**SUPPLEMENATARY DATA**

**SUPPLEMENTARY MATERIALS AND METHODS**

**Machine-aided quantitative IHC quantification**

Quantitative synaptophysin IHC analysis was performed for tumors demonstrated ≥ 5% synaptophysin immunoreactivity, including 10 of 49 trial cases and 14 of 49 untreated controls, to evaluate the extent of synaptophysin-positive cells (low-medium-high immunointensity). First, all areas of invasive tumors were annotated on the scanned slide images using 600 µm circle markers in Pannoramic Viewer, excluding benign glands, prostatic intraepithelial neoplasia (PIN), and intraductal carcinoma (IDC). Next, images corresponding to each annotation marker were captured. Segmentation, cell identification, and quantification of tumor cells that are negative or positive for synaptophysin were then performed on each captured image, using Definiens image analysis software (version 4.2, Definiens Tissue Studio).

**Calling of differentially expressed genes and pathways**

The quality of scanned array images was determined on the basis of background values, average perfect-match probe intensity, area under the curve for probe hybridization to negative and positive controls, spike in controls, scatter, MA plots and 3′ -5′ ratios of *ACTB* and *GAPD* using various BioConductor R packages. To obtain the signal values, chips were further analyzed using Robust Multichip Average (RMA) method that includes background correction, quantile normalization and summarization of signal using median polish algorithm. The array data was normalized using Entrez ID based customized CDF files from the Brainarray database (1). The expression data from Affymetrix Human 1.0 ST and HTA 1.0 arrays were combined based on common Entrez ID. Batch effects among the datasets were removed using the ComBat algorithm. After normalization and batch correction, unsupervised and supervised analysis was performed; unsupervised analysis was performed on normalized and preprocessed data using hierarchical clustering with Euclidean Distance and Ward-linkage method.

To identify differentially expressed genes between control and cases, a linear model was implemented using the linear model microarray analysis (LIMMA) software package (2). Transcripts with absolute fold change ≥ 2 between cases and controls with a multiple test corrected *P* ≤ 0.05 were considered differentially expressed. Pathway and regulatory network analyses were performed on differentially expressed genes using Ingenuity Pathway Analysis (Qiagen) platform. To identify pathways and biological processes further based on differences between cases and controls, single-sample gene set enrichment analysis (3) was performed on adjusted gene expression data, transforming gene level data to pathways and biological processes, comparing the treated to the untreated cohort. Statistical comparisons of gene expression and gene set enrichment analyses (Tukey’s test, Pearson correlation, or Spearman correlation) were performed using GraphPad Prism 7.

**Calling of genomic alterations**

Point mutation calling required no greater than 90% strand bias, with mutations present in at least 5 reads and a minimum variant allele frequency of 20%. It was also required that the benign sample (obtained by LCM of an area on the block that was histologically benign) had at least 15× coverage with no more than 1 mutant read at the same position. It should be noted that low level detection of a mutation in the benign control could reflect contamination with tumor or a field effect. Only mutations covered or adjacent to hybrid capture probe target regions are reported. To be considered exclusive to a focus, it was required that the same position be covered at least 15× with no more than 1 mutant read at the same position in the other tumor focus.

 To identify SCNAs, pre-processed and duplicate-removed BAM files were processed by the Python ngCGH package, comparing pairs of tumor BAM files to their matched benign control to generate pseudo-CGH probe estimates. Each probe consisted of a variable-length genomic window per 1,000 unique reads. Copy number change events were called from the median-centered log2 ratios of these values using the FASST2 circular binary segmentation algorithm and a hidden Markov model (HMM) with systemic quadratic correction in BioDiscovery Nexus Copy Number. To mark a region as gain or loss for a segment of the genome, 50 contiguous probes were required to be gained or lost at an HMM significance threshold of *P* < 10-6. Probes were required to be within 200 kilobases of each other for a contiguous gain or loss event. Thresholds were log2 ratios of -0.25 for shallow deletions, -0.5 for deep deletions and +0.25 for gains.

 Cancer cell fraction (CCF) computation as described previously (4) required ploidy, purity, and variant allele fraction values. Ploidy was determined by copy number call from each segment of the genome as described above. A conservative estimate of 30-50% was determined for LCM after review of images. Only mutations with raw variant allele fractions greater than approximately 10% (corresponding to CCF ≥~35%) are reported. In cases where a mutation met this criteria in one focus, the requirement for it to be considered to be shared was that the other focus/foci must harbor that mutation within 50% of the CCF for the first focus. Mutations were individually inspected using the Integrative Genome Viewer. Regions of overlapping copy number alterations were determined using BedTools intersect. Euler diagrams were generated using the VennEuler package for Bioconductor.

**Immunohistochemical and *in situ* hybridization assays**

Five-micron sections were baked at 60 ºC for an hour, followed by deparaffinization, rehydration, and epitope retrieval using the Dako PT Link platform. Immunohistochemical staining was carried out on Dako Link 48 autostainer, with antibody incubation, amplification using Envision FLEX rabbit or mouse linkers, and visualization using the Envision Flex High-sensitivity visualization system (Dako). For all cases, consecutive sections were stained using anti-AR (N-20, Santa Cruz; 1:1000), anti-AR-V7 (RM-7, RevMAb, 1:1000), anti-PSA (Dako, RTU), anti-Ki-67 (MIB-1, Dako; RTU), anti-ERG (EPR3864, Epitomics; 1:100), anti-NKX3.1 (Biocare; RTU), anti-PTEN (D4.3, Cell Signaling; 1: 50), anti-GR (D6H2L, Cell Signaling; 1:800), anti-synaptophysin (DAK-SYNAP, Dako; RTU), anti-chromogranin (DAK-A3, Dako, 1:800), pAkt Ser473 (D9E, Cell Signaling, 1:50), and anti-pRb (4H1, Cell Signaling, 1:500) antibodies. Dual immunostains for Ki-67 (MIB-1, Dako; RTU) and CK18 (DC10, Dako; 1:4) were stained on Dako Link 48 autostainer, using Dako EnVision G|2 Doublestain System following manufacturer’s protocol. PIN-4 staining was performed manually using the CK5/14 + P63 + P504S ready-to-use cocktail (Biocare) for 1 h following heat-induced epitope retrieval in Diva Decloaker (Biocare) for 30 m and detection with the MACH 2 Double Stain polymer reagent (Biocare). *In situ* hybridization (ISH) staining was performed using probes recognizing total AR (Hs-AR, Advanced Cell Diagnostics) and AR variant 7 (Hs-AR-V7, Advanced Cell Diagnostics) using RNAscope® 2.0 HD Detection Kit following manufacturer’s protocol.

**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure S1.** (A) H&E stains of RP specimens of two representative trial cases (×400 magnification), demonstrating prostatic adenocarcinoma, not otherwise specified (NOS). Different treatment effects are shown, with low (top) and high (bottom) tumor cellularity. (B) H&E stain and immunostain for synaptophysin (SYP), androgen receptor (AR), and NKX 3.1 on a trial case containing two components: adenocarcinoma, NOS (black arrows) and adenocarcinoma with Paneth-cell like neuroendocrine differentiation (red arrows) (×400 magnification). Scale bar: 30 μm. (C) A spectrum of NED, from rare to extensive, depicted by anti-synaptophysin (SYP) IHC of three example cases from treated and untreated cohorts (×400 magnification); (D-E) Quantitative image analysis of synaptophysin-positive cells in in 10 treated and 14 untreated cases that demonstrated positive synaptophysin staining in ≥ 5% tumor cells. “All cells” includes low, medium, and high immunointensity. “Medium/high intensity” excludes low immunointensity. (D) Box-and-whiskers plot of the absolute number of synaptophysin-positive tumor cells. Comparing the treated and untreated groups, *P* = 0.0484 by Mann-Whitney test for all cells, and *P* = 0.0038 for medium/high intensity cells. (E) Box-and-whiskers plot of percentage of synaptophysin-positive tumor cells. Difference in percent SYP immunointensity was not significant (n.s.) comparing the treated and untreated groups, at *P* = 0.7887 by t test with Welch’s correction for all cells, and *P* = 0.5953 for medium/high intensity cells.

**Supplementary Figure S2.** Scatter plots depicting relationships between the 267-gene AR activity ssGSEA score and the 131-gene proliferation ssGSEA score for multiple genesets. (A) Prostate TCGA primary PCa (5). *N* = 499. (B) MSKCC 2010 primary PCa (6). *N* = 131. (C) Stanbrough mCRPC (7). *N* = 33. (D) Prostate Cancer Foundation-Stand up To Cancer mCRPC (8). *N*  = 118*.* (E) FHCRC mCRPC (9). *N* = 171. Pearson *r* correlation coefficients are shown with their 95% confidence intervals.

**Supplementary Figure S3.** Scatter plot depicting correlation of the 267-gene AR activity ssGSEA score and log2-transformed microarray expression value for *AR* (A), *AKR1C3* (B), *CYP17A1* (C) and *NR3C1* (D) from each microdissected focus. Pearson *r* correlation coefficients are shown with their 95% confidence intervals. (E) Stacked bar chart of nuclear GR IHC scores. For the 12- and 24-week treatment arms, the difference was not significant at *P* = 0.8633 by Wilcoxon rank sum test. Box-and-whiskers plots of comprehensive 121-gene (F) or the selective 67-gene (G) GR activity signature comparing treated to untreated cases. ssGSEA scores were plotted using Tukey’s method for a box-and-whiskers plot, where the box depicts the 25th, 50th, and 75th percentiles, whiskers depict either the lowest and highest values or the 25th and 75th percentiles ± 1.5× IQR, and open circles depict outlier scores higher or lower than the 75th/25th percentile ± 1.5× IQR. Statistical differences in (F) and (G) were determined using the t test with Welch’s correction.

**Supplementary Figure S4.** Segmented read depth array CGH-style plots for chromosomes showing alterations to cancer-related genes. Plots were generated using Biodiscovery Nexus Copy Number for Mac. Each lettered panel represents a single case, with each column corresponding to a focus of dissected tumor. Red bars and downward arrows indicate genomic losses; blue bars and upward arrays indicate genomic gains.

**Supplementary Figure S5.** Euler diagrams depicting shared and distinct somatic genomic events. Circles are drawn to scale for all mutations and copy number alterations that passed filtering requirements. Annotations are limited to alterations overlapping with 727 curated cancer-related genes (10). Cases 003, 007, 501, 503, 506, 507, 509, 512, and 520 are shown.

**SUPPLEMENTARY TABLES**

Supplementary Table S1. Clinicopathologic characteristics of microdissected cases.

Supplementary Table S2. Genes up-regulated by 2-fold or more in the treated vs. untreated cases.

Supplementary Table S3. Fold change in expression of AR coactivators in treated versus untreated cases.

Supplementary Table S4. Genes down-regulated by 2-fold or more in the treated versus untreated cases.

Supplementary Table S5. Somatic copy number alterations.

Supplementary Table S6. Shared somatic mutations.

Supplementary Table S7. Unique somatic mutations.

Supplementary Table S8. Mutations in 3 cases with only 1 focus dissected.

Supplementary Table S9. Number of genomic alterations in each focus.

**SUPPLEMENTARY REFERENCES**

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