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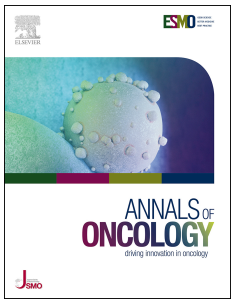

Plasma tumor gene conversions after one cycle abiraterone acetate for metastatic castration-resistant prostate cancer: a biomarker analysis of a multi-center international trial

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Original article

Plasma tumor gene conversions after one cycle abiraterone acetate for metastatic castration-resistant prostate cancer: a biomarker analysis of a multi-center international trial

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Highlights

- The rolling b-allele method applied into a clinical assay allows detection of plasma tumor DNA independent of mutation calls.
- Patients with *TP53*, *RB1* or *PTEN* alterations persisting after one cycle treatment have the worst outcomes.
- Conversion of alterations after one cycle is associated with the similar outcomes as patients negative before treatment.
- Plasma *AR* gain pre-treatment persists at progression whilst *AR* point mutations appear de novo in ~16%.

Abstract

Background

Plasma tumor DNA fraction is prognostic in metastatic cancers. This could improve risk stratification prior to commencing a new treatment. We hypothesized that a second sample collected after one cycle treatment could refine outcome prediction of patients identified as poor prognosis based on plasma DNA collected pre-treatment.

Patients and Methods

Plasma DNA (128 pre-treatment, 134 cycle 2 day 1 (C2D1), and 49 progression) from 151 chemotherapy-naïve metastatic castration resistant prostate cancer (mCRPC) patients in a phase 2 study of abiraterone acetate (NCT01867710) were subjected to custom targeted next-generation sequencing covering exons of these genes:*TP53, AR, RB1, PTEN, PIK3CA, BRCA1*, *BRCA2, ATM, CDK12, CHEK2, FANCA HDAC2, and PALB2*. We also captured 1500 pan-genome regions enriched for single nucleotide polymorphisms to allow detection of tumor DNA using the rolling B-allele method. We tested associations with overall survival (OS) and progression-free survival (PFS).

Results

Plasma tumor DNA was associated with shorter OS (hazard ratio (HR): 2.89, 95% confidence intervals (CI): 1.77-4.73, p=<0.0001) and PFS (HR: 2.05; 95% CI: 1.36-3.11, p<0.001). Using a multivariable model, including plasma tumor DNA, patients who had a *TP53* or *RB1* or *PTEN* gene alteration pre-treatment and at C2D1 had a significantly shorter OS than patients with no alteration at either time point (*TP53*, HR 7.13, 95% CI 2.37-21.47, p<0.001; *RB1*, HR 6.24, 95% CI 1.97-19.73, p=0.002; *PTEN*, HR 11.9, 95% CI 3.6-39.34, p<0.001). Patients who were positive pre-treatment and converted to undetectable had no evidence of a difference in survival compared to those who were undetectable pre-treatment (respectively p=0.48, p=0.43, p=0.5). Progression samples harbored *AR* gain in all patients who had gain pre-treatment (9/49) and de novo *AR* somatic point mutations were detected in 8/49 patients.

Conclusions

Plasma gene testing after one cycle treatment refines prognostication and could provide an early indication of treatment benefit.

Keywords

Prostate Cancer, plasma DNA, next-generation sequencing, liquid biopsies, genomic alterations, biomarkers.

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Background

Approximately 20% of metastatic castration-resistant prostate cancer (mCRPC) patients have primary resistance and a short duration of response to androgen receptor (AR) targeting agents, abiraterone acetate and enzalutamide (1, 2). Therefore, there has been considerable interest in developing a test to identify patients susceptible to resistance to these agents. Given the challenge of acquiring tumor tissue from this population, several studies have evaluated perturbations in liquid biopsies taken just prior to the start of treatment (3-6). Amongst the genomic alterations detected in plasma DNA at this stage of the disease, pathogenic events involving *TP53, RB1, AR, PTEN* and *PIK3CA* have been reported in several studies to be associated with worse outcomes (3, 4, 7-10). Approximately 20% of all mCRPC cases harbor somatic genomic alterations involved in DNA damage repair (DDR) and benefit from poly(adenosine diphosphate–ribose) polymerase (PARP) inhibitors (11). Several PARP inhibitor trials have used tissue and plasma DNA for DDR gene characterization. The prognostic relevance of DDR alterations detected in plasma from mCRPC patients starting abiraterone acetate is currently uncertain (12-15).

Plasma DNA is suited to repeat testing on treatment to determine response to therapy(16). We were interested in evaluating whether a change in plasma DNA status after one cycle of treatment is associated with a change in outcome. We tested this question in asymptomatic or minimally symptomatic, chemotherapy-naïve mCRPC patients treated with open-label abiraterone acetate plus glucocorticoid treatment in an international, multi-institutional clinical trial (PCR2023, NCT01867710). Plasma DNA was collected prior to, after one cycle of treatment, and at disease progression. We utilized an accredited, custom, targeted next-generation sequencing (NGS) assay performed in a clinical laboratory and focused on the most common aforementioned alterations detected at this stage of disease. A number of studies on plasma DNA have used recurrent somatic point mutations or genomic mono-allelic deletions to estimate tumor fraction (3, 4, 17). We implemented an orthogonal

approach leveraging genome-wide informative single nucleotide polymorphisms (SNPs) to classify patients as plasma tumor DNA positive or negative. We then aimed to interrogate whether dynamic changes in gene alterations from pre-treatment to after four weeks were associated with worse outcomes in mCRPC patients treated with abiraterone acetate.

Methods

Participants and Study design

The NCT01867710 cohort has been described previously (18). Patients were randomized to receive abiraterone acetate and 1:1:1:1 between four different glucocorticoid regimens, prednisone (P) (5mg or once daily [QD] or twice daily [BID] or 2·5mg BID) or dexamethasone (D) (0·5mg QD), to evaluate tolerability. The trial was not designed to detect differences in clinical outcomes between steroid doses; therefore, for the purposes of biomarker analysis all patients were grouped together. The clinical outcome data used for this analysis was from the final database, after completion of the main study and extension protocols with a closure date of 5 June 2018. The study obtained Institutional Review Board and Ethics Committee approvals and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines of the International Conference of Harmonization. All patients provided written informed consent for the biomarker research.

Plasma samples were collected pre-treatment (just immediately prior to cycle 1 day 1), at cycle 2 day 1 and at progression with the primary aim of studying associations between plasma DNA gene alterations and clinical outcome as secondary exploratory objectives in the PCR2023 study protocol. Serum prostate specific antigen (PSA) was assessed pre-treatment, every month for the first six months and every three months thereafter. Disease was evaluated radiographically with the use of computed tomography scans of the thorax, abdomen and pelvis (CT TAP) and technetium whole-body bone

scans pre-treatment and every 12 weeks on treatment. Serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were measured pre-treatment.

Sample Next-generation Sequencing and Analysis

Plasma DNA was extracted using the Promega bead-based automated platform and subjected to a proprietary target capture and analysis NGS pipeline (19) with a set of probes covering exons including these genes; *TP53, AR, RB1, PTEN, PIK3CA*, DDR (*BRCA1, BRCA2, ATM, CDK12, CHEK2, FANCA, HDAC2, PALB2*) and approximately 1500 commonly heterozygous, pan-genome single nucleotide polymorphisms (SNPs). Analytical validation studies using cell line titrations found the limit of detection (LOD)95 was 0.18% for somatic point mutations and at a shift of 0.2 for copy number alterations (CNAs) (19). Somatic point mutations that did not result in loss of function or were reported to be benign from two databases (ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/ or CBioPortal: https://www.cbioportal.org/) were excluded. To detect the presence of tumor using an orthogonal approach that did not rely on somatic mutations, we used the "rolling B-allele" method that detects shifts in frequencies of heterozygous SNPs using rolling windows containing either three or nine consecutive SNPs. If a significant shift was detected compared to a background noise model generated from a dataset of wild-type samples analyzed with the same targeted panel, the sample was classified as plasma tumor DNA positive. A negative result indicated that no evidence was found for plasma tumor DNA presence. This analysis cannot rule out tumor presence that was either lower than the limit of sensitivity or arose from clones that did not harbor CNAs. Cell line titration studies using a matched diluent found that the method detects dilutions down to 5%.

Outcomes

The primary activity endpoints of the trial and this biomarker analysis were overall survival (OS) and progression-free survival (PFS) calculated from randomization to a glucocorticoid regimen. OS was

calculated from randomization to death from any cause, while PFS included radiographic-progression free survival (rPFS), clinical progression or death. The association between plasma tumor DNA and time on androgen deprivation therapy (ADT) was measured from the start of uninterrupted ADT to start of abiraterone acetate plus glucocorticoids. Patients on combined androgen blockade and antiandrogens were not excluded.

Statistical analyses

Associations for plasma tumor DNA and clinical variables including time on ADT were assessed using Fisher's Exact and Mann-Whitney tests. Clinical variables were treated as binary covariates: baseline LDH (dichotomized at upper limit of normal (ULN), baseline ALP (dichotomized at ULN), baseline PSA (dichotomized at 51 ng/mL), and patient age (dichotomized at 75 years). For each gene variable and detectable tumor in plasma, hazard ratios (HR) and P-values were calculated using Cox proportional hazard models. We assessed the independent biomarker potential of each gene alteration by constructing multivariable Cox proportional hazards models assessing each individual alteration and plasma tumor DNA (positive versus negative). The association between gene alterations and time-to-event outcomes were evaluated by Kaplan-Meier (KM) analysis using the log-rank test. The association between the conversion of a gene alteration from either detectable or undetectable pre-treatment to C2D1 was examined using a multivariable Cox regression model. The HR for each variable plasma tumor DNA (rolling B-allele), *TP53, RB1, AR, PTEN, PIK3CA* and DDR was compared to the reference group: undetectable at both time points. All tests were two-sided and an α-error of 5% was considered as statistically significant. All statistical analysis was performed using SPSS (IBM SPSS statistics version 26).

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Results

Patient Disposition and Plasma Tumor DNA Detection

Between June 2013 and October 2014, 164 patients were recruited onto the PCR2023 study at 22 centers in 5 countries. Of the 164 intention-to-treat patients, 151 patients consented to the optional biomarker study. Targeted NGS data was available on 311 samples, consisting of 128 pre-treatment, 134 C2D1 and 49 progression samples (**Figure 1A**). The median follow-up time was 48.9 months. Median PFS and OS were 12.8 months (95% CI, 8.0 – 17.6) and 39.4 (95% CI, 30.3 – 48.4) months, respectively.

Using the rolling B-allele method, plasma tumor DNA was detected in 60 of 128 pre-treatment (47%), 25 of 134 C2D1 (19%) and 25 of 49 (51%) progression plasma samples. Of the 110 plasma tumor DNA positive samples, 89 of 110 (81%) had a detectable gene alteration in a target gene compared to 26 of 201 (13%) plasma tumor negative samples (Fisher's exact test, p<0.0001). In keeping with previous reports (13), plasma tumor positive pre-treatment samples were significantly associated with higher levels of baseline clinical indices of tumor volume, namely serum LDH, ALP, PSA and total plasma DNA genomic equivalents in the target fragment length range (Mann-Whitney test, p<0.0001 for all four parameters; **Supplementary Figure 1, Supplementary Table 1**). Plasma tumor DNA positive patients were also significantly associated with the presence of bone metastases (Supplementary Table 1). Start dates of primary ADT were available for 119 patients (93%). Disease stage at diagnosis was available for 92 of the 119 patients; 63 (68.5%) were stage M0 at diagnosis. We observed that patients with positive plasma tumor DNA pre-treatment had a significantly shorter time on ADT (p=0.02) (**Supplementary Figure 2**).

Positive plasma tumor DNA pre-treatment was associated with significantly shorter PFS (HR: 2.05; 95% confidence intervals (CI): 1.36 to 3.11, p=0.0002) and OS (HR: 2.89, 95% CI: 1.77 to 4.73, p=<0.0001) (**Figures 1B and 1C**). After adjusting for clinical prognostic factors that have been previously demonstrated to be strong independent predictors of OS and PFS for AR targeted

therapies(2, 20, 21), detectable plasma tumor DNA pre-treatment remained independently associated with worse outcomes (HR: 2.03, 95% CI: 1.21 to 3.41, p=0.007 for PFS; HR: 2.61, 95% CI: 1.44 to 4.72, p=0.001 for OS; **Supplementary Table 2**).

Plasma gene alterations prior to treatment and clinical outcome

We detected somatic point mutations involving *TP53*, *PTEN*, *PIK3CA* and *AR*, gene loss of *TP53*, *PTEN* and *RB1*, and copy number (CN) gain of *AR* or *PIK3CA* in pre-treatment samples (**Figure 2, Supplementary Figure 3**). Pre-treatment CNAs and/or somatic point mutations for one of these five alterations were detected in 56 of 128 patients (44%). With each additional alteration, patients demonstrated a shorter OS (median OS: no detectable alterations, 48.4 months; one alteration, 34.5 months; two alterations, 27.9 months; and three alterations, 20.9 months, p<0.0001) and PFS (median: no detectable alterations, 20.96; one alteration, 15.51; two alterations, 7.36; and three alterations, 4.73 months, respectively; p<0.0001; **Supplementary Figure 4A and B**). We first performed a univariable analysis to test whether the selected alterations in our data-set were associated with worse outcome. The detection of *AR* gain or an alteration in *TP53* was associated with shorter PFS and OS and alterations in *PTEN, RB1* and *PIK3CA* were only associated with shorter OS (**Supplementary Table 3**).

Next, we evaluated the relationship between DDR gene alterations (*BRCA1, BRCA2, ATM CDK12, CHEK2, FANCA, HDAC2 and PALB2*) and outcome. We observed 11 patients (8.6%) with biallelic DDR gene alterations (either homozygous deletions or a monoallelic deletion with a pathogenic mutation) in *BRCA2, ATM, CHEK2, FANCA*, or *HDAC2*. Additionally, in a further 11 patients, monoallelic truncating mutations were detected in *BRCA2* (4 patients), *ATM* (2 patients), *CDK12* (3 patients), *FANCA* (1 patient) and *PALB2* (1 patient). Taken together, patients with DDR alterations had

a significantly shorter PFS (HR: 2.13; 95% CI: 1.3 to 3.48, p=0.003) but there was no association with OS (**Supplementary Table 3**).

As the detection of an alteration in plasma could be confounded by circulating tumor content, we next tested each gene that had a significant association with PFS or OS individually in a multivariable model that included plasma tumor DNA positive versus negative determined by the rolling B-allele method. After adjusting for plasma tumor DNA status, only DDR alterations were associated with a shorter PFS, while for OS this was only observed for *PTEN* (**Supplementary Tables 4 and 5**).

Conversion of alterations after one cycle of abiraterone acetate and long-term outcome

We had C2D1 plasma for comparison with matched pre-treatment plasma for 111 patients. We observed a conversion from detected to not detected in pre-treatment compared to C2D1 for plasma tumor DNA in 31 of 53 patients (58%) and specifically for alterations of each gene: 63% (17 of 27) *TP53*, 70% (16 of 23) *AR* (only gain), 63% (10 of 16) *PTEN*, 63% (12 of 19) *RB1* (only loss), 55% (5 of 11) *PIK3CA* and 35% (8 of 22) DDR (**Figure 2**). We hypothesized that the conversion of a gene alteration to undetectable would be associated with a similar outcome to those patients who were undetectable at both time points. Conversely, those patients who remained or became detectable would have the worst outcomes. Alterations that have met these criteria would support our hypothesis and represent an alteration of potential interest for tracking response. For plasma tumor DNA, *TP53, RB1, PTEN* and *PIK3CA*, only one patient converted from undetectable to detectable while none did for *AR* gain and DDR genes. We therefore grouped these patients with those that were detectable at both time points as we hypothesized that these patients would have similar outcomes to the patients that remained detectable. We split patients into three groups based on whether a gene alteration was detected or not at C2D1 and, if not, whether it was detected or not pre-treatment.

First, we started by evaluating plasma tumor DNA and found that a conversion to undetectable at C2D1 resulted in a worse PFS and OS compared to being undetectable pre-treatment; not satisfying our criteria for an alteration of interest to track response (**Supplementary Tables 6 and 7**). We then used a multivariable model to test the association with outcome compared to patients who were undetectable at both time points. We ran the model independently for each gene alteration and included plasma tumor detection to confirm that associations were independent of high circulating tumor burden.

We observed that patients in whom an alteration in *TP53* was detectable at both pre-treatment and C2D1 time points had a significantly worse outcome for PFS (Cox regression: HR: 2.69, 95% CI: 1.07 - 6.77, p=0.035) and OS (HR: 7.13, 95% CI: 2.37 - 21.47, p<0.001) while those who converted did not (HR: 1.33, 95% CI:0.68 – 2.6, p=0.40, for PFS and HR:0.77, 95% CI: 0.38 - 1.58, p=0.48 for OS, **Figure 3A, 3B and 4A, Supplementary Figure 5B)**. We also observed this relationship for OS with *RB1* (converted to undetectable: HR: 0.71, 95% CI: 0.30 - 1.67, p=0.43; or remained detectable: HR: 6.24, 95% CI: 6.24, p=0.002) (**Figure 3B and Figure 4B**). Similarly, for *PTEN* (converted to undetectable: HR: 1.36, 95% CI:0.56 - 3.32, p=0.5; remained detectable HR: 11.9, 95% CI: 3.6 - 39.34, p<0.001) (**Figure 3B and Figure 4C**). A similar observation was noted for *AR* in relation to PFS (converted to undetectable: HR: 1.33, 95% CI: 0.66 - 2.70, p=0.43; remained detectable: HR: 4.61, CI: 1.76 – 12.08, p=0.002) (**Figure 3A and Supplementary figure 5A**). All results are included in **Supplementary Tables 6 and 7**.

Selection of *AR* gene changes at progression

Progression samples and matched pre-treatment or C2D1 samples were available for 49 patients (**Figure 1, Figure 5A**). Of the 45 patients with matched progression and pre-treatment samples, all nine with *AR* gain pre-treatment had gain at progression. Additionally, three of 36 patients (9%) who were *AR* normal pre-treatment had *AR* gain at progression (**Figures 5B and 5C, Supplementary Figure 6**).

We identified the two somatic point mutations in *AR*, 2105T>A (p.L702H) and 2632A>G (p.T878A), which were previously shown to result in promiscuous ligand activation and to associate with worse outcome (4, 22), in eight of 49 patients (18.4%), two patients with L702H and six with T878A) progression samples, but none collected pre-treatment or at C2D1. Overall, *AR* gain was the only aberration in our custom panel that was invariably present at progression if detected pre-treatment and functionally promiscuous AR mutations were the only ones detected solely at progression (**Figures 5B** and **5C**).

Discussion

In this pre-planned biomarker analysis in a multi-center clinical trial, we studied the plasma copy number and mutation landscape of selected genes at three time-points using a clinically accredited test. Patients with higher disease burden at the start of treatment were characterized by detection of more gene alterations in circulation that were associated with a worse outcome. Prognostic associations of specific gene alterations could be biased by total tumor burden, so we constructed multivariable models that tested each gene adjusted for tumor DNA detection using an orthogonal method designed to leverage information at genome-wide heterozygous SNPs. Notably, when compared to patients with an alteration not detected pre-treatment and at C2D1, patients in whom a *TP53* or *PTEN* or *RB1* alteration persisted after one cycle had shorter overall survival. In contrast, there was little evidence to suggest that patients with a detectable alteration pre-treatment that becomes undetectable at C2D1 have different survival outcomes to those who have no such alterations detected at both timepoints. We also observed the same association for progression-free survival with *TP53* and *AR*. Future trials could test the benefits of treatment change based on C2D1 plasma testing and incorporate composite response assessments including PSA (23), serum LDH or circulating tumor cells (24).

Due to the size of our cohort, we limited gene assessment of plasma DNA to five genes commonly aberrant in mCRPC (*AR, TP53, RB1, PTEN* and *PIK3CA*) and eight genes from the DDR pathway. Our

observation of the potential utility of a change in gene alteration status with treatment may not be restricted to the genes we described. Given that many patients harbored several alterations, we cannot dissect the contribution of individual genes in the absence of others. Nonetheless, our findings highlight the opportunities for tracking genes in plasma DNA. For the DDR analysis, we had insufficient numbers to robustly test the association of single genes with outcome. Although the effect for different genes within this family is likely to be heterogeneous, we observed an association with shorter PFS. Subsequent treatments may be more effective in this population and may explain the absence of an association with worse overall survival.

Our data suggest that testing of dynamic changes in plasma after four weeks' treatment with abiraterone acetate plus a glucocorticoid is more informative for identifying resistant clones than a pre-treatment sample. Future studies will evaluate this prospectively with treatment changes for patients who do not convert after a cycle of treatment. Emergence of *AR* gene mutations is temporally related to resistance and could constitute a therapeutic opportunity. Serial plasma DNA testing shows promise for improving the therapeutic management of mCRPC.

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Acknowledgments

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Author contributions

Conception and design: AJ and GA

Sample acquisition : AJ, CNS, RJ, AB, ASM, BT, GA

Sample processing and next-generation sequencing: AJ, AW, DW, FM, KG, LPL

Data analysis, interpretation: AJ, AW, DW, GW, FL, ML, ST, MG, DR, GA

Writing first draft: AJ, GA

Writing, review, and/or revision of the manuscript: all

All authors meet ICMJE criteria and had full access to study data. The corresponding author had final responsibility for the decision to submit for publication.

Data Sharing statement

The data sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at https://www.janssen.com/clinical-trials/transparency. As noted on this site, requests for access to the study data can be submitted through Yale Open Data Access (YODA) Project site at http://yoda.yale.edu.

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Disclosure of Potential Conflicts of Interest:

Dr Jayaram, Ms Wingate and Dr Wetterskog have no conflicts of interest to report.

Dr Wheeler has received honoraria from AstraZeneca. Dr Sternberg has consulted for Janssen, Pfizer, MSD, Merck, AstraZeneca, Astellas, Sanofi-Genzyme Roche-Genentech, Incyte, Clovis, Immunomedics, Medscape and UroToday. Dr Jones has received honoraria from Janssen, Astellas, Bayer, Clovis, and AAA, and research funding from Astellas and Bayer. Dr Berruti has consulted for and received honoraria from Janssen, Astellas and Ipsen. Dr Lefresne, Ms Lahaye, Dr Thomas, Dr Gormley and Dr Ricci are employees of Janssen and are shareholders in Johnson & Johnson. Dr Meacham, Dr Garg, and Dr Lim are employees and shareholders of Resolution Bioscience, Inc. Dr Merseburger reports personal fees, research support, and travel support from Janssen during the conduct of the study; personal fees and/or travel support from Astellas, Bayer, Ferring, Pfizer, Ipsen, Novartis, Takeda and Sanofi-Aventis; and research funding from AstraZeneca. Dr Tombal has received research funding from Astellas and Ferring, and honoraria from Janssen, Amgen, Astellas, Ferring, Bayer, and Sanofi. Dr Attard reports personal fees, research support and travel support from Janssen during the conduct of the study; personal fees and/or travel support from Astellas, Pfizer, Millennium Pharmaceuticals, Ipsen, Ventana, Veridex, Novartis, Abbott Laboratories, ESSA Pharmaceuticals, Bayer Healthcare Pharmaceuticals, Takeda and Sanofi-Aventis and research funding from AstraZeneca, Innocrin Pharma and Arno Therapeutics outside the submitted work; in addition, Dr Attard's former employer, The Institute of Cancer Research (ICR), receives royalty income from

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abiraterone acetate and Dr Attard receives a share of this income through ICR's Rewards to Discoverers Scheme.

Figure Legends

Figure 1. Plasma DNA analysis in PCR2023 trial. **(A)** Horizontal bars denote the three time points at which samples were collected, namely pre-treatment (Pre-T), cycle 2 day 1 (C2D1) and progression (PD), and each column represents a single patient included in the biomarker population; the green bars represent time-points where plasma next-generation sequencing (NGS) data were available whilst grey bars denote none was. Plasma tumor detection as determined by the rolling B allele method and associations with overall survival (**B**) or progression-free survival (**C**).

Figure 2. Landscape of plasma DNA genomic alterations. The matrix represents a summary for every patient ordered by time on abiraterone acetate plus glucocorticoid in descending order from left to right. Detection of tumor using the rolling B allele method and of alterations involving *AR, TP53, RB1*, *PTEN*, *PIK3CA* and DNA damage repair (DDR) genes prior to treatment (N=128) are shown. Inset used for alterations with both a mutation and deletion. Patients with no cycle 2 day 1 (C2D1) data are marked with an asterisk (N = 17). Patients with a conversion from positive to negative or negative to positive at C2D1 are denoted by a black or red X respectively. Superimposed PSA waterfall plots for PSA decline at C2D1 and C4D1 are plotted at the bottom of the genomic matrix. PSA levels were not available for BZ52, AP16, EB118 and FH138 at C2D1 while at C4D1, PSA samples were not available for CT98, DD82, CC55, FH138 and FL142.

Figure 3. Conversion of genomic alterations after one cycle abiraterone acetate.

Forest plots for progression-free survival (PFS) (**A**) and overall survival (OS) (**B**) showing the hazard ratios, 95% confidence intervals and p values comparing the outcomes for patients in whom the alteration was not detected at either time-point to when gene alterations converted to undetectable (no

significant differences) or remained detectable (significantly worse outcome) at cycle 2 day 1 (C2D1). Gene dynamics of interest are marked in orange. The grey triangle in the red event detected boxes is representative of the one patient that converted from undetectable to detectable at C2D1 for *TP53, RB1* and *PTEN* and plasma tumor DNA. Results for all genes are shown in **Supplementary Tables 6 and 7.**

Figure 4. Association of gene alteration conversion and overall survival (OS). Kaplan Meir curves for OS comparing the outcomes for patients in whom the alteration was not detected at either time-point to when genomic alterations converted to undetectable or remained detectable at cycle 2 day 1 (C2D1). **(A)** *TP53*: Median OS for patients who were undetectable at both pre-treatment and C2D1 was 44.5 months, 34.5 months for those who converted from detectable to undetectable and 12.9 months for those who remained detectable at both time points. **(B)** *RB1*: Median OS 35.5 months for those who were remained undetectable, 26.7 months for those who converted to undetectable and 4.3 months for those who remained detectable.**(C)** *PTEN*: Median OS 35.5 months for those who were remained undetectable, 25.2 months for those who converted to undetectable and 2.9 months for those who remained detectable.

Figure 5. Plasma DNA status of progression samples. (**A)** *AR, TP53, RB1, PTEN, PIK3CA* and DDR alterations at progression (PD) ordered by time on abiraterone acetate plus glucocorticoid in descending order from left to right (each column represents a patient, patient ID at top), including only samples with detectable alterations (N=28); results for all PD samples included in Supplementary figure 3 (N=49). (**B**) 2x2 tables of gene status for matched pre-treatment and PD samples (N=45). **(C**) *AR* aberrations in progression and matched baseline (BL) and cycle 2 day 1 (C2D1) (where available) plasma.

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References

1. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in Metastatic Prostate Cancer before Chemotherapy. New England Journal of Medicine. 2014;371(5):424-33.

2. Ryan CJ, Smith MR, Fizazi K, Saad F, Mulders PF, Sternberg CN, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. The Lancet Oncology. 2015;16(2):152-60.

3. Annala M, Vandekerkhove G, Khalaf D, Taavitsainen S, Beja K, Warner EW, et al. Circulating tumor DNA genomics correlate with resistance to abiraterone and enzalutamide in prostate cancer. Cancer discovery. 2018.

4. Romanel A, Tandefelt DG, Conteduca V, Jayaram A, Casiraghi N, Wetterskog D, et al. Plasma AR and abiraterone-resistant prostate cancer. Science translational medicine. 2015;7(312):312re10-re10.

5. Conteduca V, Wetterskog D, Sharabiani MTA, Grande E, Fernandez-Perez MP, Jayaram A, et al. Androgen receptor gene status in plasma DNA associates with worse outcome on enzalutamide or abiraterone for castration-resistant prostate cancer: a multi-institution correlative biomarker study. Ann Oncol. 2017;28(7):1508-16.

6. Wyatt AW, Azad AA, Volik SV, Annala M, Beja K, McConeghy B, et al. Genomic Alterations in Cell-Free DNA and Enzalutamide Resistance in Castration-Resistant Prostate Cancer. JAMA Oncology. 2016;2(12):1598-606.

7. Torquato S, Pallavajjala A, Goldstein A, Valda Toro P, Silberstein JL, Lee J, et al. Genetic Alterations Detected in Cell-Free DNA Are Associated With Enzalutamide and Abiraterone Resistance in Castration-Resistant Prostate Cancer. JCO Precision Oncology. 2019(3):1-14.

8. De Laere B, Oeyen S, Mayrhofer M, Whitington T, van Dam P-J, Van Oyen P, et al. TP53 outperforms other androgen receptor biomarkers to predict abiraterone or enzalutamide outcome in metastatic castration-resistant prostate cancer. Clinical cancer research. 2018.

9. Jayaram A, Wingate A, Wetterskog D, Conteduca V, Khalaf D, Sharabiani MTA, et al. Plasma Androgen Receptor Copy Number Status at Emergence of Metastatic Castration-Resistant Prostate Cancer: A Pooled Multicohort Analysis. JCO Precision Oncology. 2019(3):1-13.

10. Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, et al. Genomic correlates of clinical outcome in advanced prostate cancer. Proceedings of the National Academy of Sciences. 2019:201902651.

11. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. The New England journal of medicine. 2020;382(22):2091-102.

12. Annala M, Struss WJ, Warner EW, Beja K, Vandekerkhove G, Wong A, et al. Treatment Outcomes and Tumor Loss of Heterozygosity in Germline DNA Repair-deficient Prostate Cancer. Eur Urol. 2017;72(1):34-42.

Journal Pre-proof

13. Annala M, Vandekerkhove G, Khalaf D, Taavitsainen S, Beja K, Warner EW, et al. Circulating Tumor DNA Genomics Correlate with Resistance to Abiraterone and Enzalutamide in Prostate Cancer. Cancer Discov. 2018;8(4):444-57.

14. Castro E, Romero-Laorden N, Del Pozo A, Lozano R, Medina A, Puente J, et al. PROREPAIR-B: A Prospective Cohort Study of the Impact of Germline DNA Repair Mutations on the Outcomes of Patients With Metastatic Castration-Resistant Prostate Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2019;37(6):490-503.

15. Hussain M, Daignault-Newton S, Twardowski PW, Albany C, Stein MN, Kunju LP, et al. Targeting Androgen Receptor and DNA Repair in Metastatic Castration-Resistant Prostate Cancer: Results From NCI 9012. J Clin Oncol. 2018;36(10):991-9.

16. Conteduca V, Wetterskog D, Scarpi E, Romanel A, Gurioli G, Jayaram A, et al. Plasma tumour DNA as an early indicator of treatment response in metastatic castration-resistant prostate cancer. British journal of cancer. 2020;123(6):982-7.

17. Ulz P, Belic J, Graf R, Auer M, Lafer I, Fischereder K, et al. Whole-genome plasma sequencing reveals focal amplifications as a driving force in metastatic prostate cancer. Nat Commun. 2016;7:12008.

18. Attard G, Merseburger AS, Arlt W, Sternberg CN, Feyerabend S, Berruti A, et al. Assessment of the Safety of Glucocorticoid Regimens in Combination With Abiraterone Acetate for Metastatic Castration-Resistant Prostate Cancer: A Randomized, Open-label Phase 2 Study. JAMA Oncology. 2019;5(8):1159-67.

19. Paweletz CP, Sacher AG, Raymond CK, Alden RS, O'Connell A, Mach SL, et al. Bias-Corrected Targeted Next-Generation Sequencing for Rapid, Multiplexed Detection of Actionable Alterations in Cell-Free DNA from Advanced Lung Cancer Patients. Clinical cancer research : an official journal of the American Association for Cancer Research. 2016;22(4):915-22.

20. Rathkopf DE, Smith MR, de Bono JS, Logothetis CJ, Shore ND, de Souza P, et al. Updated interim efficacy analysis and long-term safety of abiraterone acetate in metastatic castration-resistant prostate cancer patients without prior chemotherapy (COU-AA-302). European urology. 2014;66(5):815-25.

21. Armstrong AJ, Lin P, Higano CS, Sternberg CN, Sonpavde G, Tombal B, et al. Development and validation of a prognostic model for overall survival in chemotherapy-naive men with metastatic castration-resistant prostate cancer. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2018;29(11):2200-7.

22. Conteduca V, Jayaram A, Romero-Laorden N, Wetterskog D, Salvi S, Gurioli G, et al. Plasma Androgen Receptor and Docetaxel for Metastatic Castration-resistant Prostate Cancer. European urology. 2018.

23. Rescigno P, Lorente D, Bianchini D, Ferraldeschi R, Kolinsky MP, Sideris S, et al. Prostate-specific Antigen Decline After 4 Weeks of Treatment with Abiraterone Acetate and Overall Survival in Patients with Metastatic Castration-resistant Prostate Cancer. European urology. 2016;70(5):724-31.

24. Heller G, McCormack R, Kheoh T, Molina A, Smith MR, Dreicer R, et al. Circulating Tumor Cell Number as a Response Measure of Prolonged Survival for Metastatic Castration-Resistant Prostate

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Cancer: A Comparison With Prostate-Specific Antigen Across Five Randomized Phase III Clinical Trials. Journal of Clinical Oncology. 2017;36(6):572-80.

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Figure 1

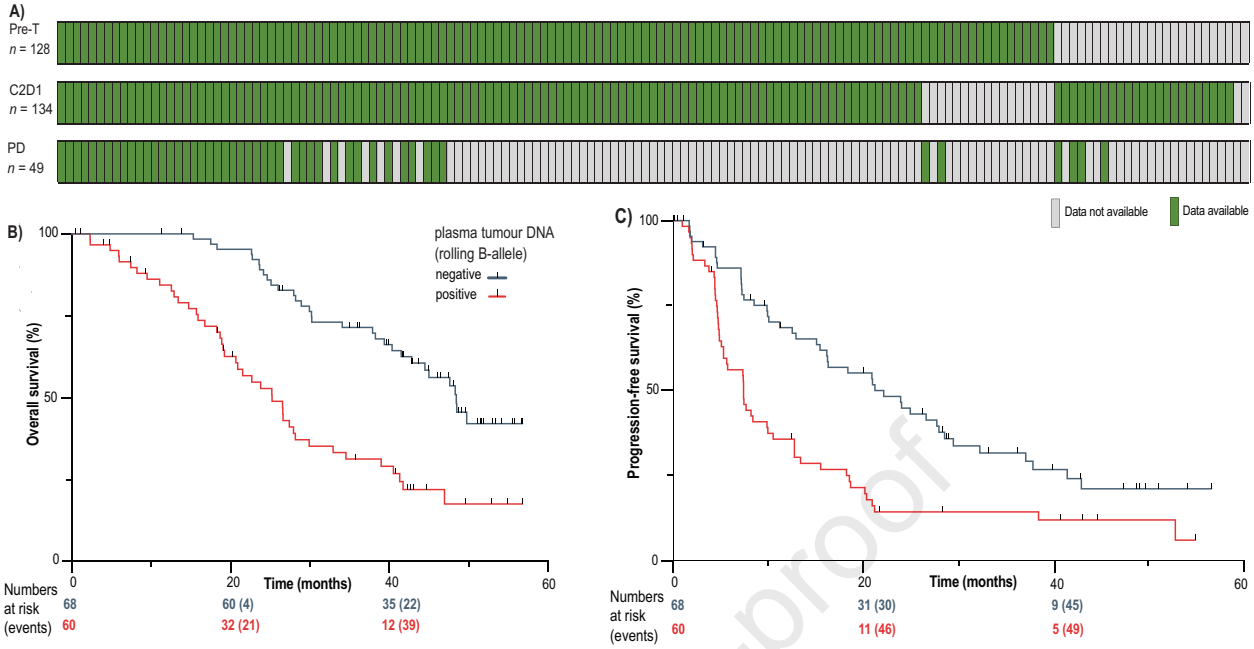

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Figure 2

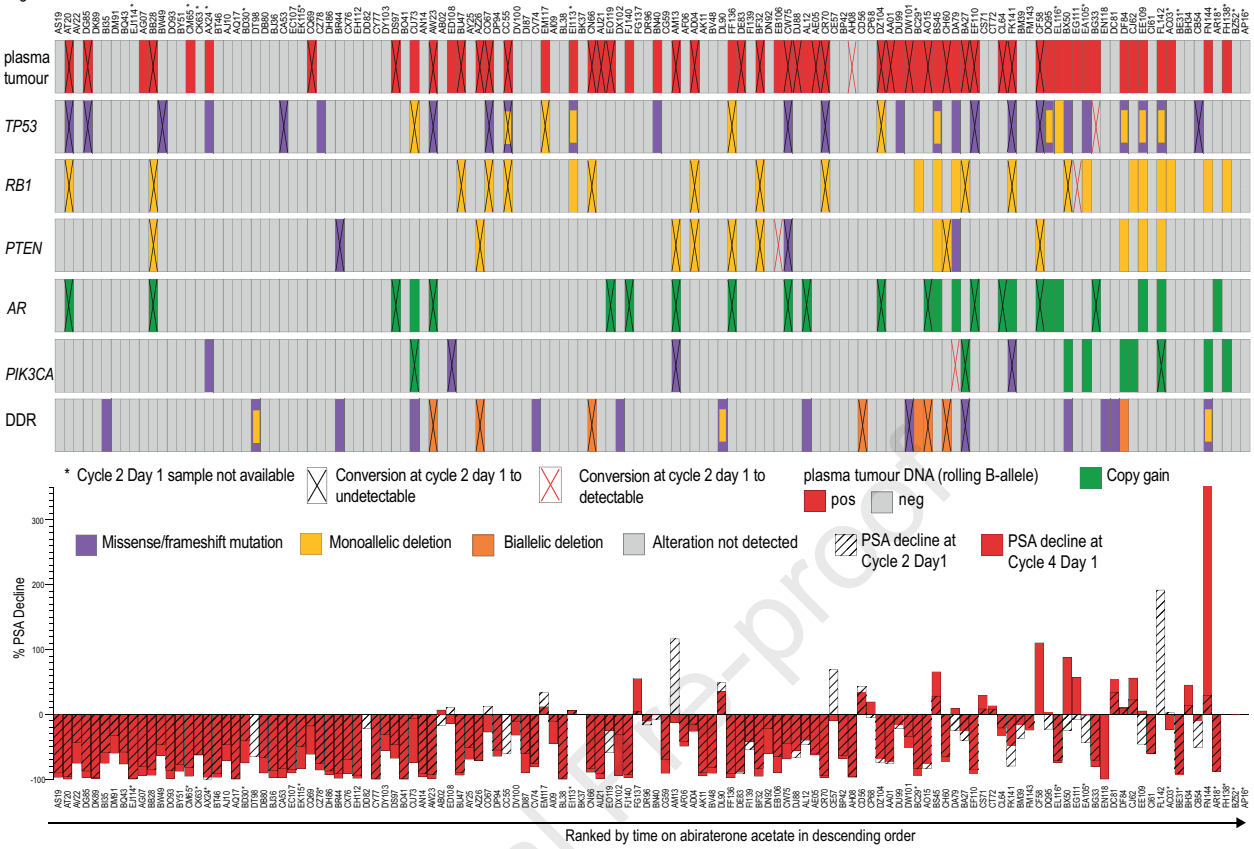

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Figure 3

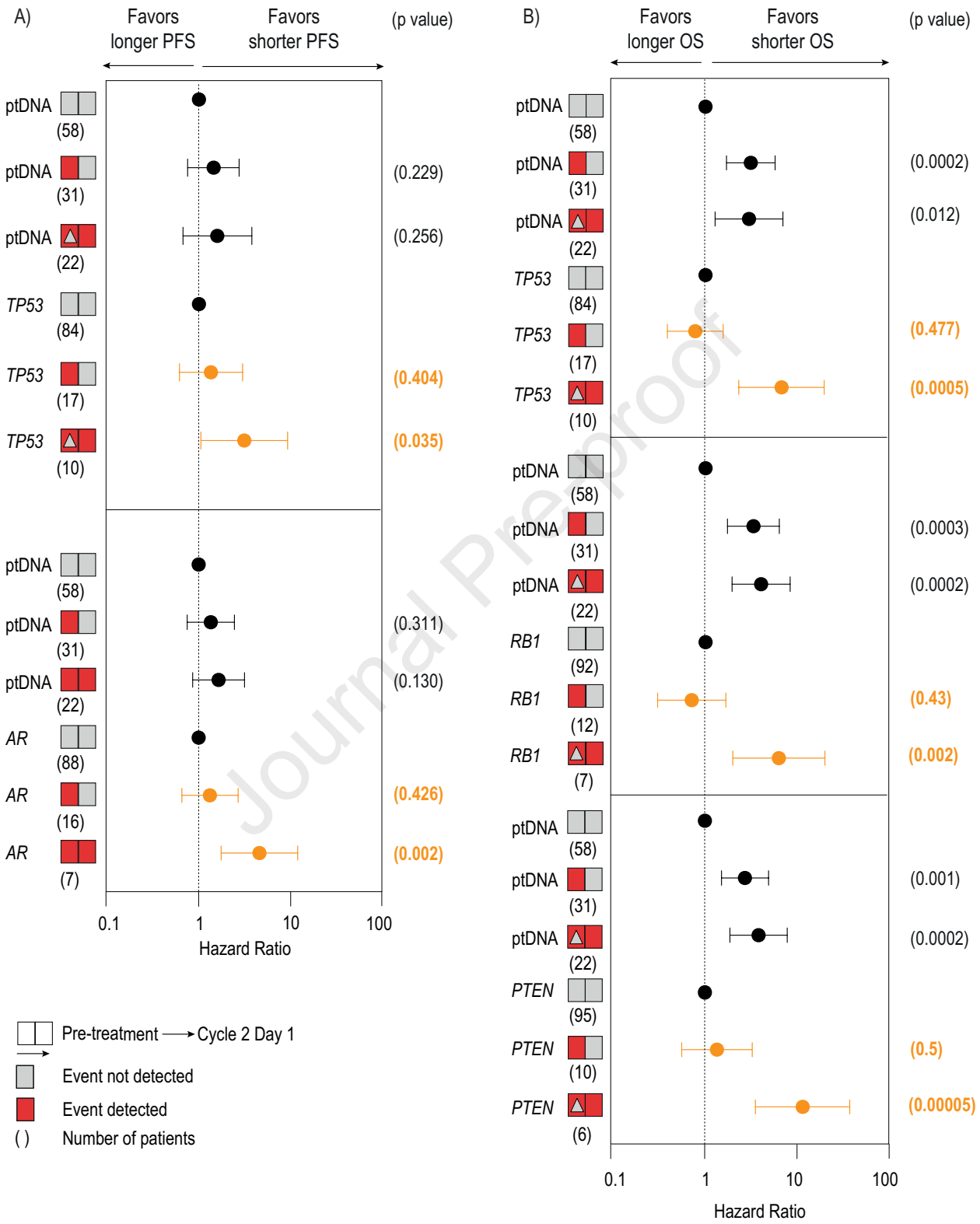

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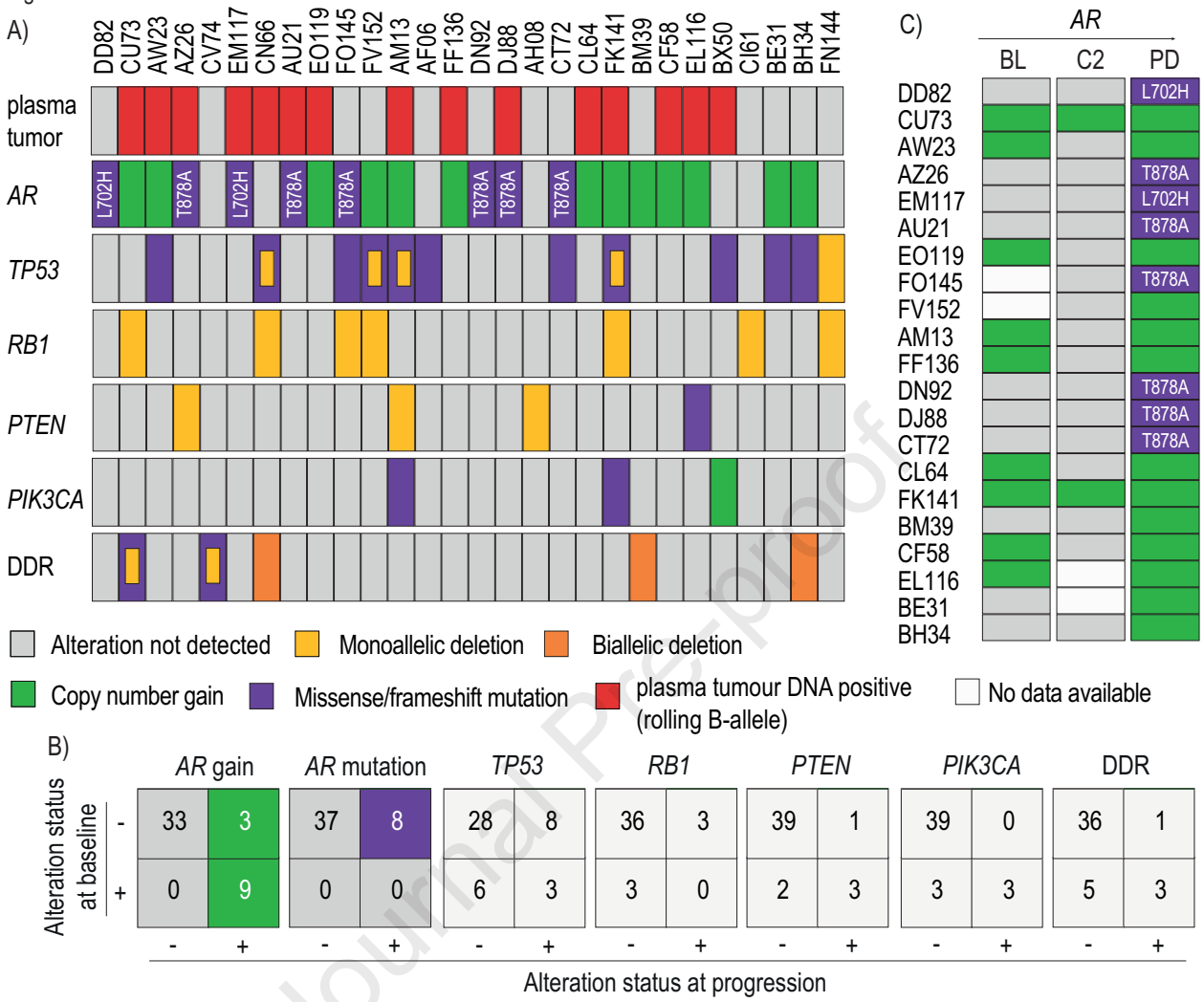
Figure 5



B)

Alteration status at baseline		*AR* gain		*AR* mutation		*TP53*		*RB1*		*PTEN*		*PIK3CA*		DDR	
		-	+	-	+	-	+	-	+	-	+	-	+	-	+
-		33	3	37	8	28	8	36	3	39	1	39	0	36	1
+		0	9	0	0	6	3	3	0	2	3	3	3	5	3

Alteration status at progression

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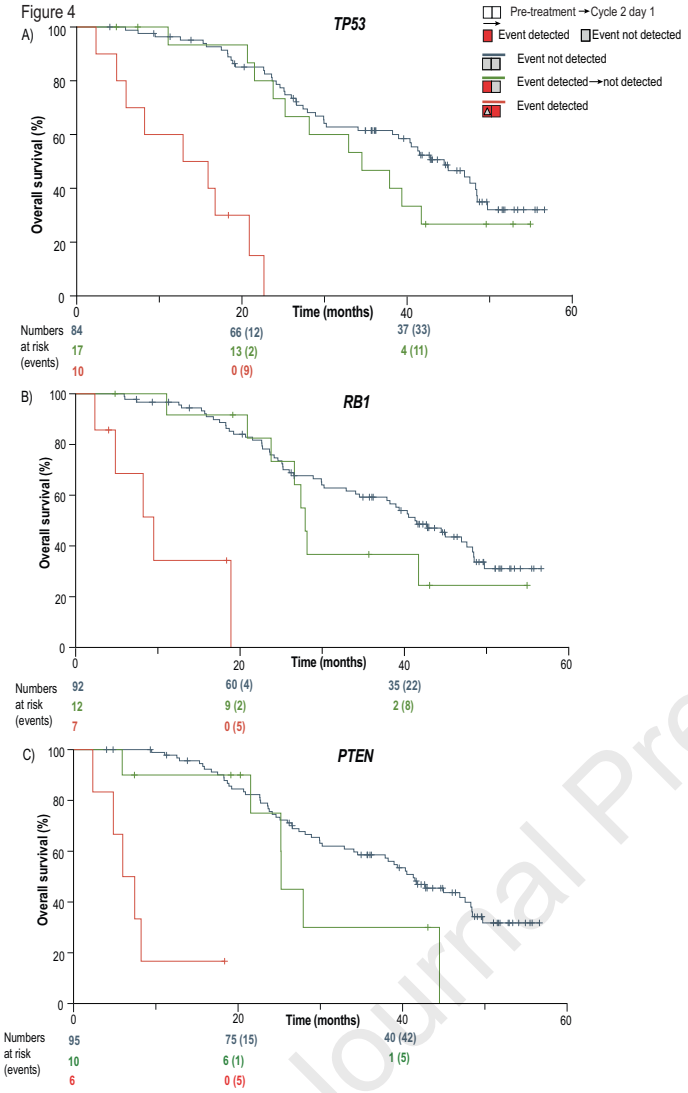
Figure 4



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