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Original articles

Liquid Biopsy in Progressing Prostate Cancer Patients Starting Docetaxel with or Without Enzalutamide: A Biomarker Study of the PRESIDE Phase 3b Trial

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Abstract

Background and objective: The PRESIDE (NCT02288247) randomized trial demonstrated prolonged progression-free survival (PFS) with continuing enzalutamide beyond progression in metastatic castration-resistant prostate cancer (mCRPC) patients starting docetaxel. This study aims to test the associations of PFS and circulating tumor DNA (ctDNA) prior to and after one cycle (cycle 2 day 1 [C2D1]) of docetaxel and with a liquid biopsy resistance biomarker (LBRB; plasma androgen receptor [*AR*] gain and/or circulating tumor cells [CTCs] expressing AR splice variant 7 [CTC-AR-V7]) prior to continuation of enzalutamide/placebo.
Methods: Patients consenting to the biomarker substudy and donating blood before starting docetaxel with enzalutamide/placebo (N = 157) were included. Sequential plasma DNA samples were characterized with a prostate-cancer bespoke next-generation-sequencing capture panel (PCF_SELECT), and CTCs were assessed for AR-V7 (Epic Sciences, San Diego, CA, USA). Cox models, Kaplan-Meier, and restricted mean survival time (RMST) at 18 mo were calculated.
Key findings and limitations: There was a significant association of worse PFS with pre-docetaxel ctDNA detection (N = 86 (55%), 8.1 vs 10.8 mo hazard ratio [HR] = 1.78, p = 0.004) or persistence/rise of ctDNA at C2D1 (N = 35/134, 5.5 vs 10.9 mo, HR = 1.95, 95% confidence interval [CI] = 1.15–3.30, p = 0.019). LBRB-positive patients (N = 62) had

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no benefit from continuing enzalutamide with docetaxel (HR = 0.78, 95% CI = 0.41–1.48, $p = 0.44$; RMST: 7.9 vs 7.1 mo, $p = 0.50$). Conversely, resistance biomarker–negative patients ($N = 87$) had significantly prolonged PFS (HR = 0.49, 95% CI = 0.29–0.82, $p = 0.006$; RMST: 11.5 vs 8.9 mo, $p = 0.005$). Eight patients were unevaluable. An exploratory analysis identified increased copy-number gains (*CDK6*/*CDK4*) at progression on docetaxel. Limitations included relatively low detection of CTC-AR-V7. Validation of impact on overall survival is required.

Conclusions and clinical implications: Liquid biopsy gives an early indication of docetaxel futility, could guide patient selection for continuing enzalutamide, and identifies cell cycle gene alterations as a potential cause of docetaxel resistance in mCRPC.

Patient summary: In the PRESIDE biomarker study, we found that detecting circulating tumor DNA in plasma after starting treatment with docetaxel (chemotherapy) for metastatic prostate cancer resistant to androgen deprivation therapy can predict early how long patients will take to respond to treatment. Patients negative for a liquid biopsy resistance biomarker (based on the status of androgen receptor (*AR*) gene and AR splice variant 7 in circulating tumor cells) benefit from continuing enzalutamide in combination with docetaxel, while patients positive for the resistance biomarker did not. Additionally, we identified alterations in the cell cycle genes *CDK6* and *CDK4* as a potential genetic cause of resistance to docetaxel, which may support testing of specific drugs targeting these alterations.

1. Introduction

Lifelong androgen deprivation therapy is standard for metastatic prostate cancer [1], while next-generation hormonal agents are discontinued at progression [2,3]. The PRESIDE trial reported a 28% statistically significant improvement in progression-free survival (PFS) with continued enzalutamide beyond progression and start of docetaxel [4], suggesting that enzalutamide-sensitive clones remain at docetaxel initiation. Concerns about the magnitude and heterogeneity of this benefit across unselected patients have limited clinical implementation.

The PRESIDE trial included a prespecified translational substudy using liquid biopsies to identify patients who derive the greatest benefit from continuing enzalutamide when starting docetaxel. Across many cancers, including metastatic castration-resistant prostate cancer (mCRPC), persistence of circulating tumor DNA (ctDNA) in plasma may be associated with worse outcome and could indicate treatment futility [5–11]. Prior nonrandomized studies of mCRPC reported that liquid biopsy detection of androgen receptor (AR) alterations identified patients with worse outcome on enzalutamide or abiraterone, but not on docetaxel [12–19].

Our primary objectives were to evaluate whether ctDNA at baseline and cycle 2 day 1 (C2D1) associate with PFS and whether a pretreatment AR-based liquid biopsy resistance biomarker (plasma *AR* gain and/or circulating tumor cells [CTCs] expressing AR splice variant 7 [CTC-AR-V7]) identifies patients who benefit from continuing enzalutamide. We also perform an exploratory analysis of progression samples to characterize docetaxel resistance (Fig. 1).

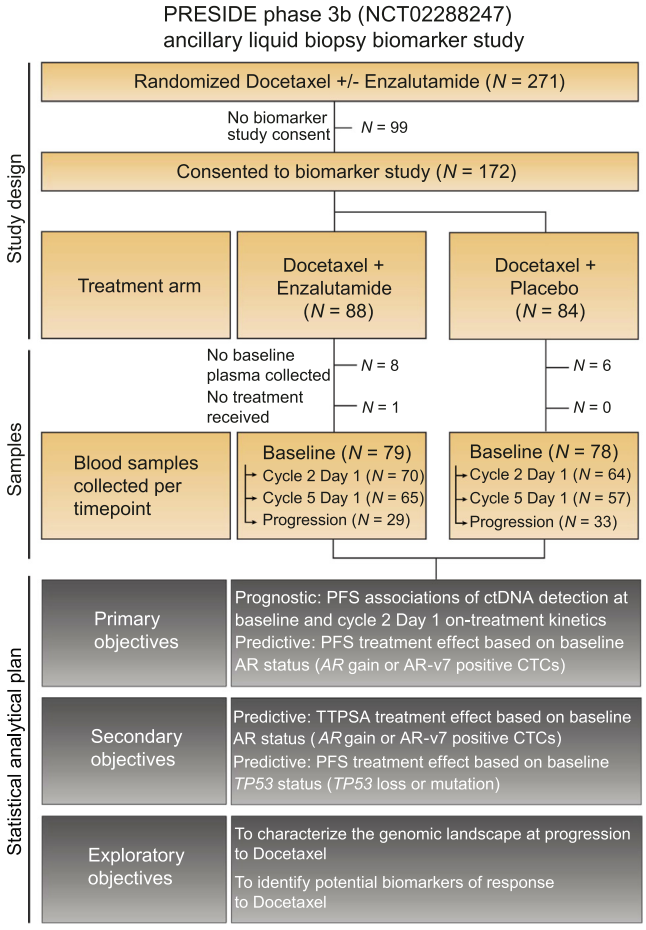


Fig. 1 – CONSORT flowchart of study design and objectives of the PRESIDE biomarker study, an overview of PRESIDE biomarker cohort flowchart, blood samples collected per time point, and objectives defined earlier in the statistical analysis plan. AR = androgen receptor; AR-V7 = androgen receptor splice variant 7; CTC = circulating tumor cell; ctDNA = circulating tumor DNA; *N* = number; PFS = progression-free survival; PSA = prostate-specific antigen; TTPSA = time to PSA progression.

2. Patients and methods

2.1. Population and study design

PRESIDE was a multicenter, two-period, double-blind, randomized, placebo-controlled phase 3b trial [4]. Eligible

patients had histologically confirmed prostate adenocarcinoma, bone or soft tissue metastases, progression despite serum testosterone ≤1.73 nmol/l, and Eastern Cooperative Oncology Group performance status 0–1. Screening occurred from December 2014 to February 2016, and data were extracted after the completion of the main study and extension protocols (April 30, 2020). The study protocol and amendments were approved by local independent review boards. It was conducted in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice and the Declaration of Helsinki, and registered at ClinicalTrials.gov (NCT02288247). All patients provided written informed consent to trial and biomarker substudy participation. REMARK guidelines were followed.

Patients initially received oral enzalutamide 160 mg/d. Those with prostate-specific antigen (PSA) decline and no radiographic progression after 12 wk continued enzalutamide until radiographic and/or PSA progression, and then were randomized 1:1 to enzalutamide 160 mg/d plus docetaxel 75 mg/m^2 3-weekly with oral prednisolone 10 mg/d, or oral placebo plus docetaxel and prednisolone, continued until disease progression, intolerable toxicity, subject withdrawal, or death. Radiographic assessments included abdominopelvic computed tomography (CT) or magnetic resonance imaging (MRI), chest x-ray (or chest CT/MRI if lung metastasis suspected/present), and whole-body technetium bone scans. Assessments were done before randomization and every 12 wk until radiographic progression, new therapy initiation, or a maximum of 112 d after treatment discontinuation.

Blood for plasma DNA and CTC analyses was collected between progression on single-agent enzalutamide and start of docetaxel (baseline), at C2D1, after 12 wk (C5D1), on progression, or at the last follow-up visit. The collection time points were predefined in the trial protocol (Supplementary material).

2.2. Liquid biopsy analysis

Blood collected in Streck tubes was processed for plasma, white blood cell (WBC) extraction, and CTC capture (Epic Sciences, San Diego, CA, USA) [20], and then stored at –80°C until analyses.

Plasma cell-free and WBC DNA was sequenced using a custom, targeted next-generation sequencing panel (PCF_SELECT) [21], aiming for ~500× and 200× coverage, respectively. PCF_SELECT targets 63 genes frequently altered in prostate cancer, and includes ~25 000 high minor allele frequency (AF) single nucleotide polymorphisms in ~100 target and control gene regions to enable allelic imbalance (AI) detection using a bespoke computational pipeline [21]. ctDNA fractions and allele-specific copy numbers (CNs) were computed by integrating read-depth estimations and AI calls within the CLONETv2 framework [22]. *AR* CN values were derived using read-depth estimations. Observed Log2-ratio values were corrected by tumor content and ploidy if consistent with a CN change, applying gene-specific thresholds accounting for regions' noise [22,23]. ABEMUS [24] was applied to detect somatic single nucleotide variants (SNVs). SNVs with local coverage >50, WBC sample AF ≤0.01, and annotated as "pathogenic/likely pathogenic" in ClinVar, the Genome Aggregation Database, Catalogue of Somatic Mutations in Cancer, or peer-reviewed PubMed articles were included. The *AR* SNV analysis focused on mutations coding for L702H, T878A, H875Y, F877L, and W742C/L.

CTC slides were subjected to the Epic Sciences automated immunofluorescent assay, staining for DNA, cytokeratins, CD45, and AR-V7 [17]. Cytokeratin + CD45 - cells with intact nucleus were identified and counted by morphology algorithms, normalized to blood volume and expressed as CTCs/ml [20]. Slides with one or more CTCs with nuclear-localized AR-V7 were scored AR-V7 positive [13].

Researchers were blinded to treatment assignment and patient clinical characteristics during sample processing and analysis.

2.3. Outcome measures

The primary endpoint was PFS, defined as the time from randomization to radiographic progression, unequivocal clinical progression, or death. The secondary endpoint was time to PSA progression (TTPSA), defined as the time from randomization to ≥25% increase and an absolute increase of ≥2 ng/ml above nadir, confirmed by a second value after ≥3 wk [4]. Other protocol-defined endpoints were not tested to minimize overtesting.

2.4. Statistical analyses

As stated earlier in the PRESIDE trial protocol, a statistical analysis plan was developed by the industry sponsor (Astellas Pharma Inc., Chuo-Ku, Tokyo, Japan) and academic partner (University College London, London, UK). ctDNA and CTCs were assessed as continuous/binary variables; parametric and nonparametric tests were applied. Fisher's exact test was used to compare grouped/categorical variables. Univariate and multivariable Cox regression models, and Kaplan-Meier analyses with Mantel-Haenszel test (median reported) evaluated the association of biomarkers with outcome. The restricted mean survival time (RMST) at 18 mo (prespecified as including follow-up or death events in 95% of patients) was calculated if nonproportional hazards were observed by the Shoenfeld residual analysis. All tests were two sided, and an alpha error of ≤5% was considered significant. R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria) and MedCalc version 22.006 (MedCalc Software Ltd., Ostend, Belgium) were used (Supplementary material).

3. Results

3.1. PRESIDE biomarker cohort and liquid biopsy analytical plan

In PRESIDE, 271 patients were randomized 1:1 at enzalutamide progression to docetaxel plus enzalutamide/-placebo, with 172 (63%) consenting to the biomarker study. Of those, 157 (enzalutamide = 79 and placebo = 78) gave a baseline blood sample and were eligible for a biomarker study analysis as per the prespecified analytical plan (Fig. 1). In addition, sequential blood samples from

these patients were collected at C2D1 (*N* = 134), C5D1 (*N* = 122), and progression (*N* = 62). The biomarker cohort showed no significant differences in baseline clinical characteristics as compared with the intention-to-treat cohort or between treatment arms (Supplementary Table 1).

3.2. Plasma ctDNA detection and kinetics on docetaxel

Plasma DNA sequencing succeeded in 474/475 samples, with one baseline sample not passing quality control. The levels of ctDNA were analyzed across time points, showing a significantly lower ctDNA detection rate at C2D1 (26.1%) than at baseline (55.1%, $p < 0.001$) and C5D1 (46.7%, $p < 0.001$), and in progression samples (61.1%, $p < 0.0001$; Fig. 2A). In ctDNA-positive samples, ctDNA fractions were significantly lower at C2D1 and C5D1 than at baseline and in progression samples ($p < 0.0001$, median ctDNA fraction = 0.05, 0.05, 0.26, and 0.25, respectively; Fig. 2B). In 109 patients, we had sequential samples allowing for further insights into ctDNA kinetics (Fig. 2C). We observed that baseline ctDNA-negative patients, versus ctDNA-positive patients, were more likely to be ctDNA negative at C2D1 (odds ratio [OR] = 6.1, 95% confidence interval [CI] = 2.0–15.6, $p = 0.0004$). Conversely, the C2D1 ctDNA-positive group, versus the ctDNA-negative group, was more likely to be ctDNA positive at C5D1 (OR = 7.2, 95% CI = 2.7–19.2, $p < 0.0001$).

3.3. ctDNA and PFS on docetaxel

As per our primary and secondary objectives, we investigated ctDNA associations with docetaxel PFS. Baseline

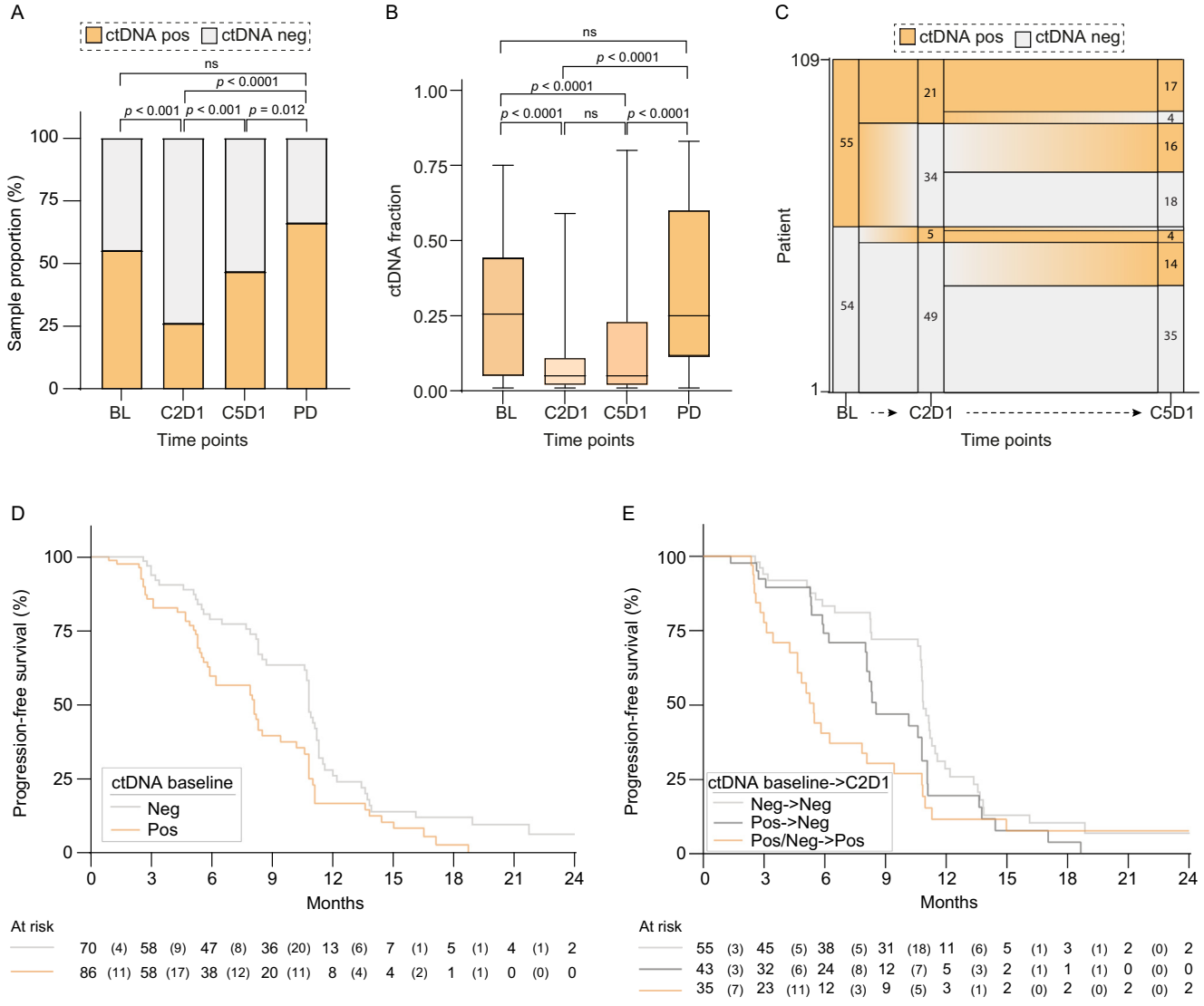


Fig. 2 – On-treatment plasma ctDNA kinetics and association with outcome. (A) Detection of ctDNA in plasma samples across time points. Patients with plasma samples collected at baseline (*N* = 156), C2D1 (*N* = 134), C5D1 (*N* = 122), and PD (*N* = 62). (B) Changes in ctDNA fraction in tumor-positive samples across time points. Patients with tumor DNA-positive samples at baseline (*N* = 86), C2D1 (*N* = 35), C5D1 (*N* = 57), and PD (*N* = 41). (C) Changes in ctDNA across baseline, C2D1, and C5D1 samples (gradients between time points are for illustration and do not represent the time of change). (D) Association of baseline ctDNA detection and progression-free survival. (E) Association of baseline to C2D1 ctDNA changes and progression-free survival after starting docetaxel. One patient withdrew at 0.07 mo due to a protocol violation. BL = baseline; ctDNA = circulating tumor DNA; C2D1 = cycle 2 day 1; C5D1 = cycle 5 day 1; D = detected; *N* = number; Neg = negative; ns = not significant; PD = progression of disease; Pos = positive.

ctDNA-positive patients had significantly shorter PFS than ctDNA-negative patients (hazard ratio [HR] = 1.78, 95% CI = 1.20–2.64, p = 0.004), with median survival of 8.1 versus 10.8 mo (Fig. 2D). The ctDNA detection rate and fractions were similar between treatment arms, and the ctDNA association with PFS was consistent regardless of treatment (Supplementary Fig. 1A–C). For ctDNA kinetics, patients who remained or converted to ctDNA positive between baseline and C2D1 (N = 35) had significantly shorter PFS than the 55 patients who were ctDNA negative at both time points (5.5 vs 10.9 mo, HR = 1.95, 95% CI = 1.15–3.30, p = 0.019). Patients converting to ctDNA negative (N = 43) showed no significant difference from patients ctDNA negative at both time points (8.3 mo, HR = 1.48, 95% CI = 0.93–2.36, p = 0.24; Fig. 2E).

3.4. Liquid biopsy resistance biomarker prevalence

At baseline, 56 patients were positive for plasma *AR* gain. CTC slides from 143 patients met quality control, with AR-V7–positive CTCs detected in 17 cases. CTC-AR-V7 positivity was significantly associated with both increased ctDNA detection (Fig. 3A) and higher ctDNA fractions (Fig. 3B). Further, we observed a significant association of AR-V7–positive CTC samples and plasma *AR* gain (Fig. 3C). Patients were classified as positive for the liquid biopsy resistance biomarker at baseline by having a plasma *AR* gain with or without AR-V7–positive CTCs (n = 56) or by having AR-V7–positive CTCs when plasma *AR* wild type (n = 6). Eighty-seven patients were both plasma *AR* wild type and AR-V7 negative. Eight patients were excluded from the outcome analysis, seven due to no CTC result while having plasma *AR* wild type and one due to no plasma sequencing data while being AR-V7 negative (Fig. 3D).

3.5. Liquid biopsy resistance biomarker and benefit from continuing enzalutamide

In our primary predictive objective, we hypothesized that the liquid biopsy resistance biomarker in circulation at baseline

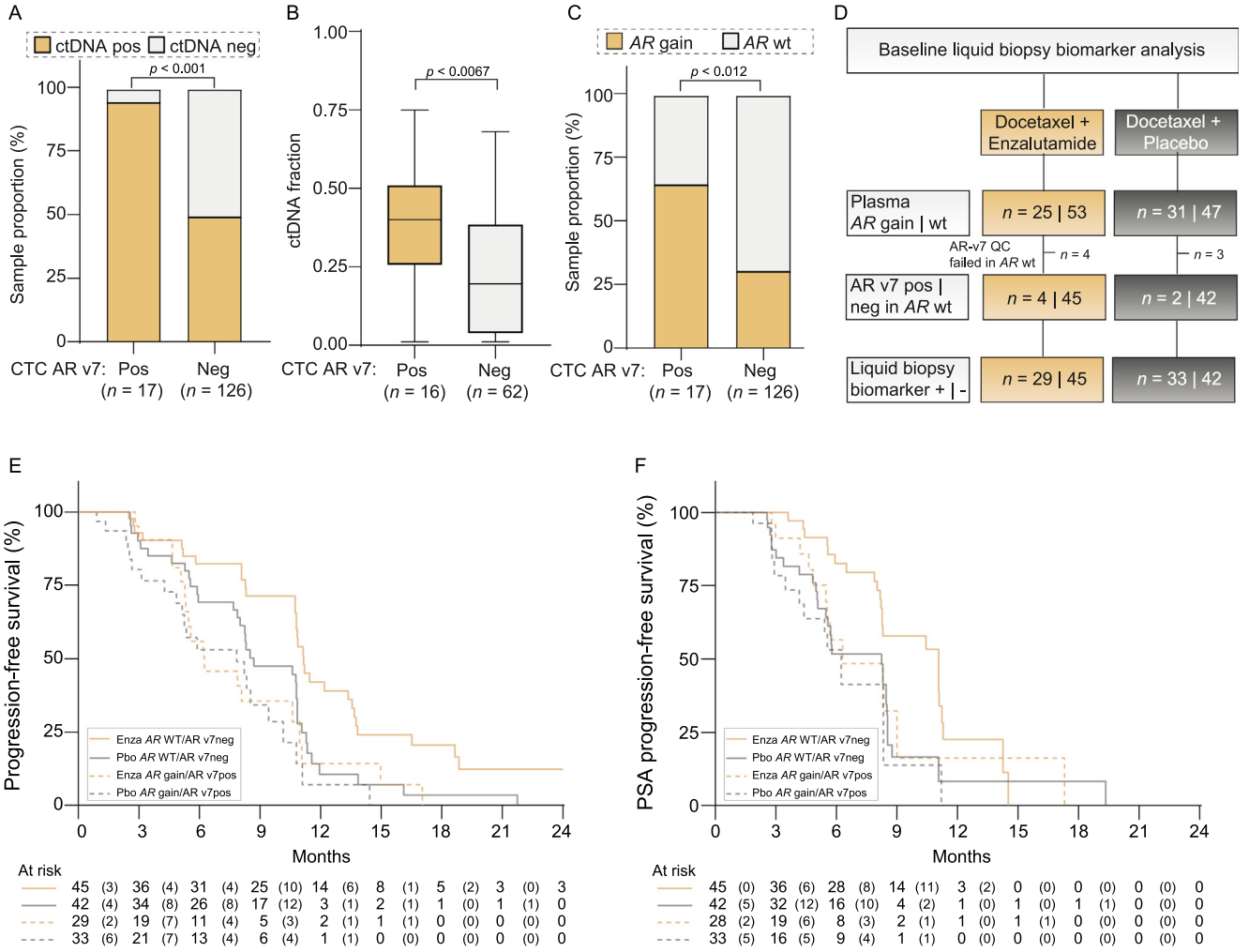


Fig. 3 – Analysis of plasma *AR* and CTC AR-V7 status at baseline, and association of pretreatment liquid biopsy biomarker-resistance status and prediction of treatment response. (A) Baseline association of CTC AR-V7 and ctDNA detection. (B) Baseline association of CTC AR-V7 detection and ctDNA fraction. (C) Baseline association of CTC AR-V7 detection and plasma *AR* gene status. (D) Overview of the liquid biopsy resistance-biomarker status at baseline and treatment arm distribution. (E) Liquid biopsy resistance-biomarker status at baseline and prediction of progression-free survival by treatment arm. (F) Liquid biopsy resistance-biomarker status at baseline and prediction of time to PSA progression by treatment arm. Time to PSA progression was not available for one patient. Seven patients did not meet the criteria for the CTC analysis. In one patient, DNA sequencing was unsuccessful at baseline. AR = androgen receptor; AR-V7 = androgen receptor splice variant 7; CTC = circulating tumor cell; ctDNA = circulating tumor DNA; Enza = enzalutamide; n/N = number; Neg = negative; Pbo = placebo; Pos = positive; PSA = prostate-specific antigen; QC = quality control; wt = wild type.

would associate with a lack of benefit in continuing enzalutamide with docetaxel. Between treatment arms we observed similar proportions of biomarker positivity (docetaxel + enzalutamide: 29 liquid biopsy resistance biomarker positive and 45 negative, docetaxel plus placebo: 33 patients positive and 42 negative; Supplementary Fig. 2A and 2B).

Resistance biomarker–negative patients had significantly prolonged PFS when continuing enzalutamide (enzalutamide 11.2 mo vs placebo 8.7 mo, HR = 0.49, 95% CI = 0.29–0.82, p = 0.006), whereas resistance biomarker–positive patients did not (enzalutamide 6.2 mo vs placebo 7.9 mo, HR = 0.78, 95% CI = 0.41–1.48, p = 0.44; Fig. 3E). Nonproportional hazards were observed and, as prespecified in our statistical analysis plan, an RMST analysis was performed. The analysis confirmed longer PFS for resistance biomarker–negative patients with enzalutamide (11.5 vs 8.9 mo, p = 0.005), with no difference for resistance biomarker–positive patients (7.9 vs 7.1 mo, p = 0.50). Resistance biomarker–negative patients also showed longer TTPSA for continuing enzalutamide (11.1 vs 8.2 mo, HR = 0.47, 95% CI = 0.26–0.83, p = 0.009; RMST: 10.0 vs 7.5 mo, p = 0.0004), but not resistance biomarker–positive patients (6.3 vs 6.2 mo, HR = 0.70, 95% CI = 0.32–1.53, p = 0.37; RMST: p = 0.50; Fig. 3F and Supplementary Table 2). Finally, as ctDNA detection was associated with the detection of the liquid biopsy resistance biomarkers, we investigated the treatment effect by ctDNA status. The association of ctDNA status and benefit from the addition of enzalutamide (ctDNA-negative PFS, enzalutamide 11.0 vs 8.7 mo, HR = 0.57, 95% CI = 0.32–1.01, p = 0.054; RMST: p = 0.058, ctDNA-positive enzalutamide 8.1 vs 8.0 mo, HR = 0.61, 95% CI = 0.352–1.06, p = 0.078;. RMST: p = 0.13) suggested that the liquid biopsy resistance biomarker may provide information that is independent of the circulating tumor burden.

3.6. Exploratory analysis of baseline clinical and ctDNA features and PFS

After characterizing the enzalutamide-resistant genomic landscape of mCRPC before starting docetaxel (Fig. 4A), we performed a univariable analysis of clinical variables, ctDNA and CTC features, and associations with outcome (Fig. 4B). ctDNA positivity, high ctDNA fractions (≥0.26), *AR* gain or mutation, *TP53* loss or mutation, *RB1* loss or mutation ,and *PTEN* loss or mutation were significantly associated with shorter PFS. We included these in multivariable analyses individually including ctDNA positivity, and identified *AR* and *TP53* alterations as independently associated with outcome (Fig. 4C and Supplementary Table 2).

3.7. TP53 alterations and benefit from continuing enzalutamide

We then investigated the association of *TP53* alterations and treatment response. Of note, unlike when patients were split by the liquid biopsy resistance biomarker, there was no significant improvement in PFS when continuing enzalutamide in the *TP53*-wildtype group (HR = 0.61, 95% CI = 0.37–1.02, p = 0.059) or the *TP53*-altered group (HR = 0.61, 95% CI = 0.32–1.13, p = 0.12; Fig. 4D and Supplementary Table 2).

3.8. Exploratory analysis of ctDNA alteration changes at progression on docetaxel

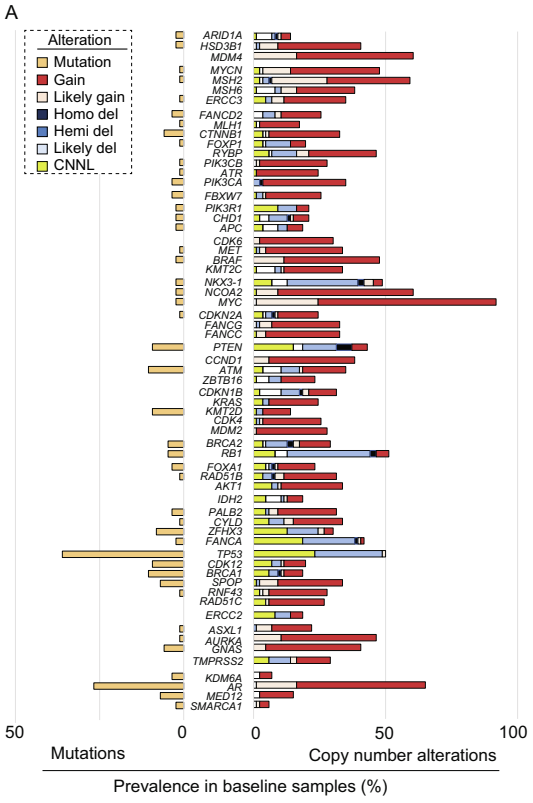
Finally, we investigated gene alterations at progression on docetaxel. This exploratory analysis first compared the prevalence of CN changes and SNVs between patient-matched ctDNA-positive baseline and progression samples (N = 25). We identified eight genes with a two-fold or more increase: grouped by biological pathway impact, four are involved in cell cycle regulation (*CDK4, CDK6, MDM2*, and *KRAS*), two in DNA repair (*FANCA* and *ERCC2*), and one in AR signaling (*NCOA2*), and one is a chromatin modifier (*KDM6A*; Fig. 4E). We then compared the prevalence of genomic alterations across all baseline (n = 86) versus progression (n = 41) ctDNA-positive samples. Significant increases at progression were observed for CN gains of cell cycle genes (OR: *CDK6*: 2.95, p = 0.006; *CCND1*: 2.78, p = 0.013; *CDK4:* 2.34, p = 0.039), the neuroendocrine-associated gene *ASXL1* (OR = 3.26, p = 0.004), and the steroid-biosynthesis gene *HSD3B1* (OR = 2.78, p = 0.013; Fig. 4F).

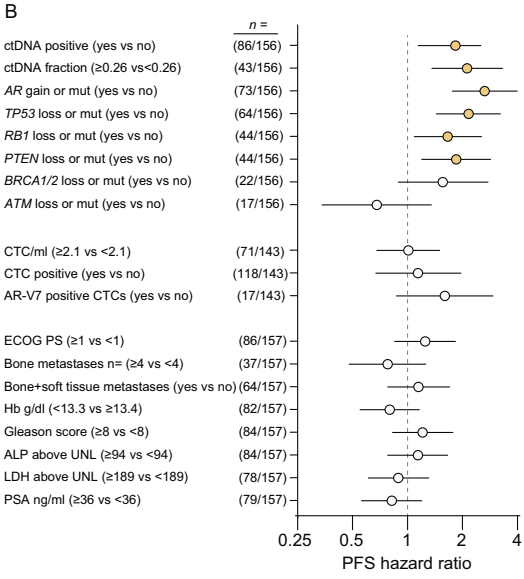
4. Discussion

In this preplanned biomarker substudy of PRESIDE, we provide strong evidence that persistence or a rise in plasma DNA prior to the second docetaxel dose is associated with a shorter time to progression. We also provide hypothesis-generating evidence that patients progressing with circulating AR alterations would not benefit from continuing enzalutamide. This translational finding is biologically plausible and, given that resistance biomarker–negative patients have an estimated 51% improvement in PFS, could offer a route to clinical implementation of continuing enzalutamide for selected patients.

One main limitation with testing circulating AR alterations is the inevitable association with the circulating tumor burden, which is also strongly prognostic [19,25–27]. Given that our population was randomized, we could

Fig. 4 – The genomic landscape at baseline and progression in the PRESIDE biomarker cohort. (A) The genomic landscape upon progression to enzalutamide alone in ctDNA-positive baseline samples (N = 86). (B) A univariable analysis of baseline clinical characteristics, CTC features, ctDNA levels, and alterations prespecified in the analytical plan with PFS. Orange circles denote significant hazard ratios. (C) Multivariable Cox regression models of gene alterations significant in the univariable analysis in combination with ctDNA detection and their association with progression-free survival at baseline. Only significant variables are displayed. (D) *TP53* status at baseline and prediction of PFS by treatment arm. (E) Baseline to progression changes in the detection of the most per gene frequent alteration in 25 patients with matched ctDNA-positive samples. (F) Baseline to progression changes in the detection of the most per gene frequent alteration in ctDNA-positive unmatched baseline (N = 86) and progression (N = 41) samples. ALP = alkaline phosphatase; AR = androgen receptor; AR-V7 = androgen receptor splice variant 7; BL = baseline; CNNL = copy number neutral loss; CTC = circulating tumor cell; ctDNA = circulating tumor DNA; ECOG PS = Eastern Cooperative Oncology Group performance status; Enza = enzalutamide; Hb = hemoglobin; Hemi del = heterozygous deletions; Homo del = homozygous deletions; LDH = lactate dehydrogenase; Likely del = Likely deletions; mut = mutation; Pbo = Placebo; PFS = progression-free survival; PSA = prostate-specific antigen; UNL = upper normal limit; wt/WT = wild type.





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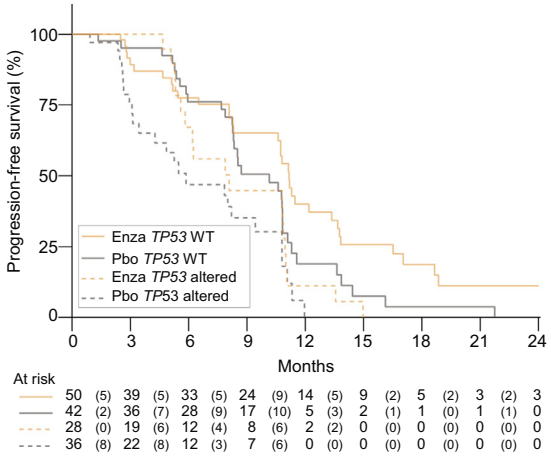
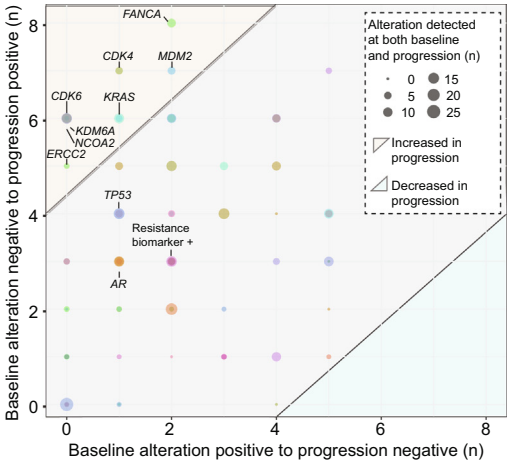
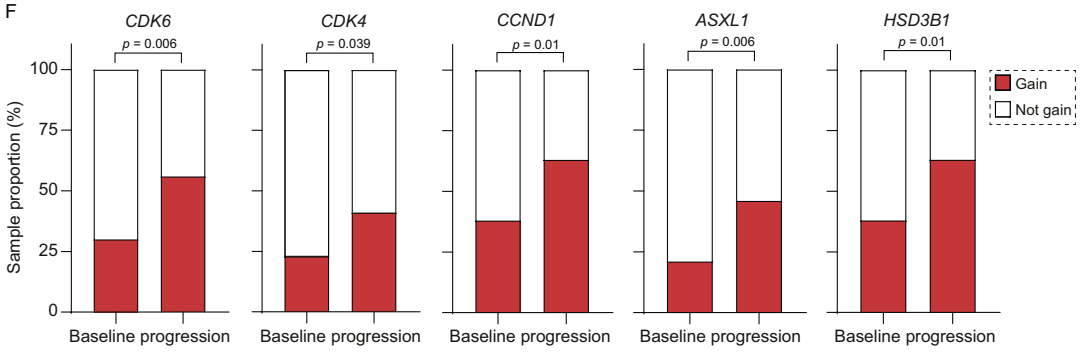
Multivariable analysis (PFS)		
Variable	HR (95% CI)	*p*-value
AR gain or mutated	2.35 (1.37-4.02)	0.002
TP53 loss or mutated	1.72 (1.10-2.67)	0.016







compare treatment effect by the resistance biomarker or plasma tumor DNA abundance. We observed a smaller numerical difference in PFS times of randomized patients split by detection of plasma tumor DNA, and neither group showed a significant benefit from continuing enzalutamide. Similarly, the benefit from continuing enzalutamide was the same in patients split by *TP53* status, another biomarker proposed for the stratification of mCRPC patients [5,28,29].

Other challenges were the higher failure rate for CTC characterization and the relatively low prevalence of CTC-AR-V7. In addition, CTC-AR-V7 patients were more likely to have an *AR* gain so the additional value of testing CTCs in this population remains uncertain. These observations, although not statistically conclusive, support the hypothesis that an AR-based biomarker could provide additional biologically relevant information over plasma tumor DNA abundance. Although our objectives were prespecified, the sample size for biomarker testing was not predefined to ensure sufficient statistical power to formally test for treatment interactions. Adjusting the sample size in the study design would have been particularly challenging given that patients entered the trial at the start of enzalutamide, and it would not have been feasible to mandate blood collection as a criterion of randomization prior to docetaxel [4]. Although our results should be considered hypothesis generating and will require validation in a larger trial that prospectively incorporates the biomarker, the confidence one assigns to our observation is greater given that we predefined the hypothesis and direction of effect based on biologically plausible evidence. Future investigation may also require confirmation of improvements in overall survival in liquid biopsy resistance biomarker–negative patients who continue enzalutamide with docetaxel.

To minimize multiple testing, we restricted our exploratory analysis to aberrations reported previously as prognostic in mCRPC [5,12,27–32]. In many cases, the coexistence of several aberrations posed a challenge in isolating the specific contribution of individual genes from others. We aimed to partially account for the confounder prognostic effect of plasma tumor DNA, selecting only ctDNA-positive cases for univariate Cox analyses. Our results underscore the prognostic associations of *TP53* and *AR* alterations. We also report new evidence that progression on docetaxel is associated with CN gain of genes involved in cell cycle regulation, notably *CDK6* and *CDK4,* which could both be inhibited by targeted cell cycle inhibitors [28].

5. Conclusions

Despite the early nature of our findings, our study provides intriguing results and addresses three unmet needs in mCRPC. First, we provide compelling data that a plasma DNA test 3 weeks after starting docetaxel could provide an early indication of treatment futility. Second, we generate new evidence to support the utility of an AR-based liquid biopsy for selecting patients for continuing enzalutamide with docetaxel. Third, we introduce the hypotheses that clones harboring genomic alterations in cell cycle genes are selected by docetaxel and that cell cycle targeted inhibition could reduce or overcome resistance to taxanes.

***Author contributions*:** Gerhardt Attard had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition of data: Ruiz-Vico, Wetterskog, Orlando, Thakali, Wingate, Martins, Gupta, Wenstrup, Attard.
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Drafting of the manuscript: Ruiz-Vico, Wetterskog, Attard.
Critical revision of the manuscript for important intellectual content: All authors.
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Supervision: Attard, Demichelis, Gonzalez-Billalabeitia.
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Data sharing: The individual-patient genomic sequencing and CTC data that support this study, plus the data dictionary, will be deposited in the European Genome-phenome Archive and will be made available for researchers with an academic project proposal, after review and approval by the data access committee. The PRESIDE clinical trial protocol and the PRESIDE biomarker study statistical analysis plan are provided in the Supplementary material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euo.2024.08.006.

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