# Plasma *AR* status and cabazitaxel in heavily-treated metastatic castration-resistant prostate cancer

Running head: Plasma *AR* status and cabazitaxel

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## Abstract

### Background

Plasma androgen receptor (*AR*) copy number status has been identified as a potential biomarker of response in metastatic castration-resistant prostate cancer (mCRPC) patients receiving docetaxel or the AR-targeted therapies abiraterone or enzalutamide. However,the relevance of plasma *AR* status in the context of cabazitaxel therapy is unknown.

### Patients and Methods

Between September 2011 and January 2018, pre-therapy plasma samples were collected from 155 patients treated with second or third-line cabazitaxel at standard or reduced-dose in different biomarker protocols. Droplet digital PCR was used to identify plasma *AR* gain and normal samples. The primary objective was to evaluate associations of plasma *AR* status with treatment outcome. In an exploratory analysis, a comparison between plasma *AR* and treatment type was investigated by incorporating updated data from our prior study of 85 post-docetaxel patients receiving abiraterone or enzalutamide.

### Results

We observed a shorter median overall survival (OS) and progression-free survival (PFS) in *AR*-gained compared to *AR*-normal patients (OS 10.5 versus 14.1months, hazard ratio (HR)=1.44, 95% confidence interval (CI) 0.98-2.13, *P*=0.064, and PFS 4.0 versus 5.0months, HR=1.47, 95%CI 1.05-2.07,*P*=0.024). In mCRPC patients receiving second-line therapies, a significant treatment interaction was observed between plasma *AR* and cabazitaxel versus AR-directed therapies for OS (*P*=0.041) but not PFS (*P*=0.244). In an exploratory analysis, *AR*-gained patients treated with initial reduced-dose of cabazitaxel had a significantly shorter median OS (7.3 versus 11.5months, HR=1.95, 95%CI 1.13-3.38,*P*=0.016), and PFS (2.7 versus 5.0months, HR=2.27, 95%CI 1.39-3.71,*P*=0.001).

**Conclusion**

Plasma *AR* status has a potential clinical utility in patients being considered for cabazitaxel. Validation of these findings in prospective trials are warranted.

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## 1. Introduction

For patients with metastatic castration-resistant prostate cancer (mCRPC) there is an urgent need for predictive and prognostic biomarkers to the androgen receptor (AR)-directed therapies, abiraterone and enzalutamide [1,2] and the taxanes, docetaxel and cabazitaxel [3,4], all approved and survival-prolonging. Molecular profiling of serial prostate cancer biopsies [5] has been proposed to be performed before each treatment to best inform therapy selection [6]. Due to the logistical challenges in performing longitudinal solid tumour assessments, liquid biopsies have been suggested as an alternative approach with the potential to improve clinical practice. Analysis of plasma DNA could provide an opportunity for real-time molecular characterization and stratification of patients for better treatment selection [7].

Plasma DNA studies of metastatic castration-resistant prostate cancer (mCRPC) patients treated with AR-directed therapies in the first and second-line setting have revealed the association between genomic changes of the androgen receptor and worse outcome [8-14]. Moreover, plasma *AR* copy number status has been identified as a potential therapy-guiding predictive and prognostic biomarker in the first-line setting for mCRPC with the clinical evidence that, for *AR*-gained patients, the preferred choice of therapy is docetaxel rather than a hormonal drug and vice versa for *AR*-normal patients [14]. However, the potential clinical utility of plasma *AR* status for the second and third-line used therapy cabazitaxel remains unknown.

Our primary objective was to evaluate associations of plasma *AR* status with progression-free/overall survival (PFS/OS) in mCRPC patients treated with cabazitaxel. In an exploratory analysis, we also aimed to compare the difference in survival by plasma *AR* copy number status for patients treated either with second-line cabazitaxel or anti-AR therapies. Determining the right therapy with the

adequate dose for the selected patient remains a significant challenge for many drugs, including cabazitaxel [15]. We therefore performed an exploratory analysis of our non-randomised cohorts of patients starting at different schedule of cabazitaxel to determine a potential role of circulating *AR* copy number in the overall management of CRPC patients receiving cabazitaxel.

## 2. Material and methods

### 2.1 Study design

In this prospective multicentre nonrandomized study, blood samples were collected from patients with mCRPC before starting therapy with cabazitaxel at standard doses in routine clinical practice (25mg/m$^2$ every three weeks together with prednisone 5mg twice daily for a maximum of ten cycles until evidence of progressive disease [PD] or unacceptable toxicity), with the aim of analysing the possible association between potential biomarkers and outcomes. Our patients were required to have histologically confirmed prostate adenocarcinoma without small cell histology and progressive disease despite "castration levels" of serum testosterone (<50 ng/dL). Additional selection criteria included Eastern Cooperative Oncology Group (ECOG) performance status ≤2, and adequate cardiac, hepatic, renal, bone marrow function and severe comorbidities. We excluded patients receiving additional concurrent anticancer therapies (standard or investigational) during the course of taxane treatment (supplementary data). All patients signed consent to an institutional review board-approved protocol before sample collection.

In this study, we also identified a comparator population composed of mCRPC post-docetaxel patients treated with abiraterone or enzalutamide who were enrolled in our previous biomarker study [13]. For each treatment cohort, we recorded clinicopathologic features, treatment outcomes. Serum prostate-

specific antigen (PSA), serum lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and cell blood count were assessed within 1 week of starting treatment and at before every therapy cycle thereafter. Documentation of progressive disease (PD) was considered radiographic evidence of new lesions by bone scintigraphy, and/or new or enlarging soft tissue lesions by computed tomography (CT) or magnetic resonance imaging (MRI), per the Prostate Cancer Clinical Trials Working Group 3 (PCWG3) guidelines [6]. We used CT and bone scan at the time of screening and every 12 weeks on treatment. As in other clinical practice studies, both the deterioration in clinical condition and/or radiologic progression according to local radiologist evaluation, were considered criteria to establish PD and discontinuation of treatment.

**2.2 Molecular analysis**

Peripheral blood samples were collected within 30 days of each treatment initiation, drawn into 10-ml EDTA tubes or in DNA preservation tubes (StreckTM) (for samples that could not be processed within 2 hours from collection), maintained at room temperature, processed within 30 min and stored at -80°C. Circulating DNA was extracted from 1 to 2 ml of plasma with the QIAamp Circulating Nucleic Acid Kit (Qiagen) and quantified with the Quant-iT high sensitivity PicoGreen double-stranded DNA Assay Kit (Invitrogen) or by spectrophotometric evaluation (NanoDrop® ND-1000, Celbio, Milan, Italy). We assessed plasma *AR* copy number with a multiplex digital droplet PCR (ddPCR) assay [13], using three reference genes: *NSUN3*, *EIF2C1*, and *AP3B1*, and *ZXDB* at Xp11.21 as a control gene not involving the whole arm of chromosome. Each PCR reaction was prepared with 1-2 ng DNA, 10ul 2xSupermix and a total volume of primer probe assays of 2ul in a total volume of 20ul. PCR reactions were partitioned into ~20,000 droplets per sample with an Automated Droplet generator (Bio-Rad). Emulsified PCR reactions were run on a Mastercycler Nexus GSX1 (Eppendorf). Digital PCR analysis was performed with QuantaSoft v1.3.2.0 software to evaluate the number of positive droplets. At least

two negative control wells with no DNA and positive control wells with known *AR* copy number were included in every run.

### 2.3 Statistical analysis

In this study, data were summarized by frequency for categorical variables and by median and range for continuous variables. Association between categorical variables was assessed using the Chi-Square test or the Fisher's exact test, as appropriate. The primary endpoint of the study was OS and the secondary endpoints were radiographic PFS and PSA response. OS was calculated from the start of therapy until death or last follow-up. PFS was calculated from the first day of each therapy to the date of progression disease or death, whichever occurred first, or last tumor evaluation. Radiographic progression was defined using Response Evaluation Criteria in Solid Tumors version 1.1. PSA decline was evaluated according to PCWG3 guidelines [6]. Survival curves were estimated by the Kaplan-Meier method and were compared using the log-rank test. Univariate and multivariate Cox regression models were used to investigate potential predictors of PFS and OS and to estimate hazard ratios (HR) and their 95% confidence intervals (95%CI). Odds ratios (OR) and 95%CI of PSA response were assessed using a logistic regression analysis. All *P*-values were two-sided and a $P<0.05$ was considered as statistically significant. Statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary, NC, USA). We then incorporated updated data on OS and PFS from our prior study of post-docetaxel patients treated with abiraterone or enzalutamide [13] to compare the impact of plasma *AR* copy number in the context of cabazitaxel versus *AR*-directed therapy. Specifically, we tested the interaction in second-line therapy between *AR* copy number (gain or normal) and treatment type (cabazitaxel versus enzalutamide or abiraterone) with respect to OS and PFS.

## 3. Results

### 3.1 Patient characteristics

This was a multi-institution study of associations between baseline plasma *AR* copy number status[13] and outcome in 155 mCRPC patients who started treatment with cabazitaxel between September 2011 and January 2018 (NCT03381326 trial for Italian patient cohort). Of these, 49 (31.6%) and 106 (68.4%) received cabazitaxel as second-line and third-line treatment, respectively. Based on plasma *AR* copy number status 65 (41.9%) cabazitaxel-treated patients were classified as *AR* gain (23 in second-line and 42 in third-line). When comparing the baseline characteristics of the patient groups according to *AR* status, *AR*-gained patients displayed a greater incidence of bone and liver metastases as well as higher levels of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) and PSA (Table 1).

### 3.2 Clinical outcomes in cabazitaxel-treated patients according to plasma *AR* status

For patients receiving cabazitaxel as second-line or third-line treatment median follow-up at time of analysis was 24 months (range 0.5-47), and the median survival 12.2 months (95%CI 10.1-15.2) and 4.4 months (95%CI 3.7-5.4) for OS and radiographic PFS, respectively. Univariate analysis showed that presence of visceral metastasis, liver metastasis and baseline levels of serum PSA, hemoglobin and ALP associated with worse OS. Presence of visceral metastasis, baseline levels of serum PSA and ALP associated with worse PFS (Supplementary Table 1). When comparing *AR*-gained to *AR*-normal patients we observed a trend for a shorter median OS (10.5 versus 14.1 months, HR 1.44, 95%CI 0.98-2.13, *P*=0.064), and a significantly shorter median PFS (4.0 versus 5.0 months, HR 1.47, 95%CI 1.05-2.07, *P*=0.026) (Fig. 1A and 1B). No impact of *AR* status was observed on PSA decline ≥50% (OR 1.00, 95%CI 0.99-1.00, *P*=0.882) (Fig. 1C).

### 3.3 Exploratory analysis for the comparison of the impact of *AR* status in men treated with second-line cabazitaxel or *AR*-directed therapies

In the 49 patients treated with second-line cabazitaxel therapy the median follow-up was 25 months (range 0.8-46) with a median OS and radiographic PFS of 13.0 months (95%CI 8.5-18.7) and 5.3 months (95%CI 3.7-7.1), respectively. In this subpopulation, no difference was observed in either OS or PFS between *AR*-gained and *AR*-normal patients (Fig. 2A and B). In an exploratory analysis, we compared the cabazitaxel-treated patients with 85 previously described patients treated with second-line abiraterone or enzalutamide [13] with an updated median follow-up of 40 months (range 1-67). The baseline characteristics of plasma *AR*-normal and *AR*-gained patients receiving either second-line cabazitaxel or abiraterone or enzalutamide were compared (Table 2). Cabazitaxel-treated patients with plasma *AR*-gained patients had a significantly decreased concentration of hemoglobin while abiraterone or enzalutamide treated patients with *AR* gain had a higher incidence of bone metastasis and higher baseline levels of serum LDH and ALP levels. In a multivariate Cox proportional hazard model, we found statistically significant interaction between type of treatment (abiraterone or enzalutamide versus cabazitaxel) and AR status (AR normal versus AR-gained) for OS (P=0.041) but not PFS (P=0.244) (Table 3). The Kaplan-Meier estimates of outcomes based on treatment and *AR* copy number status in patients treated in the second-line setting showed a significant difference in OS and PFS between treatments stratified by *AR* status (Fig. 2A, B and Supplementary Table 2). Multivariable analysis including treatment type, plasma *AR* copy number and other pre-treatment characteristics showed that plasma *AR* gain was independently associated with worse OS (HR 2.87, 95%CI 1.30-6.32, *P*=0.009) and with a trend for PFS (HR 1.70, 95%CI 0.82-3.56, *P*=0.156) (Table 3).

### 3.4 The prognostic impact of initial cabazitaxel dose on survival

As an additional exploratory endpoint, we studied the impact of plasma *AR* gain on treatment outcome in 71 (45.8%) patients treated with initial reduced dose of cabazitaxel based on the physician's choice and supported by PROSELICA study results [14]. For OS and PFS, no difference was seen between plasma *AR* normal and gain patients treated with full dose cabazitaxel (Fig. 3A and 3B). However, in the initial reduced dose sub-group, *AR*-gained patients had a worse median OS and PFS compared to *AR*-normal patients (7.3 versus 11.5 months, HR 1.95, 95%CI 1.13-3.38, *P*=0.016, and 2.7 versus 5.0 months, HR 2.27, 95%CI 1.39-3.71, *P*=0.001, respectively) (Fig. 3C and 3D). We performed a forest plot of survival hazard ratios from the multivariable analysis of the most common features considered for physician's initial choice about dose reduction (age, ECOG performance status, site of metastasis, pre-treatment hemoglobin, line of therapy) as well as *AR* status. Plasma *AR* gain was identified as independently associated with worse OS and PFS in patients treated with reduced dose of cabazitaxel (HR 1.61, 95%CI 0.80-3.23) for OS and HR 2.32 (95%CI 1.26-4.28) for PFS (Fig. 3E and 3F).

## 4. DISCUSSION

*AR* copy number detection in plasma has been shown to have a potential clinical utility for predicting treatment response and guiding treatment choice between abiraterone or enzalutamide and docetaxel [15]. We here report in our multivariable analysis that plasma *AR* gain associates with significantly shorter OS in patients receiving cabazitaxel as third-line therapy. This is in agreement with our previous result for *AR* gain and docetaxel [15] and further highlights the need to identify treatments and treatment settings where *AR* gain does not associate with a worse survival.

Previous studies have suggested *AR* status as a potential treatment selection biomarker where the detection of the AR splice variant 7 (AR-V7) messenger RNA (mRNA) transcript in circulating tumor cells (CTCs) associated with resistance to AR-targeted therapies but not taxanes in mCRPC patients

254 [16-19]. In addition, we previously observed that plasma *AR*-gained patients were likely to benefit more
255 from docetaxel therapy than AR-directed therapies [15]. The recently presented randomised trial of
256 cabazitaxel with AR-targeting agents [20] suggests a benefit for cabazitaxel in *AR* gain. Our results from
257 the exploratory analysis on second-line therapies in this study supports these observations where our
258 analysis suggests that *AR*-normal patients survived longer on AR-targeted treatments post-docetaxel
259 treatment. The lack of differences seen for *AR* gain between the treatments could be a reflection of two
260 equally non-functional treatments in this setting and for the case of cabazitaxel could be a result of
261 cross resistance with the previous docetaxel treatment in this group. In addition, in our study there was
262 no *a priori* selection of cabazitaxel-treated patients based on poor clinical prognostic factors, as
263 performed in the recent phase 2 trial [20].. Consequently, our biomarker study primarily underlines the
264 importance to distinguish between treatment stage and sequencing when we evaluate the association
265 of *AR* status with outcome. Moreover, *in vivo* and *in vitro* studies [21-23] have demonstrated that the AR
266 pathway can confer cross-resistance with docetaxel but not cabazitaxel in enzalutamide-treated CRPC.
267 Thus, a better understanding of cross-resistance and association with *AR* status in different treatment
268 settings should help guide treatment sequencing efforts.
269
270 Our additional exploratory analysis aimed to explore the impact of *AR* status and initial cabazitaxel
271 dose which might be of use considering the recent non-inferiority PROSELICA trial that randomized
272 between 20 and 25mg/m$^2$ cabazitaxel [14]. We observed that patients treated with a reduced dose had
273 worse survival and, specifically, *AR*-gained patients appeared to have a worse outcome when
274 compared to *AR* normal. These data are hypothesis-generating but could suggest that the response of
275 *AR*-gained clones to cabazitaxel is dose dependent.
276
277 Overall, we recognize some limitations of our study such as the relatively modest sample size of the
278 cohorts and its retrospective non-randomised design. In addition, as most patients included in our study

were not treated under trial setting, radiological assessment was not always carried out at pre-determined interval likely influencing the evidence between *AR* status and PFS in cabazitaxel cohort; even a trend for PFS was reported. Lastly, as we only consider *AR* copy number gain and not other AR aberrations such as mutations or splice variant expression a complete picture of the AR status landscape and its association with outcome in mCRPC is lacking. Nevertheless, our results suggest that *AR* gain associates with worse outcome to cabazitaxel but that this association seems restricted to patients receiving an initial reduced dose, and that *AR*-normal patients might benefit more from AR-directed therapies than cabazitaxel in the second line setting.

## 5. Conclusion

This study provides evidence that plasma *AR* status has a potential clinical utility in patients being considered for cabazitaxel, and suggests that outcomes with chemotherapy or hormonotherapy in mCRPC may be different according to the *AR* status. Prospective trials to validate these findings and further elucidate the clinical utility of liquid biopsies are warranted for CRPC patients starting new systemic treatments.

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**Legend to Figures**

**Figure 1.** Association of plasma *AR* status with outcome in CRPC patients treated with cabazitaxel. Overall (A) and progression-free survival (B) for *AR* copy number normal and gain in CRPC patients treated with cabazitaxel. Waterfall plot (C) showing prostate-specific antigen (PSA) declines by *AR* copy number normal and gain. Bars clipped at maximum 100%.

**Figure 2.** Association of plasma *AR* status with outcome in CRPC patients treated with second-line cabazitaxel. Interaction between *AR* status and treatment type, after including data from abiraterone or enzalutamide-treated patient, for overall survival (A) and progression-free survival (B).

**Figure 3**. The impact of plasma *AR* status on clinical outcomes in patients treated with initial standard and reduced dose of cabazitaxel. Kaplan Maier estimates of overall survival (OS) (A) and progression-free survival (PFS) (B) in *AR*-gained and *AR*-normal patients treated with initial full dose cabazitaxel. Kaplan Maier estimates of OS (C) and PFS (D) in *AR*-gained and *AR*-normal patients treated with initial reduced dose cabazitaxel. Forest plots of hazard ratios derived from Cox model multivariable analysis for OS (E) and PFS (F) and initial cabazitaxel dose according to *AR* status. *Abbreviations. AR, androgen receptor; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HB, haemoglobin; OS, overall survival; PFS, progression-free survival; PS, performance status.*

**Conflict of interest statement**

G. Attard certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: G. Attard reports receiving commercial research grants from Janssen, Arno Therapeutics, and Innocrin Pharma; has received honoraria and/or travel support from the speakers' bureaus of Janssen, Astellas, Sanofi-Aventis, and Roche/Ventana; has received travel support from Pfizer, Abbott Laboratories, Bayer Healthcare, and Essa Pharmaceuticals; has ownership interest (including patents) in The Institute of Cancer Research

Rewards to Inventors; and is a consultant for/advisory board member of Janssen-Cilag, Veridex, Bayer Healthcare, Roche/Ventana, Astellas, Medivation, Pfizer, Novartis, Millennium Pharma, Abbott Laboratories, and Essa Pharma. V. Conteduca, E. Gonzalez-Billalabeitia, and U. De Giorgi received speaker honoraria or travel support from Astellas, Janssen-Cilag, and Sanofi-Aventis. V. Conteduca received consulting fee from Bayer. D. Olmos has received research funding from Janssen and Bayer. E. Castro reports receiving commercial research grants from Astra Zeneca, Bayer, Janssen; has received honoraria and/or travel support from the speakers' bureaus of Astra Zeneca, Astellas, Bayer, Janssen, Pfizer, Bristol-Myers and Roche; and is an advisory board member of Astellas, Bayer and Janssen. N. Romero-Laorden has received honoraria and/or travel support from Bayer, Astellas, Janssen-Cilag, and Sanofi-Aventis. D. Olmos has a compensated advisory role for Astellas, Astra-Zeneca, Bayer, Clovis, Genetech/Roche, Janssen, and uncompensated advisory role for BioOncotech, Tokai; has received a speaker fee from Astellas, Bayer, Janssen, Sanofi, and travel support from Astellas, Bayer, Janssen, Roche; has received research funding (to the institution): Astra-Zeneca, BioOncotech, Bayer, Janssen. M. I. Sáez has an advisory role with Sanofi. J. Puente reports receiving commercial research grants from Pfizer and Astellas; has received honoraria and/or travel support from the speakers' bureaus of Pfizer, Astellas, Janssen, MSD, Roche, Bristol, AstraZeneca, Boehringer, Pierre Fabre, Kyowa, Celgene, Lilly, Merck, Ipsen and Eisai; and is a consultant for Pfizer, Astellas, Janssen, MSD, Bayer, Roche, Bristol, AstraZeneca, Boehringer, Novartis, Clovis, Ipsen, EssaPharma, Eisai and Sanofi. A. Medina has received honoraria and /or travel support from BMS, Janssen-Cilag, Bayer healthcare, Sanofi Aventis, Astellas Medivation, Roche, Novartis and Pfizer. R. Querol-Niñerola has received speaker honoraria or travel support from Astellas, Janssen-Cilag, and Sanofi-Aventis. M. Marín-Aguilera has received travel support from Bristol-Myers Squibb. J. A. Arranz has received honoraria from Novartis, MSD Oncology, Janssen-Cilag, EUSA Pharma; has a compensated consulting or advisory role from Pfizer, Astellas Pharma, Janssen-Cilag, Novartis, Bayer, Ipsen, MSD Oncology, Bristol-Myers Squibb, EUSA Pharma; has received research funding from Novartis, Pierre Fabre,

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Figure 1

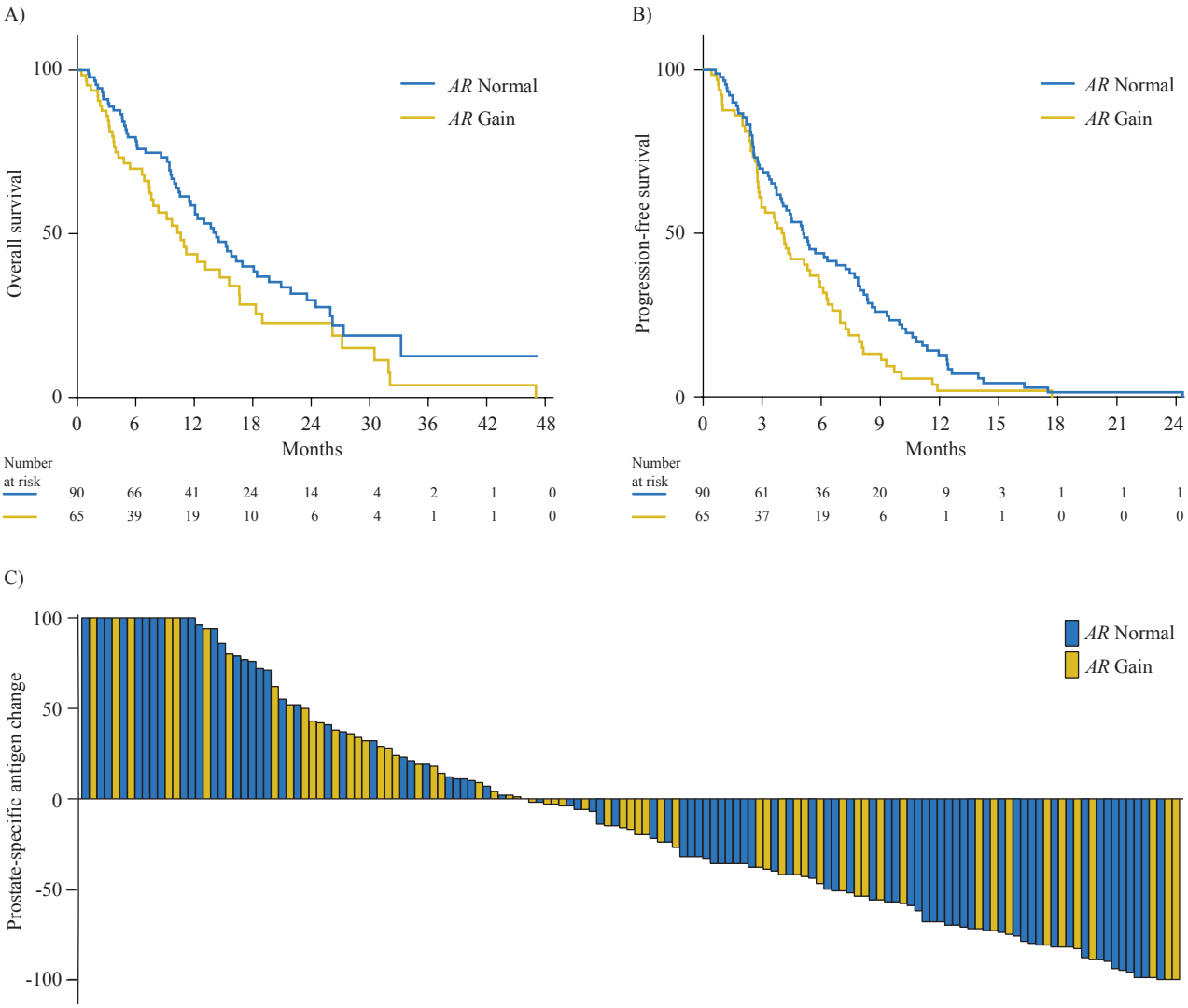

A)
100
50
0
Overall survival
AR Normal
AR Gain
0 6 12 18 24 30 36 42 48
Months
Number at risk
90 66 41 24 14 4 2 1 0
65 39 19 10 6 4 1 1 0
B)
100
50
0
Progression-free survival
AR Normal
AR Gain
0 3 6 9 12 15 18 21 24
Months
Number at risk
90 61 36 20 9 3 1 1 1
65 37 19 6 1 1 0 0 0
C)
100
50
0
-50
-100
Prostate-specific antigen change
AR Normal
AR Gain

Figure 2

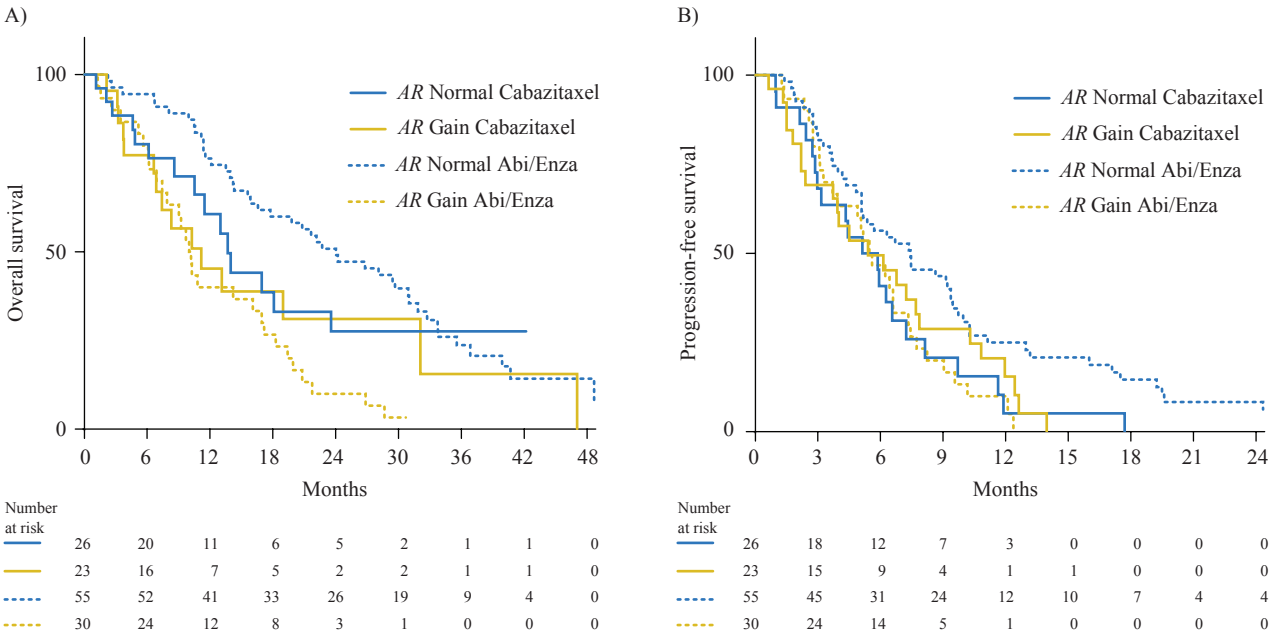
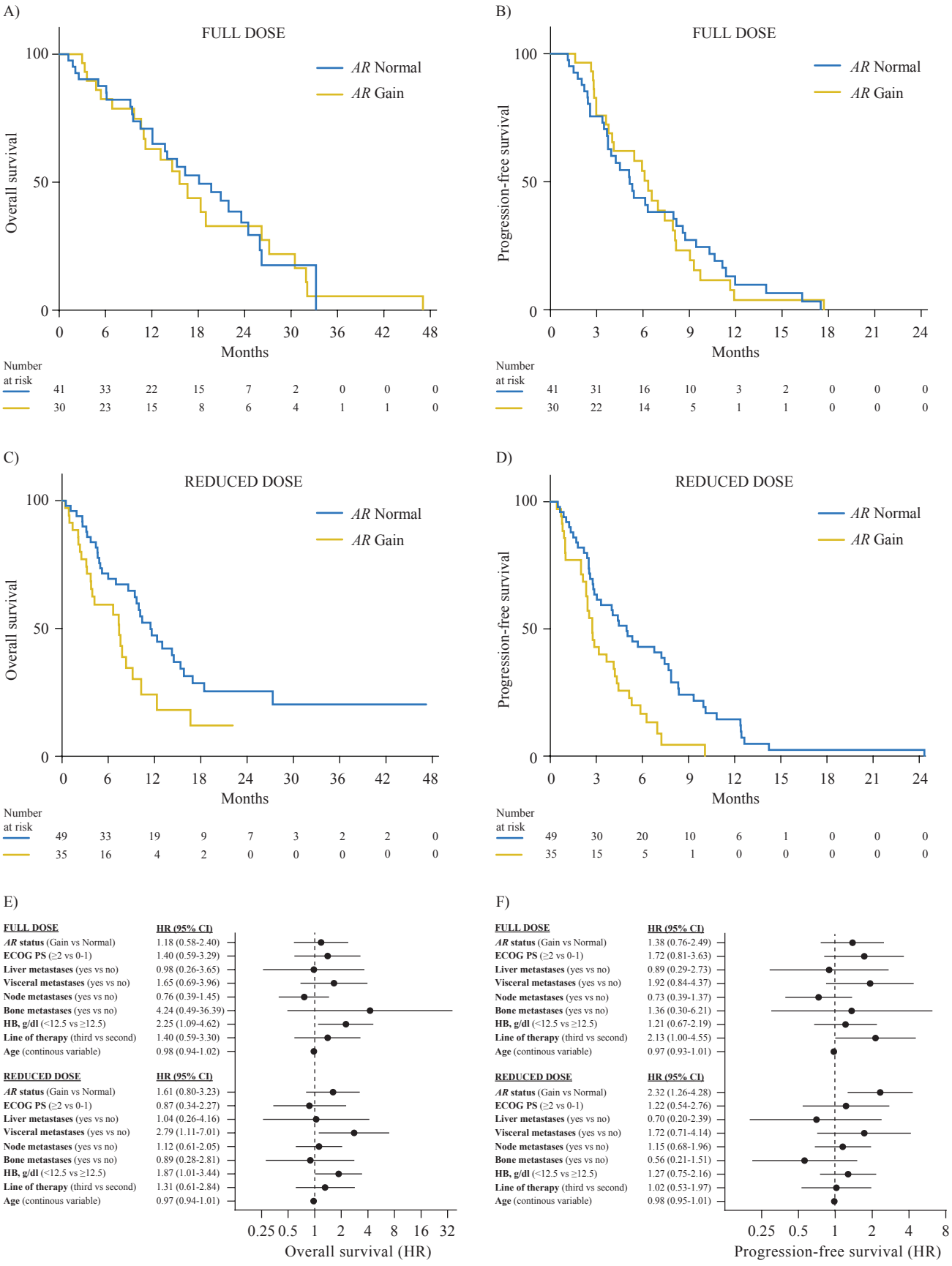

A)
100
50
0
Overall survival
0 6 12 18 24 30 36 42 48
Months
AR Normal Cabazitaxel
AR Gain Cabazitaxel
AR Normal Abi/Enza
AR Gain Abi/Enza
Number at risk
26 20 11 6 5 2 1 1 0
23 16 7 5 2 2 1 1 0
55 52 41 33 26 19 9 4 0
30 24 12 8 3 1 0 0 0
B)
100
50
0
Progression-free survival
0 3 6 9 12 15 18 21 24
Months
AR Normal Cabazitaxel
AR Gain Cabazitaxel
AR Normal Abi/Enza
AR Gain Abi/Enza
Number at risk
26 18 12 7 3 0 0 0 0
23 15 9 4 1 1 0 0 0
55 45 31 24 12 10 7 4 4
30 24 14 5 1 0 0 0 0

Figure 3


A)
FULL DOSE
AR Normal
AR Gain
Overall survival
100
50
0
0 6 12 18 24 30 36 42 48
Months
Number at risk
41 33 22 15 7 2 0 0 0
30 23 15 8 6 4 1 1 0
B)
FULL DOSE
AR Normal
AR Gain
Progression-free survival
100
50
0
0 3 6 9 12 15 18 21 24
Months
Number at risk
41 31 16 10 3 2 0 0 0
30 22 14 5 1 1 0 0 0
C)
REDUCED DOSE
AR Normal
AR Gain
Overall survival
100
50
0
0 6 12 18 24 30 36 42 48
Months
Number at risk
49 33 19 9 7 3 2 2 0
35 16 4 2 0 0 0 0 0
D)
REDUCED DOSE
AR Normal
AR Gain
Progression-free survival
100
50
0
0 3 6 9 12 15 18 21 24
Months
Number at risk
49 30 20 10 6 1 0 0 0
35 15 5 1 0 0 0 0 0
E)
FULL DOSE HR (95% CI)
AR status (Gain vs Normal) 1.18 (0.58-2.40)
ECOG PS (≥2 vs 0-1) 1.40 (0.59-3.29)
Liver metastases (yes vs no) 0.98 (0.26-3.65)
Visceral metastases (yes vs no) 1.65 (0.69-3.96)
Node metastases (yes vs no) 0.76 (0.39-1.45)
Bone metastases (yes vs no) 4.24 (0.49-36.39)
HB, g/dl (<12.5 vs ≥12.5) 2.25 (1.09-4.62)
Line of therapy (third vs second) 1.40 (0.59-3.30)
Age (continous variable) 0.98 (0.94-1.02)
REDUCED DOSE HR (95% CI)
AR status (Gain vs Normal) 1.61 (0.80-3.23)
ECOG PS (≥2 vs 0-1) 0.87 (0.34-2.27)
Liver metastases (yes vs no) 1.04 (0.26-4.16)
Visceral metastases (yes vs no) 2.79 (1.11-7.01)
Node metastases (yes vs no) 1.12 (0.61-2.05)
Bone metastases (yes vs no) 0.89 (0.28-2.81)
HB, g/dl (<12.5 vs ≥12.5) 1.87 (1.01-3.44)
Line of therapy (third vs second) 1.31 (0.61-2.84)
Age (continous variable) 0.97 (0.94-1.01)
0.25 0.5 1 2 4 8 16 32
Overall survival (HR)
F)
FULL DOSE HR (95% CI)
AR status (Gain vs Normal) 1.38 (0.76-2.49)
ECOG PS (≥2 vs 0-1) 1.72 (0.81-3.63)
Liver metastases (yes vs no) 0.89 (0.29-2.73)
Visceral metastases (yes vs no) 1.92 (0.84-4.37)
Node metastases (yes vs no) 0.73 (0.39-1.37)
Bone metastases (yes vs no) 1.36 (0.30-6.21)
HB, g/dl (<12.5 vs ≥12.5) 1.21 (0.67-2.19)
Line of therapy (third vs second) 2.13 (1.00-4.55)
Age (continous variable) 0.97 (0.93-1.01)
REDUCED DOSE HR (95% CI)
AR status (Gain vs Normal) 2.32 (1.26-4.28)
ECOG PS (≥2 vs 0-1) 1.22 (0.54-2.76)
Liver metastases (yes vs no) 0.70 (0.20-2.39)
Visceral metastases (yes vs no) 1.72 (0.71-4.14)
Node metastases (yes vs no) 1.15 (0.68-1.96)
Bone metastases (yes vs no) 0.56 (0.21-1.51)
HB, g/dl (<12.5 vs ≥12.5) 1.27 (0.75-2.16)
Line of therapy (third vs second) 1.02 (0.53-1.97)
Age (continous variable) 0.98 (0.95-1.01)
0.25 0.5 1 2 4 8
Progression-free survival (HR)

## Table 1. Baseline characteristics of overall cabazitaxel-treated patients

| | Total (n = 155) | *AR* normal (n = 90) | *AR* gain (n = 65) | *P* Value |
|---|---|---|---|---|
| **Age**, years Median (range) | 70 (43-87) | 70 (43-84) | 70 (55-87) | 0.952 |
| **ECOG PS,** n (%) | | | | |
| 0-1 | 118 (84.3) | 73 (85.9) | 45 (81.8) | |
| 2 | 22 (15.7) | 12 (14.1) | 10 (18.2) | |
| Unknown/missing | 15 | 5 | 10 | 0.520 |
| **Gleason score,** n (%) | | | | |
| <8 | 33 (23.6) | 19 (22.9) | 14 (24.6) | |
| ≥8 | 107 (76.4) | 64 (77.1) | 43 (75.4) | |
| Unknown/missing | 15 | 7 | 8 | 0.820 |
| **Bone metastases,** n (%) | | | | |
| No | 10 (6.5) | 9 (10.0) | 1 (1.5) | |
| Yes | 145 (93.5) | 81 (90.0) | 64 (98.5) | 0.046 |
| **Visceral metastases,** n (%) | | | | |
| No | 124 (80.5) | 77 (85.6) | 47 (73.4) | |
| Yes | 30 (19.5) | 13 (14.4) | 17 (26.6) | |
| Unknown/missing | 1 | 0 | 1 | 0.062 |
| **Liver metastases,** n (%) | | | | |
| No | 142 (91.6) | 87 (96.7) | 55 (84.6) | |
| Yes | 13 (8.4) | 3 (3.3) | 10 (15.4) | 0.008 |
| **Nodal metastases,** n (%) | | | | |
| No | 73 (47.1) | 45 (50.0) | 28 (43.1) | |
| Yes | 82 (52.9) | 45 (50.0) | 37 (56.9) | 0.396 |
| **Serum PSA**, mg/l | | | | |
| Median (range) | 80 (0.05-5000) | 60 (0.05-5000) | 123 (0.18-2871) | 0.001 |
| **Serum LDH**, n (%) | | | | |
| <225 U/l | 52 (42.6) | 37 (52.1) | 15 (29.4) | |
| ≥225# U/l | 70 (57.4) | 34 (47.9) | 36 (70.6) | |
| Unknown/missing | 33 | 19 | 14 | 0.013 |
| **Hemoglobin**, n (%) | | | | |
| ≥12.5# g/l | 73 (47.1) | 48 (53.3) | 25 (38.5) | |
| <12.5 g/l | 82 (52.9) | 42 (46.7) | 40 (61.5) | 0.068 |
| **ALP**, n (%) | | | | |
| <129 U/l | 49 (41.2) | 37 (53.6) | 12 (24.0) | |
| ≥129# U/l | 70 (58.8) | 32 (46.4) | 38 (76.0) | |
| Unknown/missing | 36 | 21 | 15 | 0.001 |
| **Previous abi or enza**, n (%) | | | | |
| No | 49 (30.7) | 26 (28.9) | 23 (35.4) | |
| Yes | 106 (69.3) | 64 (71.1) | 42 (64.6) | 0.392 |

# Upper normal value

*Abbreviations*. Abi, abiraterone; ALP, alkaline phosphatase; *AR*, androgen receptor; ECOG, Eastern Cooperative Oncology Group; enza, enzalutamide; LDH, lactate dehydrogenase; n, number; PS, performance status; PSA, prostate-specific antigen.

**Table 2. Baseline patient characteristics of cabazitaxel or *AR*-directed therapies according to plasma *AR* status**

| | Cabazitaxel (n = 49) | | | Abi or Enza (n = 85) | | |
|---|---|---|---|---|---|---|
| | ***AR* Normal (n = 26)** | ***AR* Gain (n = 23)** | ***P* Value** | ***AR* Normal (n = 55)** | ***AR* Gain (n = 30)** | ***P* Value** |
| **Age**, years Median (range) | 71 (48-81) | 71 (57-87) | 0.741 | 75 (41-87) | 73 (41-91) | 0.433 |
| **Gleason score,** n (%) | | | | | | |
| <8 | 5 (20.8) | 5 (29.4) | | 15 (30.6) | 9 (36.0) | |
| ≥8 | 19 (79.2) | 12 (70.6) | | 34 (69.4) | 16 (64.0) | |
| Unknown/missing | 2 | 6 | 0.714 | 6 | 5 | 0.642 |
| **Bone metastases,** n (%) | | | | | | |
| No | 1 (3.8) | 1 (4.4) | | 10 (18.2) | 1 (3.3) | |
| Yes | 25 (96.2) | 22 (95.7) | 0.930 | 45 (81.8) | 29 (96.7) | 0.088 |
| **Visceral metastases,** n (%) | | | | | | |
| No | 20 (76.9) | 14 (60.9) | | 47 (87.0) | 23 (82.1) | |
| Yes | 6 (23.1) | 9 (39.1) | | 7 (13.0) | 5 (17.9) | |
| Unknown/missing | 0 | 0 | 0.228 | 1 | 2 | 0.533 |
| **Liver metastases,** n (%) | | | | | | |
| No | 25 (96.1) | 19 (82.6) | | 51 (94.4) | 23 (88.5) | |
| Yes | 1 (3.9) | 4 (17.4) | | 3 (5.6) | 3 (11.5) | |
| Unknown/missing | 0 | 0 | 0.173 | 1 | 4 | 0.384 |
| **Nodal metastases,** n (%) | | | | | | |
| No | 13 (50.0) | 10 (43.5) | | 27 (49.1) | 15 (50.0) | |
| Yes | 13 (50.0) | 13 (56.5) | 0.651 | 28 (50.9) | 15 (50.0) | 0.936 |
| **Serum PSA**, mg/l Median (range) | 75.65 (0.05-5000) | 210 (0.18-2871) | 0.098 | 31 (1.01-3211) | 162 (1.99-3150) | 0.019 |
| **Serum LDH**, n (%) | | | | | | |
| <225 U/l | 8 (38.1) | 5 (23.8) | | 47 (85.5) | 17 (56.7) | |
| ≥225[#] U/l | 13 (61.9) | 16 (76.2) | | 8 (14.5) | 13 (43.3) | |
| Unknown/missing | 5 | 2 | 0.322 | 0 | 0 | 0.003 |
| **Hemoglobin**, n (%) | | | | | | |
| ≥12.5[#] g/dl | 19 (73.1) | 8 (34.8) | | 25 (78.1) | 11 (84.6) | |
| <12.5 g/dl | 7 (26.9) | 15 (65.2) | | 7 (21.9) | 2 (15.4) | |
| Unknown/missing | 0 | 0 | 0.008 | 23 | 17 | 0.626 |
| **ALP**, n (%) | | | | | | |
| <129 U/l | 8 (40.0) | 4 (19.0) | | 37 (67.3) | 11 (36.7) | |
| ≥129[#] U/l | 12 (60.0) | 17 (81.0) | | 18 (32.7) | 19 (63.3) | |
| Unknown/missing | 6 | 2 | 0.145 | 0 | 0 | 0.007 |

# Upper normal value

*Abbreviations*. Abi, abiraterone; ALP, alkaline phosphatase; *AR*, androgen receptor; ECOG, Eastern Cooperative Oncology Group; enza, enzalutamide; LDH, lactate dehydrogenase; n, number; PSA, prostate-specific antigen.

**Table 3 Multivariable analysis of OS and PFS in mCRPC patients treated with second- line therapy**

| | OS | | PFS | |
|---|---|---|---|---|
| | **HR (95% CI)** | ***P* Value** | **HR (95% CI)** | ***P* Value** |
| **Age** (continuous variable) | 0.979 (0.940-1.018) | 0.286 | 0.986 (0.951-1.022) | 0.431 |
| **Visceral metastases** | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 1.73 (0.89-3.39) | 0.108 | 1.23 (0.65-2.33) | 0.515 |
| **Liver metastases** | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 0.67 (0.21-2.14) | 0.504 | 0.70 (0.25-1.94) | 0.494 |
| **Nodal metastases** | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 0.92 (0.52-1.63) | 0.770 | 0.90 (0.55-1.47) | 0.667 |
| **Baseline PSA**, mg/l (continuous variable) | 1.001 (1.000-1.001) | 0.030 | 1.001 (1.000-1.001) | 0.001 |
| **LDH**, U/l | | | | |
| <225 | 1.00 | | 1.00 | |
| ≥225 | 0.68 (0.30-1.54) | 0.359 | 0.81 (0.39-1.65) | 0.557 |
| **Hemoglobin**, g/dl | | | | |
| ≥12.5 | 1.00 | | 1.00 | |
| <12.5 | 2.31 (1.22-4.38) | 0.010 | 1.57 (0.88-2.80) | 0.128 |
| **ALP**, U/l | | | | |
| <129 | 1.00 | | 1.00 | |
| ≥129 | 1.18 (0.61-2.28) | 0.624 | 1.29 (0.72-2.30) | 0.391 |
| ***Plasma AR* status** | | | | |
| Normal | 1.00 | | 1.00 | |
| Gain | 2.87 (1.30-6.32) | 0.009 | 1.70 (0.82-3.56) | 0.156 |
| **Therapy** | | | | |
| Abi/Enza | 1.00 | | 1.00 | |
| Cabazitaxel | 1.91 (0.76-4.77) | 0.167 | 1.59 (0.72-3.51) | 0.253 |
| **Plasma *AR* status therapy interaction** | 0.28 (0.08-0.95) | 0.041 | 0.53 (0.18-1.54) | 0.244 |

*Abbreviations*. Abi, abiraterone; ALP, alkaline phosphatase; *AR*, androgen receptor; CI, confidence interval; enza, enzalutamide; HR, hazard ratio; LDH, lactate dehydrogenase; n, number; OS, overall survival; PFS, progression-free survival; PSA, prostate-specific antigen.

**Data Supplement**

**Eligibility Criteria**

**Inclusion Criteria**

1. Patients must have histologically-confirmed adenocarcinoma of prostate without neuroendocrine differentiation or small cell histology.
2. Patients have progressive disease despite “castration levels” of serum testosterone (<50 ng/dL) (≤1.73 nmol/L), and ongoing LHRH analogue treatment or prior surgical castration.
3. Progression as defined by at least two of the following: a rise in PSA, worsening symptoms, or radiological progression, namely, progression in soft tissue lesions measured by computed tomography imaging according to the modified Response Evaluation Criteria in Solid Tumors (RECIST) or progression on bone scanning according to criteria adapted from the Prostate Cancer Working Group (PCWG3) criteria.
4. Patients have not received radiotherapy, chemotherapy, or immunotherapy at least 30 days prior to the treatment.
5. Male, aged ≥18 years.
6. Life expectancy of greater than three months.
7. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤2.
8. Willing to use a method of birth control with adequate barrier protection.
9. Patients must have normal organ and marrow function as defined below:
    a. leukocytes >3,000/mL
    b. absolute neutrophil count >1,500/mL
    c. platelets >100,000/mL
    d. total bilirubin within normal institutional limits
    e. AST(SGOT)/ALT(SGPT) <2.5 X institutional upper limit of normal
    f. creatinine within normal institutional limits
10. No evidence (within five years) of prior malignancies (except successfully treated basal cell or squamous cell carcinoma of the skin).
11. Participant is willing and able to give informed consent for participation in the study.

**Exclusion Criteria**

1. Concurrent use of other anticancer agents or treatments, with the following exceptions:
    a. LHRH agonists or antagonists
    b. denosumab or bisphosphonate (e.g., zoledronic acid).
2. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
3. Have known allergies, hypersensitivity or intolerance to cabazitaxel, prednisone, or their excipients.

8. Other primary tumor (other than CRPC) including hematological malignancy present within the last five years (except non-melanoma skin cancer or low-grade superficial bladder cancer).

**Supplementary Table 1. Univariate analysis of overall survival and progression-free survival in cabazitaxel-treated patients**

| | OS | | PFS | |
|---|---|---|---|---|
| | **HR (95% CI)** | ***P* Value** | **HR (95% CI)** | ***P* Value** |
| **Age**, years (continuous variable) | 0.989 (0.967-1.012) | 0.341 | 0.991 (0.971-1.012) | 0.406 |
| **ECOG PS,** n (%) | | | | |
| 0-1 | 1.00 | | 1.00 | |
| 2 | 1.35 (0.80-2.29) | 0.259 | 1.51 (0.95-2.40) | 0.078 |
| **Gleason score,** n (%) | | | | |
| <8 | 1.00 | | 1.00 | |
| ≥8 | 1.08 (0.68-1.72) | 0.744 | 0.81 (0.54-1.20) | 0.292 |
| **Bone metastases,** n (%) | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 1.23 (0.57-2.67) | 0.592 | 0.90 (0.47-1.73) | 0.761 |
| **Visceral metastases,** n (%) | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 1.92 (1.22-3.00) | 0.005 | 1.58 (1.05-2.37) | 0.027 |
| **Liver metastases,** n (%) | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 1.95 (1.01-3.75) | 0.047 | 1.56 (0.88-2.77) | 0.130 |
| **Nodal metastases,** n (%) | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 1.12 (0.76-1.65) | 0.574 | 1.09 (0.78-1.53) | 0.609 |
| **Serum PSA**, mg/l (continuous variable) | 1.001 (1.001-1.001) | <0.0001 | 1.001 (1.001-1.001) | <0.0001 |
| **Serum LDH**, n (%) | | | | |
| <225 U/l | 1.00 | | 1.00 | |
| ≥225[#] U/l | 1.53 (0.99-2.35) | 0.055 | 1.11 (0.76-1.62) | 0.593 |
| **Hemoglobin**, n (%) | | | | |
| ≥12.5[#] g/l | 1.00 | | 1.00 | |
| <12.5 g/l | 2.09 (1.41-3.10) | 0.0003 | 1.33 (0.96-1.86) | 0.089 |
| **ALP**, n (%) | | | | |
| <129 U/l | 1.00 | | 1.00 | |
| ≥129[#] U/l | 1.75 (1.14-2.70) | 0.011 | 1.48 (1.01-2.17) | 0.043 |
| **Plasma *AR* status** | | | | |
| Normal | 1.00 | | 1.00 | |
| Gain | 1.44 (0.98-2.13) | 0.064 | 1.47 (1.05-2.07) | 0.026 |

# Upper normal value

*Abbreviations*. ALP, alkaline phosphatase; *AR*, androgen receptor; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; LDH, lactate dehydrogenase; n, number; OS, overall survival; PFS, progression-free survival; PS, performance status; PSA, prostate-specific antigen.

**Supplementary Table 2. Univariate analysis of OS and PFS according to plasma *AR* status and type of second-line treatment**

| | OS | | | | | |
|---|---|---|---|---|---|---|
| | **N pts** | **N events** | **Median value (95% CI)** | ***P* Value** | **HR (95% CI)** | ***P* value** |
| *AR* Normal Abi/Enza | 55 | 44 | 23.7 (15.7-30.6) | | 1.00 | |
| *AR* Gain Abi/Enza | 30 | 29 | 10.0 (7.3-16.7) | | 2.93 (1.78-4.81) | |
| *AR* Normal Caba | 26 | 15 | 13.5 (8.5-23.3) | | 1.49 (0.82-2.71) | |
| *AR* Gain Caba | 23 | 15 | 11.0 (6.5-31.7) | 0.0002 | 1.97 (1.08-3.58) | 0.0003 |
| | **PFS** | | | | | |
| | **No. pts** | **No. events** | **Median value (95% CI)** | ***P* Value** | **HR (95% CI)** | ***P* Value** |
| *AR* Normal Abi/Enza | 55 | 51 | 7.3 (5.0-9.2) | | 1.00 | |
| *AR* Gain Abi/Enza | 30 | 29 | 5.5 (3.7-7.2) | | 1.88 (1.16-3.04) | |
| *AR* Normal Caba | 26 | 24 | 5.3 (2.4-7.8) | | 1.61 (0.97-2.65) | |
| *AR* Gain Caba | 23 | 21 | 5.4 (2.8-7.1) | 0.025 | 1.85 (1.09-3.12) | 0.028 |

*Abbreviations*. Abi, abiraterone; *AR*, androgen receptor; caba, cabazitaxel; CI, confidence interval; enza, enzalutamide; HR, hazard ratio; N, number; OS, overall survival; PFS, progression-free survival.