments, the method further comprises detecting one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and 1-DOPA Decarboxylase (DDC), or any combination thereof, wherein the presence any one or more neuroendocrine markers indicates a likelihood of CRPC and/or the development of super-resistant prostate tumors.

25 In additional embodiments, assaying the gene expression levels is performed using a method comprising at least one of: PCR, RNA sequencing, and microarray.

In additional embodiments, assaying the protein levels is performed using a method comprising at least one of the following methods: quantitative Western blot, immunoblot, quantitative mass spectrometry, enzyme-linked immunosorbent assays (ELISAs),
30 radioimmunoassays (RIA), immunoradiometric assays (IRMA), and immunoenzymatic assays (IEMA) and sandwich assays using monoclonal and polyclonal antibodies.

In certain embodiments, a combination of gene expression and protein expression is performed to arrive at a differential profile, wherein the differential profile indicates CRPC

and/or the development of super-resistant prostate tumors, and/or castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE) or CRPC-NE likelihood.

In certain embodiments, the present invention relates to a method for detecting castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE) or CRPC-NE likelihood in a patient sample comprising obtaining a sample comprising prostate cancer tumor tissue from the patient and detecting one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and l-DOPA Decarboxylase (DDC), or any combination thereof, in the sample and diagnosing the patient with CRPC-NE or prognosing the patient as having an increased likelihood of prostate cancer progression (CRPC-NE likelihood) when one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and l-DOPA Decarboxylase (DDC), or any combination thereof is detected in the sample.

In additional embodiments, traditional anti-androgen treatment is counter-indicated when the patient is diagnosed with CRPC-NE or CRPC-NE likelihood.

When anti-androgen treatment is counter-indicated as determined, then alternative treatments should be considered for treatment. Examples of such alternative treatments, that are expected to be less likely to lead to development of CRPC or super-resistant prostate tumors include at least the following: a therapeutically effective amount of treatment selected from the group consisting of an autologous active cellular immunotherapy (e.g. Sipuleucel-T), a poxvirus based vaccine, a T-cell checkpoint inhibiting monoclonal antibody (anti-CTL4 or anti-PD-1 or anti-PDL1), and cabazitaxel, and further comprising monitoring the subject at least monthly. It is noted that the following alternative androgen synthesis inhibitors (TAK700), androgen receptor inhibitors (ARN-509, ODM-201, and EZN-4176), AR DNA binding domain inhibitors (EPI-001), selective AR downregulators or SARDs (AZD-3514), including agents that inhibit both androgen synthesis and receptor binding (TOK-001/galeterone), may be appropriate treatment when the gene expression or protein levels indicate susceptible or likely to develop CRPC or super CRPC, or the likelihood of CRPC-NE.

In certain embodiments, the present invention also provides methods for stratifying subjects prior to treatment and/or monitoring subjects for their responses to treatment, e.g., administration of agents, both oral and topical, life style alterations such as diet and exercise, and non-traditional treatment such as acupuncture. This is useful in both patient care as well as clinical trials. In certain embodiments, the methods comprise obtaining the expression of

at least one gene in at least one gene signature, or the corresponding protein level, in a subject prior to any treatment. In alternative embodiments, the methods comprise obtaining the expression of at least one gene in at least one gene signature (or the corresponding protein) in a normal subject that serves as a reference expression value. After a course of treatment at a particular time period that a person of skill in the art can determine, the measurement of expression of the same gene or genes (or corresponding proteins) is measured, and differential expression or protein level, when compared to the reference (e.g. prior to treatment levels, control levels, or NES) would indicate that the subject is susceptible or likely to develop CRPC or to develop super-resistant tumors. In certain embodiments, the expression of at least one gene in at least one gene signature, or corresponding protein level, would be measured before and after treatment. In an alternative embodiment, more than one gene from each signature, or corresponding protein would be measured. In an additional embodiment, a combination of genes and other markers such as one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), Neuron-specific enolase (NSE), and l-DOPA Decarboxylase (DDC), and any combination thereof, can be detected and form a diagnostic or signature group for determine whether the subject is susceptible or likely to develop CRPC or to develop super-resistant tumors, or the likelihood of CRPC-NE.

The present invention also provides a method for determining target genes or proteins for drug development.

The invention also contemplates that the protein products of any of the genes in the gene signatures found for example in Table S4, Table S8, or in dataset 1and/or described in any of the Tables or Figures herein may have diagnostic value, as well as to serve as potential therapeutic targets for patient monitoring, stratification, or drug development.

Assays and Methods to Detect Proteins

In certain embodiments, a sample of biological tissue or bodily fluid from a subject with prostate cancer, is obtained.

In certain embodiments, the sample is tested for protein levels (for protein corollaries of any of the genes listed in Tables S4 or S8, for example, or any of the Tables or Figures herein. The protein sample can be obtained from any biological tissue. Preferred biological tissues include, but are not limited to, epidermal, whole blood, and plasma. The protein sample can be obtained from any biological fluid. Preferred fluids include, but are not limited to, plasma, saliva, and urine. Protein can be isolated and/or purified from the sample using any method known in the art, including but not limited to immunoaffinity chromatography.

While any method known in the art can be used, preferred methods for detecting and measuring increase levels of the proteins in a protein sample include quantitative Western blot, immunoblot, quantitative mass spectrometry, enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIA), immunoradiometric assays (IRMA), and immunoenzymatic assays (IEMA) and sandwich assays using monoclonal and polyclonal antibodies.

Antibodies are a preferred method of detecting and measuring target or desired proteins in a sample. Such antibodies are available commercially or can be made by conventional methods known in the art. Such antibodies can be monoclonal or polyclonal and fragments thereof, and immunologic binding equivalents thereof. The term “antibody” means both a homologous molecular entity as well as a mixture, such as a serum product made up of several homologous molecular entities.

In a preferred embodiment, such antibodies will immunoprecipitate the desired proteins from a solution as well as react with desired/target proteins on a Western blot, immunoblot, ELISA, and other assays listed above.

Antibodies for use in these assays can be labeled covalently or non-covalently with an agent that provides a detectable signal. Any label and conjugation method known in the art can be used. Labels, include but are not limited to, enzymes, fluorescent agents, radiolabels, substrates, inhibitors, cofactors, magnetic particles, and chemiluminescent agents. A number of fluorescent materials are known and can be utilized as detectable labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate. Any desired targets or binding partner(s) can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{125}I , ^{131}I , and ^{186}Re . Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. In embodiments the enzymes can be peroxidase, β -glucuronidase, β -D-glucosidase, β -D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and

4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

The terms "sample" or "biological sample" as used herein, refers to a sample of biological fluid, tissue, or cells, in a healthy and/or pathological state obtained from a subject.

5 Such samples include, but are not limited to, blood, bronchial lavage fluid, sputum, saliva, urine, amniotic fluid, lymph fluid, tissue or fine needle biopsy samples, peritoneal fluid, cerebrospinal fluid, and includes supernatant from cell lysates, lysed cells, cellular extracts, and nuclear extracts. In some embodiments, the whole blood sample is further processed into serum or plasma samples. In some embodiments, the sample includes blood spotting tests.

10

Kits

It is contemplated that all of the assays disclosed herein can be in kit form for use by a health care provider and/or a diagnostic laboratory.

15 Assays for the detection and quantitation of one or more of the gene signatures can be incorporated into kits. Such kits may include probes for one or more of the genes from one or more signatures, as described herein, reagents for isolating and purifying nucleic acids from biological tissue or bodily fluid, reagents for performing assays on the isolated and purified nucleic acid, instructions for use, and reference values or the means for obtaining reference values in a control sample for the included genes.

20 A preferred kit for patient classification with regard to disease activity and clinical manifestations would include probes for at least one gene from each of the MR signatures described herein.

In a further embodiment, the kit would include reagents for testing for Synaptophysin, for example. Such a kit could include antibodies that recognize the peptide of interest, 25 reagents for isolating and/or purifying protein from a biological tissue or bodily fluid, reagents for performing assays on the isolated and purified protein, instructions for use, and reference values or the means for obtaining reference values for the quantity or level of peptides in a control sample.

A preferred kit for monitoring treatment to disease activity would include probes from 30 at least one gene from each of the MR signatures described herein. Such a kit could include antibodies that recognize the peptide of interest, reagents for isolating and/or purifying protein from a biological tissue or bodily fluid, reagents for performing assays on the isolated and purified protein, instructions for use, and reference values or the means for obtaining reference values for the quantity or level of peptides in a control sample.

A preferred kit for diagnosing or prognosing CRPC and/or the development of super-resistant prostate tumors, and/or castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE) or CRPC-NE likelihood, would include probes for at least one gene from each of the determinative signatures, such as any combination of those from Table S4 or Table S8, or corresponding protein. Additional kits may further or optionally, include one or more antibodies for detecting one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and l-DOPA Decarboxylase (DDC), and any combination thereof, and optionally, any one or more of *TP53*, *PTEN*, *SOX11* and/or *RB1*, and further optionally, wherein any one or more of the antibodies or probes are detectably labeled.

A preferred embodiment of these kits would have the probes attached to a solid state. Another embodiment would have the probes in a microarray format wherein nucleic acid probes for one or more of the genes from one or more of the gene signatures would be in an ordered arrangement on a surface or substrate.

In a further embodiment of this invention, commercial test kits suitable for use by a medical specialist may be prepared to determine the presence or amount of a desired gene or protein activity, expression or signature gene amplification in suspected cancer cells or biopsy or tumor samples. One class of such kits will contain at least the labeled target or its binding partner, for instance an antibody specific thereto, and directions, of course, depending upon the method selected, e.g., "competitive," "sandwich," "DASP" and the like. The kits may also contain peripheral reagents such as buffers, stabilizers, etc. In embodiments the kits comprise one or more PCR primers described herein.

Accordingly, a test kit may be prepared for the determination and quantitation of a desired target or protein in cells or a cellular or biopsy sample, comprising:

(a) a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of the target or a specific binding partner thereto, to a detectable label;

(b) other reagents; and

(c) directions for use of said kit.

More specifically, the diagnostic test kit may comprise:

(a) a known amount of the target as described above (or a binding partner) generally bound to a solid phase to form an immunosorbent, or in the alternative, bound to a suitable tag, or plural such end products, etc. (or their binding partners) one of each;

(b) if necessary, other reagents; and

(c) directions for use of said test kit.

In a further variation, the test kit may be prepared and used for the purposes stated above, and comprises:

5 (a) a labeled component which has been obtained by coupling the target to a detectable label;

(b) one or more additional immunochemical reagents of which at least one reagent is a ligand or an immobilized ligand, which ligand is selected from the group consisting of:

(i) a ligand capable of binding with the labeled component (a);

10 (ii) a ligand capable of binding with a binding partner of the labeled component (a);

(iii) a ligand capable of binding with at least one of the component(s) to be determined; and

15 (iv) a ligand capable of binding with at least one of the binding partners of at least one of the component(s) to be determined; and

(c) directions for the performance of a protocol for the detection and/or determination of one or more components of an immunochemical reaction between the target and a specific binding partner thereto.

In accordance with the above, an assay system for screening potential drugs effective
20 to modulate the activity or expression of the target or gene signature may be prepared and is provided. The target may be introduced into a test system, and the prospective drug may also be introduced into the resulting cell culture, and the culture thereafter examined to observe any changes in the target activity of the cells, or in the proliferation or division of the cells, due either to the addition of the prospective drug alone, or due to the effect of added
25 quantities of the known target.

As referenced herein “target” can include any of the following: any of the genes (including any single or combinations) identified in Table S4 and/or Table S8, any corresponding protein of these genes; alone or in combination with one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and L-DOPA Decarboxylase (DDC), and any combination thereof,
30 and optionally, any one or more of *TP53*, *PTEN*, *SOX11* and/or *RBI*.

Drug Screening Assays and Research Tools

All of the biomarkers disclosed herein can be used as the basis for drug screening assays and research tools.

In one embodiment, polypeptides and proteins encoded by the transcripts in the gene signatures, described herein, can be used in drug screening assays, free in solution, or affixed to a solid support. All of these forms can be used in binding assays to determine if agents being tested form complexes with the peptides, proteins or fragments, or if the agent being tested interferes with the formation of a complex between the peptide or protein and a known ligand.

Thus, the present invention provides for methods and assays for screening agents for treatment of prostate cancer, comprising contacting or incubating the test agent with a polypeptide or protein encoded by a gene in one of the gene signatures described herein and detecting the presence of a complex between the polypeptide and the agent or the presence of a complex between the polypeptide and a ligand, by methods known in the art. In such competitive binding assays, the polypeptide or fragment is typically labeled. Free polypeptide is separated from that in the complex, and the amount of free or uncomplexed polypeptide is measured. This measurement indicates the amount of binding of the test agent to the polypeptide or its interference with the binding of the polypeptide to a ligand.

High throughput screening can also be used to screen for therapeutic agents. Small peptides or molecules can be synthesized and bound to a surface and contacted with the polypeptides encoded by the gene signature transcripts, and washed. The bound peptide is visualized and detected by methods known in the art.

Antibodies to the polypeptides can also be used in competitive drug screening assays. The antibodies compete with the agent being tested for binding to the polypeptides. The antibodies can be used to find agents that have antigenic determinants on the polypeptides, which in turn can be used to develop monoclonal antibodies that target the active sites of the polypeptides.

The invention also provides for polypeptides to be used for rational drug design where structural analogs of biologically active polypeptides can be designed. Such analogs would interfere with the polypeptide *in vivo*, such as by non-productive binding to target. In this approach the three-dimensional structure of the protein is determined by any method known in the art including but not limited to x-ray crystallography, and computer modeling. Information can also be obtained using the structure of homologous proteins or target-specific antibodies.

Using these techniques, agents can be designed which act as inhibitors or antagonists of the polypeptides, or act as decoys, binding to target molecules non-productively and blocking binding of the active polypeptide.

Polypeptides encoded by any of the differentially expressed transcripts of the gene signatures described herein can be used. Additionally, testing can be done as described above using the proteins or fragments of the proteins described herein.

A further embodiment of the present invention is gene constructs comprising any one of the differentially expressed transcripts and a vector. These gene construct can be used for testing of therapeutic agents as well as basic research regarding prostate cancer, the development of CRPC, the development of super-resistant tumors, and tumor metastasis in general. These gene constructs can also be used to transform host cells can be transformed by methods known in the art.

The resulting transformed cells can be used for testing for therapeutic agents as well as basic research regarding prostate cancer. Specifically, cells can be transformed with any one of the differentially expressed transcripts, and contacted with a test agent. The resulting expression of the transcript can be detected and compared to the expression of the transcript in the cell before contact with the agent.

The expression of the transcripts in host cells can be detected and measured by any method known in the art, including but not limited to, reporter gene assays.

These gene constructs as well as the host cells transformed with these gene constructs can also be the basis for transgenic animals for testing both as research tools and for therapeutic agents. Such animals would include but are not limited to, nude mice. Phenotypes can be correlated to the genes and looked at in order to determine the genes effect on the animals as well as the change in phenotype after administration or contact with a potential therapeutic agent.

The gene constructs and host cells transformed with the gene constructs can also be administered to murine models of prostate cancer, for analyzing test agents as well as basic research. Any of the differentially expressed transcripts of the gene signatures found in Tables and described herein can be used.

This invention will be better understood from the Experimental Details, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

EXAMPLES

A refined GEM model of CRPC is conserved with human CRPC

The most common somatic alterations in human CRPC are predicted to result in loss-of-function of *TP53* and *PTEN*. Whole-exome sequencing of biopsies from men with metastatic CRPC as reported by the Stand Up to Cancer (SU2C) consortium revealed that alterations of *TP53*, including missense mutations, deletions and truncations, occurred in 50% of cases (n=150 patients), while those affecting *PTEN*, which were mostly deletions, occurred in 40% of cases (n=150) (Fig. 1A, B; Table S1) (17). Similarly, whole-exome sequencing of metastatic CRPC tumors obtained at autopsy have reported frequent somatic alterations of *TP53* and *PTEN* (54% for *TP53* and 50% for *PTEN*, n = 48 patients) (15) (Table S1). Notably, the prevalence of *TP53* alterations in CRPC contrasts with their infrequent occurrence in primary tumors (< 10%, n = 333 patients; Fig. 1B; Table S1) (14, 16, 18). Furthermore, although co-mutation of *TP53* and *PTEN* is rare in primary tumors (<2%), such co-mutations are highly prevalent in CRPC biopsies (23% co-occurrence, n=150; p<0.0001; Fisher exact test) (Fig. 1B) and tumors (33%, n=48; p<0.0001; Fisher exact test) (17). Therefore, somatic co-mutation of *TP53* and *PTEN* distinguishes human CRPC from primary tumors.

Given the prevalence of their co-inactivation in human CRPC, we sought to investigate the consequences of combined loss-of-function of *Pten* and *Trp53* for treatment of CRPC using a genetically-engineered mouse (GEM) model based on an inducible *Nkx3.1^{CreERT2}* driver to delete these genes in adult prostate epithelium (19). Our approach has distinct advantages over previous GEM models of *Pten* and *Trp53* loss-of-function in prostate, which used a constitutive Cre driver that is mostly specific to prostate, but is not restricted to adults or a specific epithelial cell type (20,21). In contrast, the *Nkx3.1^{CreERT2}* driver is a knock-in allele in which a tamoxifen-inducible CreERT2 cassette is placed under the transcriptional control of the endogenous *Nkx3.1* promoter, resulting in heterozygous inactivation of *Nkx3.1* and thereby resulting in pre-invasive phenotypes (19). Induction of Cre activity by tamoxifen administration enables temporal control of gene deletion in mature adult prostate, as well as spatial restriction to prostatic luminal epithelial cells (19,22), which are a cell of origin for prostate cancer (23), and enables lineage-tracing to define the cellular origin of tumor phenotypes.

Because GEM models with loss-of-function of *Trp53* alone have modest prostate cancer phenotypes (20,24,25), whereas those with loss-of-function of *Pten* alone develop prostate adenocarcinoma and CRPC (21,26), we compared the phenotype of *Trp53* and *Pten* compound mutant mice with that of *Pten* single mutant mice. In particular, we analyzed the prostate phenotype of two different inducible GEM models, corresponding to *Nkx3.1^{CreERT2/+}; Pten^{flox/flox}*; *Trp53^{flox/flox}* mice (*NPp53* mice) and *Nkx3.1^{CreERT2/+}; Pten^{flox/flox}* mice (*NP* mice, previously described (26)) under normal androgen conditions (hormonally-intact mice), or following androgen-ablation by surgical castration (Fig. 1C; Table S2). As expected, castration resulted in profound reduction of testosterone (T) and dihydrotestosterone (DHT) in both *NP* and *NPp53* mice to barely detectable levels (Fig. 7A). Furthermore, following castration, both the *NP* and *NPp53* mice develop CRPC that retains features of adenocarcinoma (Fig. 1C; Table S2), thereby resembling human CRPC-Adeno, similar to previously reported GEM models based on loss-of-function of *Pten* and *Trp53* (20,21). Notably, the CRPC phenotype in the *NP* and *NPp53* mice shows appropriate expression of epithelial cytokeratins, consistent with the luminal phenotype of prostate adenocarcinoma, and has a high proliferative index (Fig. 1C; Table S2). As expected, AR displays primarily nuclear localization in prostate tumor cells of hormonally-intact mice, but is more diffusely localized in *NP* and *NPp53* CRPC (Fig. 1C).

To extrapolate preclinical studies from these GEM models to human CRPC, we sought to establish whether the molecular pathways that drive CRPC in *NP* and *NPp53* mice are conserved with those that drive CRPC in humans. Toward this end, we used the MARINA computational algorithm to identify master regulators (MRs) that drive CRPC in these GEM models, and then performed cross-species computational analyses to evaluate their conservation with MRs that drive human CRPC (see detailed experimental methods and (27)). For this purpose, we generated mouse MR signatures representing the transition to CRPC in the *NP* or *NPp53* mice (*NP* CRPC versus *N* and *NPp53* CRPC versus *N*, respectively, with *N* representing the control *Nkx3.1^{CreERT2}* mice), or comparing the CRPC in *NPp53* and *NP* mice (*NPp53* CRPC versus *NP* CRPC). We then performed cross-species gene set enrichment analysis (GSEA) by querying these mouse MR signatures with several independent human MR signatures that are indicative of specific biological phenotypes of prostate cancer and/or CRPC-Adeno (Table S1).

First, we analyzed whether molecular drivers associated with the transition to CRPC in these GEM models are conserved with those in humans by querying the relevant mouse MR signatures (*NP* CRPC versus *N* and *NPp53* CRPC versus *N*) with a human MR signature

of treatment-naïve androgen-independent primary tumors (n=10) versus androgen-dependent tumors (n = 10) from Best *et al.* (28) (Table S1). Cross-species GSEA revealed a striking enrichment of MR signatures for both mouse models (p<0.001; Fig. 1D), indicating a strong conservation of the molecular drivers of CRPC between the mouse and human tumors.

5 Next, we asked whether CRPC in *NPp53* versus *NP* mice is representative of more advanced, metastatic CRPC in humans, as might be anticipated given the additional *Trp53* loss-of-function. For this we compared a mouse MR signature of *NPp53* CRPC versus *NP* CRPC with a human MR signature from metastatic CRPC (n=35) versus localized prostate cancer (n = 59) from Grasso *et al.* (15) (Table S1). Cross-species GSEA revealed a strong
10 enrichment particularly of the activated MR signatures (NES = 8.66, p < 0.001; Fig. 1E), demonstrating that the molecular drivers of mouse *NPp53* CRPC are conserved with metastatic CRPC in humans.

 Lastly, we asked whether molecular drivers of *NPp53* CRPC are conserved specifically with those of human prostate tumors with reduced levels of *TP53* and *PTEN*. For
15 this purpose, we generated two independent human MR signatures that compare cases having low *PTEN*/low *TP53* (n=5) versus low *PTEN*/high *TP53* (n=5) in primary tumors (from TCGA) and metastatic CRPC (from SU2C) (16,17) (Table S1). We then performed cross-species GSEA using these human signatures to query the analogous mouse MR signature (*NPp53* CRPC versus *NP* CRPC), which revealed a significant enrichment for both human
20 MR signatures (p < 0.001; Figs. 1F, 7B), indicating a high degree of molecular conservation of *NPp53* CRPC-Adeno with human tumors having low *PTEN*/ low *TP53*. Based on the histological and molecular similarity of *NPp53* CRPC to human CRPC and particularly to CRPC with co-mutation of *TP53* and *PTEN*, we reasoned that these GEM models would be informative for preclinical investigations of treatment response for human CRPC.

25 **Preclinical analyses of abiraterone reveals acceleration of the tumor phenotype in *NPp53* CRPC**

 While abiraterone is widely used for treatment of advanced prostate cancer (1), it has not been extensively investigated in preclinical studies *in vivo*. Furthermore, although originally described as a CYP17A1 inhibitor that blocks androgen biosynthesis, abiraterone
30 has also been shown to be metabolized to a potent AR antagonist (29,30), and thus can potentially inhibit androgen signaling at multiple levels. To perform preclinical studies, we first optimized dosing and scheduling parameters using strain-matched mice (Figs. 8A-C), which led us to select a dose of 200 mg/kg/day, which is similar to the dosage used for other mouse strain backgrounds (e.g., (31)). Mass spectrometry analyses confirmed that abiraterone

was both taken up and processed to its active form in wild-type mouse prostate as well as *NPp53* CRPC (Figs. 8C-D). However, since most of the relevant steroid metabolites are below or near the limits of detection of these assays, we were not able to determine whether the AR antagonist form (29,30) is present in the mouse prostate.

5 Preclinical analyses revealed that response to treatment with abiraterone differed considerably between *NP* CRPC and *NPp53* CRPC (Fig. 2A-E). In particular, abiraterone treatment resulted in a modest but significant inhibition of *NP* CRPC, as revealed by analyses of tumor histopathology, reduced cellular proliferation ($p=0.006$, t-test), and reduced tumor volume (an average of 12.5% decrease after treatment) ($n=15$ vehicle-treated and 21
10 abiraterone-treated; Figs. 2B-E, 9A; Table S2). In contrast, *NPp53* CRPC was not inhibited by abiraterone treatment, as evident from their histopathology and lack of reduced cellular proliferation or tumor volume following treatment ($n=21$ vehicle-treated and 28 abiraterone-treated; Fig. 2B-E, Fig. 9A-C; Table S2). Since it has been proposed that the consequences of p53 loss-of-function in prostate cancer are at least partially due to its inactivation in stroma
15 (32), we examined whether the disparate effects of abiraterone treatment on *NP* and *NPp53* CRPC could be attributed to non-cell autonomous effects of stroma by generating epithelial cell lines from treatment-naïve tumors ($n=2$ independent cell lines for each genotype; Fig. 10A). The responses of these epithelial tumor cells to abiraterone *in vitro* and *in vivo* were analogous to those observed for the corresponding *NP* and *NPp53* CRPC tumors (Fig. 10B-
20 D), indicating that the impaired response of *NPp53* CRPC to abiraterone is, at least in part, cell-autonomous.

Strikingly, not only did abiraterone treatment fail to reduce CRPC tumor growth in the *NPp53* mice, a subset of these mice displayed accelerated tumor phenotypes following treatment. In particular, MRI analyses of *NPp53* CRPC prior to and following abiraterone
25 treatment revealed an approximately 2-fold increase in tumor volume after treatment (Table S2). Furthermore, while some of the *NPp53* CRPC mice analyzed by MRI had minimal change in volume ($<10\%$), consistent with their lack of response to treatment ($n=4/16$; Fig. 2D, E; Table S2), most others displayed increased tumor volume ($>10\%$) after treatment, in some cases up to 400% ($n=12/16$; Fig. 2D,E; Table S2); this difference was not due to
30 differential tumor volumes prior to the initiation of treatment (Fig. 9B,C). Moreover, although metastasis was rare in the *NPp53* CRPC mice, several of the abiraterone-treated cases, but none of the vehicle-treated ones, displayed overt metastasis to visceral tissues; notably, these metastatic cases solely occurred in the *NPp53* CRPC mice that displayed increased tumor volume by MRI ($n=4/28$; Table S2).

Furthermore, several of the *NPp53* CRPC tumors displayed variant histologies, including areas of squamous, sarcomatoid, small-cell neuroendocrine-like, and other non-adenocarcinoma phenotypes, which were most prevalent in the abiraterone-treated *NPp53* CRPC mice with increased tumor volume (Figs. 2B, 11; Tables S2, S3). These areas of variant histopathology had reduced expression of AR and epithelial cytokeratins and significantly higher levels of proliferation (>50%) compared with regions of adenocarcinoma in the vehicle-treated mice (p=0.013) or abiraterone-treated mice (p=0.03; Fig. 2B,C; Tables S2,S3). Notably, similar regions of non-adenocarcinoma histopathology were observed in several of the vehicle-treated *NPp53* CRPC tumors, as well as the intact (non-castrated) abiraterone-treated *NPp53* mice (Fig. 11; Tables S2,S3), suggesting that *NPp53* CRPC has a latent potential for non-adenocarcinoma phenotypes that is augmented by abiraterone treatment.

To understand the molecular bases for their distinct phenotypic responses to abiraterone treatment, we performed expression profiling analyses comparing abiraterone- and vehicle-treated CRPC from the *NP* and *NPp53* mice. Non-supervised Principal Component Analysis (PCA) showed that expression profiles from the abiraterone- and vehicle-treated *NP* CRPC clustered separately (Fig. 12A), consistent with their phenotypic response to abiraterone. Furthermore, PCA using expression profiles from abiraterone-treated *NPp53* CRPC tumors, showed that a subset corresponding to those that did not display accelerated tumor phenotypes (Group 1) clustered with the vehicle-treated cases, consistent with their lack of phenotypic response to abiraterone treatment (Fig. 2F). However, the other abiraterone-treated *NPp53* CRPC cases, corresponding to those that displayed accelerated tumor phenotypes (Group 2), were widely dispersed (Fig. 2F), indicating that this distinct phenotypic sub-group has divergent molecular profiles.

To investigate the diversity of response of these distinct subgroups of abiraterone-treated *NPp53* CRPC to human CRPC, we performed single-sample GSEA (ssGSEA). In particular, we first compared each individual abiraterone-treated *NP* or *NPp53* CRPC tumor sample to the pool of corresponding vehicle-treated tumors (*NP* or *NPp53*, respectively), defining a signature for each individual sample. We then performed cross-species ssGSEA comparing these individual signatures to a gene expression signature of human androgen-independent versus androgen-dependent prostate cancer from Best *et al.* (Table S1 (28)). These analyses revealed that the Group 2 abiraterone-treated *NPp53* CRPC with accelerated tumor phenotypes has a strong positive enrichment in human CRPC, whereas the Group 1 *NPp53* CRPC and the *NP* CRPC were not strongly enriched (Fig. 2G).

Cross-species computational analyses of adverse treatment response

The data described above define a subgroup of abiraterone-treated *NPp53* CRPC with accelerated tumor phenotypes and distinct molecular profiles that are highly enriched for genes expressed in human CRPC. Based on their distinct histopathological and molecular phenotypes, we term these Group 2 abiraterone-treated *NPp53* CRPC as “exceptional non-responders” to distinguish them from the Group 1 “non-responder” *NPp53* CRPC tumors and the “responder” *NP* CRPC tumors. Thus, in subsequent analyses, we consider these *NPp53* CRPC “exceptional non-responder” and “non-responder” groups separately, and compare these to the *NP* CRPC “responder” group.

In previous studies, it was demonstrated that cross-species analysis of treatment response in GEM models can identify candidate molecular drivers (master regulators, MRs) that are informative for stratifying human patients based on treatment response and/or disease outcome (33). In particular, the data illustrated that expression profiles comparing pre- versus post-treatment of GEM models can be used as a quantitative measure of drug response, such that treatments that inhibit tumor phenotypes (in “responders”) result in reversion of the MR signatures in the post-treatment group, which can be quantified by the down-regulation of activated target genes and up-regulation of repressed target genes (33) (Fig. 3A, Scenario 1). In contrast, “non-responders” are phenotypically and molecularly similar to the vehicle-treated controls, and have signatures that are essentially unchanged post-treatment (Fig. 3A, Scenario 2). Experiments were designed to illustrate that “exceptional non-responders” would display an enhancement of the MR signature (Fig. 3A, Scenario 3), which might be associated with adverse treatment response and/or adverse disease outcome in human CRPC.

To test this concept, cross-species analyses were performed to query MR signatures from relevant human CRPC datasets, using reference signatures based on these distinct groups of “responder”, “non-responder”, and “exceptional non-responder” mouse tumors. In particular, we performed MARINA to generate mouse MR signatures corresponding to: (i) the “responder” abiraterone- versus vehicle-treated *NP* tumors (Scenario 1); (ii) the “non-responder” (group 1) abiraterone- versus vehicle-treated *NPp53* tumors (Scenario 2); and (iii) the “exceptional non-responder” (group 2) abiraterone- versus vehicle-treated *NPp53* tumors (Scenario 3). We then performed cross-species GSEA using each of these reference mouse MR signatures to query two independent human CRPC MR signatures: (i) a signature comparing bone metastases from CRPC to hormone-naïve prostate primary tumors from the Balk dataset (34), and (ii) a signature comparing androgen-independent and androgen-dependent prostate tumors from the Best dataset, as described above (28) (Table S1).

We found that the MR signature of the “exceptional non-responder” *NPp53* tumors was strongly positively enriched in the human MR signatures from both the Balk (NES = 7.22, $p < 0.001$) and Best (NES = 5.12 $p < 0.001$) datasets (Fig. 3B; Fig. 12B). In contrast, the MR signature of “responder” *NP* tumors was reverted in both human MR signatures (Balk NES = 7.45, $p < 0.001$; Best NES = 7.79 $p < 0.001$), whereas the “non-responder” *NPp53* signature displayed minimal enrichment in either human MR signature (Fig. 3B; Fig. 12B). These findings are consistent with our hypothesis (Fig. 3A), and indicate that the exceptional non-responders have molecular drivers that are conserved with human CRPC.

To extend these analyses to treatment response, we generated a human MR signature comparing CRPC tumors with neuroendocrine differentiation (CRPC-NE; $n=15$), most of which were treatment-related, to CRPC tumors with adenocarcinoma (CRPC-Adeno; $n=34$), as reported by Beltran *et al.* (5). We queried this human signature with a mouse MR signature comparing the “exceptional non-responder” abiraterone-treated *NPp53* tumors to the “responder” *NP* tumors, which revealed a significant enrichment in both the up-regulated and down-regulated MRs (NES=3.36 $p = 0.006$, and NES = -4.03 $p < 0.001$, respectively) (Fig. 3C). These findings indicate strong conservation of adverse treatment response in mouse CRPC with drivers of treatment failure and neuroendocrine differentiation in human prostate cancer, and identify conserved MRs that drive the CRPC-NE phenotype, which we refer to as “adverse treatment response MRs” (Table S4). To evaluate whether these adverse treatment response MRs are associated with disease outcome, we used the Sboner *et al.* dataset, which is one of the few published cohorts with extensive clinical outcome data, including disease-specific death due to prostate cancer (Table S1) (35). Kaplan-Meier survival analysis using this dataset revealed that patients with higher activity levels of the adverse treatment-response MRs had a shorter time to prostate cancer-specific death compared to those with lower activity levels (log-rank p -value = 8.32×10^{-6}) (Fig. 3D), providing clinical validation that these adverse treatment response MRs are relevant for disease outcome in human prostate cancer.

Cumulatively, these phenotypic and computational analyses define a sub-group of “exceptional non-responders” in the mouse that are conserved with more aggressive variants of human CRPC, including CRPC-NE. Furthermore, the molecular drivers of this phenotype, namely the adverse treatment-response MRs, are conserved with human CRPC, enriched in patients that develop CRPC-NE, and associated with adverse outcome for human prostate cancer. Therefore, these adverse treatment-response MRs may help identify patients with aggressive prostate cancer and/or who are predisposed to fail treatment with abiraterone.

Neuroendocrine differentiation in *TP53*-deficient CRPC is mediated in part by *SOX11*

These findings suggest that the treatment-related *NPp53* CRPC phenotype shares molecular features in common with human CRPC-NE. Indeed, we found that expression profiles of *NPp53* “exceptional non-responders” displayed significant up-regulation of genes that have been shown to be expressed in human CRPC-NE (6) ($p < 0.05$, t-test) (Fig. 4A). Therefore, we queried the adverse treatment response MRs (Table S4) to identify candidate MRs that might contribute to the CRPC-NE phenotype. Among these, we focused on *SOX11*, a member of the SoxC subclass of HMG-box transcriptional regulators that functions in a wide range of neural and mesenchymal progenitors during organogenesis and is also a pan-neuronal differentiation factor (36-38). Notably, *SOX11* has been shown to be regulated by *TP53* in other contexts (39), and was one of the top up-regulated genes between mouse *NP* and *NPp53* CRPC (p -value = 0.0003, t-test; Dataset 1). Furthermore, *Sox11* was also the most up-regulated gene in the TRAMP mouse model of prostate cancer in the transition from adenocarcinoma to neuroendocrine disease (40).

We found that *SOX11* expression is significantly up-regulated in comparing higher versus lower Gleason grade primary tumors ($p = 0.028$, t-test), as reported by TCGA (16), as well as in CRPC-NE relative to CRPC-Adeno ($p = 0.012$, t-test), as reported by Beltran *et al.* (5) (Fig. 4B). Furthermore, target genes that are predicted to be up-regulated by *SOX11* in the human prostate cancer interactome are significantly enriched in CRPC-NE versus CRPC-Adeno (NES 4.31, $p < 0.001$; Fig. 4C). We found that *Sox11* expression was also up-regulated in mouse *NPp53* CRPC relative to *NP* CRPC ($p < 0.01$, t-test), and particularly in the exceptional non-responders (Fig. 13A). In addition, *Sox11* expression was up-regulated in mouse epithelial cell lines established from these *NPp53* tumors relative to lines from *NP* tumors ($p < 0.01$, t-test), and was correlated with expression of the neuroendocrine marker neuron-specific enolase (NSE) ($p < 0.001$, t-test; Fig. 4D). Furthermore, we found that shRNA-mediated knock-down of *Sox11* in the *NPp53* mouse cell lines resulted in down-regulation of neuroendocrine markers, such as *NSE* and *Synaptophysin* ($p < 0.01$, t-test; Fig. 4E), while not resulting in reduced expression of other *Sox* genes, such as *Sox2* or *Sox7* (Fig. 13B). Taken together, these findings suggest that neuroendocrine differentiation in treatment-related *NPp53* CRPC is mediated at least in part by *Sox11*.

Focal and overt neuroendocrine differentiation arise through transdifferentiation of luminal prostate epithelial cells

Given these molecular findings showing that *NPp53* CRPC shares features in common with human CRPC-NE, we investigated whether the *NPp53* CRPC tumors display

neuroendocrine differentiation by immunostaining for Synaptophysin, a neuroendocrine marker that is rarely expressed in hormonally-intact prostate adenocarcinoma. In particular, we determined the relative abundance of Synaptophysin-positive (Syn⁺) cells in tumors from *NP* and *NPp53* mice that were hormonally-intact, castrated, and castrated with abiraterone treatment (Figs. 5A-B; Table S5A). Although Syn⁺ cells were very rare in *NP* tumors in all cases (less than 1% in all contexts, n = 3 to 7/group) as well as in *NPp53* intact tumors (0.34%; n=7, one-way ANOVA), they were significantly more abundant in both the castrated (2.54%; n=11; p<0.05, one-way ANOVA) and castrated and abiraterone-treated *NPp53* mice (4.03%; n=12, p<0.001, one-way ANOVA). In some cases, however, the Syn⁺ cells were extremely abundant, comprising up to 99% of the total cells in the region (Figs. 5B-C; Table S5A).

In the non-responder mice, we mostly observed small patches of Syn⁺ cells within regions of adenocarcinoma (Fig. 5A), consistent with focal neuroendocrine differentiation. These Syn⁺ cells also co-expressed other neuroendocrine markers such as Chromogranin A and Foxa2; they also expressed the luminal marker cytokeratin 8 (CK8), albeit at lower levels, but not the basal cell marker CK5, and expressed low levels of AR compared to the surrounding non-Syn⁺ cells (Fig. 5C). Notably, in these regions of focal neuroendocrine differentiation in castrated or castrated plus abiraterone-treated mice, the Syn⁺ cells never co-expressed the proliferation marker Ki67 (0/357 and 0/560 cells, respectively; Fig. 5C; Table S5B).

In contrast, exceptional non-responder tumors displayed some regions in which Syn⁺ cells comprised the bulk of tumor cells (>70% of tumor cells) (Fig. 5C; Table S5B). In these regions of overt neuroendocrine differentiation, the Syn⁺ cells phenotypically resemble those found in focal differentiation, but were completely lacking AR expression (Fig. 5C). Notably, however, the Syn⁺ cells in areas of overt neuroendocrine differentiation are highly proliferative, as shown by Ki67 co-expression (44%, n=461/1038 Ki67 positive cells; Fig. 5C; Table S5B).

Finally, we sought to determine the cellular origin of the Syn⁺ cells in both the focal and overt regions of neuroendocrine differentiation. For this purpose, we performed lineage-tracing using *NPp53* mice that also carried a *R26R-YFP* reporter allele. Since the inducible *Nkx3.1^{CreERT2}* driver is specific for luminal epithelial cells in the adult prostate (19,22), YFP is only expressed by luminal cells and their descendants in *NPp53* tumors. We found that nearly all Syn⁺ cells in *NPp53* tumors co-expressed YFP, demonstrating that these Syn⁺ cells were derived from luminal cells, and not from neuroendocrine or basal cells (n=346/347 cells; Fig.

5C; Table S5B). Furthermore, this was the case for Syn⁺ cells in both the focal and overt regions of neuroendocrine differentiation (n=519/521 in the latter; Fig. 5C; Table S5B), despite the considerable differences in the proliferative status of the Syn⁺ cells and tumor phenotype between these groups. Therefore, these findings provide direct genetic evidence in a mouse model of CRPC-NE that both focal and overt neuroendocrine differentiation arises by transdifferentiation of luminal prostate adenocarcinoma cells.

Discussion

The elucidation of the biological and molecular processes that underlie adverse treatment response and identification of patients that are likely to fail treatment represent fundamental clinical challenges that are particularly germane for prostate cancer. In the current study, we have used an integrative approach that combines phenotypic and molecular analyses of mouse and human CRPC to investigate the underlying causes of treatment failure for abiraterone, an anti-androgen that is now widely used in the clinic (1). Our findings demonstrate that the *NPp53* mouse model of CRPC recapitulates key phenotypic and molecular characteristics of human CRPC. Further, preclinical analyses of *NPp53* CRPC reveal that these tumors fail to respond to abiraterone, suggesting that CRPC with co-inactivation of *PTEN* and *TP53*, which is frequent in humans, may be inherently less responsive to abiraterone. Moreover, we observed that many of the *NPp53* CRPC tumors were actually accelerated in their phenotype by abiraterone treatment. These “exceptional non-responders” display highly aggressive histopathological phenotypes that share molecular and phenotypic features in common with treatment-related CRPC with neuroendocrine differentiation (CRPC-NE) in humans (Fig. 6). Furthermore, cross-species computational analysis has identified treatment response regulators that are associated with adverse disease outcome in human CRPC-NE, which may enable identification of patients prior to treatment who are at risk for developing CRPC-NE.

To date, it has been unclear whether focal versus overt neuroendocrine differentiation in CRPC represent two distinct entities at the phenotypic and/or molecular level. Although neuroendocrine differentiation is rare in primary prostate cancer, it can be occasionally observed in CRPC as sporadic small foci of cells expressing neuroendocrine markers (41,42). However, the recent widespread clinical use of anti-androgens has selected for the emergence of CRPC-NE, which features large regions of overt neuroendocrine differentiation typically having small cell histology and neuroendocrine marker expression (4,5). Our current results model focal and overt neuroendocrine differentiation in the “non-responder” and “exceptional non-responder” *NPp53* CRPC mice, respectively. We find that a distinguishing feature of

focal neuroendocrine differentiation is that the neuroendocrine-like cells are non-proliferative, in striking contrast to overt neuroendocrine differentiation, which is highly proliferative. Based on these observations, we suggest that a stochastic event during tumor progression promotes proliferation of neuroendocrine-like cells, which are consequently selected by abiraterone treatment since they completely lack AR expression (Fig. 6). We propose that such a “proliferative switch” might represent a key molecular event in the emergence of CRPC-NE.

Our study provides new insights into the roles of *TP53* and *PTEN*, and their relationship to other relevant drivers, in suppressing cellular plasticity and neuroendocrine differentiation in prostate cancer, which is significant since co-mutation of *TP53* and *PTEN* is considerably more prevalent than that of *RB* and *TP53* in advanced prostate cancer. Indeed, previous studies have reported that dysregulation of *TP53* and/or *RB* and/or *PTEN* is associated with the transition to small-cell neuroendocrine-like tumors in human prostate cancer (7). Notably, there has been little precedent for a role of *PTEN* in modulating cellular plasticity, which deserves further investigation. In contrast, a general role for the p53 pathway in regulating cellular reprogramming has been previously suggested by studies showing that inhibition of p53 pathway activity results in increased efficiency in the formation of induced pluripotent stem cells (43-47). Moreover, the specific functional relevance of *RB* and *TP53* in neuroendocrine differentiation has been previously demonstrated in a GEM model based on their combined loss-of-function (24), as well as in the TRAMP and LADY mouse models, in which inactivation of *RB* and *TP53* results in adenocarcinoma and neuroendocrine differentiation (48,49). In the current study, we find that *Rb1* is significantly reduced in expression in *NPp53* CRPC and particularly those cases with overt neuroendocrine differentiation (Fig. 13A). Furthermore, two recent studies have shown that combined loss of *RB* and *TP53* in human and mouse models leads to altered sensitivity to anti-androgen treatment and up-regulation of *SOX2*, which promotes epithelial plasticity (50,51). Interestingly, combined loss of *RB* and *TP53* facilitates neuroendocrine differentiation via up-regulation of *SOX2* in a model of small cell lung cancer (52), suggesting that there may be common mechanisms in the transition to neuroendocrine disease across cancer types.

The findings of our study suggest that *SOX11*, a known target of *TP53*, is likely to be a key modulator of neuroendocrine differentiation in CRPC-NE. Notably, distinct Sox transcription factors are believed to act sequentially during neurogenesis, with members of the SoxB1 subclass such as Sox2 functioning to maintain the neural progenitor state and

inhibit differentiation, whereas SoxC factors such as Sox11 function later to promote neuronal differentiation (36,53). By analogy, we propose that Sox2 may act early in the emergence of CRPC-NE to promote epithelial plasticity (51), whereas Sox11 may act at subsequent stages to promote neuroendocrine differentiation. Interestingly, we find that Sox2 expression is reduced in the *NPp53* CRPC relative to *NP* CRPC (Fig. 13A). Among the predicted targets of SOX11 are *POU3F2* (*BRN2*), which can drive a neuroendocrine phenotype in prostate cancer xenografts (54), as well as *MYCN* (6), which promotes the formation of a CRPC-NE phenotype in relevant human and mouse models in collaboration with *Pten* loss-of-function or activation of AKT kinase activity (55,56). Interestingly, these activities of *MYCN* contrast with those of *MYC* (*c-Myc*), which collaborates with *Pten* loss-of-function in mouse models to generate highly aggressive adenocarcinoma and metastasis but not neuroendocrine differentiation (57).

Our analyses provide definitive and quantitative evidence that both focal and overt neuroendocrine differentiation in prostate cancer occurs through transdifferentiation from luminal tumor cells (Fig. 6). In particular, we have demonstrated transdifferentiation by lineage-tracing, which represents the “gold standard” approach for analyses of cell fate specification *in vivo* (58). Thus, our findings extend and greatly strengthen the conclusions of previous work that had suggested that CRPC-NE arises from luminal adenocarcinoma cells, based upon sequence analyses and detection of the *TMPRSS2-ERG* translocation by fluorescent *in situ* hybridization (5,6). Furthermore, the common origin of both focal and overt neuroendocrine differentiation from luminal cells may be consistent with a linear pathway for their emergence (Fig. 6). However, it remains conceivable that focal versus overt neuroendocrine differentiation can arise from distinct luminal subpopulations within CRPC-Adeno tumors.

Interestingly, transdifferentiation has been implicated as a potential cause of drug resistance in a clinical setting for non-small cell lung cancer with alterations of *TP53* and *RB1* (59,60). Our current findings provide direct experimental evidence for transdifferentiation in mediating drug resistance, and extend the generality of this mechanism to other tumor types and cancer drivers. Thus, perturbations of key conserved pathways that regulate cellular plasticity and differentiation in normal developmental contexts may represent significant mechanisms for driving drug resistance in cancer.

Methods

Preclinical and phenotypic analyses of genetically engineered mouse (GEM) models

All experiments using animals were conducted according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Columbia University Medical Center. Mouse alleles were obtained from the NCI Mouse Models of Human Cancer Consortium Repository on the world wide web at (mouse.ncifcrf.gov/) or the Jackson Laboratory (www.jax.org) and maintained in a mixed C57BL/6/129S strain background. Abiraterone-acetate was provided by Johnson & Johnson. Optimal dosage, pharmacokinetic profile, and optimal scheduling were determined using non-tumor bearing littermates (Fig. 8). Preclinical studies were done using tamoxifen-induced *NP* and *NPp53* mice that had been surgically castrated. Treatment was initiated two months after castration and continued for four weeks following which prostate tissues were collected for analyses (see detailed experimental procedures).

Semi-quantitative analysis of histological phenotypes is summarized in Table S3. Immunostaining was done as described (22); quantification was done using at least 5 sections per mouse and from at least 3 independent mice per group, and summarized in Table S5. Magnetic Resonance Imaging (MRI) was done using a Bruker Biospec 9.4T Tesla Small Animal MR Imager. Levels of steroids and abiraterone in prostate tissues and serum were determined by mass spectrometry. Allograft studies were done using cell lines generated from treatment-naïve *NP* or *NPp53* tumors (see detailed experimental procedures), which were implanted into the flank of immunodeficient NCr nude mice (Taconic) followed by treatment with abiraterone. All antibodies used for this study are provided in Table S6; all primers are described in Table S7.

Gene expression profiling and computational analyses

Gene expression profiling of mouse CRPC was done using RNA sequencing on an Illumina HiSeq 2500 platform; a complete list of differentially expressed genes is provided in Dataset 1 (61). Published human datasets used in these studies for cross-species analyses are described in Table S1. Master regulator analysis was performed using the MAster Regulator INference algorithm (MARINa) to interrogate a human prostate cancer interactome, as described (27). Cross-species gene GSEA and single sample GSEA (ssGSEA) were done using “humanized” mouse signatures and human MR signatures, as described (27).

Statistical analyses

Statistical analyses were performed using a two-tailed t-test, one-way ANOVA, X2 test, and Fisher’s Exact test as appropriate. GraphPad Prism software (Version 6.0) and R-

studio 0.99.902, R v3.3.0, were used for statistical calculations and data visualization. COX proportional hazard model and Kaplan-Meier analysis were done with the surv and coxph functions from survcomp package (Bioconductor).

Accession numbers

- 5 Mouse expression profiling data are deposited in the Gene Expression Omnibus (GEO) database (GSE92721) (61).

References

1. Attard G, Parker C, Eeles RA, Schroder F, Tomlins SA, Tannock I, *et al.* Prostate cancer. *Lancet* **2016**;387(10013):70-82 doi 10.1016/S0140-6736(14)61947-4.
- 10 2. Scher HI, Solo K, Valant J, Todd MB, Mehra M. Prevalence of Prostate Cancer Clinical States and Mortality in the United States: Estimates Using a Dynamic Progression Model. *PLoS One* **2015**;10(10):e0139440 doi 10.1371/journal.pone.0139440.
3. Scher HI, Sawyers CL. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. *J Clin Oncol* 15 **2005**;23(32):8253-61.
4. Watson PA, Arora VK, Sawyers CL. Emerging mechanisms of resistance to androgen receptor inhibitors in prostate cancer. *Nat Rev Cancer* **2015**;15(12):701-11 doi 10.1038/nrc4016.
- 20 5. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, *et al.* Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat Med* **2016**;22(3):298-305 doi 10.1038/nm.4045.
6. Beltran H, Rickman DS, Park K, Chae SS, Sboner A, MacDonald TY, *et al.* Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. *Cancer Discov* **2011**;1(6):487-95 doi 10.1158/2159-8290.CD-11-0130.
- 25 7. Aparicio AM, Shen L, Tapia EL, Lu JF, Chen HC, Zhang J, *et al.* Combined Tumor Suppressor Defects Characterize Clinically Defined Aggressive Variant Prostate Cancers. *Clin Cancer Res* **2016**;22(6):1520-30 doi 10.1158/1078-0432.CCR-15-1259.
- 30 8. Aggarwal R, Zhang T, Small EJ, Armstrong AJ. Neuroendocrine prostate cancer: subtypes, biology, and clinical outcomes. *J Natl Compr Canc Netw* **2014**;12(5):719-26.

9. Humphrey PA. Histological variants of prostatic carcinoma and their significance. *Histopathology* **2012**;60(1):59-74 doi 10.1111/j.1365-2559.2011.04039.x.
10. Berman-Booty LD, Knudsen KE. Models of neuroendocrine prostate cancer. *Endocr Relat Cancer* **2015**;22(1):R33-49 doi 10.1530/ERC-14-0393.
- 5 11. Terry S, Beltran H. The many faces of neuroendocrine differentiation in prostate cancer progression. *Front Oncol* **2014**;4:60 doi 10.3389/fonc.2014.00060.
12. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, *et al.* Integrative genomic profiling of human prostate cancer. *Cancer Cell* **2010**;18(1):11-22 doi 10.1016/j.ccr.2010.05.026.
- 10 13. Baca SC, Prandi D, Lawrence MS, Mosquera JM, Romanel A, Drier Y, *et al.* Punctuated evolution of prostate cancer genomes. *Cell* **2013**;153(3):666-77 doi 10.1016/j.cell.2013.03.021.
14. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, *et al.* Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nat Genet* **2012**;44(6):685-9 doi 10.1038/ng.2279.
- 15 15. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, *et al.* The mutational landscape of lethal castration-resistant prostate cancer. *Nature* **2012**;487(7406):239-43 doi 10.1038/nature11125.
16. Network CGAR. The Molecular Taxonomy of Primary Prostate Cancer. *Cell* **2015**;163(4):1011-25 doi 10.1016/j.cell.2015.10.025.
- 20 17. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, *et al.* Integrative clinical genomics of advanced prostate cancer. *Cell* **2015**;161(5):1215-28 doi 10.1016/j.cell.2015.05.001.
18. Fraser M, Sabelnykova VY, Yamaguchi TN, Heisler LE, Livingstone J, Huang V, *et al.* Genomic hallmarks of localized, non-indolent prostate cancer. *Nature* **2017**;541(7637):359-64 doi 10.1038/nature20788.
- 25 19. Wang X, Kruthof-de Julio M, Economides KD, Walker D, Yu H, Halili MV, *et al.* A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature* **2009**;461(7263):495-500 doi 10.1038/nature08361.
- 30 20. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, *et al.* Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature* **2005**;436(7051):725-30.
21. Lunardi A, Ala U, Epping MT, Salmena L, Clohessy JG, Webster KA, *et al.* A co-clinical approach identifies mechanisms and potential therapies for androgen

- deprivation resistance in prostate cancer. *Nat Genet* **2013**;45(7):747-55 doi 10.1038/ng.2650.
22. Toivanen R, Mohan A, Shen MM. Basal Progenitors Contribute to Repair of the Prostate Epithelium Following Induced Luminal Anoikis. *Stem Cell Reports* **2016**;6(5):660-7 doi 10.1016/j.stemcr.2016.03.007.
 23. Wang ZA, Toivanen R, Bergren SK, Chambon P, Shen MM. Luminal cells are favored as the cell of origin for prostate cancer. *Cell Rep* **2014**;8(5):1339-46 doi 10.1016/j.celrep.2014.08.002.
 24. Zhou Z, Flesken-Nikitin A, Corney DC, Wang W, Goodrich DW, Roy-Burman P, *et al.* Synergy of p53 and Rb deficiency in a conditional mouse model for metastatic prostate cancer. *Cancer Res* **2006**;66(16):7889-98 doi 10.1158/0008-5472.CAN-06-0486.
 25. Couto SS, Cao M, Duarte PC, Banach-Petrosky W, Wang S, Romanienko P, *et al.* Simultaneous haploinsufficiency of Pten and Trp53 tumor suppressor genes accelerates tumorigenesis in a mouse model of prostate cancer. *Differentiation* **2009**;77(1):103-11 doi 10.1016/j.diff.2008.09.010.
 26. Floc'h N, Kinkade CW, Kobayashi T, Aytes A, Lefebvre C, Mitrofanova A, *et al.* Dual targeting of the Akt/mTOR signaling pathway inhibits castration-resistant prostate cancer in a genetically engineered mouse model. *Cancer Res* **2012**;72(17):4483-93 doi 10.1158/0008-5472.CAN-12-0283.
 27. Aytes A, Mitrofanova A, Lefebvre C, Alvarez MJ, Castillo-Martin M, Zheng T, *et al.* Cross-species regulatory network analysis identifies a synergistic interaction between FOXM1 and CENPF that drives prostate cancer malignancy. *Cancer Cell* **2014**;25(5):638-51 doi 10.1016/j.ccr.2014.03.017.
 28. Best CJ, Gillespie JW, Yi Y, Chandramouli GV, Perlmutter MA, Gathright Y, *et al.* Molecular alterations in primary prostate cancer after androgen ablation therapy. *Clin Cancer Res* **2005**;11(19 Pt 1):6823-34 doi 10.1158/1078-0432.CCR-05-0585.
 29. Li Z, Alyamani M, Li J, Rogacki K, Abazeed M, Upadhyay SK, *et al.* Redirecting abiraterone metabolism to fine-tune prostate cancer anti-androgen therapy. *Nature* **2016**;533(7604):547-51 doi 10.1038/nature17954.
 30. Li Z, Bishop AC, Alyamani M, Garcia JA, Dreicer R, Bunch D, *et al.* Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. *Nature* **2015**;523(7560):347-51 doi 10.1038/nature14406.

31. Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM, *et al.* Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. *Clin Cancer Res* **2011**;17(18):5913-25 doi 10.1158/1078-0432.CCR-11-0728.
- 5 32. Hill R, Song Y, Cardiff RD, Van Dyke T. Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. *Cell* **2005**;123(6):1001-11 doi 10.1016/j.cell.2005.09.030.
33. Mitrofanova A, Aytes A, Zou M, Shen MM, Abate-Shen C, Califano A. Predicting Drug Response in Human Prostate Cancer from Preclinical Analysis of In Vivo
10 Mouse Models. *Cell Rep* **2015**;12(12):2060-71 doi 10.1016/j.celrep.2015.08.051.
34. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, *et al.* Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res* **2006**;66(5):2815-25 doi 10.1158/0008-5472.CAN-05-4000.
- 15 35. Sbomer A, Demichelis F, Calza S, Pawitan Y, Setlur SR, Hoshida Y, *et al.* Molecular sampling of prostate cancer: a dilemma for predicting disease progression. *BMC Med Genomics* **2010**;3:8 doi 1755-8794-3-8 [pii] 10.1186/1755-8794-3-8.
36. Bergsland M, Werme M, Malewicz M, Perlmann T, Muhr J. The establishment of neuronal properties is controlled by Sox4 and Sox11. *Genes Dev* **2006**;20(24):3475-
20 86 doi 10.1101/gad.403406.
37. Bhattaram P, Penzo-Mendez A, Sock E, Colmenares C, Kaneko KJ, Vassilev A, *et al.* Organogenesis relies on SoxC transcription factors for the survival of neural and mesenchymal progenitors. *Nat Commun* **2010**;1:9 doi 10.1038/ncomms1008.
38. Sock E, Rettig SD, Enderich J, Bosl MR, Tamm ER, Wegner M. Gene targeting
25 reveals a widespread role for the high-mobility-group transcription factor Sox11 in tissue remodeling. *Mol Cell Biol* **2004**;24(15):6635-44 doi 10.1128/MCB.24.15.6635-6644.2004.
39. Akdemir KC, Jain AK, Allton K, Aronow B, Xu X, Cooney AJ, *et al.* Genome-wide profiling reveals stimulus-specific functions of p53 during differentiation and DNA
30 damage of human embryonic stem cells. *Nucleic Acids Res* **2014**;42(1):205-23 doi 10.1093/nar/gkt866.
40. Kela I, Harmelin A, Waks T, Orr-Urtreger A, Domany E, Eshhar Z. Interspecies comparison of prostate cancer gene-expression profiles reveals genes associated with aggressive tumors. *Prostate* **2009**;69(10):1034-44 doi 10.1002/pros.20950.

41. Berruti A, Mosca A, Porpiglia F, Bollito E, Tucci M, Vana F, *et al.* Chromogranin A expression in patients with hormone naive prostate cancer predicts the development of hormone refractory disease. *The Journal of urology* **2007**;178(3 Pt 1):838-43; quiz 1129 doi 10.1016/j.juro.2007.05.018.
- 5 42. Hirano D, Okada Y, Minei S, Takimoto Y, Nemoto N. Neuroendocrine differentiation in hormone refractory prostate cancer following androgen deprivation therapy. *Eur Urol* **2004**;45(5):586-92; discussion 92 doi 10.1016/j.eururo.2003.11.032.
43. Utikal J, Polo JM, Stadtfeld M, Maherali N, Kulalert W, Walsh RM, *et al.* Immortalization eliminates a roadblock during cellular reprogramming into iPS cells.
10 *Nature* **2009**;460(7259):1145-8 doi 10.1038/nature08285.
44. Marion RM, Strati K, Li H, Murga M, Blanco R, Ortega S, *et al.* A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature* **2009**;460(7259):1149-53 doi 10.1038/nature08287.
45. Kawamura T, Suzuki J, Wang YV, Menendez S, Morera LB, Raya A, *et al.* Linking
15 the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature* **2009**;460(7259):1140-4 doi 10.1038/nature08311.
46. Rasmussen MA, Holst B, Tumer Z, Johnsen MG, Zhou S, Stummann TC, *et al.* Transient p53 suppression increases reprogramming of human fibroblasts without affecting apoptosis and DNA damage. *Stem Cell Reports* **2014**;3(3):404-13 doi
20 10.1016/j.stemcr.2014.07.006.
47. Yi L, Lu C, Hu W, Sun Y, Levine AJ. Multiple roles of p53-related pathways in somatic cell reprogramming and stem cell differentiation. *Cancer Res* **2012**;72(21):5635-45 doi 10.1158/0008-5472.CAN-12-1451.
48. Greenberg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD, Aspinall JO, *et al.*
25 *Prostate cancer in a transgenic mouse. Proc Natl Acad Sci U S A* **1995**;92(8):3439-43.
49. Masumori N, Thomas TZ, Chaurand P, Case T, Paul M, Kasper S, *et al.* A probasin-large T antigen transgenic mouse line develops prostate adenocarcinoma and neuroendocrine carcinoma with metastatic potential. *Cancer Res* **2001**;61(5):2239-49.
50. Ku SY, Rosario S, Wang Y, Mu P, Seshadri M, Goodrich ZW, *et al.* Rb1 and Trp53
30 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. *Science* **2017**;355(6320):78-83 doi 10.1126/science.aah4199.
51. Mu P, Zhang Z, Benelli M, Karthaus WR, Hoover E, Chen CC, *et al.* SOX2 promotes lineage plasticity and antiandrogen resistance in TP53- and RB1-deficient prostate cancer. *Science* **2017**;355(6320):84-8 doi 10.1126/science.aah4307.

52. Ferone G, Song JY, Sutherland KD, Bhaskaran R, Monkhorst K, Lambooi JP, *et al.* SOX2 Is the Determining Oncogenic Switch in Promoting Lung Squamous Cell Carcinoma from Different Cells of Origin. *Cancer Cell* **2016**;30(4):519-32 doi 10.1016/j.ccell.2016.09.001.
- 5 53. Bergsland M, Ramskold D, Zaouter C, Klum S, Sandberg R, Muhr J. Sequentially acting Sox transcription factors in neural lineage development. *Genes Dev* **2011**;25(23):2453-64 doi 10.1101/gad.176008.111.
54. Bishop JL, Thaper D, Vahid S, Davies A, Ketola K, Kuruma H, *et al.* The Master Neural Transcription Factor BRN2 Is an Androgen Receptor-Suppressed Driver of
10 Neuroendocrine Differentiation in Prostate Cancer. *Cancer Discov* **2017**;7(1):54-71 doi 10.1158/2159-8290.CD-15-1263.
55. Dardenne E, Beltran H, Benelli M, Gayvert K, Berger A, Puca L, *et al.* N-Myc Induces an EZH2-Mediated Transcriptional Program Driving Neuroendocrine Prostate Cancer. *Cancer Cell* **2016**;30(4):563-77 doi 10.1016/j.ccell.2016.09.005.
- 15 56. Lee JK, Phillips JW, Smith BA, Park JW, Stoyanova T, McCaffrey EF, *et al.* N-Myc Drives Neuroendocrine Prostate Cancer Initiated from Human Prostate Epithelial Cells. *Cancer Cell* **2016**;29(4):536-47 doi 10.1016/j.ccell.2016.03.001.
57. Hubbard GK, Mutton LN, Khalili M, McMullin RP, Hicks JL, Bianchi-Frias D, *et al.* Combined MYC Activation and Pten Loss Are Sufficient to Create Genomic
20 Instability and Lethal Metastatic Prostate Cancer. *Cancer Res* **2016**;76(2):283-92 doi 10.1158/0008-5472.CAN-14-3280.
58. Kretzschmar K, Watt FM. Lineage tracing. *Cell* **2012**;148(1-2):33-45 doi 10.1016/j.cell.2012.01.002.
59. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, *et al.* Genotypic and histological evolution of lung cancers acquiring resistance to EGFR
25 inhibitors. *Sci Transl Med* **2011**;3(75):75ra26 doi 10.1126/scitranslmed.3002003.
60. Niederst MJ, Sequist LV, Poirier JT, Mermel CH, Lockerman EL, Garcia AR, *et al.* RB loss in resistant EGFR mutant lung adenocarcinomas that transform to small-cell lung cancer. *Nat Commun* **2015**;6:6377 doi 10.1038/ncomms7377.
- 30 61. Zou, Min *et al.* Transdifferentiation as a Mechanism of Treatment Resistance in a Mouse Model of Castration-resistant Prostate Cancer. *Cancer Discovery*; April 14 2017 DOI: 10.1158/2159-8290.CD-16-1174.

Table 31: Description of human datasets used in this study				
Name	Description and uses	n*	Platform	Geo/Ref
TCGA	<u>Description:</u> - Surgical resection biospecimens from prostate adenocarcinoma without prior treatment <u>Use:</u> - To demonstrate genomic alterations of <i>PTEN</i> and <i>TP53</i> in localized prostate cancer - To generate MR signatures from primary tumors having low <i>PTEN</i>low <i>TP53</i> versus low <i>PTEN</i>high <i>TP53</i>	333	Illumina HiSeq 2500	TCGA Data Portal (7)
SU2C	<u>Description:</u> - Bone or soft tissue tumor biopsies from metastatic castration-resistant prostate cancer (CRPC) <u>Uses:</u> - To demonstrate genomic alterations of <i>PTEN</i> and <i>TP53</i> in CRPC - To generate MR signatures from CRPC having low <i>PTEN</i>low <i>TP53</i> versus low <i>PTEN</i>high <i>TP53</i>	150	Illumina HiSeq 2500	dbGap: phs000915.v1.p1 (8)
Best et al.	<u>Description:</u> - Treatment naïve androgen-independent primary tumors from biopsies - Treatment naïve androgen-dependent primary tumors from prostatectomies <u>Use:</u> - To generate MR signatures of treatment naïve androgen-independent versus androgen-dependent primary disease - For single-sample GSEA analysis	10 10	Affymetrix Human Genome U133A Array	Geo: GSE2443 (9)
Grasso et al.	<u>Description:</u> - Rapid autopsy specimens from metastatic CRPC - Prostatectomy specimens from localized prostate cancer - Rapid autopsy specimens from matched benign prostate tissues <u>Use:</u> - To generate MR signatures of metastatic CRPC versus primary tumors	35 59 28	Agilent Human Genome 44K Array	Geo: GSE35988 (10)
Balk et al.	<u>Description:</u> - Bone metastases from CRPC obtained from bone marrow biopsies - Hormone treatment naïve prostate tumors isolated from frozen biopsies <u>Use:</u> - To generate MR signatures of metastatic CRPC bone metastasis versus primary tumors	29 22	Affymetrix Human Genome U133A Array	Geo: GSE32269 (11)
Beltran et al.	<u>Description:</u> - Adenocarcinoma CRPC - Neuroendocrine CRPC <u>Use:</u> - To generate MR signatures of CRPC-NE versus CRPC-Adeno	34 15	Illumina HiSeq 2500	dbGap: phs000909.v.p1 (12)

Sporer et al.	<u>Description:</u> - Transurethral specimens of patients from the Swedish waiting-watchful cohort - One of the few datasets that has extensive clinical follow-up data for a 20-year period including the outcome of death due to prostate cancer. <u>Use:</u> - For clinical validation, relative to disease-specific survival	261	Human 6K Transcription-Informative Panel for DASL	Geo: GSE16560 (13)
*n = number of cases in the cohort				

Table S2: Summary of preclinical studies of GEM models											
GEM	Treatment Group	Total N	Weight (grams)	MRI (mm ³)			Frequency and % area non-Adenocarcinoma	% Ki67+ cells			Mets
				N	Pre-treat	Post-treat		N	%Adeno	% Other	
NP	Intact w/ vehicle	9	0.62 ± 0.31	4	1120.05 ± 891.73	1283.97 ± 772.45	11.11% of cases and 0.4% of area	N.D.	N.D.	N.D.	0/9
	Intact w/ abiraterone	11	0.56 ± 0.31	4	1098.38 ± 839.80	1116.18 ± 870.38	0% of cases and 0% of area	N.D.	N.D.	N.D.	0/11
	CRPC w/ vehicle	15	0.97 ± 0.80	8	1248.98 ± 948.76	1564.10 ± 1537.28	6.67 % of cases and 0.5% of area	3	29.80 ± 3.76	N.D.	0/15
	CRPC w/ abiraterone	21	0.66 ± 0.26	12	1270.53 ± 705.00	1082.19 ± 555.54	14.29 % of cases and 0.9% of area	3	10.18 ± 1.55	N.D.	0/21
NPp53	Intact w/ vehicle	11	0.93 ± 0.39	5	853.90 ± 306.60	925.56 ± 94.22	18.18% of cases and 1.7% of area	N.D.	N.D.	N.D.	0/11
	Intact w/ abiraterone	10	1.33 ± 1.42	4	870.38 ± 283.01	1638.77 ± 1736.67	50% of cases and 36.6% of area	N.D.	N.D.	N.D.	0/10
	CRPC w/ vehicle	21	0.52 ± 0.35	10 ^a	706.98 ± 266.18	806.95 ± 413.63	42.86% of cases and 13.5% of area	10	17.57 ± 4.49	48.24 ± 8.04	0/21
	CRPC w/ abiraterone	28	0.92 ± 1.07	16 ^b	709.78 ± 711.97	1284.83 ± 1692.94	39.29% of cases and 18.0% of area	6	25.35 ± 3.80	50.68 ± 9.43	4/28

a) 7/13 of the vehicle-treated mice cases were increased in size (>10% increase); 6/13 were unchanged (<10%)

b) 12/16 of the abiraterone-treated mice cases were increased in size (>10% increase); 3/16 were unchanged (<10% change); 1/16 decreased (>10% decrease).

Table S3: Detailed analyses of alternative histopathology in individual experimental mice

NP intact (vehicle)		Frequency and percentage of alternative histopathologies		
Mouse ID		Squamous	Sarcomatoid	Other
51		0%	0%	0%
52		0%	0%	0%
125		0%	0%	0%
176		0%	0%	0%
652		0%	0%	0%
653		0%	0%	0%
1763		0%	0%	0%
2753		0%	4%	0%
2927		0%	0%	0%
Totals:				
# of cases	1/9	0/9	1/9	0/9
% area affected	0.4	0%	0.4%	0%

NP intact (abiraterone)				
Mouse ID		Squamous	Sarcomatoid	Other
35		0%	0%	0%
38		0%	0%	0%
133		0%	0%	0%
137		0%	0%	0%
654		0%	0%	0%
1755		0%	0%	0%
1756		0%	0%	0%
1757		0%	0%	0%
1764		0%	0%	0%
2750		0%	0%	0%
9616		0%	0%	0%
Total				
# of cases	0/11	0/11	0/11	0/11
% affected area	0	0%	0%	0%

NPCRPC (vehicle)				
Mouse ID		Squamous	Sarcomatoid	Other
0308		0%	0%	0%
128		0%	0%	0%

163	0%	0%	0%
164	0%	0%	0%
165	0%	0%	0%
166	0%	0%	0%
167	0%	0%	0%
306	0%	0%	0%
308	7%	0%	0%
401	0%	0%	0%
423	0%	0%	0%
2141	0%	0%	0%
6026	0%	0%	0%
7594	0%	0%	0%
7605	0%	0%	0%
Total			
# of cases	1/15	1/15	0/15
% affected area	0.5	0.5%	0%

NP CRPC (abiraterone)			
Mouse ID	Squamous	Sarcomatoid	Other
72	0%	0%	0%
73	0%	0%	0%
74	0%	0%	0%
205	0%	0%	0%
206	0%	0%	0%
321	0%	0%	0%
405	0%	0%	0%
451	5%	0%	0%
452	0%	0%	0%
453	0%	0%	0%
735	0%	0%	0%
742	0%	0%	0%
965	0%	0%	0%
1006	0%	0%	0%
1012	0%	0%	0%
1026	0%	0%	0%
1029	10%	0%	0%
2144	4%	0%	0%
2155	0%	0%	0%

7592	0%	0%	0%
7748	0%	0%	0%
Total			
# of cases 3/21	3/21	0/21	0/21
% affected area 0.9	0.9%	0%	0%

NPp53 intact (vehicle)			
Mouse ID	Squamous	Sarcomatoid	Other
2503	0%	0%	0%
2504	0%	0%	0%
2505	0%	0%	0%
2537	0%	0%	0%
2539	0%	0%	0%
4304	0%	0%	0%
4305	18%	0%	0%
4306	0%	0%	0%
4307	0%	0%	0%
5528	1%	0%	0%
5529	0%	0%	0%
Total			
# of cases 2/11	2/11	0/11	0/11
% affected area 1.7	1.7%	0%	0%

NPp53 intact (abiraterone)			
Mouse ID	Squamous	Sarcomatoid	Other
2365	0%	0%	0%
2547	0%	6%	75% ^{NEPC}
2548	0%	0%	0%
2549	0%	0%	92% ^{NEPC}
2550	0%	0%	0%
4309	0%	0%	0%
4310	0%	0%	0%
5530	5%	0%	0%
5531	0%	88%	0%
5532	2%	98%	0%
Total			
# of cases 5/10	2/10	3/10	2/10
% affected area 36.6	0.7%	19.2%	16.7%

NPp53 CRPC (vehicle)			
Mouse ID	Squamous	Sarcomatoid	Other
1760	0%	0%	0%
1762	0%	0%	0%
2332	0%	15%	0%
2333	66%	0%	0%
2340	20%	0%	0%
2392	0%	12%	0%
2393	0%	26%	0%
4353	10%	0%	0%
5523	0%	0%	0%
5524	12%	0%	0%
5525	0%	0%	0%
7366	0%	0%	0%
7390	0%	0%	0%
7391	0%	0%	0%
7392	0%	0%	0%
9045	0%	0%	100% ^{NEPC}
9313	0%	0%	0%
9314	0%	0%	0%
9315	0%	0%	0%
R660	22%	0%	0%
R662	0%	0%	0%
Total			
# of cases	9/21	5/21	3/21
% affected area	13.5	6.2%	2.5%
			4.8%

NPp53 CRPC (abiraterone)			
Mouse ID	Squamous	Sarcomatoid	Other
1767	0%	0%	0%
1768	0%	23%	0%
2341	0%	26%	0%
2381	0%	0%	0%
2384	0%	0%	0%
2385	0%	0%	0%
2386	0%	0%	0%

2389	0%	94%	0%
3044	8%	0%	0%
3046	0%	0%	0%
4146	6%	29%	65% ^{HGC}
4315	0%	0%	0%
5519	0%	0%	0%
5521	0%	0%	0%
5522	0%	0%	0%
5527	0%	0%	0%
7386	0%	0%	0%
7387	16%	0%	0%
7559	8%	0%	0%
7805	0%	0%	0%
7831	0%	0%	0%
9043	0%	95%	0%
9044	0%	0%	0%
9309	0%	0%	0%
9310	5%	0%	0%
R550	0%	0%	0%
R552	0%	98% ^{NE}	0%
R561	0%	0%	57% ^{HGC}
Total			
# of cases	11/28	5/28	6/28
% affected area	18.9	1.5%	13%
			4.4%

HGC – High grade invasive carcinoma; NE- sarcomatoid cells express synaptophysin in 78% of cells;
NEPC – Neuroendocrine prostate cancer

Table S4: Human master regulators from NEPC versus CPRC from Beltran et al.

MR	NES	p-val
FOXM1	7.318345946	2.51E-13
MYBL2	7.189060611	6.52E-13
UHRF1	5.595421566	2.20E-08
ZNF367	5.331317288	9.75E-08
BRCA2	5.183969152	2.17E-07
BRCA1	4.97232119	6.62E-07
MLF1IP	4.801564361	1.57E-06
MTA1	4.679705696	2.87E-06
TRIP13	4.401653731	1.07E-05
ZNF165	4.398927458	1.09E-05
E2F1	4.351791211	1.35E-05
ASF1B	4.331682031	1.46E-05
TIMELESS	3.991349187	6.57E-05
PGRC1	3.97684828	6.98E-05
CENPF	3.931479516	8.44E-05
E2F8	3.875318107	0.000106485
CENPK	3.81679967	0.000135194
DNMT3B	3.71263622	0.000205112
MLL4	3.575059897	0.000350148
PTTG1	3.560119071	0.000370667
NFKBIL2	3.474195458	0.000512368
SIN3B	3.472627672	0.00051539
CCNA2	3.464375179	0.000531485
ZBTB45	3.381379717	0.000721228
MLXIPL	3.298133977	0.000873297
E2F7	3.241800461	0.001167772
PTGES2	3.23037085	0.001236297
SOLH	3.224116366	0.001263611
SNAPC4	3.195306194	0.001398826
MLLT1	3.185304222	0.001549289
RBCK1	3.122117578	0.001795562
C11orf9	3.08294859	0.002049606
PRDM16	3.020764192	0.002521376
HDAC10	3.014238968	0.002576248
CNOT3	3.013407756	0.002583316
ZFR2	3.012340636	0.002592415

TNFRSF4	2.949067991	0.003187132
HOXC13	2.856822035	0.004279057
MYT1L	2.853334959	0.004326299
SOX11	2.847739809	0.004409091
PAX7	2.839029163	0.004525102
HOXB9	2.824785039	0.004731235
SIRT6	2.822763368	0.004760873
RNF6	-2.80841567	0.004978589
NCOA2	-2.821131134	0.004785464
RB1CC1	-2.833570303	0.004603118
HOXB13	-2.841482892	0.004490426
BZW1	-2.861063205	0.003963362
AFF4	-2.86589543	0.003902981
MORF4L1	-2.89361875	0.003808303
USP16	-2.898083993	0.0037545
ZNF24	-2.89823236	0.003752724
MORC3	-2.921654216	0.003461779
TFEC	-2.95698836	0.003106598
ATF6	-2.959583519	0.003080552
AFF1	-2.965705989	0.00301989
IKZF5	-2.970206558	0.002975996
ZNF260	-2.972444473	0.002954386
GATB1	-2.979590586	0.002886339
AKNA	-3.003722366	0.002666986
NCOA7	-3.006349583	0.002644048
TLR2	-3.01313391	0.002585648
C19orf2	-3.020512341	0.002523474
ZNF615	-3.050720798	0.002282928
ELP2	-3.057464441	0.002232181
CAMKK2	-3.067774548	0.002156563
ZDHHC21	-3.103434394	0.001912886
ICAM1	-3.118721714	0.001816374
NR4A1	-3.145901931	0.001655755
CNOT6	-3.157822463	0.001589523
HMG20B	-3.193815893	0.001404056
GLRX2	-3.193901343	0.001403641
CCNC	-3.195695131	0.001394944
ZFYVE16	-3.208045807	0.001336402

MNDA	-3.241806275	0.001187739
FOXA1	-3.322669334	0.000891605
TRIP11	-3.33384343	0.000856549
AFF3	-3.374304221	0.000740025
ATP6B1	-3.440477844	0.000580688
MAPK1	-3.476555589	0.000507899
CREB3L4	-3.501666922	0.000462357
RLIM	-3.524858716	0.000423703
VAV1	-3.535492099	0.000407016
LASS4	-3.644558758	0.000267841
MORF4L2	-3.681088608	0.00023224
MSC	-3.695551373	0.00021941
ZDHHC6	-3.73693619	0.000186276
ATF1	-3.747491872	0.000178612
ZCCHC6	-3.764456401	0.000166912
ZEB2	-3.806606297	0.000140887
ELL2	-3.82485005	0.000130852
SETD7	-3.827607621	0.000129395
NLRP3	-3.980194141	6.89E-05
UBE2N	-3.988478688	6.65E-05
SP140	-4.006178605	6.12E-05
BMI1	-4.009145158	6.09E-05
RUNX3	-4.13464316	3.56E-05
ZDHHC20	-4.231557456	2.32E-05
RIPK2	-4.234225018	2.29E-05
UBA3	-4.236066009	2.27E-05
ETS1	-4.37246377	1.23E-05
SLC30A9	-4.393337137	1.12E-05
IRF4	-4.627804077	3.70E-06
XBP1	-4.644131717	3.42E-06
SPDEF	-4.732856475	2.21E-06
IKZF3	-4.757419536	1.96E-06
PRDM1	-4.76830259	1.86E-06
RAB18	-4.773868799	1.81E-06
IRF8	-4.907108083	9.24E-07
IL16	-4.962979651	6.26E-07
SKAP1	-5.036583959	4.74E-07
ZNF532	-5.058969121	4.22E-07

IFI116	-5.066475319	4.05E-07
CD80	-5.184664174	2.16E-07
CIITA	-5.670076527	1.43E-08
RHOH	-5.706616738	1.15E-08
FLI1	-6.015645114	1.79E-09
STAT4	-6.114788049	9.67E-10
CD86	-6.216251278	5.09E-10
PARP15	-6.955954049	3.50E-12
HCLS1	-7.123012789	1.06E-12

Table S5. Quantitation of Synaptophysin-positive cells

Table S5A: Percentage of Synaptophysin-positive tumor cells			
<i>NP</i> intact (vehicle)		% Synaptophysin ⁺ tumor cells	
Mouse ID	Syn ⁺	Syn ⁻	Percentage
51	0	4414	0.00%
52	0	2997	0.00%
125	2	1445	0.14%
Mean $\pm$ SD			0.05 $\pm$ 0.08
<i>NP</i> intact (abiraterone)		% Synaptophysin ⁺ tumor cells	
Mouse ID	Syn ⁺	Syn ⁻	Percentage
35	0	2495	0.00%
38	0	1824	0.00%
133	3	1430	0.21%
Mean $\pm$ SD			0.07 $\pm$ 0.12
<i>NP</i> castrated (vehicle)		% Synaptophysin ⁺ tumor cells	
Mouse ID	Syn ⁺	Syn ⁻	Percentage
128	0	1805	0.00%
308	0	1593	0.00%
401	0	1204	0.00%
2141	53	1915	2.69%
7594	0	417	0.00%
7605	23	1905	1.19%
Mean $\pm$ SD			0.65 $\pm$ 1.11
<i>NP</i> castrated (abiraterone)		% Synaptophysin ⁺ tumor cells	
Mouse ID	Syn ⁺	Syn ⁻	Percentage
405	8	1389	0.57%
735	2	2570	0.08%
1012	7	2139	0.33%
2144	72	2107	3.30%
2155	14	1293	1.07%
7592	13	1264	1.02%
7745	8	1570	0.51%
Mean $\pm$ SD			0.98 $\pm$ 1.08

<i>NPp53</i> intact (vehicle)		% Synaptophysin ⁺ tumor cells	
Mouse ID	Syn ⁺	Syn ⁻	Percentage
2503	1	2650	0.04%
2504	8	1255	0.63%
2505	2	1825	0.11%
2537	12	1855	0.64%
2539	8	1691	0.47%
4306	7	4294	0.16%
4307	13	4036	0.32%
Mean ± SD		0.34 ± 0.25	

<i>NPp53</i> intact (abiraterone)		% Synaptophysin ⁺ tumor cells	
Mouse ID	Syn ⁺	Syn ⁻	Percentage
4310	29	3492	0.82%
2550	32	3429	0.92%
2365	1	1362	0.07%
2547	3	1592	0.19%
2548	0	981	0.00%
Mean ± SD		0.4 ± 0.44	

<i>NPp53</i> castrated (vehicle)		% Synaptophysin ⁺ tumor cells	
Mouse ID	Syn ⁺	Syn ⁻	Percentage
2332	25	1906	1.29%
2333	118	2194	5.10%
2340	32	1699	1.85%
2393	28	2103	1.31%
4353	47	2931	1.58%
7366	87	2624	3.21%
7391	77	2631	2.84%
7392	118	2568	4.39%
9314	29	1811	1.58%
1760	57	2301	2.42%
1762	55	2300	2.33%
Mean ± SD		2.54 ± 1.26	
9045 (Overt CRPC-NE)		2968	5
		99.83%	

<i>NPp53</i> castrated (abiraterone)		% Synaptophysin ⁺ tumor cells	
Mouse ID	Syn ⁺	Syn ⁻	Percentage
2381	39	1581	2.41%
2384	88	1888	4.46%
2385	39	1598	2.38%
2386	209	1774	10.54%
3044	83	3477	2.33%
3046	68	2972	2.24%
7386	62	1896	3.17%
7559	112	3402	3.19%
9309	32	1875	1.68%
9044	123	2527	4.64%
1767	161	2557	5.92%
1768	152	2596	5.53%
Mean $\pm$ SD			4.03 $\pm$ 2.47
R852 (Overt CRPC-NE)	2191	629	77.69%

Table S5B. Percentage of Synaptophysin-positive cells co-expressing GFP or Ki67			
<i>NPp53</i> castrated (vehicle)		% Synaptophysin ⁺ GFP ⁺ cells	
Mouse ID	Syn ⁺ GFP ⁺	Syn ⁺ GFP ⁻	Total
7366	116	0	116/116
7391	130	6	130/136
7392	121	16	121/137
Total			367/389
9045 (Overt CRPC-NE)	1472	9	1472/1481

<i>NPp53</i> castrated (abiraterone)		% Synaptophysin ⁺ GFP ⁺ cells	
Mouse ID	Syn ⁺ GFP ⁺	Syn ⁺ GFP ⁻	Total
2384	102	1	102/103
2385	125	0	125/125
2386	119	0	119/119
Total			346/347
R652 (Overt CRPC-NE)	519	2	519/521

<i>NPp53</i> castrated (vehicle)		% Synaptophysin ⁺ Ki67 ⁺ cells	
Mouse ID	Syn ⁺ Ki67 ⁺	Syn ⁺ Ki67 ⁻	Total
7366	0	89	0/89
7391	0	115	0/115
7392	0	108	0/108
9314	0	45	0/45
Total			0/357
9045 (Overt CRPC-NE)	1094	830	1094/1924

<i>NPp53</i> castrated (abiraterone)		% Synaptophysin ⁺ Ki67 ⁺ cells	
Mouse ID	Syn ⁺ Ki67 ⁺	Syn ⁺ Ki67 ⁻	Total
2384	0	106	0/106
2386	0	125	0/125
9046	0	113	0/113
7559	0	216	0/216
Total			0/560
R652 (Overt CRPC-NE)	461	577	461/1038

Table S6: List of antibodies used in this study

Antigen	Company	Catalog #	Type	Use and dilution	
				IF/IHC	Western
β -actin	Cell Signaling	4970	Rabbit mAb		1:2000
AR	Sigma-Aldrich	A9853	Rabbit pAb		1:2000
AR	Abcam	ab133273	Rabbit mAb	1:100	1:1000
Chromogranin A	Abcam	ab15150	Rabbit	1:500	
Cytokeratin 5	Covance	SIG-3475	Chicken pAb	1:500	
	Covance	PRB-160P	Rabbit pAb	1:500	1:1000
Cytokeratin 8	Covance	MMS-162P	Mouse mAb		1:1000
	DSHB	TROMA-1	Rat mAb	1:100	
Foxa2	Abnova	H00003170-M12	Mouse mAb	1:50	
GFP	Abcam	ab13970	Chicken pAb	1:1000	
Ki67	eBiosciences	14-5698-82	Rat IgG2	1:500	
Pan-Cytokeratin	Dako	Z0622	Rabbit pAb	1:500	
Phospho-Akt (Ser473)	Cell Signaling	4060	Rabbit mAb		1:1000
Phospho-Rb	Santa Cruz	sc-16670-R	Rabbit pAb		1:200
Phospho-S6 (Ser235/236)	Cell Signaling	2211	Rabbit pAb		1:1000
Synaptophysin	BD Biosc.	611880	Mouse mAb	1:500	
Synaptophysin	Zymed	18-0130	Rabbit pAb	1:250	
Secondary antibodies used:					
Antibody	Fluorochrome	Company	Catalog #	Dilution	
Goat anti-rabbit IgG	Alexa Fluor® 488	Life Technologies	A11008	1/1000	
Goat anti-rabbit IgG	Alexa Fluor® 555	Invitrogen	A21426	1/1000	
Goat anti-mouse IgG	Alexa Fluor® 488	Life Technologies	A11001	1/1000	
Goat anti-mouse IgG	Alexa Fluor® 555	Life Technologies	A21424	1/1000	
Goat anti-mouse IgG	Alexa Fluor® 594	Invitrogen	A11032	1/500	
Goat anti-chicken IgG	Alexa Fluor® 488	Invitrogen	A11039	1/1000	
Goat anti-rat IgG	Alexa Fluor® 594	Invitrogen	A11007	1/500	
Biotinylated anti-mouse IgG	None	Vector Laboratories	BA-8200	1/1000	
Biotinylated anti-rabbit IgG	None	Vector Laboratories	BA-1000	1/300	
Biotinylated anti-rat IgG	None	BD Biosciences	559286	1/300	

Supplementary Table S7: Description of primers and shRNA used in this study		
Real Time qPCR	Forward	Reverse
Mouse genes		
Sox2	TTTGTCGAGACCGAGAAGC (SEQ ID NO: 1)	CTCCGGGAAGGGTGTACTTA (SEQ ID NO: 2)
Sox7	GTGGGCCAAGGATGAGAG (SEQ ID NO: 3)	CCATGACTTTCCAGCATC (SEQ ID NO: 4)
Sox11	CGACGACCTCATGTTGAGC (SEQ ID NO: 5)	GACAGGGATAGGTTCCCG (SEQ ID NO: 6)
NSE	AGGTGGATCTCTATACTGCCAAA (SEQ ID NO: 7)	GTCCCATCCCTTAGTCCAG (SEQ ID NO: 8)
Synaptophysin	CATTCAGGCTGCACCAAGTG (SEQ ID NO: 9)	TGGTAGTGCCCTTTAAG (SEQ ID NO: 10)
Rb1	TGCATCTTTATCGCAGCAGTT (SEQ ID NO: 11)	GTTACACGTCCGTTCTAATTTG (SEQ ID NO: 12)
GAPDH	AGGTCGGTGTGAACGCATTTG (SEQ ID NO: 13)	TGTAGACCATGTAGTTGAGSTCA (SEQ ID NO: 14)
shRNA		
shSox11 #1	TRCN0000012102	CCGGGTTTCGACCTGAGCTTGAATTTCTCGAGAAA TTCAAGCTCAGGTGGAACCTTTT (SEQ ID NO: 15)
shSox11 #2	TRCN0000012099	CCGGGCTGTACTACAGCTTCAAGAACTCGAGTTC TTGAAGCTGTAGTACAGCTTTT (SEQ ID NO: 16)

Supplemental Table S8—subset of markers associated with CRPC exceptional non-responders

MR	cox p-value
FOXMI	3.26E-09
BRCA2	5.90E-06
BRCA1	1.05E-08
MLF1IP	2.78E-07
MYBL2	3.80E-07
UHRF1	1.15E-06
TRIP13	6.99E-08
CENPK	1.86E-07
CENPF	1.51E-09
CCNA2	7.99E-08
NARG1	0.001546956
PBRM1	0.109719767
DR1	0.207186097
TCEB1	0.021800778
RC3H2	0.023467142
UBA3	0.376537609
SMARCA1	0.691479772
XRN2	0.012219889
MCM4	6.96E-09
AHCTF1	0.014005712
TAF2	0.007411543

SOX11	0.001

With reference to Supplemental Table S4, positive numbers indicate an increase in expression compared with the NES; while negative numbers indicate a decrease in expression compared to the NES. Supplemental Table S8 is a subset of differentially expressed genes associated with super resistant prostate tumors, or “exceptional non-responders”. Detecting differential expression of any one or combination of these genes (from Table S4 and/or Table S8) or their corresponding protein/s will be useful for diagnosing or prognosing a prostate cancer patient with CRPC, or with super resistant prostate tumors, or castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE) or CRPC-NE likelihood, such that the appropriate treatment options can then be made. Such treatment options include alternatives to anti-androgen treatment when CRPC and/or the development of super-resistant prostate tumors is prognosed. In response to such a diagnosis it may be determined to administer to the patient an effective amount of one or any combination of the following: androgen synthesis inhibitors (TAK700), androgen receptor (AR) inhibitors (ARN-509, ODM-201, and EZN-4176), AR DNA binding domain inhibitors (EPI-001), selective AR downregulators or SARDs (AZD-3514), including agents that inhibit both androgen synthesis and receptor binding (TOK-001/galeterone).

In certain embodiments, detecting one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and 1-DOPA Decarboxylase (DDC), or any combination thereof, can be combined with detecting differential expression from any of the markers from Table S4 or Table S8 (or the corresponding protein/s) for diagnosing or prognosing a prostate cancer patient with CRPC, with super resistant prostate tumors, or castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE) or CRPC-NE likelihood.

In certain embodiments, detecting a decrease in expression or loss of *RBI*, *TP53*, *PTEN*, or any combination of these markers may also be useful in combination with any of the above described markers for diagnosing or prognosing a prostate cancer patient with CRPC, with super resistant prostate tumors, or castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE) or CRPC-NE likelihood.

In certain embodiments, detecting expression of *SOX11*, may also be useful in combination with any of the above described markers for diagnosing or prognosing a prostate

cancer patient with CRPC, with super resistant prostate tumors, or castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE) or CRPC-NE likelihood.

Experimental Procedures

Genetically engineered mouse (GEM) models

5 All experiments using animals were conducted according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Columbia University Medical Center. The *Nkx3.1^{CreERT2/+}* allele (N mice) contains a tamoxifen-inducible Cre under the control of the *Nkx3.1* promoter, resulting in heterozygous deletion of *Nkx3.1* in the germline and, following delivery of tamoxifen, conditional gene recombination specifically in prostate
10 luminal epithelial cells (14,15). The *Nkx3.1^{CreERT2/+}* allele was crossed with a conditional *Pten* allele to generate the *Nkx3.1^{CreERT2/+}; Pten^{flox/flox}* mice (NP mice) as described in (16), and a conditional *Trp53* allele (17) to generate the *Nkx3.1^{CreERT2/+}; Pten^{flox/flox}; Trp53^{flox/flox}* mice (NPp53 mice) described herein. For lineage tracing, NP and NPp53 mice were crossed with a *Rosa-CAG-LSL-EYFP-WPRE* reporter allele, which contains a conditionally-activatable
15 enhanced YFP (EYFP) under the control of the Rosa locus (18). Mouse alleles were obtained from the NCI Mouse Models of Human Cancer Consortium Repository (<http://mouse.ncifcrf.gov/>) or the Jackson Laboratory (<https://www.jax.org>) and maintained in a mixed C57BL/6/129S strain background. Cre activity was induced in mice at 3 months of age by oral delivery of tamoxifen (Sigma; 100 mg/kg/day in corn oil) for 4 consecutive days
20 as described previously (6,16). Notably, in published studies using this *Nkx3.1^{CreERT2}* driver, we have controlled for potential transient effects of tamoxifen (which is an estrogen analog) on the prostate phenotype (see (14)). Where indicated, mice were androgen-ablated by surgical castration as in (16). Tumor-bearing mice were monitored daily to assess body condition as described in (19), and were euthanized if their Body Condition Score was <1.5,
25 as per IACUC guidelines.

Preclinical and phenotypic analysis of NP and NPp53 mice.

Abiraterone-acetate was provided by Johnson & Johnson. Optimal dosage, pharmacokinetic profile, and optimal scheduling were determined using non-tumor bearing littermates to control for strain background, based on published studies in other mouse strain
30 backgrounds (2,3). To determine dosage tolerance, abiraterone-acetate was diluted to the desired concentration in corn oil, and administered by oral gavage at a dose of 50 or 200 mg/kg once daily for 3 weeks. Body weight and animal condition were recorded daily

throughout the dosing period and compared to the vehicle control group. At the conclusion of the study, androgen dependent tissues from the urogenital system (e.g., prostate and seminal vesicle) and androgen independent ones (e.g., kidney) were isolated and their wet weights were determined. To determine the pharmacokinetic profile, mice were administered a single
5 dose of abiraterone-acetate (200 mg/kg) at time 0, and then sacrificed at individual time points up to 24 hours following drug delivery. At the time of sacrifice, prostate tissues and serum were collected and levels of abiraterone, the active form of abiraterone-acetate, were determined by mass spectrometry. Based on these studies and published work (2,3), preclinical studies were performed by administration of abiraterone-acetate once daily on a
10 Monday to Friday schedule by oral gavage at a dose of 200 mg/kg/day for a period of four weeks. Cohorts of *NP* and *NPp53* mice were induced with tamoxifen at 3 months of age; after tumor induction, the mice underwent castration (or mock surgery) at 10 or 7 months, respectively. Mice were randomly enrolled in the vehicle or abiraterone treatment groups, which was initiated two months following castration. Treatment was continued for four
15 weeks, except in cases where the mouse had to be euthanized early due to poor body condition score (as above). During the last week of treatment, mice were treated for 7 consecutive days and dissected within 24 hours of the last treatment.

Tumor volume was monitored by Magnetic Resonance Imaging (MRI) using a Bruker Biospec 9.4T Tesla Small Animal MR Imager located within the mouse barrier in the Herbert
20 Irving Cancer Center Small Animal Imaging facility. In some cases, MRI imaging was done using a Bruker ICON 1 Tesla desktop MRI scanner, which was also located within the mouse barrier. For determination of the change in tumor volume as a consequence of treatment, MRI was done immediately prior to initiation of treatment and then immediately following the last drug treatment. Images were generated using ParaVision 6 software for preclinical MRI
25 (Bruker) and reviewed by an MRI physicist (YS). Volumetric analysis was done using Analyze software (AnalyzeDirect); 3D model reconstruction was done using 3DSlicer software (on the world wide web at: www.slicer.org). At the time of sacrifice, full necropsy was performed on each experimental mouse. Mice were also inspected for signs of overt metastasis, guided by visual inspection of YFP expression from the reporter allele, as
30 described in (6). Prostate tissues were collected, photographed, and weights determined. Individual prostatic lobes (anterior, dorsolateral, and ventral) were fixed in 10% formalin and paraffin-embedded, cryopreserved in Optimal Cutting Temperature (OCT) compound, or snap-frozen in liquid nitrogen; serum was collected and stored in -80°C. Tissues with visible

metastases were fixed in 10% formalin and paraffin-embedded for histopathological validation. Pathological grading was done on hematoxylin-eosin (H&E) stained slides; cases were reviewed blinded by the pathologist (MR). For each individual experimental mouse analyzed, a minimum of 2 and up to 12 H&E sections through the tumor mass were examined to determine the presence of adenocarcinoma and alternate histopathologies. The percentage area of these phenotypes in each experimental animal was determined using ImageJ 1.50i (NIH). These data are summarized in Table S3.

Immunohistochemical and immunofluorescence staining were done on 3 µm paraffin sections as previously described (15). Briefly, sections were deparaffinized in xylene, followed by antigen retrieval in antigen unmasking solution (Vector Labs). Slides were blocked in 10% normal goat serum, then incubated with primary antibodies overnight at 4°C, followed by incubation with secondary antibodies for 1 hour.

All antibodies used in this study are described in Table S6. For immunohistochemical staining, the signal was enhanced using the Vectastain ABC system and visualized with NovaRed Substrate Kit (Vector Labs). The slides for immunohistochemistry were counterstained with Hematoxylin and mounted with Clearmount (American Master*Tech Scientific). Immunofluorescence staining was mounted with Vectashield mounting medium for fluorescence with DAPI (Vector Labs). Immunohistochemical images were captured using an Olympus VS120 whole-slide scanning microscope, and immunofluorescence was captured on a Leica TCS5 spectral confocal microscope. For quantification of immunohistochemical and immunofluorescence staining, histograms from at least 5 sections per mouse and from at least 3 independent mice were counted as described previously (15). These data are summarized in Table S5. Western blot analyses were done using total protein lysates (10 µg per lane) from dorsolateral prostate or prostate tumors using antibodies as described in Table S6. Levels of steroids and abiraterone were determined in serum and prostate tissues that were snap-frozen in liquid nitrogen at the time of sacrifice. Metabolite levels were determined by mass spectrometry as previously reported (20,21). Briefly, frozen tissues were weighed and added to 60°C water containing deuterated internal standards, heated to 60°C for 10 minutes, and homogenized using a tissue homogenizer (Precellys; Bertin, Rockville, MD); supernatant was extracted twice with hexane (ethyl acetate [80:20 v/v]), and the organic layer was dried (SpeedVac; Thermo Scientific, Waltham, MA), derivatized with 0.025 M hydroxylamine hydrochloride for 24 hours at room temperature to form oximes, and quantified using liquid chromatography electrospray-ionization tandem

mass spectrometry. The lower limit of detection for steroids was 0.49 pg/sample for progesterone, androstenedione, testosterone and DHT; 0.98 pg/sample for DHEA and androsterone, and 1.96 pg/sample for pregnenolone. The lower limit of detection for abiraterone was 0.06 ng/sample in serum and 1.2 ng/sample for tissue. Limits of quantification in serum were 0.02 ng/ml for progesterone, androstenedione, testosterone and DHT; 0.04 ng/ml for DHEA and androsterone, 0.08 ng/ml for pregnenolone, and 0.005 ng/dL for abiraterone. Limits of quantification in tissue were 0.02 pg/mg for progesterone, androstenedione, DHT, and testosterone, 0.05 ng/ml for DHEA and androsterone, 0.11 pg/mg for pregnenolone, and 0.06 pg/gm for abiraterone.

10 **Generation and analyses of cell lines from NP and NPp53 mice.**

Cell lines were generated from *NP* or *NPp53* prostate tumors in our laboratory in the Fall of 2013 as described (22). Two independent cell lines were obtained from two independent tumors each from *NP* and *NPp53* mice; to avoid clonal variation, we used a pooled cell population for these studies. Each cell line was confirmed to be negative for a multi-species mycoplasma test (Mycoplasma Detection Kit, Cat# MP70114, Fisher), which was performed in early 2014. The cell lines were confirmed to have the correct genotypes using a commercial source (TransnetYX, Inc). Further characterization of the cell lines included analyses of growth rate using MTT assays, and expression of relevant markers by Western blot analyses. Cell line stocks were established at passage 5, and have been used for experimental assays within 3 passages following thawing. Once established, cells were maintained in RPMI-1640 with 10% FBS. For *in vitro* analysis of drug treatment, cells were seeded into 96-well plates at 5000 cells/well in growth medium supplemented with 10% charcoal-stripped serum in the presence or absence of 20nM dihydrotestosterone. At 24 hours following plating, abiraterone (50 mM in ethanol or ethanol alone) was added to a final concentration of 10 μ M and incubated for 24 to 78 hours. Relative viability was determined using the Cell Proliferation Kit I (MTT, Sigma). Data was analyzed using GraphPad Prism software version 6.0f (Graphpad Software, Inc.). For *in vivo* analysis of drug treatment, 1×10^6 cells were mixed with Matrigel (1:1 vol/vol) and injected into the flank of immunodeficient NCr nude mice (Taconic). Tumors were monitored 3 times weekly for approximately 4 weeks by caliper measurement and abiraterone treatment was initiated when the tumor size reached 100-300mm³. At the time of euthanasia, subcutaneous tumors were harvested and weighed. For shRNA-mediated silencing, we used the PLKO.1 lentiviral vector with short hairpin RNA (Sigma-Aldrich) targeting two independent sequences in the

coding region of mouse *Sox11* gene (2 independent shRNA). As a control, we used the PLKO.1 lenti viral vector with shRNA targeting the luciferase gene (SHC007, Sigma-Aldrich). Lentiviral particles were made using the 2nd generation packaging vectors, psPAX2 and pMD2.G (Addgene) in HEK-293T cells (ATCC), and concentrated using the Lenti-X Concentrator reagent (Clontech), as described previously (6). Mouse cell lines were infected with the lentiviruses and selected for 5 days using 6 µg/ml of puromycin. For quantitative real-time PCR (qPCR) analyses, total RNA was isolated using a MagMAX-96 Total RNA Isolation Kit (Life Technologies, Grand Island, NY). cDNA was prepared using SuperScript III First-Strand Synthesis SuperMix for qRT-PCR kit from Thermo Fisher Scientific according to manufacturer's instructions. Real-time PCR were performed using QuantiTect SYBR Green PCR Kit from Qiagen and Gapdh was used as an internal control. Relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method as described (6). Table S7 provides a list of all primers and shRNA sequences used in this study.

Gene expression profiling and computational analyses For analyses of SOX11 expression levels in human datasets, mRNA expression (zscores) classified by Clinical Gleason category in TCGA (7) or Cancer Type Detailed in Beltran et al. (12) was exported through cBioPortal (23,24), and plotted using GraphPad Prism software (Version 6.0). For gene expression profiling of mouse tumors, total RNA was isolated from prostate tissues/tumors using a MagMAX-96 Total RNA Isolation Kit (Life Technologies, Grand Island, NY). RNA sequencing was performed at the JP Sulzberger Columbia Genome Center at Columbia University Medical Center. A TruSeq RNA Sample Prep Kit v2 (Illumina) was used for library preparation followed by sequencing (30 million single-end reads) on an Illumina HiSeq 2500. RNAseq data raw counts were normalized and the variance was stabilized using DESeq2 package (Bioconductor) in R-studio 0.99.902, R v3.3.0 (The R Foundation for Statistical Computing, ISBN 3-900051-07-0). The raw and normalized data files are deposited in Gene Expression Omnibus (GEO) GSE92721. Differentially expressed genes are listed in Dataset 1. To align RNA-Seq reads to human genome (hg19) in the SU2C and Beltran et al. datasets, we used STAR aligner v2.5.2a and then counted mapped reads using HTSeq v0.6.1. Differential gene expression signatures were defined as a list of genes ranked by their differential expression between any two phenotypes of interest (e.g., abiraterone treated vs. vehicle treated; castration-resistant vs. intact etc.), using 2-tailed Welch t-test. For comparison of mouse gene signatures with human gene signatures, mouse genes were mapped to their corresponding human orthologs based on the homoloGene

database (NCBI); all analyses comparing mouse and human genes were done using mouse “humanized” signatures. For single sample GSEA (ssGSEA), individual abiraterone-treated samples were compared to the pooled group of vehicle-treated samples, such that the fold-change value for each gene was estimated as the gene’s expression in a single treated sample over the average of the gene’s expression levels in the vehicle-treated samples. If fold change (FC) fell below 1, the resulting fold change was defined as $-1/FC$. Single-sample signatures were then defined by a list of genes ranked by their fold-change between a single treated sample and a pooled group of vehicle-treated samples. Each single-sample signature was compared to the human Best et al. signature (9) using Gene set enrichment analyses (GSEA).

Master regulator analysis was performed using the MAster Regulator INference algorithm (MARINA) (25) by interrogating a human prostate cancer interactome (26) with “humanized” mouse signatures or with human signatures. Gene set enrichment analyses (GSEA) was done using “humanized” mouse signatures or “humanized” mouse master regulator signatures as described (27) or using human gene or human MR signatures, where appropriate. For analyses of clinical outcomes, we used the Sboner et al. dataset (13), in which cancer specific survival data (i.e., death due to prostate cancer) is available. Corresponding survival analysis was estimated using the transcriptional activity levels of MRs, which were estimated as enrichment of MR transcriptional targets (from the human prostate cancer interactome) in each data sample from the Sboner et al. dataset. For Kaplan-Meier survival analysis, k-means clustering was done on the activity levels of the MRs to cluster patients into two groups: one group having similarity in overall activity to the adverse response MRs and one group having no similarity. Kaplan-Meier analysis was done using the “surv” and “coxph” functions from the survcomp package in R-studio 0.99.902, R v3.3.0; the p-value was estimated using log-rank test.

Statistical analyses Statistical analyses were performed using a two-tailed Welch t-test, one-way ANOVA, X^2 test, and Fisher’s Exact test as appropriate. GraphPad Prism software (Version 6.0) and R-studio 0.99.902, R v3.3.0, were used for statistical calculations and data visualization. Kaplan-Meier survival analysis was done with the surv and coxph functions from survcomp package (Bioconductor). Gene set enrichment analysis (GSEA) was performed as described (27, 28).

Accession numbers Raw and normalized RNA sequencing data are publically available through Gene Expression Omnibus (GEO) database (GSE92721) (28).

References for Supplemental Tables, Experimental Methods, and Figures 7-13.

1. Lunardi A, Ala U, Epping MT, Salmena L, Clohessy JG, Webster KA, et al. A co-clinical approach identifies mechanisms and potential therapies for androgen deprivation resistance in prostate cancer. *Nat Genet* 2013;45(7):747-55 doi 10.1038/ng.2650.
- 5 2. Lam HM, McMullin R, Nguyen HM, Coleman I, Gormley M, Gulati R, et al. Characterization of an Abiraterone Ultraresponsive Phenotype in Castration-Resistant Prostate Cancer Patient-Derived Xenografts. *Clin Cancer Res* 2016 doi 10.1158/1078-0432.CCR-16-2054.
- 10 3. Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM, et al. Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. *Clin Cancer Res* 2011;17(18):5913-25 doi 10.1158/1078-0432.CCR-11-0728.
- 15 4. Li Z, Alyamani M, Li J, Rogacki K, Abazeed M, Upadhyay SK, et al. Redirecting abiraterone metabolism to fine-tune prostate cancer anti-androgen therapy. *Nature* 2016;533(7604):547-51 doi 10.1038/nature17954.
5. Li Z, Bishop AC, Alyamani M, Garcia JA, Dreicer R, Bunch D, et al. Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. *Nature* 2015;523(7560):347-51 doi 10.1038/nature14406.
- 20 6. Aytes A, Mitrofanova A, Kinkade CW, Lefebvre C, Lei M, Phelan V, et al. ETV4 promotes metastasis in response to activation of PI3-kinase and Ras signaling in a mouse model of advanced prostate cancer. *Proc Natl Acad Sci U S A* 2013;110(37):E3506-15 doi 10.1073/pnas.1303558110.
7. Network CGAR. The Molecular Taxonomy of Primary Prostate Cancer. *Cell* 2015;163(4):1011-25 doi 10.1016/j.cell.2015.10.025.
- 25 8. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell* 2015;161(5):1215-28 doi 10.1016/j.cell.2015.05.001.
- 30 9. Best CJ, Gillespie JW, Yi Y, Chandramouli GV, Perlmutter MA, Gathright Y, et al. Molecular alterations in primary prostate cancer after androgen ablation therapy. *Clin Cancer Res* 2005;11(19 Pt 1):6823-34 doi 10.1158/1078-0432.CCR-05-0585.
10. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. *Nature* 2012;487(7406):239-43 doi 10.1038/nature11125.
- 35 11. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res* 2006;66(5):2815-25 doi 10.1158/00085472.CAN-05-4000.

12. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat Med* 2016;22(3):298-305 doi 10.1038/nm.4045.
13. Sboner A, Demichelis F, Calza S, Pawitan Y, Setlur SR, Hoshida Y, et al.
5 Molecular sampling of prostate cancer: a dilemma for predicting disease progression. *BMC Med Genomics* 2010;3:8 doi 1755-8794-3-8 [pii] 10.1186/1755-8794-3-8.
14. Wang X, Kruithof-de Julio M, Economides KD, Walker D, Yu H, Halili MV, et al. A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature* 2009;461(7263):495500 doi 10.1038/nature08361.
- 10 15. Toivanen R, Mohan A, Shen MM. Basal Progenitors Contribute to Repair of the Prostate Epithelium Following Induced Luminal Anoikis. *Stem Cell Reports* 2016;6(5):660-7 doi 10.1016/j.stemcr.2016.03.007.
16. Floc'h N, Kinkade CW, Kobayashi T, Aytes A, Lefebvre C, Mitrofanova A, et al. Dual targeting of the Akt/mTOR signaling pathway inhibits castration-resistant prostate
15 cancer in a genetically engineered mouse model. *Cancer Res* 2012;72(17):4483-93 doi 10.1158/0008-5472.CAN-12-0283.
17. Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A. Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat Genet* 2001;29(4):418-25 doi 10.1038/ng747.
- 20 18. Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 2010;13(1):133-40 doi 10.1038/nn.2467.
19. Easterly ME, Foltz CJ, Paulus MJ. Body condition scoring: comparing newly trained scorers and micro-computed tomography imaging. *Lab Anim (NY)* 2001;30(3):46-9.
- 25 20. Taplin ME, Montgomery B, Logothetis CJ, Bubley GJ, Richie JP, Dalkin BL, et al. Intense androgen-deprivation therapy with abiraterone acetate plus leuprolide acetate in patients with localized high-risk prostate cancer: results of a randomized phase II neoadjuvant study. *J Clin Oncol* 2014;32(33):3705-15 doi 10.1200/JCO.2013.53.4578.
21. Cho E, Mostaghel EA, Russell KJ, Liao JJ, Konodi MA, Kurland BF, et al.
30 External beam radiation therapy and abiraterone in men with localized prostate cancer: safety and effect on tissue androgens. *Int J Radiat Oncol Biol Phys* 2015;92(2):236-43 doi 10.1016/j.ijrobp.2015.01.020.
22. Speirs V, Green AR, Walton DS, Kerin MJ, Fox JN, Carleton PJ, et al. Short-term
35 primary culture of epithelial cells derived from human breast tumours. *Br J Cancer* 1998;78(11):1421-9.

23. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012;2(5):401-4 doi 10.1158/2159-8290.CD-12-0095.
24. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative
5 analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013;6(269):p11 doi 10.1126/scisignal.2004088.
25. Lefebvre C, Rajbhandari P, Alvarez MJ, Bandaru P, Lim WK, Sato M, et al. A human Bcell interactome identifies MYB and FOXM1 as master regulators of proliferation in germinal centers. *Mol Syst Biol* 2010;6:377 doi msb201031 [pii] 10.1038/msb.2010.31.
- 10 26. Aytes A, Mitrofanova A, Lefebvre C, Alvarez MJ, Castillo-Martin M, Zheng T, et al. Crossspecies regulatory network analysis identifies a synergistic interaction between FOXM1 and CENPF that drives prostate cancer malignancy. *Cancer Cell* 2014;25(5):638-51 doi 10.1016/j.ccr.2014.03.017.
- 15 27. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;102(43):15545-50 doi 0506580102 [pii] 10.1073/pnas.0506580102.
- 20 28. Zou, Min *et al.* Transdifferentiation as a Mechanism of Treatment Resistance in a Mouse Model of Castration-resistant Prostate Cancer. *Cancer Discovery*; April 14 2017 DOI: 10.1158/2159-8290.CD-16-1174.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The invention is defined by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled. The specific embodiments described herein, including the
25 following examples, are offered by way of example only, and do not by their details limit the scope of the invention.

All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by
30 reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. §1.57(b)(1), to relate to each and every individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. §1.57(b)(2), even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion
35 of dedicated statements of incorporation by reference, if any, within the specification does

not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

5 The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

10 The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

CLAIMS

1. A method of detecting castration-resistant prostate cancer (CRPC) and/or super resistant prostate tumors in a subject diagnosed with prostate cancer, comprising:
 - 5 a. assaying gene expression levels of one or more genes listed in the gene signatures of Table S4 or Table S8, or corresponding protein levels, in a sample from the subject to obtain a test expression profile,
 - b. comparing the test expression profile of the genes or proteins with a reference expression profile for the genes or proteins, wherein the reference expression profile
10 comprises the expression level of the same genes or proteins in a control;
 - c. detecting gene expression levels of one or more genes listed in Table S4 or Table S8 or corresponding protein levels, in the test expression profile compared to the gene expression levels of one or more genes in the reference expression profile, or corresponding protein levels, wherein differential expression or protein levels is
15 indicative of CRPC and/or the development of super-resistant prostate tumors as a prognosis and wherein anti-androgen treatment is counter-indicated under these conditions.
2. The method of claim 1, wherein the control is selected from the group consisting of a healthy control, an untreated subject, and a composite reference value for
20 human prostate tumors.
3. The method of claim 1, wherein CRPC and/or the development of super-resistant prostate tumors is prognosed, further comprising treating the subject with one or more alternatives to anti-androgen treatment, comprising administering to the subject a therapeutically effective amount of treatment selected from the group consisting of an
25 autologous active cellular immunotherapy (e.g. Sipuleucel-T), a poxvirus based vaccine, a T-cell checkpoint inhibiting monoclonal antibody (anti-CTL4 or anti-PD-1 or anti-PDL1), and cabazitaxel, and optionally further comprising monitoring the subject at least monthly.
4. The method of claim 1, further comprising administering to the subject one or more of chemotherapy, radiation therapy or surgery based on the prognosis.
- 30 5. The method of claim 1, further comprising treating the subject with one or more alternatives to anti-androgen treatment when CRPC and/or the development of super-resistant prostate tumors is prognosed, wherein the method further comprises administering to the subject an effective amount of one or any combination of the following: androgen synthesis inhibitors (TAK700), androgen receptor (AR) inhibitors (ARN-509, ODM-201, and EZN-

4176), AR DNA binding domain inhibitors (EPI-001), selective AR downregulators or SARDs (AZD-3514), including agents that inhibit both androgen synthesis and receptor binding (TOK-001/galeterone).

5 6. A method of monitoring the treatment of a subject diagnosed with prostate cancer, comprising:

- a. assaying gene expression of one or more genes from Table S4 or Table S8 or corresponding protein levels, in a sample from the subject to obtain a test expression profile,
- 10 b. comparing the test expression profile of the genes or corresponding proteins with a reference expression profile, wherein the reference expression profile comprises the expression level of the same genes or corresponding proteins obtained from the subject prior to treatment;
- c. 15 detecting gene expression levels of one or more genes from Table S4 or Table S8, or corresponding proteins in the test expression profile that are differential compared to the gene expression of the same one or more genes in the reference expression profile or corresponding proteins, wherein the differential expression or protein levels is indicative of CRPC and/or the development of super-resistant prostate tumors and wherein anti-androgen treatment is counter-indicated under these conditions.

20 7. The method of claim 6, further comprising treating the subject with one or more alternatives to anti-androgen treatment when CRPC and/or the development of super-resistant prostate tumors is prognosed, comprising administering to the subject a therapeutically effective amount of treatment selected from the group consisting of an autologous active cellular immunotherapy (e.g. Sipuleucel-T), a poxvirus based vaccine, a T-cell checkpoint 25 inhibiting monoclonal antibody (anti-CTL4 or anti-PD-1 or anti-PDL1), and cabazitaxel, and further comprising monitoring the subject at least monthly.

8. The method of claim 1 or 6, further comprising detecting one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and l-DOPA Decarboxylase (DDC), or any combination thereof, wherein the 30 presence any one or more neuroendocrine markers indicates a likelihood of CRPC and/or the development of super-resistant prostate tumors.

9. The method of claim 1 or 6, wherein assaying comprises detecting gene expression levels and is performed using a method comprising at least one of: PCR, RNA sequencing, and microarray.

10. The method of claim 1 or 6, wherein assaying comprises detecting protein levels and is performed using a method comprising at least one of the following methods: quantitative Western blot, immunoblot, quantitative mass spectrometry, enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIA), immunoradiometric assays (IRMA), and
5 immunoenzymatic assays (IEMA) and sandwich assays using monoclonal and polyclonal antibodies.
11. A method for detecting castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE) or CRPC-NE likelihood in a patient sample comprising obtaining a sample comprising prostate cancer tumor tissue from the patient and detecting one or more
10 neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase (NSE), and l-DOPA Decarboxylase (DDC), or any combination thereof, in the sample and diagnosing the patient with CRPC-NE or prognosing the patient as having an increased likelihood of prostate cancer progression (CRPC-NE likelihood) when one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-
15 specific enolase (NSE), and l-DOPA Decarboxylase (DDC), or any combination thereof is detected in the sample.
12. The method of claim 11, wherein anti-androgen treatment is counter-indicated when the patient is diagnosed with CRPC-NE or CRPC-NE likelihood.
13. The method of claim 3 or 6, further comprising administering to the subject one or
20 more of chemotherapy, radiation therapy or surgery based on the prognosis.
14. The method of claim 3, 6, or 11, further comprising administering to the subject one or more of chemotherapy alternatives to anti-androgen treatment, radiation, or surgery based on the prognosis.
15. The method of claim 1, 6, or 11, wherein the sample is selected from the group
25 consisting of blood, bronchial lavage fluid, sputum, saliva, urine, amniotic fluid, lymph fluid, tissue or fine needle biopsy samples, peritoneal fluid, and cerebrospinal fluid.
16. The method of claim 1, 6, or 11, wherein the sample also exhibits a decrease in expression or loss of *RB1*, *TP53*, *PTEN*, or a combination thereof; and optionally further exhibits an increase in expression of *SOX11*, or any combination thereof.
- 30 17. The method of claim 11, further comprising assaying gene expression levels of one or more genes listed in the gene signatures of Table S4 or Table S8, or corresponding protein levels, in a sample from the subject to obtain a test expression profile; comparing the test expression profile of the genes or proteins with a reference expression profile for the genes or proteins, wherein the reference expression profile comprises the expression level of the same

genes or proteins in a control; detecting gene expression levels of one or more genes listed in Table S4 or Table S8 or corresponding protein levels, in the test expression profile compared to the gene expression levels of one or more genes in the reference expression profile, or corresponding protein levels, wherein differential expression or protein levels is indicative of CRPC and/or the development of super-resistant prostate tumors as a prognosis and wherein anti-androgen treatment is counter-indicated under these conditions.

18. A method of screening or identifying a test agent for the prevention and/or treatment of prostate cancer comprising:

- a. contacting or incubating a test agent with a cell comprising a gene listed in Table S4 or Table S8, or corresponding proteins;
- b. detecting differential expression of the gene or corresponding protein in the host cell;
- c. contacting the host cell with the test agent or compound; and
- d. detecting restored differential expression of the gene or corresponding protein in the host cell after contact with the test agent or compound, wherein restoration to a reference or normal level of the differential expression of the gene or corresponding protein in the host cell after contact with the test agent identifies the test agent as a therapeutic or preventative for prostate cancer.

19. The method of claim 18, wherein the test agent is therapeutic or preventative for castration-resistant prostate cancer (CRPC) and/or super resistant prostate tumors, or for castration resistant prostate cancer with neuroendocrine differentiation (CRPC-NE).

20. A kit comprising one or more probes for detecting expression levels of one or more genes listed in the gene signatures of Table S4 or Table S8 or corresponding proteins, and optionally, any one or more of *TP53*, *PTEN*, *SOX11* and/or *RBI*, and further optionally, wherein any one or more probes are detectably labeled.

21. The kit of claim 20, further comprising at least one sealed container which contains the one or more probes, and optionally, the kit further comprising printed material providing instructions.

22. The kit of claim 20, further comprising any one or combination of antibodies for detecting one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), Neuron-specific enolase (NSE), and l-DOPA Decarboxylase (DDC), and any combination thereof.

23. A kit comprising one or more antibodies for detecting one or more neuroendocrine markers including Synaptophysin (Syn), chromogranin A (CgA), neuron-specific enolase

(NSE), and l-DOPA Decarboxylase (DDC), and any combination thereof, and optionally, any one or more of *TP53*, *PTEN*, *SOX11* and/or *RBI*, and further optionally, wherein any one or more of the antibodies or probes are detectably labeled.

24. The kit of claim 23, further comprising at least one sealed container which contains
5 the one or more probes or antibodies, and optionally, the kit further comprising printed material providing instructions.

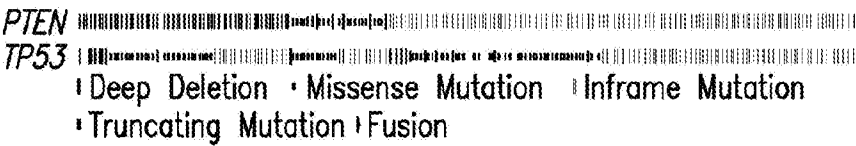


FIG.1A

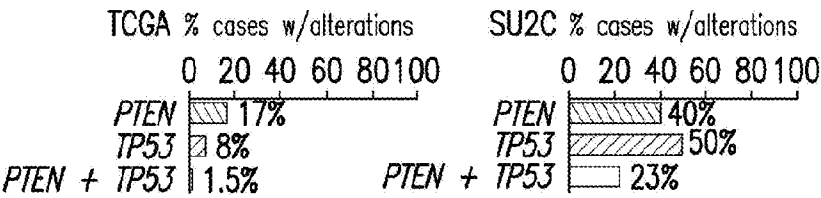


FIG.1B

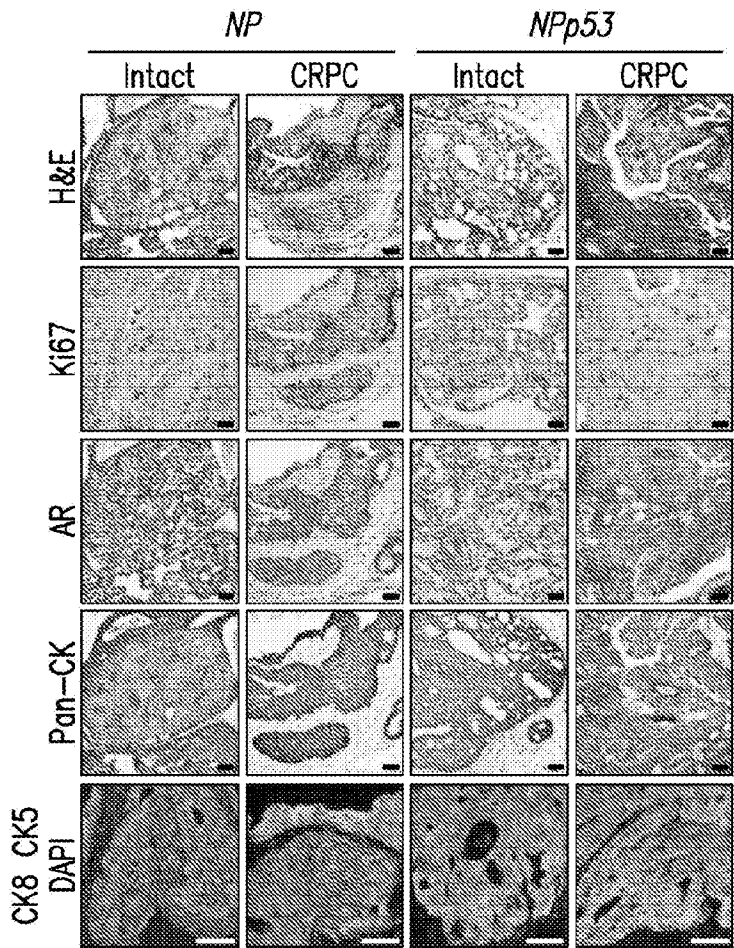


FIG.1C

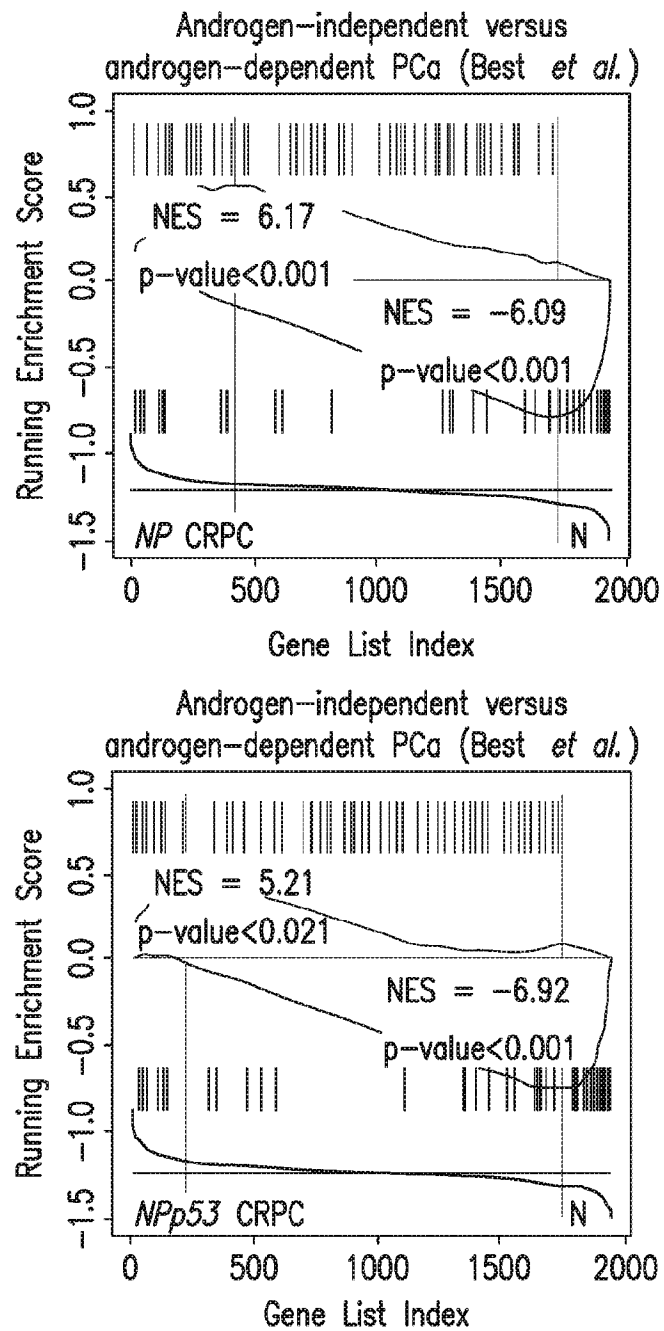


FIG.1D

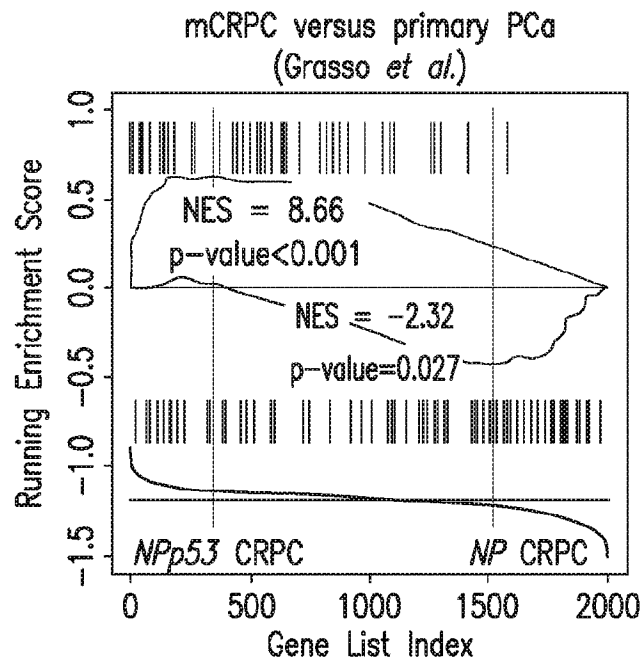


FIG. 1E

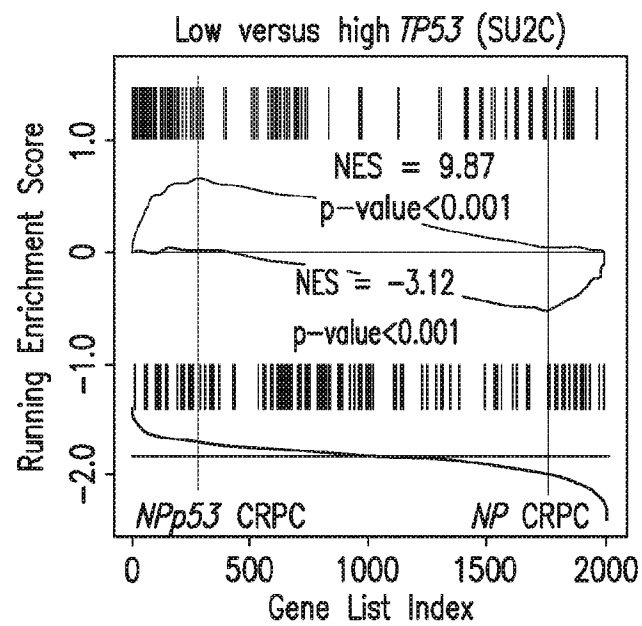


FIG. 1F

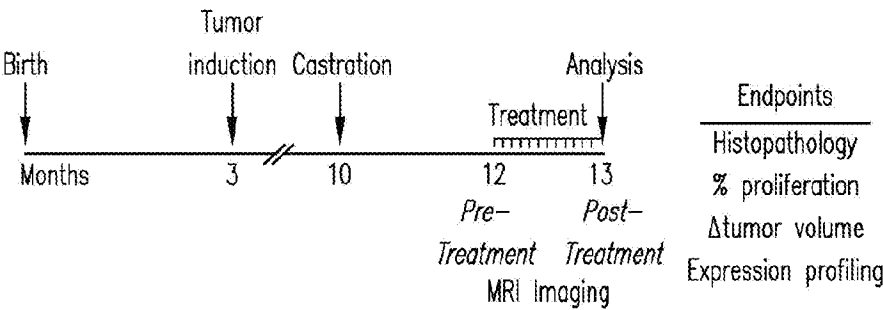


FIG.2A

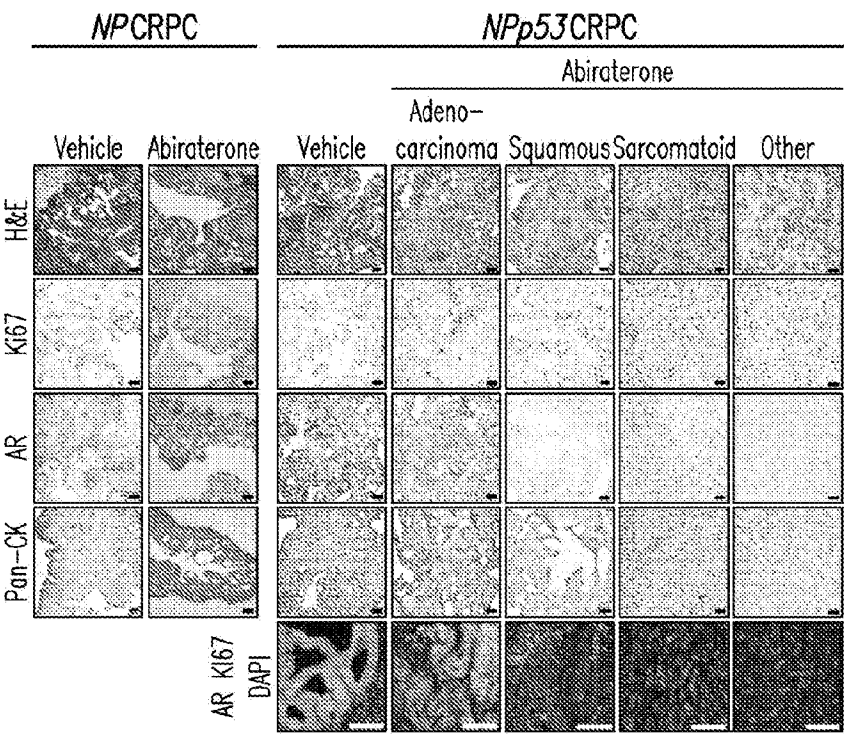


FIG.2B

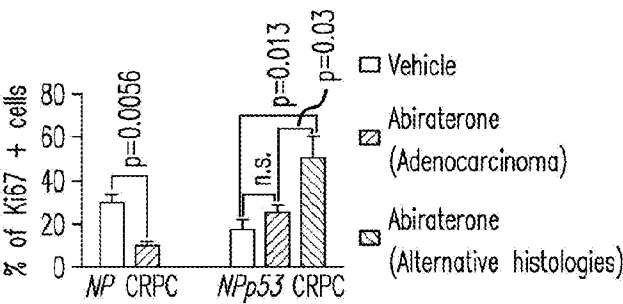


FIG.2C

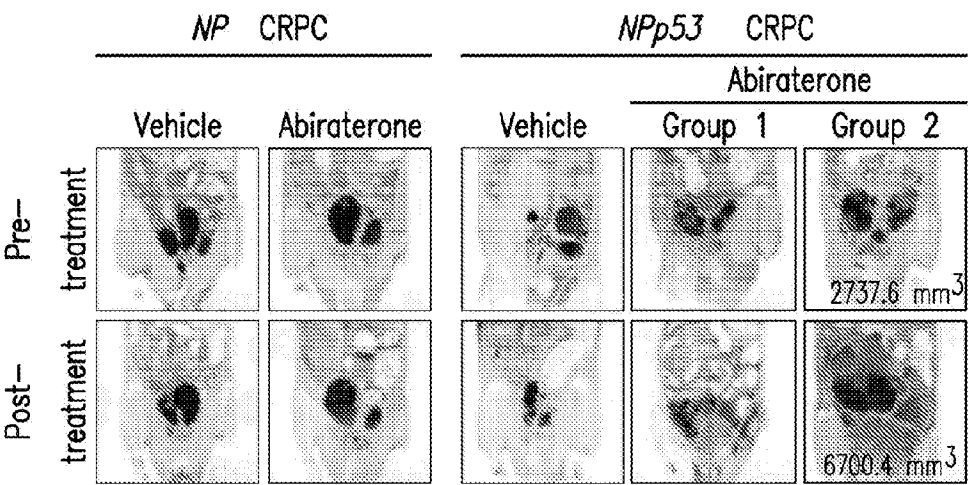


FIG.2D

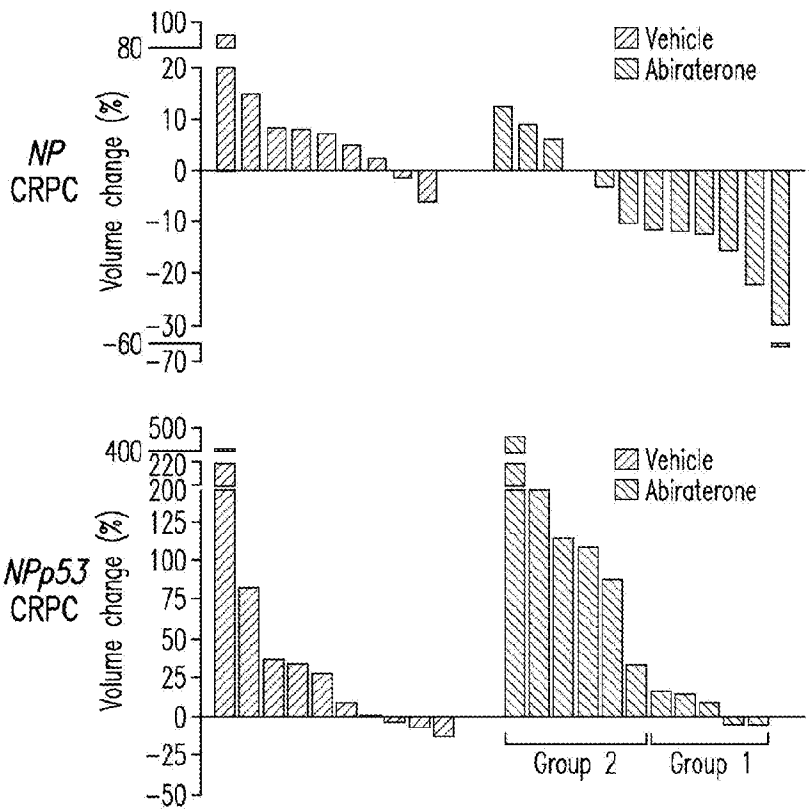


FIG.2E

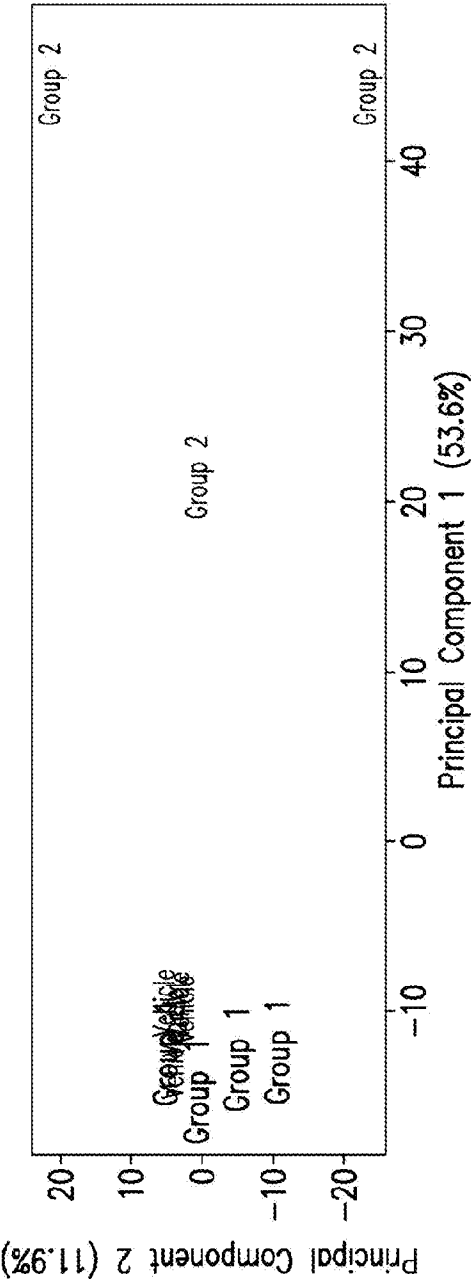


FIG.2F

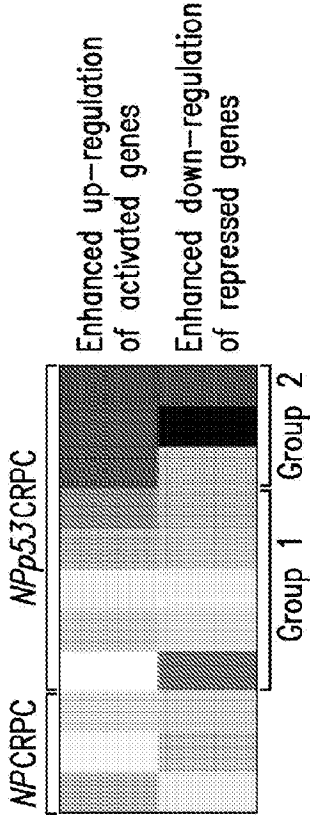


FIG.2G

7/26

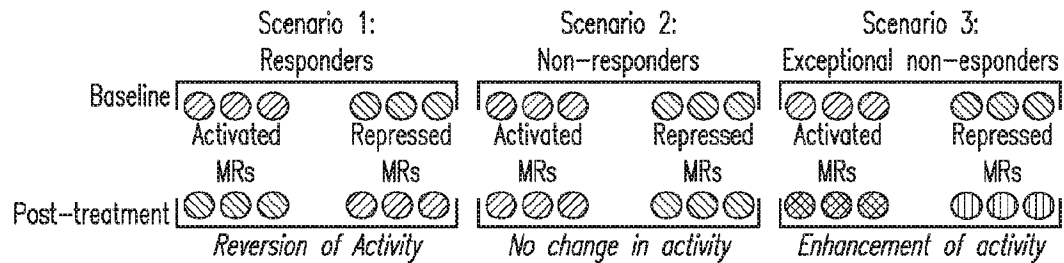


FIG.3A

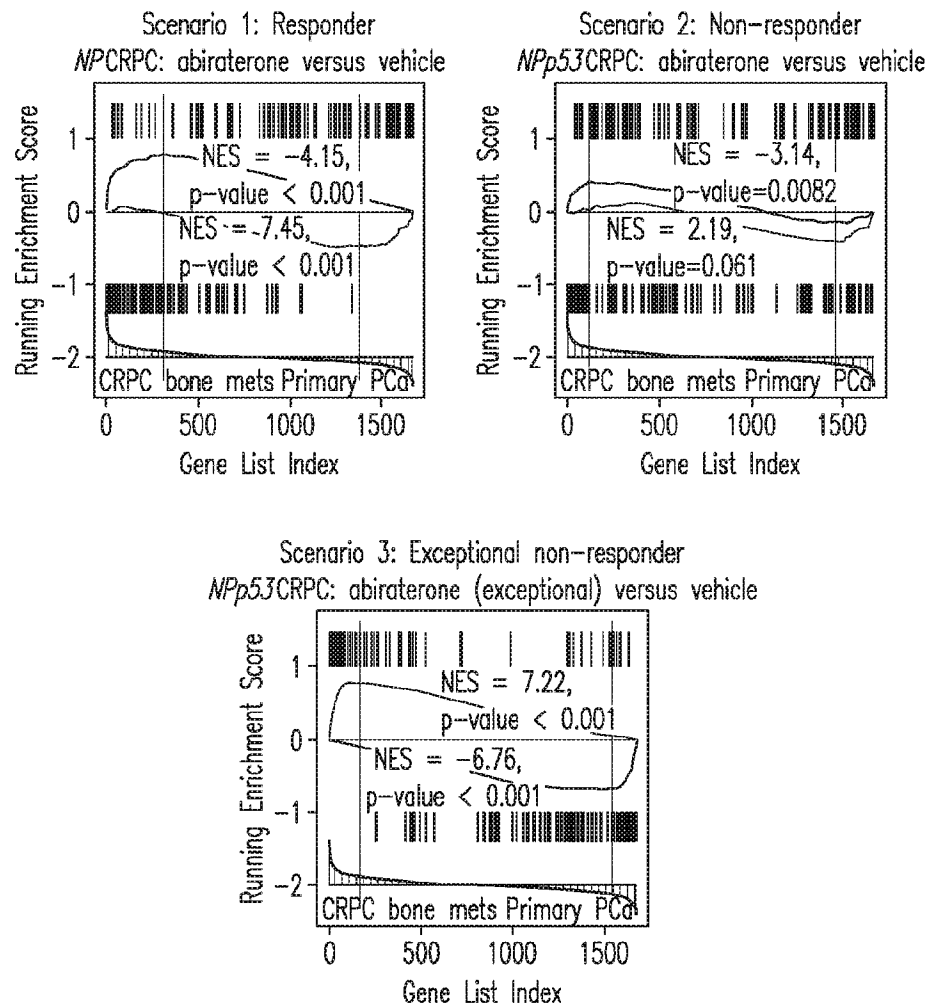
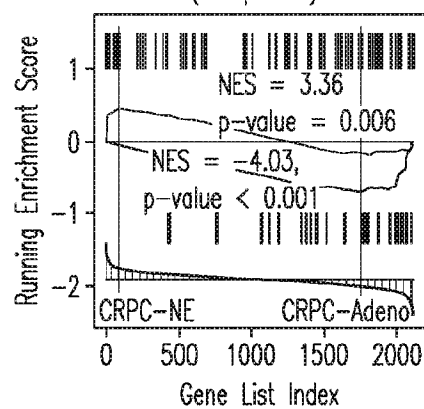
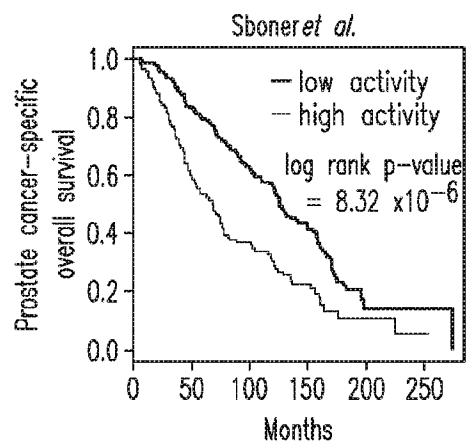


FIG.3B

Exceptional non-responder versus Responder
NPp53 CRPC-abiraterone (exceptional) vs. *NP* CRPC-abiraterone

**FIG.3C****FIG.3D**

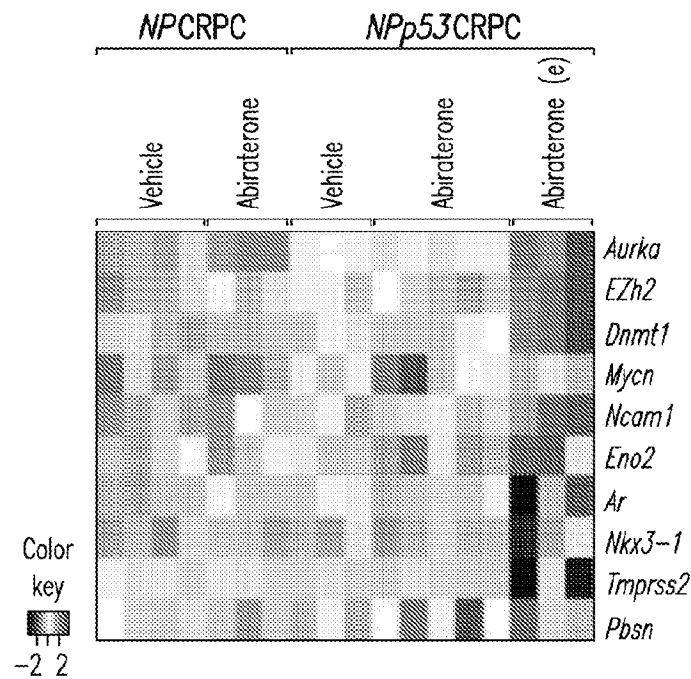


FIG.4A

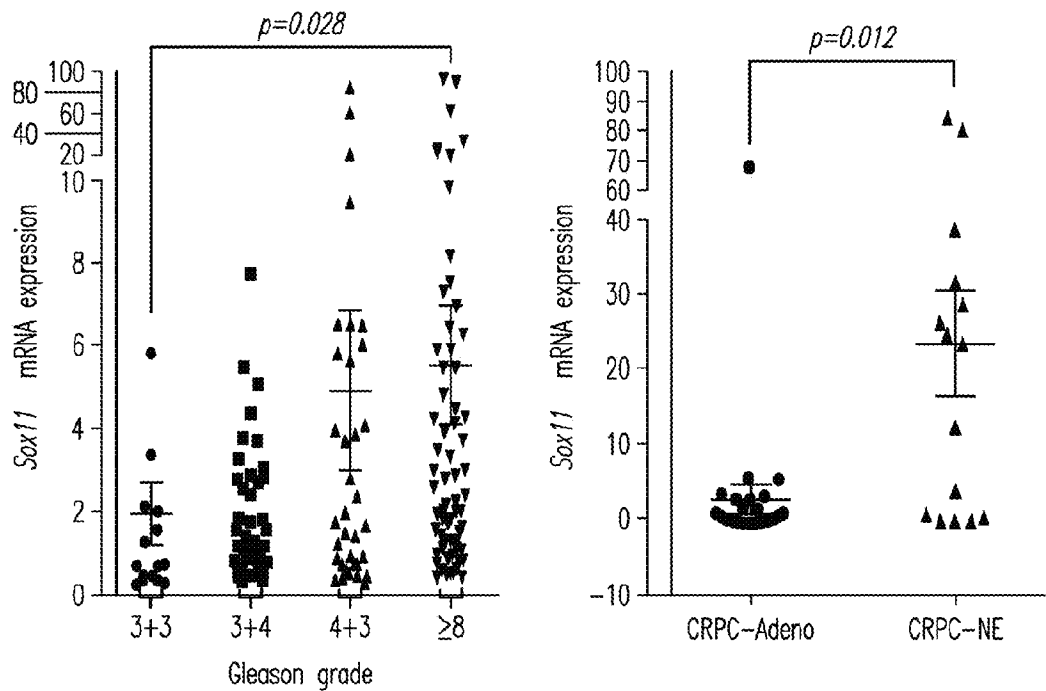


FIG.4B

10/26

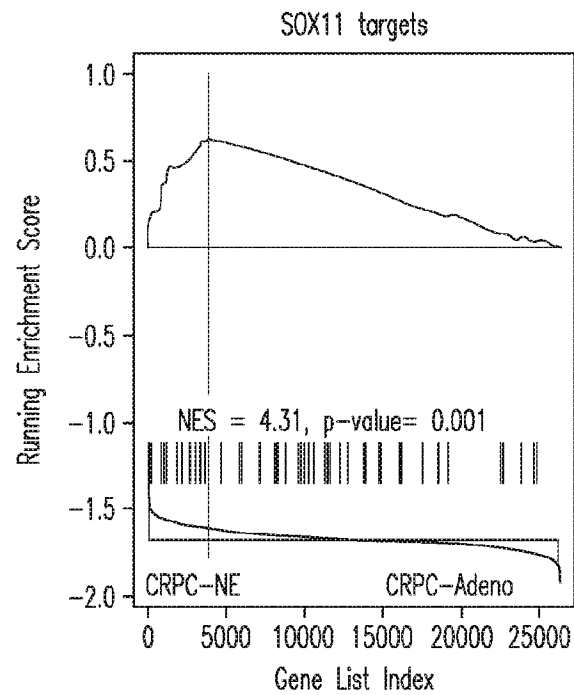


FIG.4C

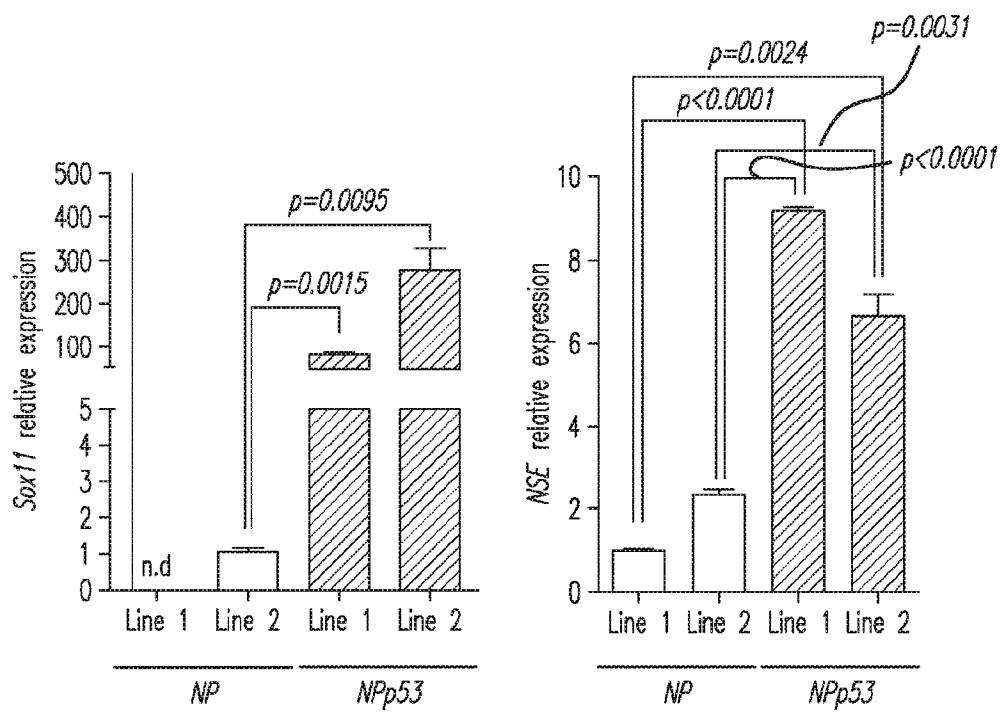


FIG.4D

11/26

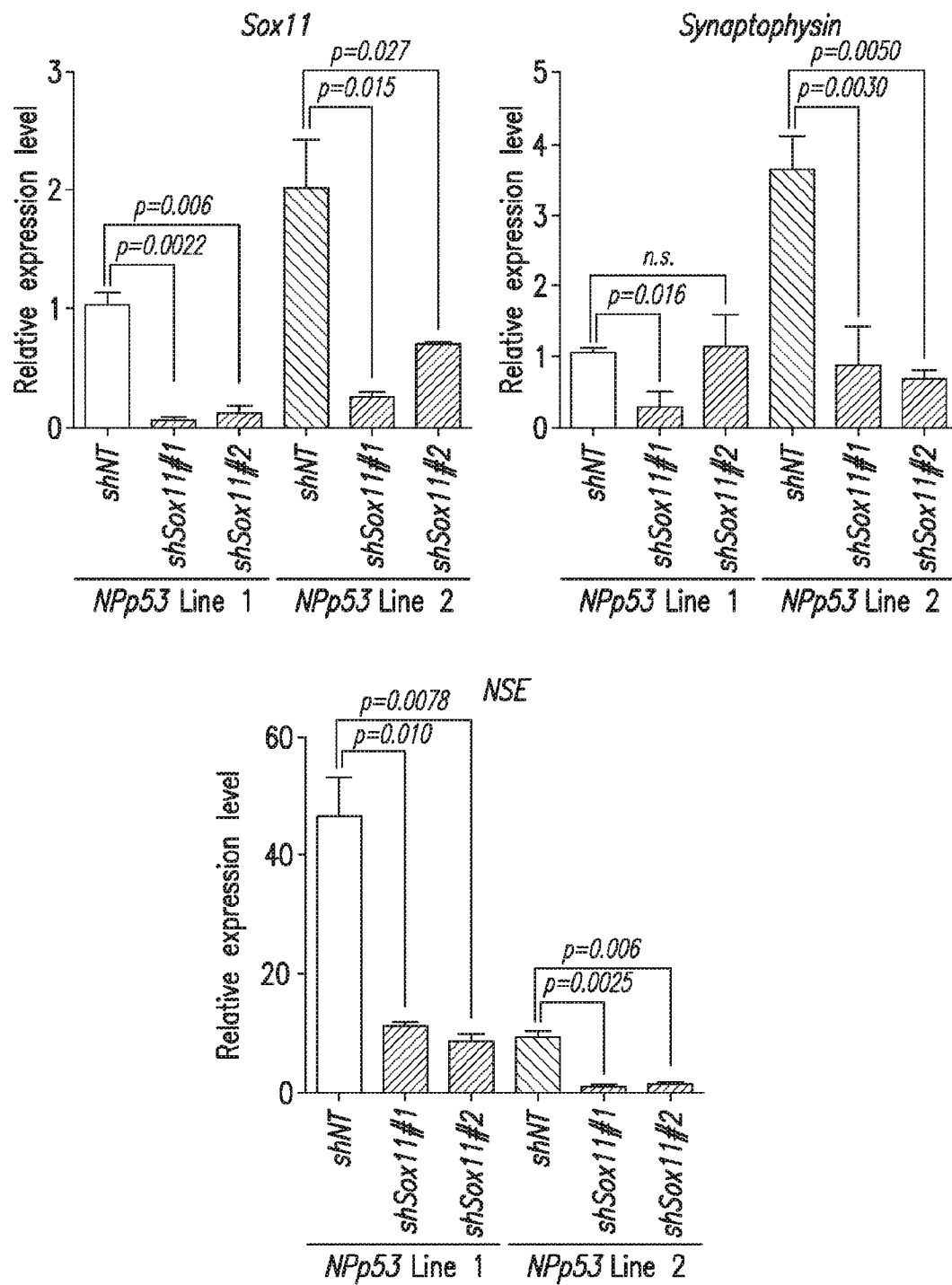


FIG.4E

12/26

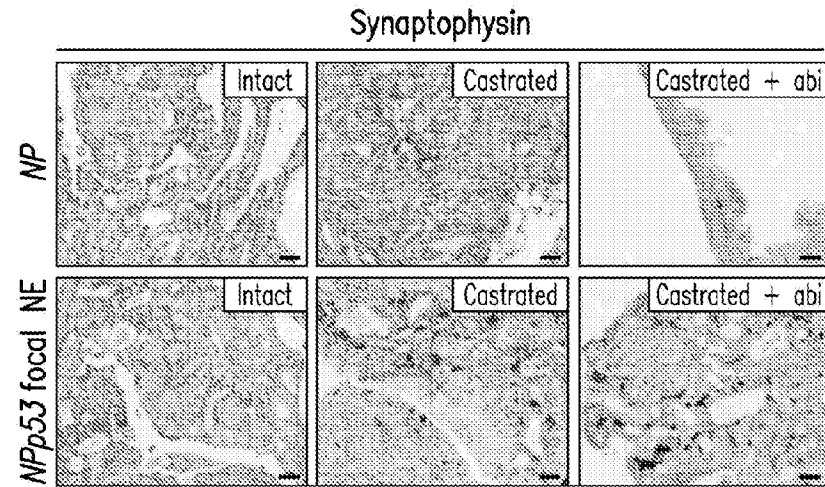


FIG.5A

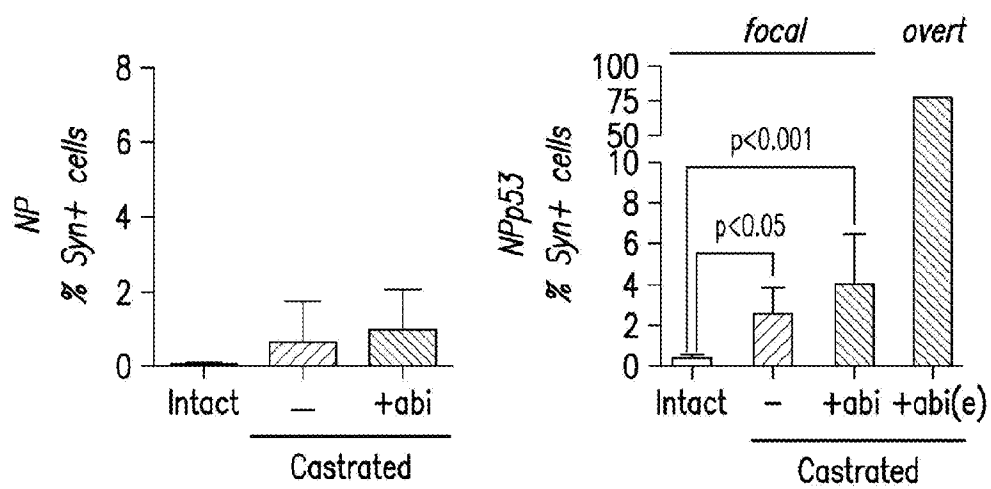


FIG.5B

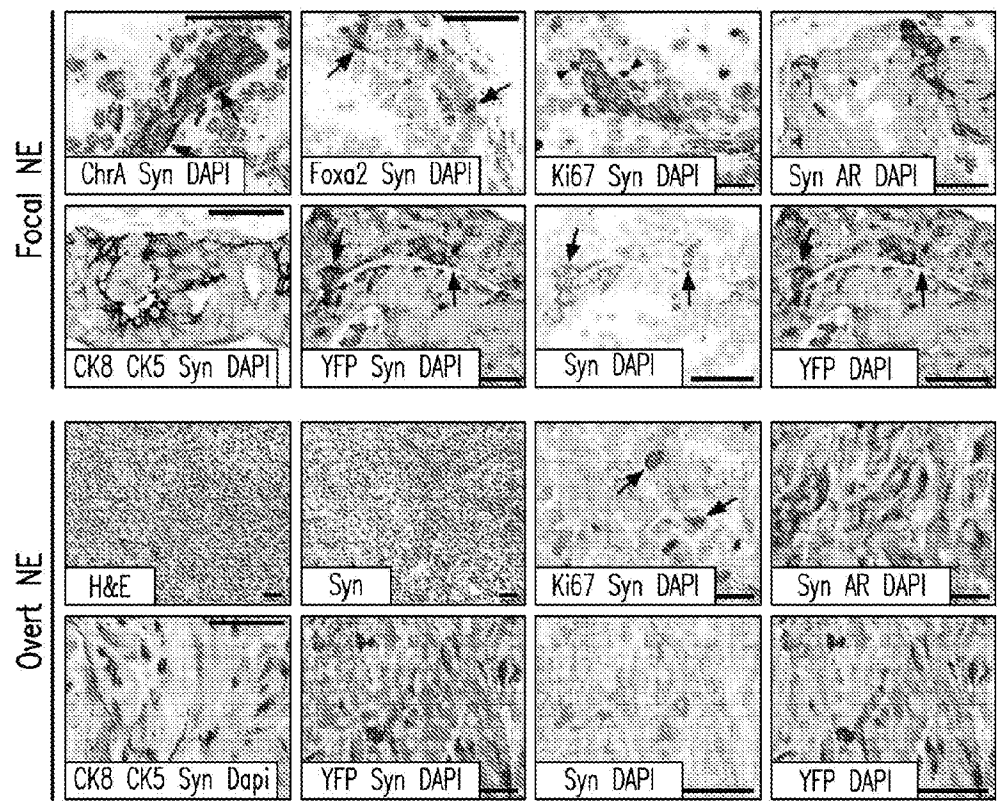


FIG.5C

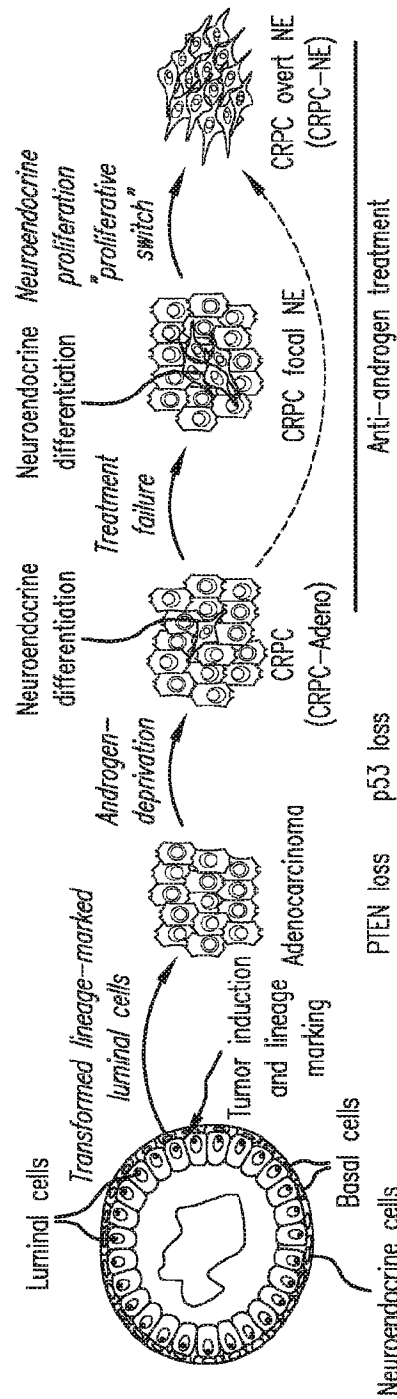


FIG.6

15/26

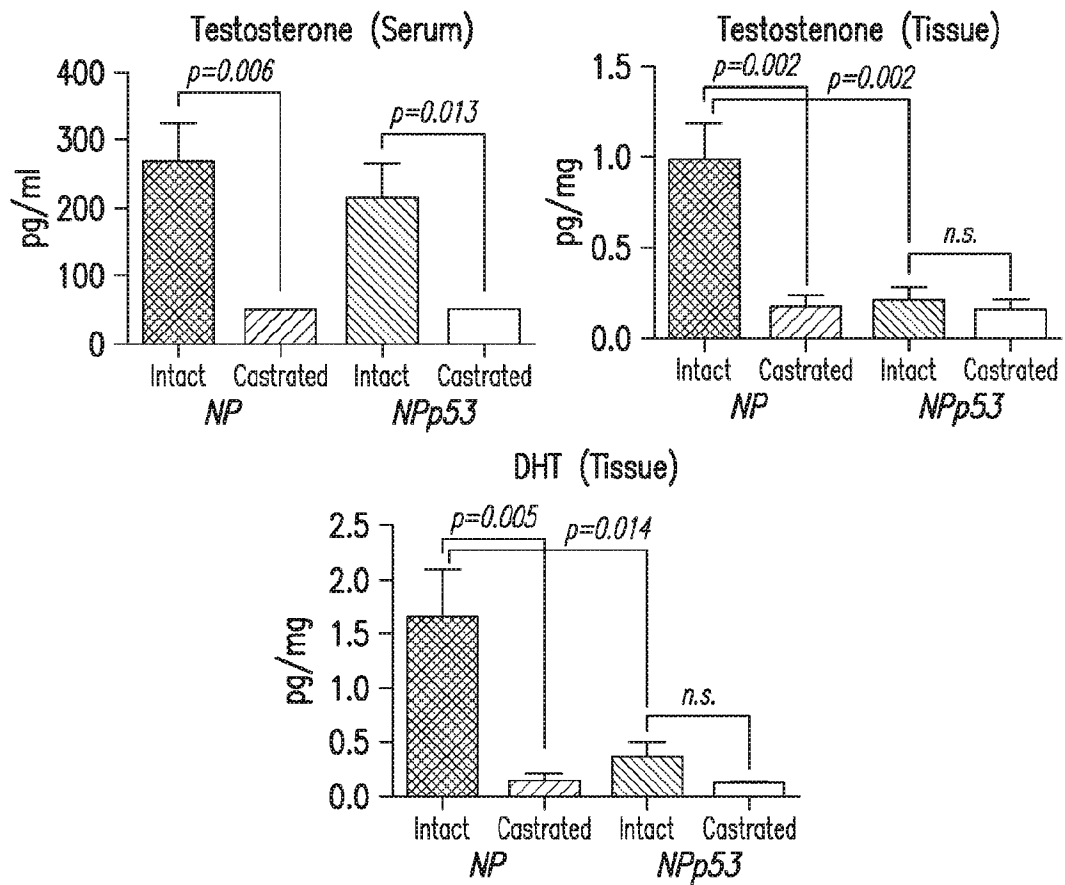


FIG.7A

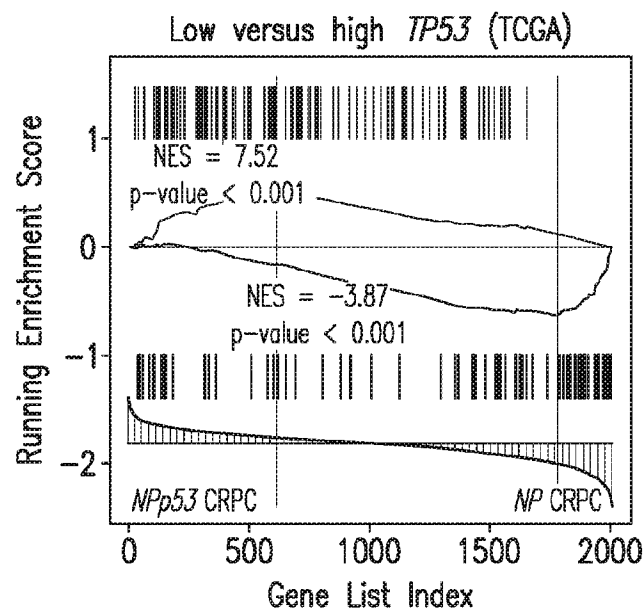


FIG.7B

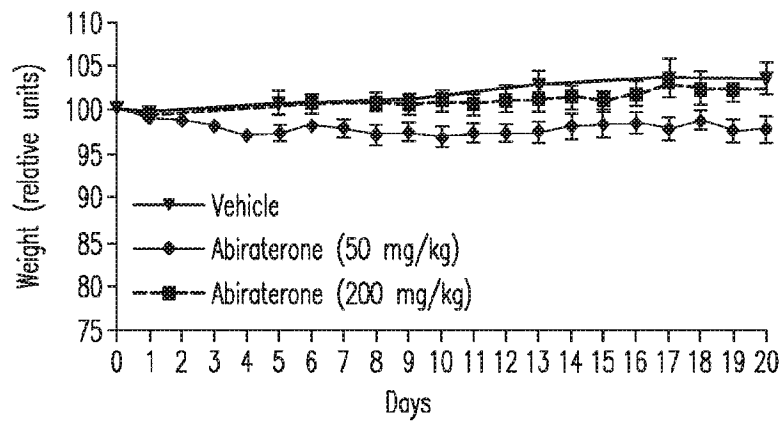


FIG.8A

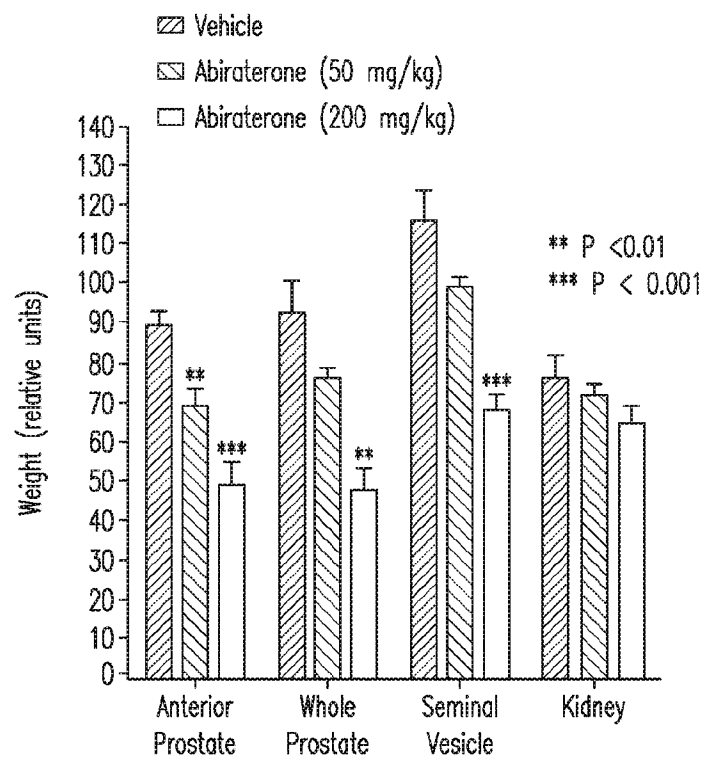


FIG.8B

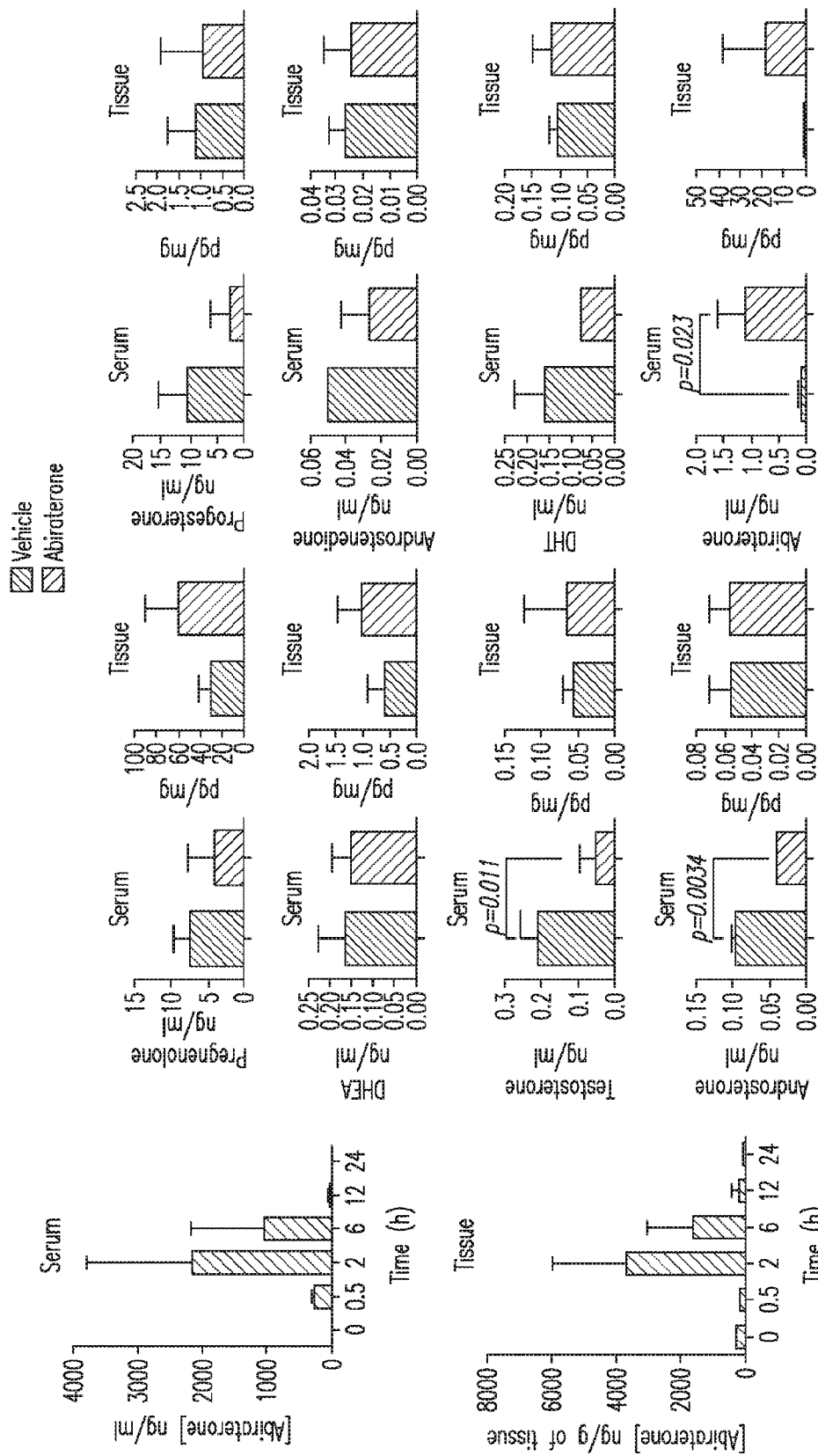


FIG. 8D

FIG. 8C

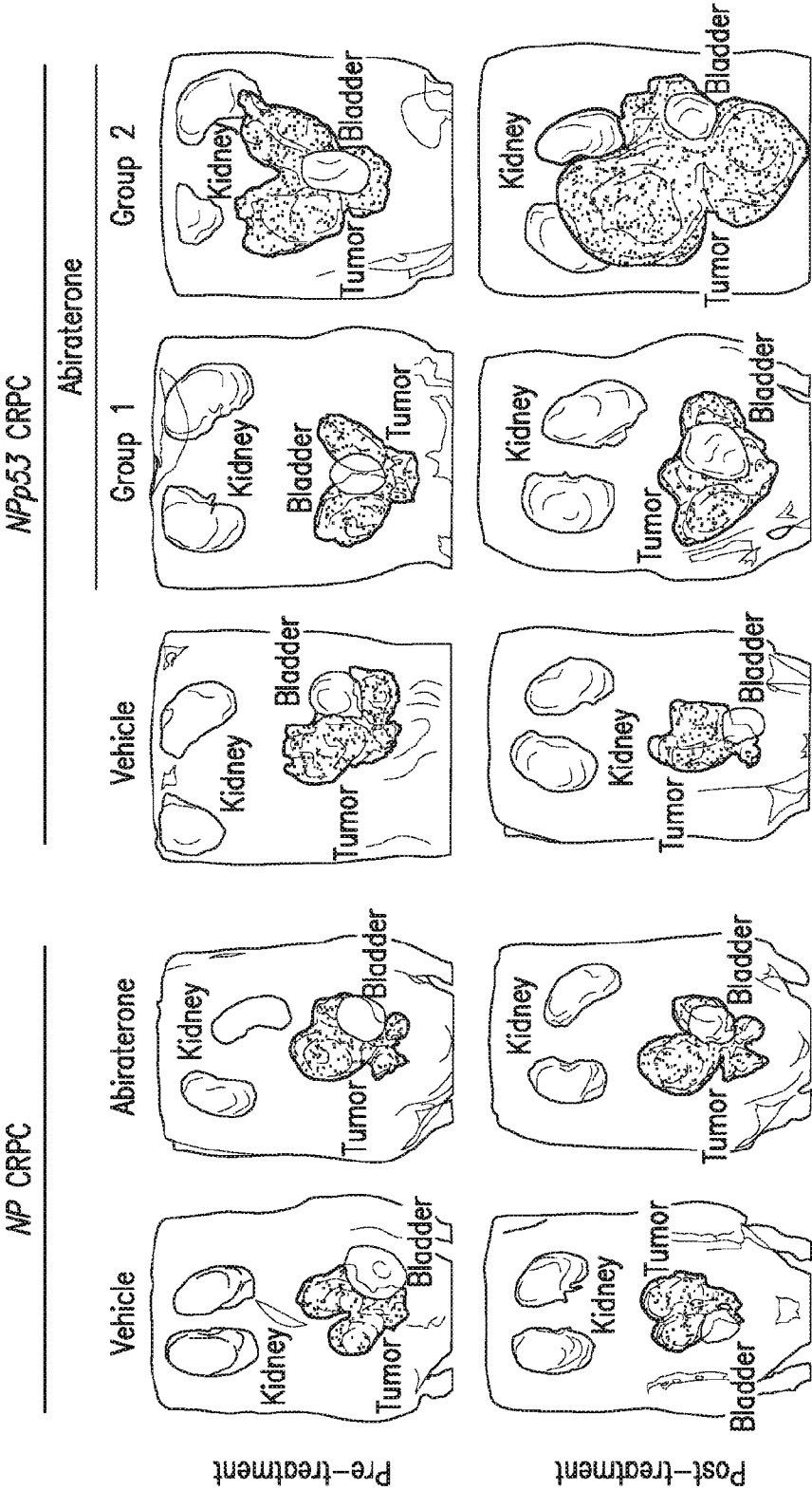


FIG.9A

19/26

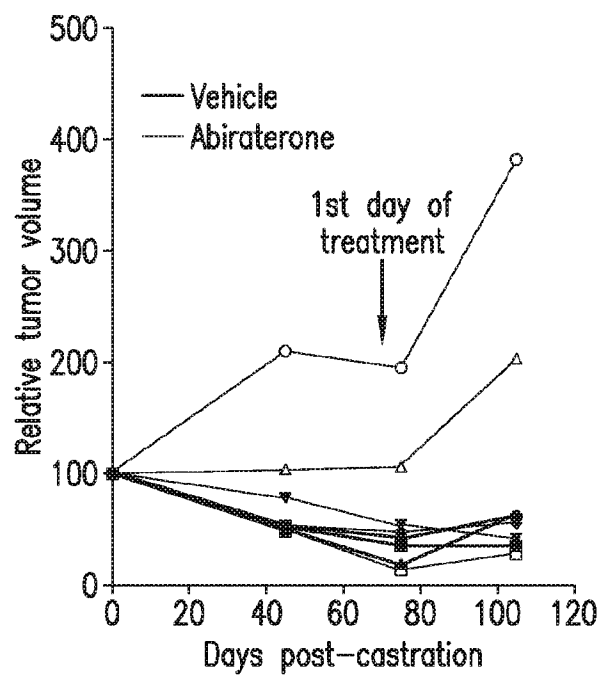


FIG.9B

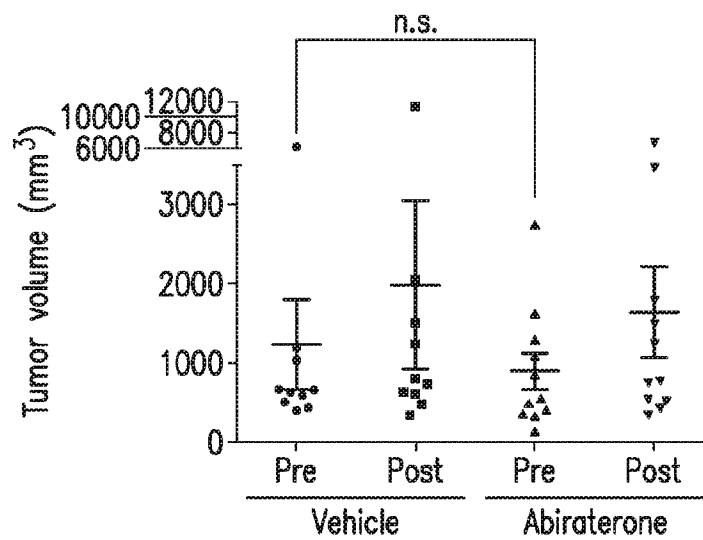


FIG.9C

20/26

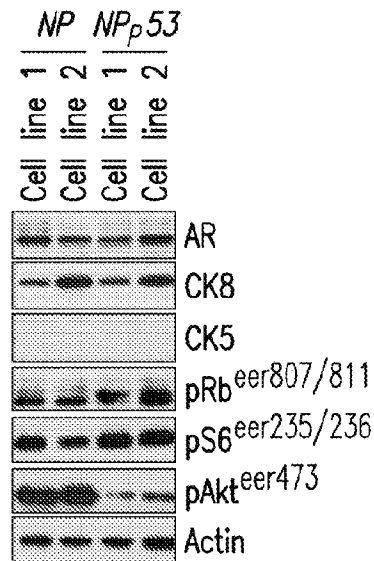


FIG. 10A

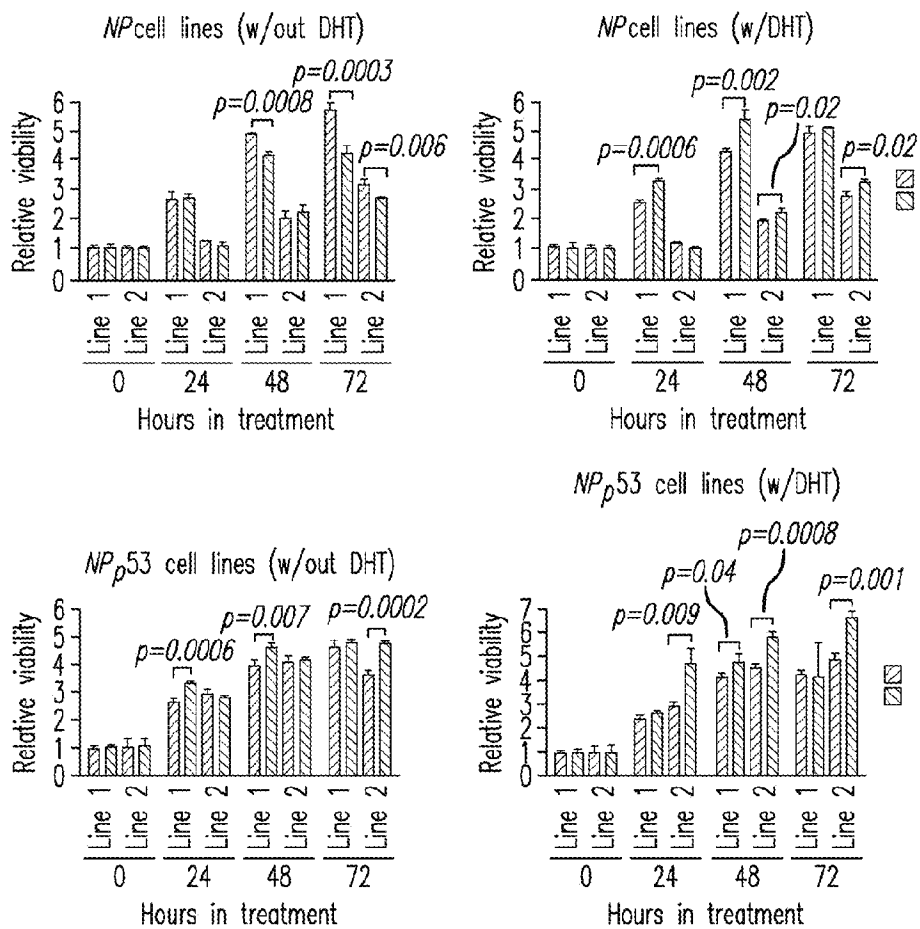


FIG. 10B

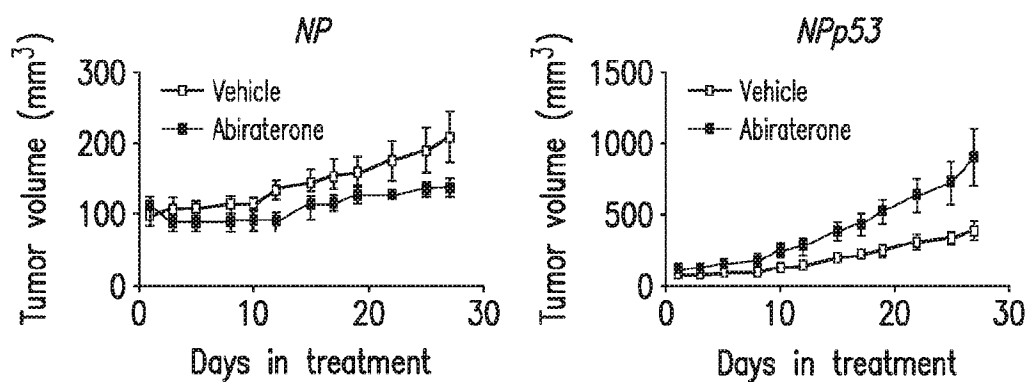


FIG.10C

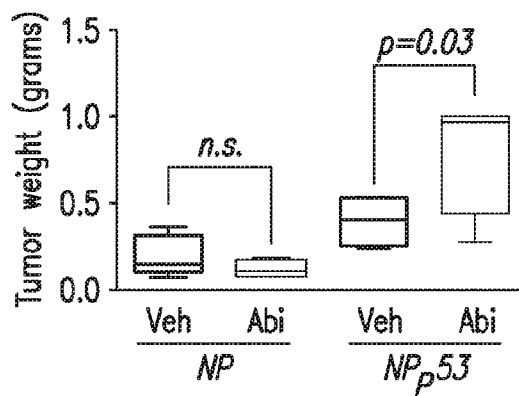


FIG.10D

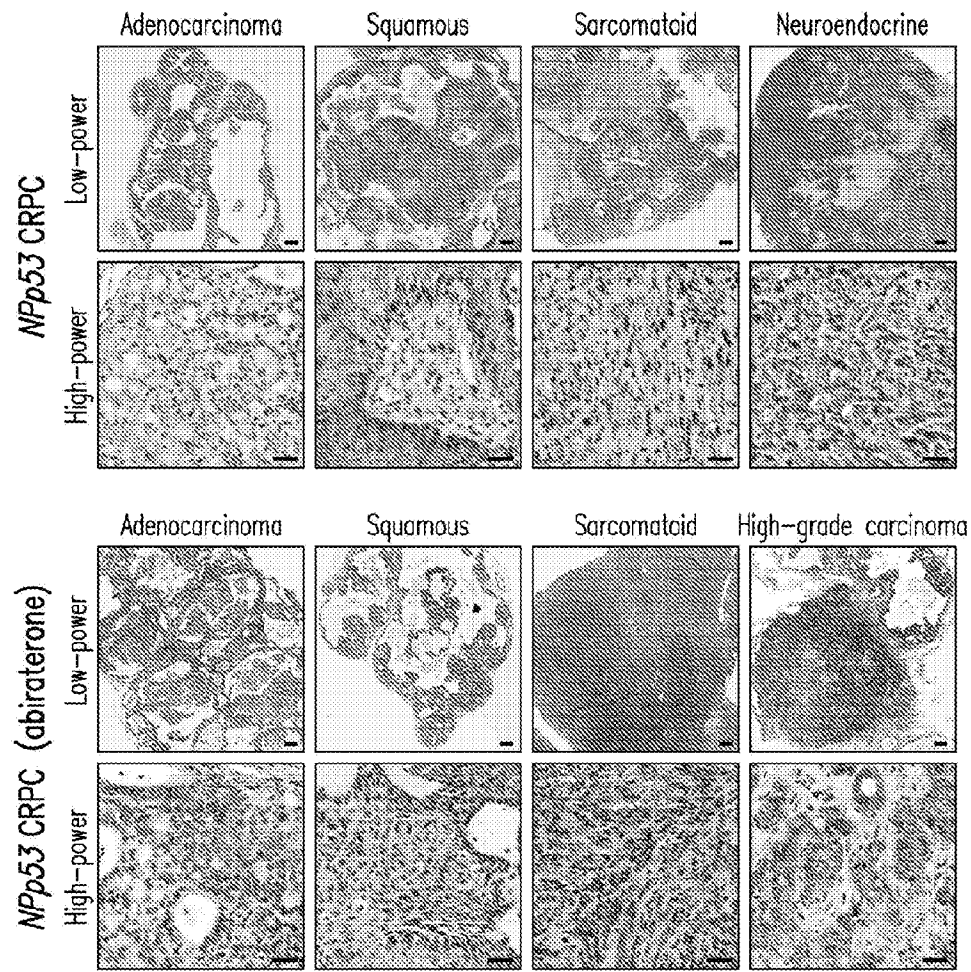


FIG.11

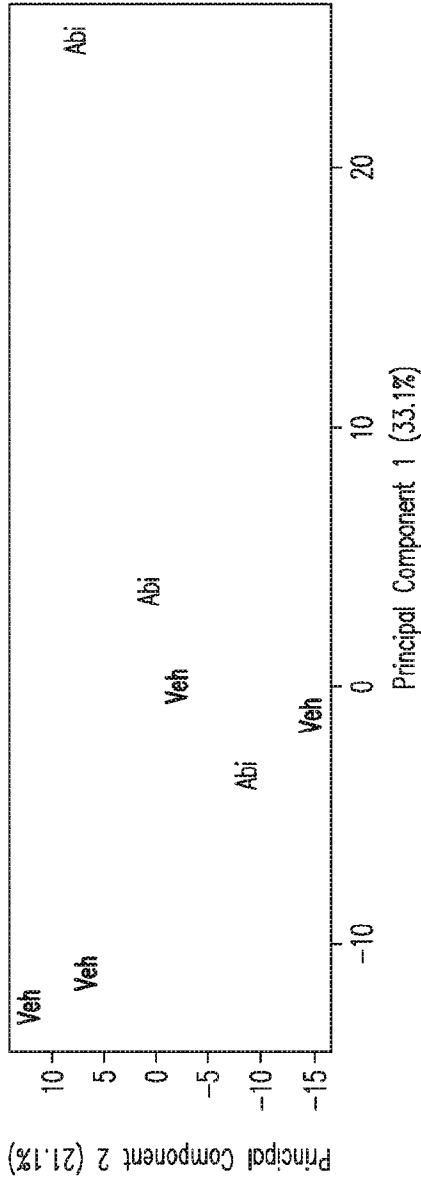


FIG.12A

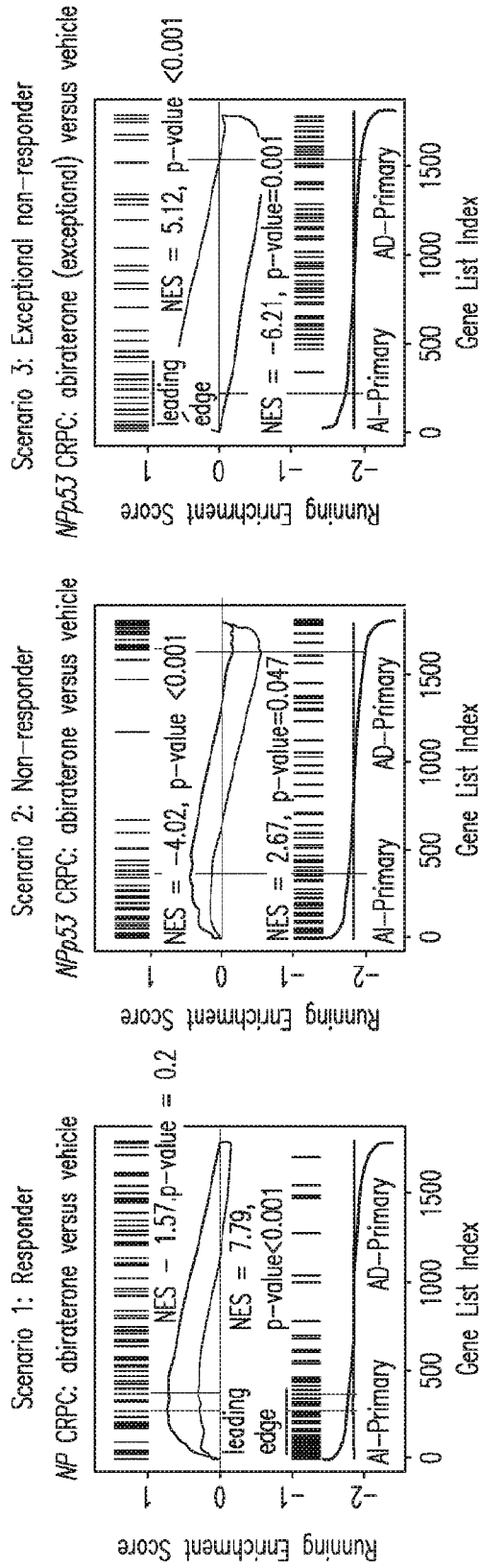


FIG.12B

24/26

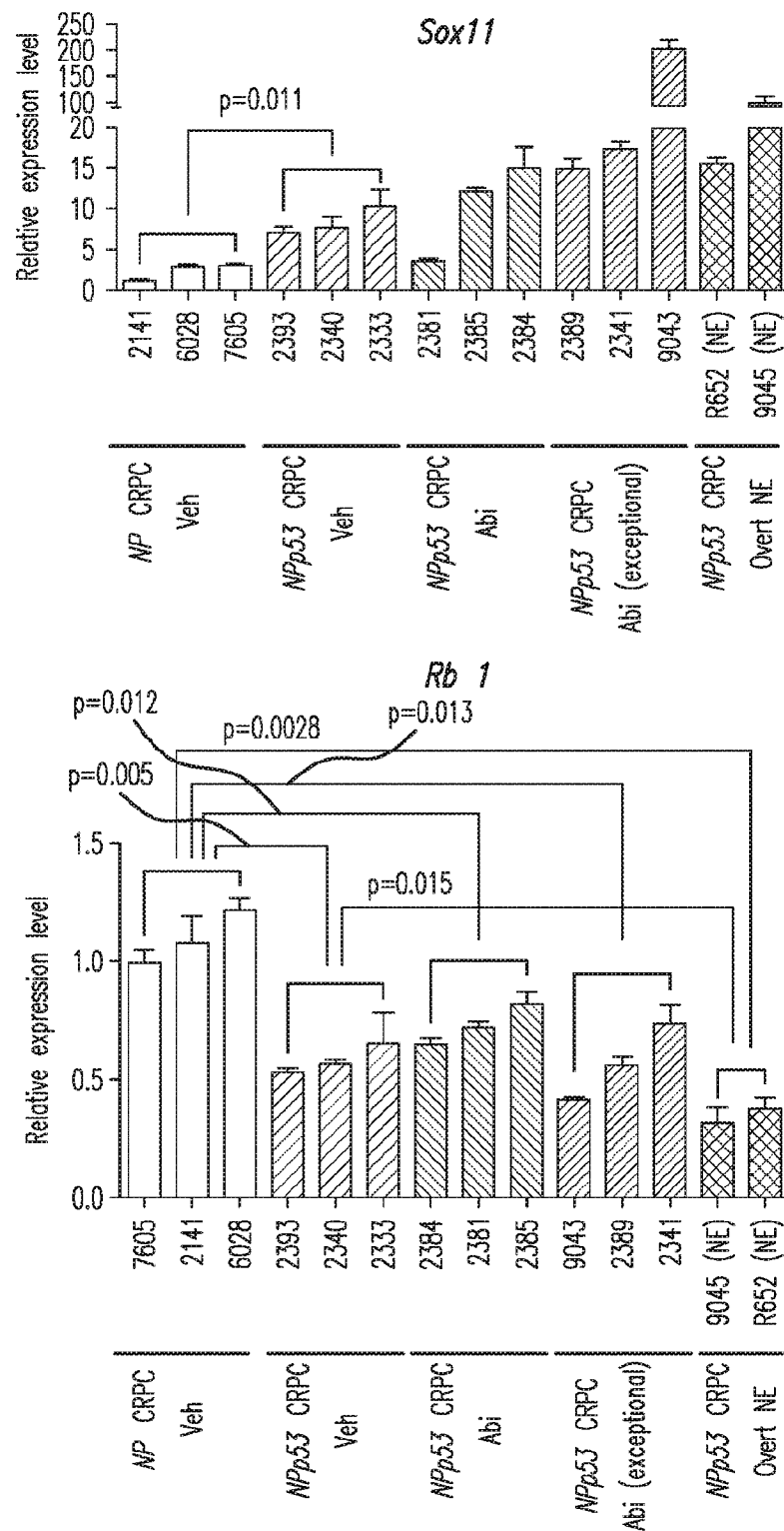


FIG. 13A-1

25/26

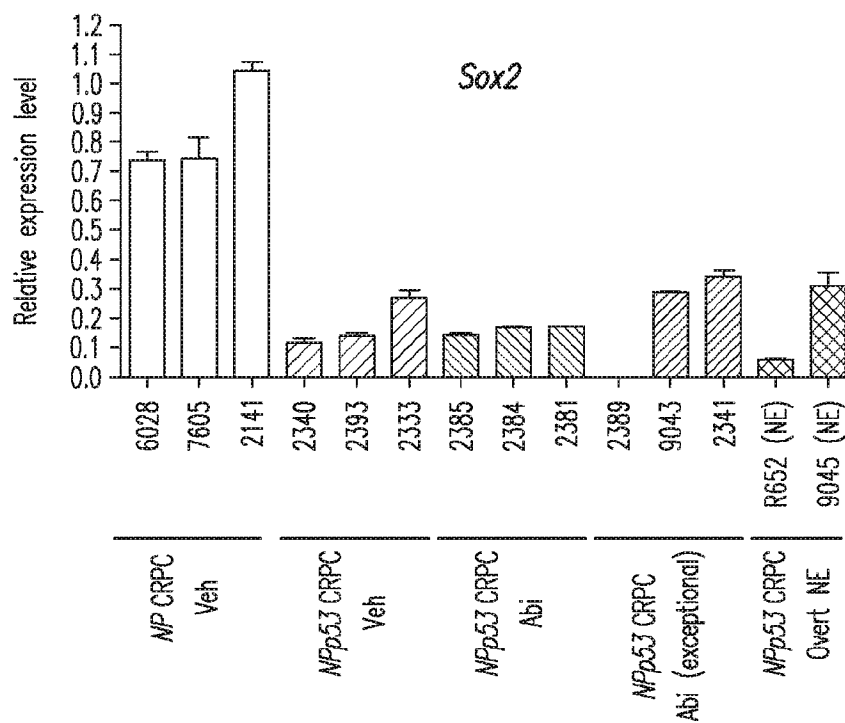


FIG.13A-2

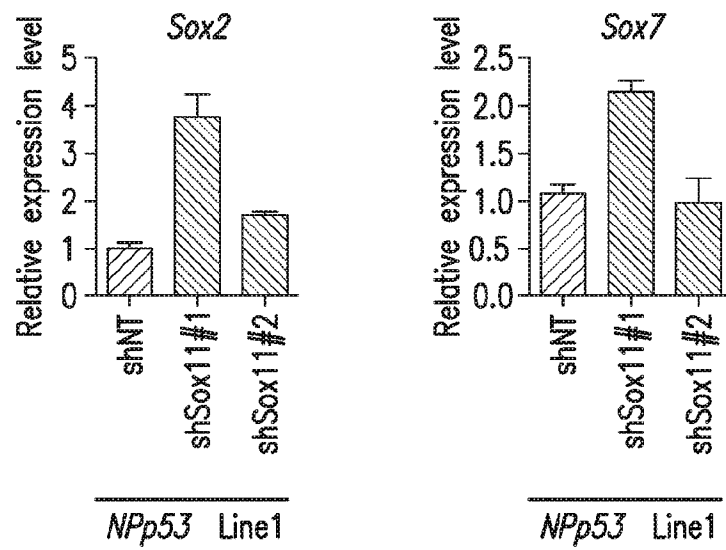


FIG.13B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/038803

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/113; C12Q 1/68; G01N 33/50; G01N 33/574; G06F 19/10; G06F 19/18 (2017.01)

CPC - C12N 15/113; C12N 2310/14; C12N 2320/32; C12Q 1/6886; C12Q 2600/112; C12Q 2600/118; C12Q 2600/158; G01N 33/57434; G01N 33/57484; G01N 2333/47; G01N 2800/52; G06F 19/10; G06F 19/18; G06F 19/22; G06F 19/3431 (2017.08)

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)

See Search History document

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

USPC - 424/156.1; 435/7.23; 702/19 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	BELTRAN et al. "Divergent clonal evolution of castration resistant neuroendocrine prostate cancer," Nat Med, 08 February 2016 (08.02.2016), Vol. 22, Pgs. 298-305 and Supplemental Information. entire document	1, 2, 4, 6, 8-10, 15, 16 ----- 3, 5, 7, 13, 14
X — Y	US 2015/0147339 A1 (PSMA DEVELOPMENT COMPANY, LLC) 28 May 2015 (28.05.2015) entire document	11, 12, 17, 23, 24 ----- 3, 5, 7, 13, 14, 21, 22
X — Y	WO 2015127101 A1 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 27 August 2015 (27.08.2015) entire document	18-20 ----- 21, 22
A	US 2013/0225433 A1 (CHINNAIYAN et al) 29 August 2013 (29.08.2013) entire document	1-24
A	KANTOFF et al. "Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer," New England Journal of Medicine, 29 July 2010 (29.07.2010), Vol. 363, Pgs. 411-422. entire document	1-24

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 August 2017

Date of mailing of the international search report

22 SEP 2017

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774