

# **Genetic Alterations Detected in Cell-free DNA are Associated with Enzalutamide and Abiraterone Resistance in Castration-resistant Prostate Cancer**

**Torquato, et al**

## **SUPPLEMENTAL MATERIALS AND METHODS**

### **Patients**

This prospectively collected biomarker study was approved by the Johns Hopkins Medicine Institutional Review Board (IRB). All patients provided written informed consent prior to enrollment. Patients had histologically confirmed prostate adenocarcinoma, progressive disease despite ADT, and documented metastatic disease by computed tomography (CT) or bone scan with technetium-99mm-labeled methylene diphosphonate. Eligibility criteria included patients diagnosed with mCRPC who were about to begin either abiraterone or enzalutamide therapy. 70 patients were prospectively enrolled between September 2014 and June 2017 at the Johns Hopkins Hospital (Baltimore, MD) and Sibley Memorial Hospital (Washington, D.C.) and followed through April 2018. Of the 70 enrolled patients with mCRPC who were about to begin either abiraterone or enzalutamide therapy, three patients were excluded due to concurrent treatment with other therapies (Veliparib or Docetaxel). Five eligible patients were excluded due to the absence of clinical follow-up data. Blood and clinical follow-up data were obtained for the remaining 62 patients. Blood was collected prior to initiation of therapy for all patients with most pre-therapy samples collected within one week prior to start of therapy (median collection day was on the day of therapy initiation). Patients were treated with enzalutamide (n=25), abiraterone + prednisone (n=35), or concurrent enzalutamide plus abiraterone + prednisone (n=2).

### **Study Endpoints**

Study endpoints were PSA response, PFS, and OS. PSA response was defined by a  $\geq 50\%$  decline in PSA from pre-therapy baseline PSA. A  $\geq 30\%$  decline in PSA from pre-therapy baseline PSA

was also evaluated. Progression was defined by an increase in PSA by  $\geq 25\%$  above the baseline or nadir PSA, radiographic progression, or death from prostate cancer. PSA increase by  $\geq 25\%$  above the baseline or nadir PSA was confirmed by a subsequent PSA increase, radiographic progression, death from prostate cancer, or physician-determine change of therapy. PFS was defined by the time to the first of the following events: an increase in PSA by  $\geq 25\%$  compared to baseline or nadir PSA, radiographic progression, or death from prostate cancer. Blood was also collected following PSA increase from nadir prior to change in therapy for 26 of the 35 patients who had a decrease in PSA following therapy and then progressed as determined by an increase in PSA by  $\geq 25\%$  above the baseline or nadir PSA, radiographic progression, or death from prostate cancer.

### **Sample Collection**

Three, 10mL blood samples were collected in Streck BCT tubes prior to therapy initiation and if applicable, at progression. Blood was stored at room temperature and then processed for plasma isolation within 24 hours. To optimize patient sample integrity and to limit DNA contamination, plasma was extracted in a bleach- and ultraviolet (UV)-cleaned hood specifically for plasma extraction in a room dedicated for blood processing, storage, and cfDNA isolation. Plasma was extracted from blood by centrifugation for 10 minutes at 1,500 x g followed by a second centrifugation for 10 minutes at 3,000 x g as previously described<sup>1-4</sup>. Plasma was stored at -80°C in 1.5mL aliquots. In a dedicated bleach- and UV-cleaned hood, cfDNA was extracted from 3mL plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen) per the manufacturer's protocol. To limit cross-contamination, samples were processed individually.

## Deep NGS

GeneRead™ DNaseq Custom Mix and Match Targeted Panel V2 (Qiagen) (46 genes; 352,096 bases; Data Supplement) was used to prepare libraries from cfDNA for NGS as per the manufacturer's protocol. NGS libraries were prepared in a dedicated bleach- and UV-cleaned hood. cfDNA was quantified using Qiagen's QIAseq™ DNA QuantiMIZE Assay (DNQC-100Y-F) as per the manufacturer's protocol. Between 0.5 and 40.0ng of cfDNA was used for library generation. Samples were PCR-amplified using Qiagen's GeneRead™ DNaseq Panel PCR Reagent V2 (Cat. #181942) for 18-22 cycles depending on input concentration as per the manufacturer's protocol. PCR panel amplicons were purified using AMPure XP beads (Beckman Coulter, Cat. #A63880) and then quantified by the Agilent 2100 Bioanalyzer. Qiagen's GeneRead™ DNA Library Prep I Kit (Cat. #180435) and GeneRead™ Adapter I Set A 12-plex (Cat. #180985) were used to construct all libraries according to the manufacturer's instructions. Purified libraries were amplified using Qiagen HiFi PCR Master Mix for 5 cycles according to the manufacturer's instructions. Prepared libraries were quantified using QIAseq™ Library Quant Assay (Qiagen; QSTF-ILZ-F or NGTF-ILZ-R). NGS was performed on the Illumina Hi-Seq with a median on-target coverage of 6,631x.

## Sequence Alignment and Analysis of Variants

Raw sequencing data was aligned to Human Genome (build GRCH37.p13/hg19) reference<sup>5</sup> using BWA aligner (v0.7.10)<sup>6</sup>. Post-alignment data was passed through Picard Tools (V1.125)<sup>7</sup> to assess the alignment quality. Quality-controlled alignment data was employed to call the variants using an in-house variant caller, MDLVC, which scans through the alignment data for raw variants. Resulting raw variant calls were further applied with various filters including

minimum base quality of q25, minimum base depth of 25, strand bias threshold, and allele frequency of  $\geq 1\%$  with the exception of *AR* which had an allele frequency threshold of  $\geq 0.5\%$  as validated previously<sup>3</sup>. In addition to the above filters, false positive variant calls arising due to the given sequencing run were assessed, tracked, and filtered out using 11 negative controls sequenced in this study. Variant calls that were coding silent or that were designated as common in populations by dbSNP<sup>8</sup>, EXAC<sup>9</sup>, TCGA<sup>10</sup>, and ClinVar<sup>11</sup> reference databases were excluded from analyses. ClinVar was used to determine pathogenicity of missense mutations<sup>11</sup>. Missense mutations that were pathogenic or likely pathogenic by ClinVar were used for analyses. High-confidence somatic variants were further annotated with information from COSMIC<sup>12</sup>, Mutation Assessor, and cBioPortal<sup>13</sup>. Final variant calls were visualized and assessed further for validity using Integrated Genome Viewer (IGV)<sup>14</sup>. For patients with allelic fractions of ClinVar pathogenic or likely pathogenic *BRCA1* or *BRCA2* mutations that were  $\geq 20\%$ , germline information was obtained through clinical records or by sequencing germline leukocyte DNA obtained from isolated buffy coat. Finally, samples with low input for NGS, the lack of genetic alteration detection was considered indeterminate as opposed to negative.

## **CN Variation**

NGS-based CN detection was performed using an in-house developed algorithm, TMM-CNV and third-party tool, CNVKit<sup>15</sup>. TMM-CNV calculates trimmed mean and corresponding two standard deviations from mean relative coverage depths obtained for the sample and a mean of multiple references including sequence of 9 healthy controls (pool of controls). Data distribution at the gene level was used to apply a trimmed mean of 0.2, 0.1 and corresponding standard deviation to calculate and set the lower and upper thresholds for calling focal deletions and focal

amplifications respectively. For autosomes a  $\log_2$  value of mean relative coverage depth between sample and pool of controls was used, whereas for chromosome X, a logarithmic value of mean relative coverage depth between sample and pool of controls was used. Final copy number calls between TMM-CNV and CNVKit were compared for consensus. Final copy number calls were visualized and assessed further for validity using the in-house MDLVC. Conservative thresholds based on heterozygous SNP fractions of control cfDNA were used to call CN gain and deletion. Given that it is targeted, gene panel sequencing and tumor cellularity varies in cfDNA sequencing, two thresholds were applied to detect copy gains and losses. CN gain for autosomes was defined by a  $\log_2$  ratio of mean relative sequencing coverage at the gene level  $\geq 0.4$  and  $\geq 1.2$ . CN loss for autosomes was defined by a  $\log_2$  ratio of mean relative sequencing coverage at the gene level  $\leq -0.4$  and  $\leq -1.0$ . For chromosome X, CN gain was defined by a logarithmic ratio of mean relative sequencing coverage at the gene level  $\geq 0.7$  and  $\geq 1.2$  and CN loss was defined by a logarithmic ratio of mean relative sequencing coverage at the gene level  $\leq -0.4$  and  $\leq -1.0$ .

### **Estimation of ctDNA Fraction**

Estimation of high versus low ctDNA fraction was based upon both mutant allele fraction (MAF) and CN alterations. The fraction of ctDNA (% ctDNA) was estimated based upon the highest autosomal variant allele fraction. ctDNA burden was dichotomized into high and low for statistical analyses. Patients with **low** ctDNA burden had a ClinVar-annotated pathogenic or likely pathogenic missense mutation or truncating mutation **MAF < 7%**. Conversely, patients with a **high ctDNA** burden had a ClinVar-annotated pathogenic or likely pathogenic missense mutation or truncating mutation **MAF  $\geq 7\%$**  and/or a **CN loss  $\leq -0.55$  and/or a CN gain  $\geq 1.0$** .

## **Statistical Analyses**

Sample size estimate of 60 informative patients was calculated prior to study initiation. Power and confidence bounds were calculated using PASS 11 (NCSS Software). Chi-squared tests and logistic regression were used to determine associations between genomic status and PSA response. Kaplan-Meier methods and log-rank tests were used to estimate survival functions. Cox proportional-hazard modeling was used to estimate PFS and OS. Due to small sample size, clinical variables were dichotomized. PSA was dichotomized at 20ng/mL based on the median PSA of 19.3ng/mL. Age was dichotomized at 72 years based on the median age of 71.5 years. Prior abiraterone and enzalutamide was dichotomized as yes or no, visceral metastasis was dichotomized as yes or no, and ctDNA burden was dichotomized as low or high. Multivariable models included only two variables to reduce the likelihood of overfitting. Statistical analyses were performed using STATA SE/15.1, and GraphPad Prism 5 was used for figure generation.

## **REFERENCES**

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## SUPPLEMENTAL FIGURE LEGENDS

**Supplemental Figure S1.** PSA response and PFS were similar between patients on abiraterone + prednisone and patients on enzalutamide. A, Waterfall plot of best PSA response for patients receiving abiraterone + prednisone therapy (n=35) as determined by best percentage fold change in PSA. B, Waterfall plot of best PSA response for patients receiving enzalutamide therapy (n=25) as determined by best percentage fold change in PSA. Chi-squared analysis of PSA response in patients receiving abiraterone + prednisone compared to patients on enzalutamide (P=0.583). C, Kaplan-Meier method and log-rank test to determine median time to progression for patients who were treated with abiraterone + prednisone (n=35) compared to patients who were treated with enzalutamide (n=25) therapy (26.1 weeks vs. 24.7 weeks; P=0.645). D, Kaplan-Meier method and log-rank test to determine median time to progression for patients who had prior abiraterone + prednisone or enzalutamide therapy (n=15) compared to patients who were abiraterone + prednisone and enzalutamide naïve (n=47) (17.9 weeks vs. 26.0 weeks; P=0.660).

**Supplemental Figure S2.** Genetic alterations detected in cfDNA following progression. Genetic alterations (CN status, ClinVar pathogenic/likely pathogenic missense and germline mutations, and truncating mutations) in 46 genes detected by NGS of cfDNA from 26 patients following progression on abiraterone + prednisone or enzalutamide therapy.

**Supplemental Figure S3.** A, Pre-therapy PSA is associated with cfDNA concentration prior to therapy as determined using Pearson correlation ( $r=0.40$ ;  $P=0.002$ ). Pre-therapy PSA was

compared with the amount of cfDNA isolated per 1.0mL plasma prior to therapy. B, PSA for a patient progressing on abiraterone + prednisone with a detectable *AR* L702H mutation and then switched to abiraterone + dexamethasone.

**Supplemental Figure S4:** Mutations detected in *BRCA1*, *BRCA2*, and *ATM* prior to therapy and at progression. A-C, Gene schematics illustrating deleterious germline and somatic mutations in (A) *BRCA2* (B=BRC repeats, Helical=helical domain, Oligo=oligonucleotide binding domain, T=tower domain), (B) *BRCA1* (R=Ring finger domain, Serine=Serine rich domain associated with BRCT, BRCT=BRCA1 C terminus domain) and (C) *ATM* (TAN=Telomere length maintenance and DNA damage repair, FAT=FAT domain, PI3,4K=Phosphatidylinositol 3- and 4-kinase, FC=FATC domain) and as detected by deep, targeted NGS of cfDNA prior to abiraterone + prednisone and enzalutamide therapies and at progression while on therapy.

| Supplemental Table S1: Qiagen Mix and Match Panel |       |       |        |
|---------------------------------------------------|-------|-------|--------|
| AR                                                | BRCA1 | POLH  | CDH1   |
| NCOA2                                             | BRCA2 | POLE  | MET    |
| TP53                                              | ATM   | POLD1 | SPOP   |
| RB1                                               | ATR   | MLH1  | POU6F2 |
| MYC                                               | CHEK2 | MLH3  | SCN11A |
| CDKN1B                                            | PALB2 | MSH2  | GNAS   |
| PTEN                                              | NBN   | MSH3  | MED12  |
| PIK3CA                                            | CDK12 | PMS2  | ZFHX3  |
| AKT1                                              | FANCG | KDM6A | IDH1   |
| AKT2                                              | FANCM | KMT2C | IDH2   |
| APC                                               | ERCC3 | KMT2D |        |
| CTNNB1                                            | ERCC5 | GLI1  |        |

**Supplemental Table S2. Patient Samples with AR Genetic Alterations**

|                                         |  | Pre-therapy (n=62) | Progression (n=26) |
|-----------------------------------------|--|--------------------|--------------------|
| AR Genetic Alteration, n (%)            |  | 34 (54.8)          | 15 (57.7)          |
| AR Copy Number Gain, n (%)              |  | 32 (51.6)          | 15 (57.7)          |
| AR Mutation, n (%)                      |  | 8 (12.9)           | 4 (15.4)           |
| ≥2 AR Mutations, n (%)                  |  | 0 (0)              | 1 (3.8)            |
| AR Copy Number Gain and Mutation, n (%) |  | 6 (9.7)            | 4 (15.4)           |

|         | Pre-therapy     |                      |                    |         | Progression     |                      |                    |
|---------|-----------------|----------------------|--------------------|---------|-----------------|----------------------|--------------------|
| Patient | AR LBD Mutation | Allelic Fraction (%) | ctDNA Low vs. High | Therapy | AR LBD Mutation | Allelic Fraction (%) | ctDNA Low vs. High |
| 26      | W742C           | 1.4                  | Low                | ENZA    | No Response     |                      |                    |
| 38      | -               | -                    |                    | ABI     | L702H<br>T878A  | 1.5<br>9.4           | High               |
| 52      | -               | -                    |                    | ABI     | L702H           | 18.5                 | High               |
| 54      | T878I           | 0.8                  | Low                | ABI     | No Response     |                      |                    |
| 63      | H875Y           | 0.6                  | Low                | ABI     | No Sample       |                      |                    |
| 70      | L702H           | 74.6                 | High               | ENZA    | No Response     |                      |                    |
| 82      | T878A           | 1.3                  | High               | ABI     | No Response     |                      |                    |
| 83      | L702H           | 27.3                 | High               | ENZA    | No Response     |                      |                    |
| 86      | -               | -                    |                    | ENZA    | H875Y           | 1.3                  | Low                |
| 91      | -               | -                    |                    | ABI     | T878A           | 1.7                  | Low                |
| 97      | V731M           | 1.5                  | High               | ABI     | No Response     |                      |                    |
| 105     | H875Y           | 5.3                  | Low                | ABI     | No Sample       |                      |                    |

AR: androgen receptor; LBD: ligand binding domain.

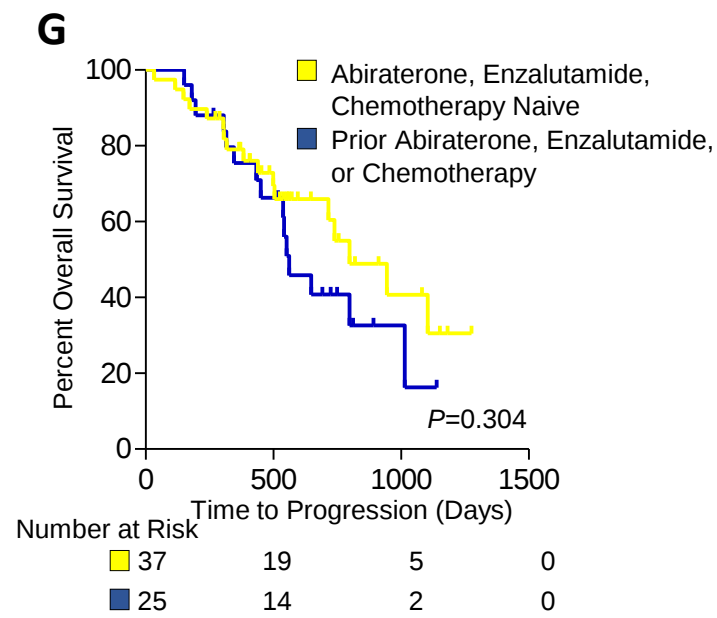
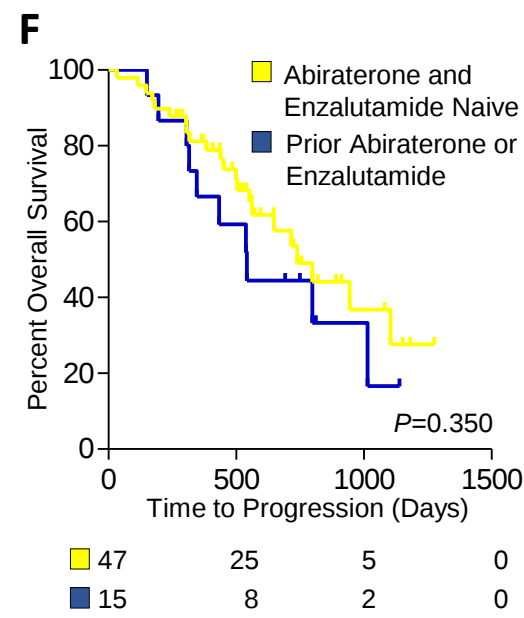
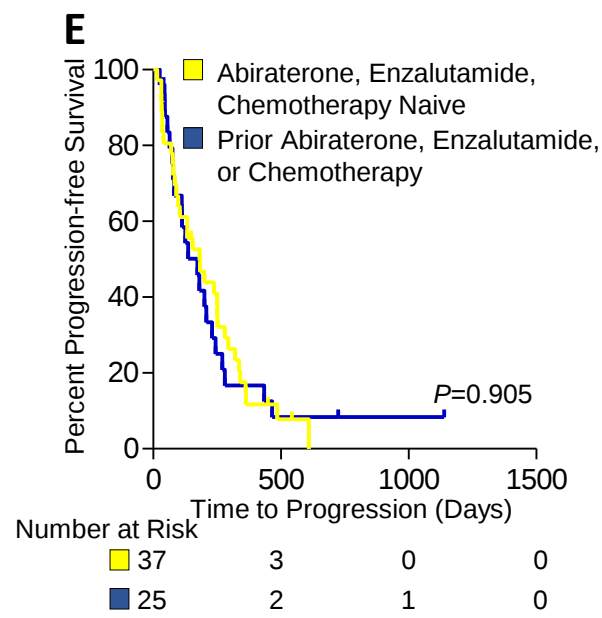
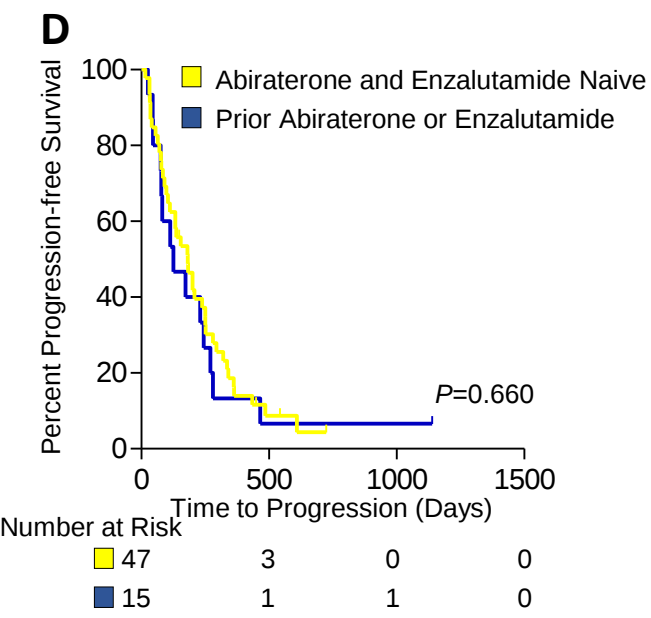
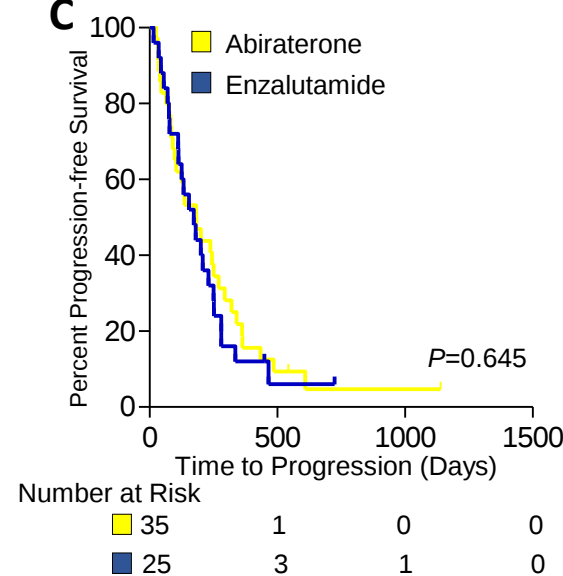
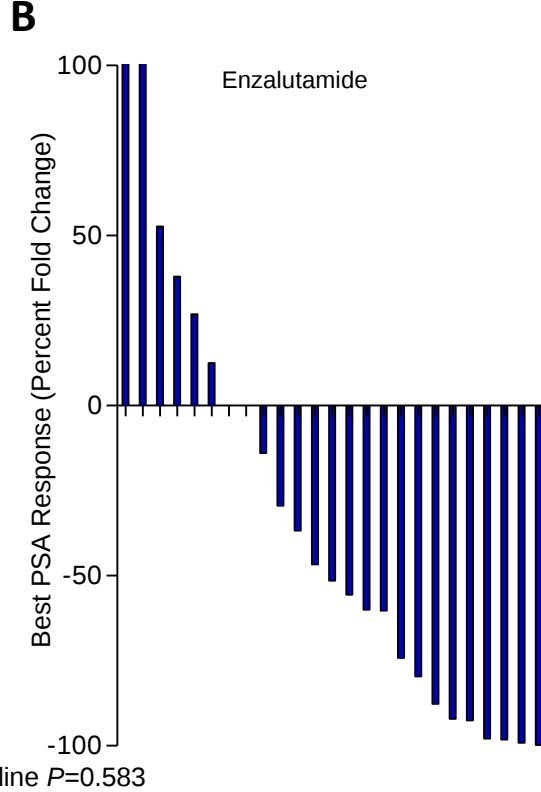
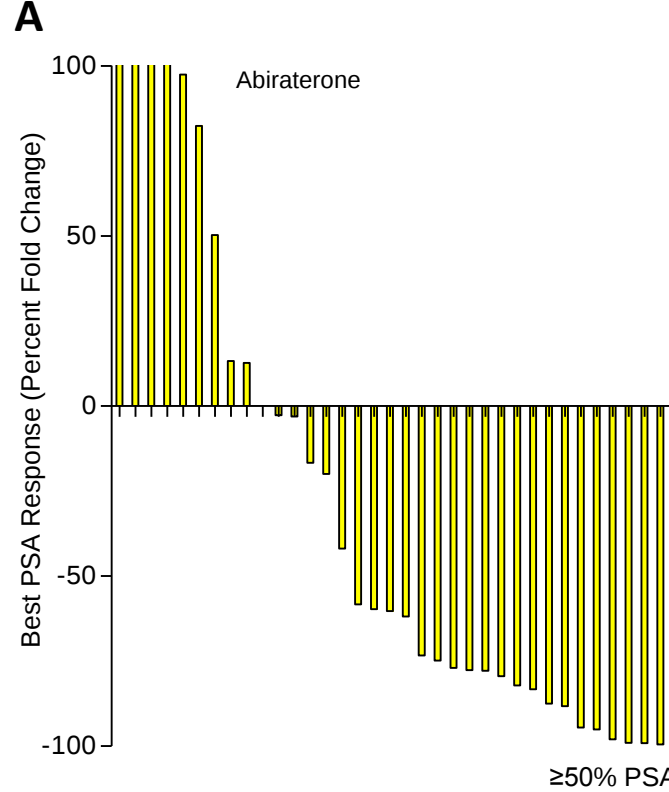
| Supplemental Table S3. Response to Therapy: Univariate Logistic Regression Analyses (n=62)                                                                                                                                                                                                                                                                                                                                           |                                    |      |            |              |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|------|------------|--------------|
|                                                                                                                                                                                                                                                                                                                                                                                                                                      | Univariate                         |      |            |              |
|                                                                                                                                                                                                                                                                                                                                                                                                                                      | ≥30% Decrease in PSA from Baseline |      |            |              |
|                                                                                                                                                                                                                                                                                                                                                                                                                                      | Patients (n)                       | OR   | 95% CI     | <i>P</i>     |
| Prior Abiraterone or Enzalutamide                                                                                                                                                                                                                                                                                                                                                                                                    | 15                                 | 1.54 | 0.48-5.01  | 0.469        |
| PSA ≥20 ng/mL                                                                                                                                                                                                                                                                                                                                                                                                                        | 30                                 | 1.46 | 0.52-4.07  | 0.470        |
| Age ≥72 years                                                                                                                                                                                                                                                                                                                                                                                                                        | 32                                 | 1.18 | 0.42-3.29  | 0.749        |
| Visceral Metastasis                                                                                                                                                                                                                                                                                                                                                                                                                  | 6                                  | 0.77 | 0.13-4.58  | 0.776        |
| ctDNA High                                                                                                                                                                                                                                                                                                                                                                                                                           | 27                                 | 3.61 | 1.24-10.56 | <b>0.019</b> |
| AR CN Gain and/or LBD Mutation                                                                                                                                                                                                                                                                                                                                                                                                       | 34                                 | 1.67 | 0.59-4.73  | 0.337        |
| AR LBD Mutation                                                                                                                                                                                                                                                                                                                                                                                                                      | 8                                  | 6.00 | 1.10-32.76 | <b>0.039</b> |
| AR CN Gain                                                                                                                                                                                                                                                                                                                                                                                                                           | 32                                 | 2.06 | 0.72-5.85  | 0.176        |
| TP53 Mutation and/or CN Loss                                                                                                                                                                                                                                                                                                                                                                                                         | 23                                 | 1.37 | 0.48-3.94  | 0.554        |
| RB1 Mutation and/or CN Loss                                                                                                                                                                                                                                                                                                                                                                                                          | 17                                 | 2.25 | 0.72-7.01  | 0.162        |
| TP53 and RB1 Mutation and/or CN Loss                                                                                                                                                                                                                                                                                                                                                                                                 | 6                                  | 9.74 | 1.06-89.40 | <b>0.044</b> |
| PI3K Pathway Defect                                                                                                                                                                                                                                                                                                                                                                                                                  | 15                                 | 7.19 | 1.94-26.68 | <b>0.003</b> |
| WNT Pathway Defect                                                                                                                                                                                                                                                                                                                                                                                                                   | 9                                  | 3.89 | 0.87-17.39 | 0.076        |
| BRCA1/BRCA2/ATM Mutation and/or CN Loss                                                                                                                                                                                                                                                                                                                                                                                              | 24                                 | 1.63 | 0.57-4.63  | 0.362        |
| BRCA1/BRCA2/ATM Truncating Mutations                                                                                                                                                                                                                                                                                                                                                                                                 | 14                                 | 1.25 | 0.37-4.19  | 0.718        |
| Significant <i>P</i> values in bold. OR: odds ratio; CI: confidence interval; PSA: prostate-specific antigen; ctDNA: cell-free tumor DNA; AR: androgen receptor; CN: copy number; LBD: ligand binding domain; TP53: tumor protein 53; RB1: retinoblastoma-associated protein 1; PI3K: phosphoinositide 3-kinase; WNT: wingless-type MMTV integration site; BRCA1/2: breast cancer gene 1/2; ATM: ataxia-telangiectasia mutated gene. |                                    |      |            |              |

| Supplemental Table S4. Response to Therapy: Multivariable Logistic Regression Analyses (n=62)                                                                                                |                                    |      |            |              |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|------|------------|--------------|
|                                                                                                                                                                                              | Multivariable                      |      |            |              |
|                                                                                                                                                                                              | ≥30% Decrease in PSA from Baseline |      |            |              |
|                                                                                                                                                                                              | Patients (n)                       | OR   | 95% CI     | <i>P</i>     |
| AR LBD Mutation                                                                                                                                                                              | 8                                  | 6.52 | 1.10-38.67 | <b>0.039</b> |
| ctDNA High                                                                                                                                                                                   | 27                                 | 3.81 | 1.23-11.78 | <b>0.020</b> |
| AR LBD Mutation                                                                                                                                                                              | 8                                  | 5.76 | 1.02-32.60 | <b>0.048</b> |
| Prior Abiraterone or Enzalutamide                                                                                                                                                            | 15                                 | 1.15 | 0.32-4.12  | 0.827        |
| AR LBD Mutation                                                                                                                                                                              | 8                                  | 6.28 | 1.13-34.79 | <b>0.036</b> |
| Prior Abiraterone, Enzalutamide, and/or Chemotherapy                                                                                                                                         | 25                                 | 0.73 | 0.24-2.21  | 0.578        |
| AR LBD Mutation                                                                                                                                                                              | 8                                  | 5.78 | 1.05-31.79 | <b>0.044</b> |
| PSA ≥ 20 ng/mL                                                                                                                                                                               | 30                                 | 1.32 | 0.45-3.84  | 0.616        |
| Significant <i>P</i> values in bold. PSA: prostate-specific antigen; OR: odds ratio; CI: confidence interval; AR: androgen receptor; ctDNA: cell-free tumor DNA; LBD: ligand binding domain. |                                    |      |            |              |

| Supplemental Table S5. Progression-free Survival: Multivariable Logistic Regression Analyses (n=62)                                                                                             |               |      |             |              |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|------|-------------|--------------|
|                                                                                                                                                                                                 | Multivariable |      |             |              |
|                                                                                                                                                                                                 | Patients (n)  | HR   | 95% CI      | <i>P</i>     |
| AR LBD Mutation                                                                                                                                                                                 | 8             | 2.51 | 1.15-5.45   | <b>0.020</b> |
| ctDNA High                                                                                                                                                                                      | 27            | 1.81 | 1.05-3.10   | <b>0.032</b> |
| AR LBD Mutation                                                                                                                                                                                 | 8             | 2.40 | 1.11-5.17   | <b>0.025</b> |
| Prior Abiraterone or Enzalutamide                                                                                                                                                               | 15            | 1.18 | 0.64-2.17   | 0.600        |
| AR LBD Mutation                                                                                                                                                                                 | 8             | 2.45 | 1.13-5.31   | <b>0.024</b> |
| Prior Abiraterone, Enzalutamide, and/or Chemotherapy                                                                                                                                            | 25            | 1.13 | 0.65-1.95   | 0.674        |
| AR LBD Mutation                                                                                                                                                                                 | 8             | 2.42 | 1.12-5.23   | <b>0.024</b> |
| PSA ≥ 20 ng/mL                                                                                                                                                                                  | 30            | 1.00 | 0.999-1.001 | 0.666        |
| Significant <i>P</i> values in bold. PSA: prostate-specific antigen; HR: hazards ratio; CI: confidence interval; AR: androgen receptor; ctDNA: cell-free tumor DNA; LBD: ligand binding domain. |               |      |             |              |

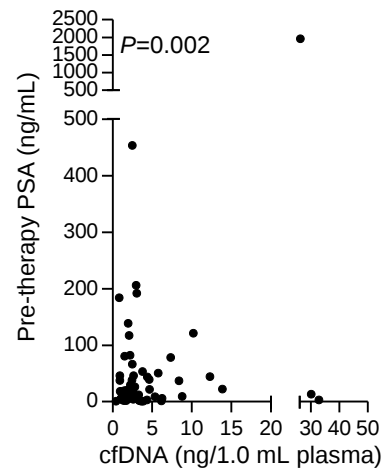
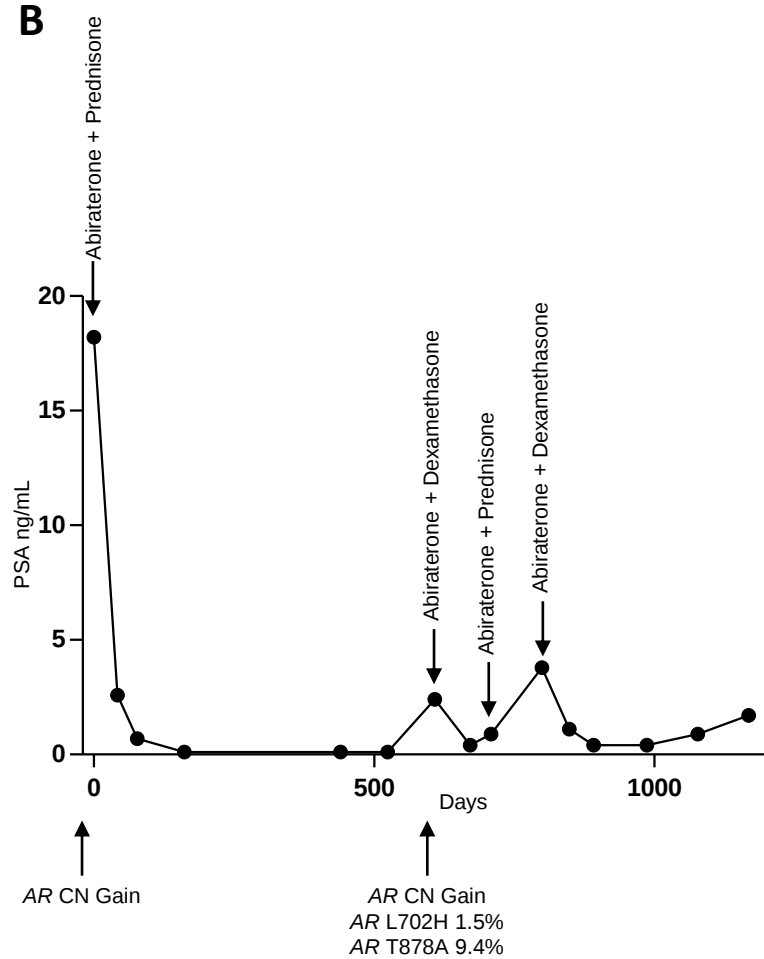
| Supplemental Table S6. Overall Survival: Multivariable Logistic Regression Analyses (n=62)                                                                                               |                  |      |            |              |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|------|------------|--------------|
|                                                                                                                                                                                          | Overall Survival |      |            |              |
|                                                                                                                                                                                          | Patients<br>(n)  | HR   | 95% CI     | <i>P</i>     |
| <i>TP53</i> Mutation and/or CN Loss                                                                                                                                                      | 23               | 2.70 | 1.27-5.72  | <b>0.009</b> |
| ctDNA High                                                                                                                                                                               | 27               | 2.42 | 1.13-5.18  | <b>0.022</b> |
| <i>TP53</i> Mutation and/or CN Loss                                                                                                                                                      | 23               | 3.19 | 1.53-6.65  | <b>0.002</b> |
| Prior Abiraterone or Enzalutamide                                                                                                                                                        | 15               | 1.52 | 0.71-3.25  | 0.283        |
| <i>TP53</i> Mutation and/or CN Loss                                                                                                                                                      | 23               | 3.05 | 1.44-6.46  | <b>0.003</b> |
| Prior Abiraterone, Enzalutamide, and/or Chemotherapy                                                                                                                                     | 25               | 1.24 | 0.59-2.59  | 0.592        |
| <i>TP53</i> Mutation and/or CN Loss                                                                                                                                                      | 23               | 3.10 | 1.49-6.48  | <b>0.003</b> |
| PSA ≥ 20 ng/mL                                                                                                                                                                           | 30               | 1.29 | 0.62-2.67  | 0.497        |
| <i>TP53</i> and <i>RB1</i> Mutation and/or CN Loss                                                                                                                                       | 6                | 4.56 | 1.78-11.71 | <b>0.002</b> |
| ctDNA High                                                                                                                                                                               | 27               | 2.99 | 1.40-6.36  | <b>0.005</b> |
| <i>TP53</i> and <i>RB1</i> Mutation and/or CN Loss                                                                                                                                       | 6                | 4.41 | 1.57-12.41 | <b>0.005</b> |
| Prior Abiraterone or Enzalutamide                                                                                                                                                        | 15               | 1.04 | 0.44-2.46  | 0.935        |
| <i>TP53</i> and <i>RB1</i> Mutation and/or CN Loss                                                                                                                                       | 6                | 4.14 | 1.55-11.04 | <b>0.004</b> |
| Prior Abiraterone, Enzalutamide, and/or Chemotherapy                                                                                                                                     | 25               | 1.20 | 0.56-2.58  | 0.643        |
| <i>TP53</i> and <i>RB1</i> Mutation and/or CN Loss                                                                                                                                       | 6                | 4.24 | 1.64-11.00 | <b>0.003</b> |
| PSA ≥ 20 ng/mL                                                                                                                                                                           | 30               | 1.19 | 0.56-2.51  | 0.656        |
| Significant <i>P</i> values in bold.<br>HR: hazards ratio; CI: confidence interval; PSA: prostate-specific antigen; ctDNA: cell-free tumor DNA; CN: copy number; TP53: tumor protein 53. |                  |      |            |              |

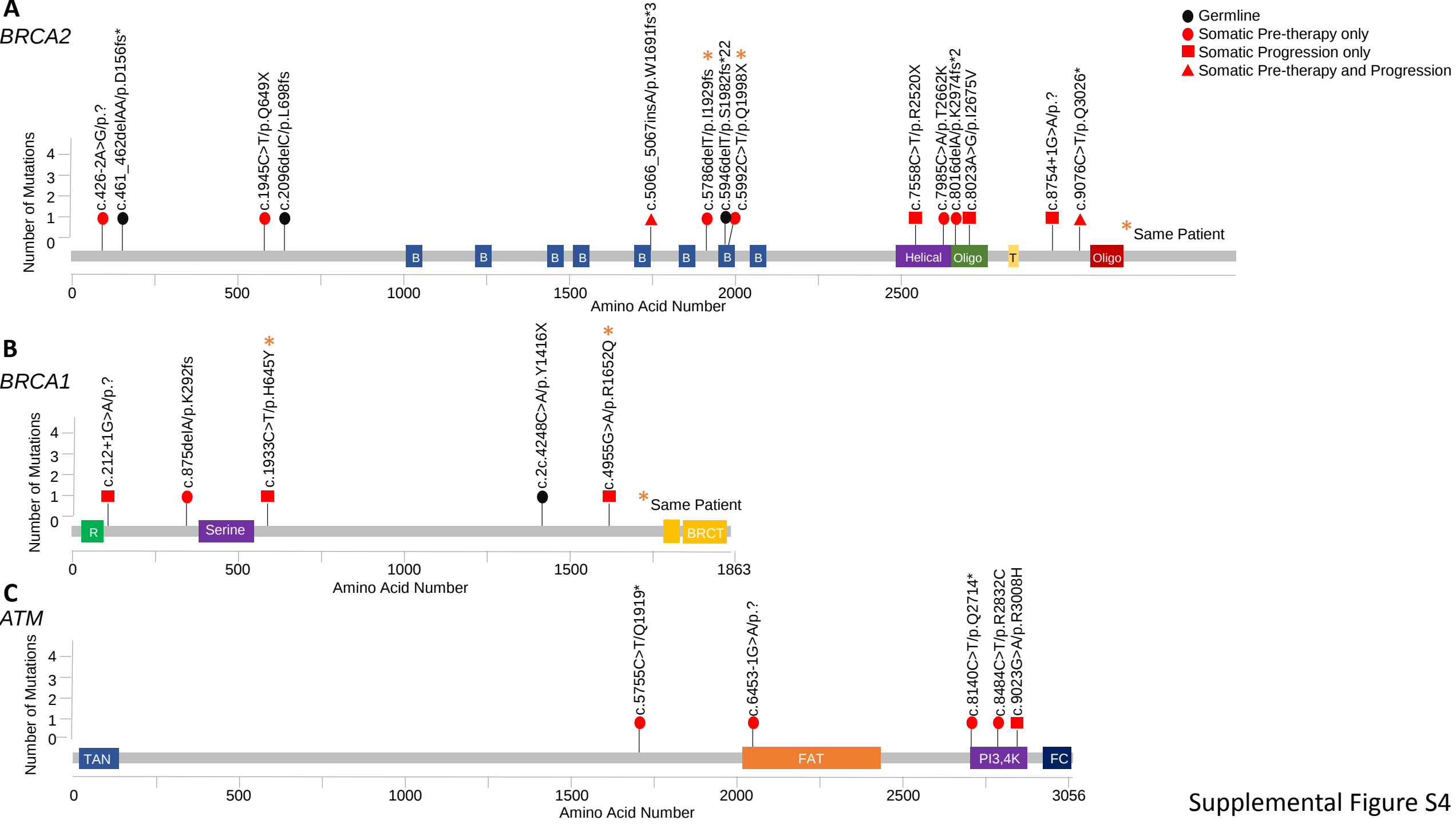
| Supplemental Table S7. Overall Survival: Multivariable Logistic Regression Analyses (n=62)                                                                                                        |                  |      |           |              |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|------|-----------|--------------|
|                                                                                                                                                                                                   | Overall Survival |      |           |              |
|                                                                                                                                                                                                   | Patients<br>(n)  | HR   | 95% CI    | <i>P</i>     |
| PI3K Pathway Defect                                                                                                                                                                               | 15               | 2.62 | 1.12-6.10 | <b>0.026</b> |
| ctDNA High                                                                                                                                                                                        | 27               | 2.17 | 0.96-4.92 | 0.063        |
| PI3K Pathway Defect                                                                                                                                                                               | 15               | 3.50 | 1.59-7.71 | <b>0.002</b> |
| Prior Abiraterone or Enzalutamide                                                                                                                                                                 | 15               | 1.89 | 0.54-2.62 | 0.669        |
| PI3K Pathway Defect                                                                                                                                                                               | 15               | 3.45 | 1.58-7.54 | <b>0.002</b> |
| Prior Abiraterone, Enzalutamide, and/or Chemotherapy                                                                                                                                              | 25               | 1.29 | 0.62-2.68 | 0.502        |
| PI3K Pathway Defect                                                                                                                                                                               | 15               | 3.51 | 1.61-7.64 | <b>0.002</b> |
| PSA ≥ 20 ng/mL                                                                                                                                                                                    | 30               | 1.23 | 0.59-2.57 | 0.579        |
| Significant <i>P</i> values in bold.<br>HR: hazards ratio; CI: confidence interval; PSA: prostate-specific antigen; ctDNA: cell-free tumor DNA; CN: copy number; PI3K: phosphoinositide 3-kinase. |                  |      |           |              |



Progression



**A****B**



Supplemental Figure S4