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Original Research

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

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KEYWORDS
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Biomarker

Abstract ***Background:*** Plasma androgen receptor (AR) copy number status has been identified as a potential biomarker of response in patients with metastatic castration-resistant prostate cancer (mCRPC) 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, pretherapy 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 polymerase chain reaction 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 14.1 months, hazard ratio (HR) = 1.44, 95% confidence interval [CI] 0.98–2.13, $P = 0.064$ and PFS 4.0 versus 5.0 months, HR = 1.47, 95% CI 1.05–2.07, $P = 0.024$). In patients with mCRPC 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 11.5 months, HR = 1.95, 95% CI 1.13–3.38, $P = 0.016$) and PFS (2.7 versus 5.0 months, 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 is warranted.

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]. Owing 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 characterisation and stratification of patients for better treatment selection [7].

Plasma DNA studies of patients with mCRPC treated with AR-directed therapies in the first- and second-line setting have revealed the association between genomic changes of the AR 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 cabazitaxel therapy remains unknown.

Our primary objective was to evaluate associations of plasma *AR* status with progression-free survival/overall survival (PFS/OS) in patients with mCRPC 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 schedules of cabazitaxel to determine a potential role of the circulating *AR* copy number in the overall management of patients with CRPC receiving cabazitaxel.

2. Material and methods

2.1. Study design

In this prospective multicentre non-randomised study, blood samples were collected from patients with mCRPC before starting therapy with cabazitaxel at standard doses in a routine clinical practice (25 mg/m^2 every three weeks together with prednisone 5 mg twice daily for a maximum of ten cycles until evidence of progressive disease [PD] or unacceptable toxicity), with

Table 1
Baseline characteristics of overall cabazitaxel-treated patients.

Characteristics	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 (0.1 −5000)	60 (0.1–5000)	123 (0.18 −2871)	0.001
Serum LDH, n (%)				
<225 U/l	52 (42.6)	37 (52.1)	15 (29.4)	
≥225[a] U/l	70 (57.4)	34 (47.9)	36 (70.6)	
Unknown/missing	33	19	14	0.013
Haemoglobin, n (%)				
≥12.5[a] 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[a] 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

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.
[a] Upper normal value.

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 PD, 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 postdocetaxel 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 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, 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 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 ethylene diamine tetra-acetic acid tubes or in DNA preservation tubes (StreckTM) (for samples that could not be processed within 2 h 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 the plasma *AR* copy number with a multiplex digital droplet polymerase chain reaction (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 the chromosome. Each PCR reaction was prepared with 1–2 ng DNA, 10 μl 2xSupermix and a total volume of primer probe assays of 2 μl 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 a known *AR* copy number were included in every run.

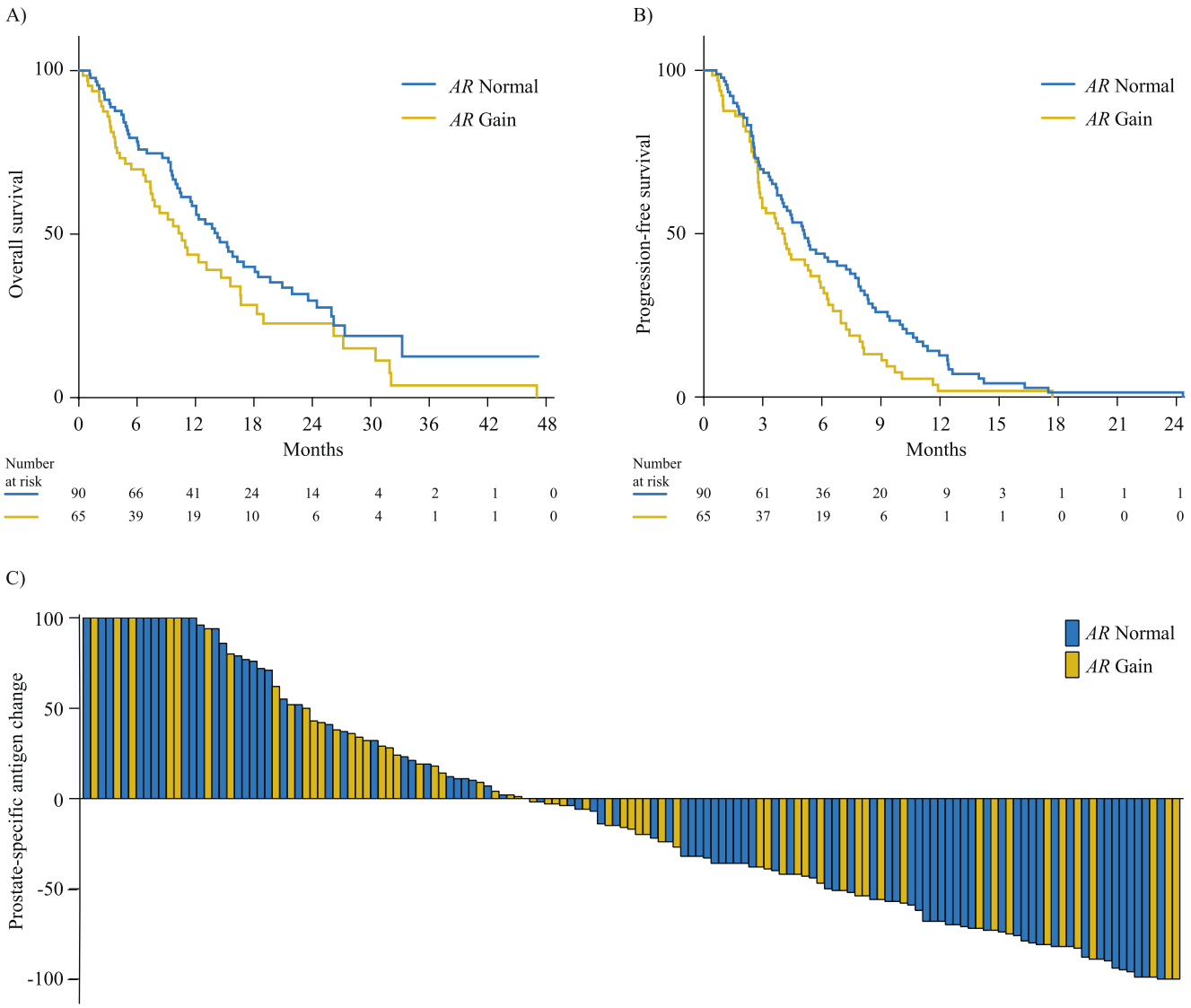

Fig. 1. Association of plasma *AR* status with outcome in patients with CRPC treated with cabazitaxel. Overall (A) and progression-free survival (B) for *AR* copy number normal and gain in patients with CRPC treated with cabazitaxel. Waterfall plot (C) showing prostate-specific antigen declines by *AR*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

2.3. Statistical analysis

In this study, data were summarised 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 end-point of the study was OS, and the secondary end-points were radiographic PFS and PSA response. OS was calculated from the start of therapy until death or the 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 tumour evaluation. Radiographic progression was defined using Response Evaluation Criteria in Solid Tumours, 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 (HRs) and their 95% confidence intervals (95% CIs). Odds ratios (ORs) 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 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 postdocetaxel patients treated with abiraterone or enzalutamide [13] to compare the impact of the plasma *AR* copy number on the context of cabazitaxel versus AR-directed therapy. Specifically, we

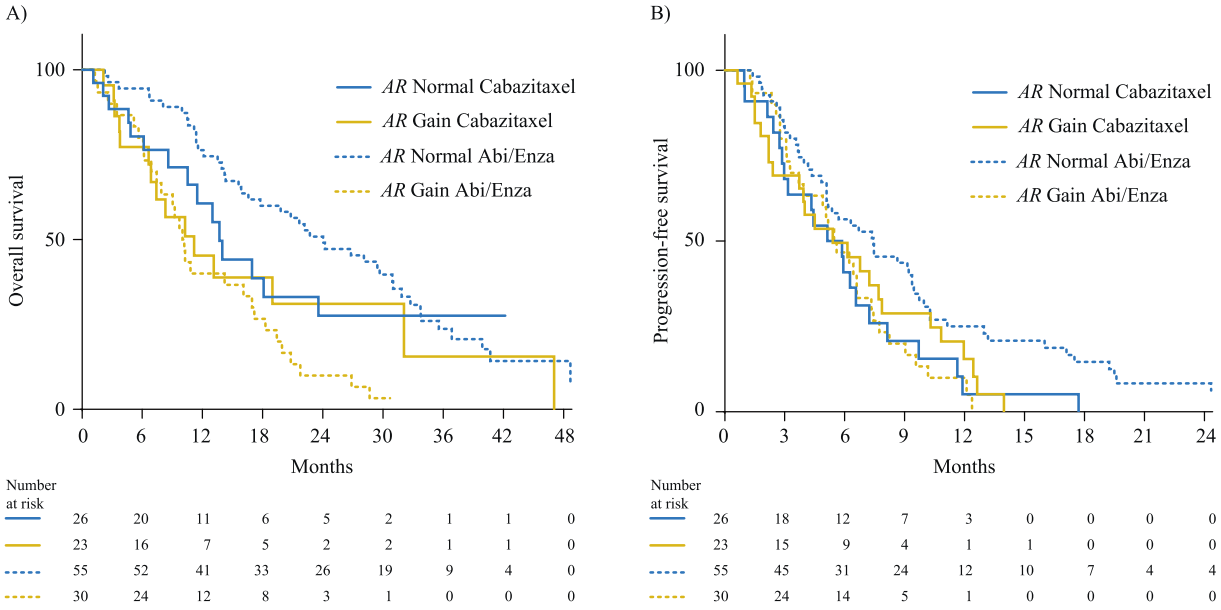

Fig. 2. Association of plasma *AR* status with outcome in patients with CRPC treated with second-line cabazitaxel. Interaction between *AR* status and treatment type, after including data from abiraterone- or enzalutamide-treated patient, for overall survival (A) and progression-free survival (B). CRPC,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

tested the interaction in second-line therapy between the *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 patients with mCRPC who started treatment with cabazitaxel between September 2011 and January 2018 (NCT03381326 trial for the 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 LDH, 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 was 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, haemoglobin and ALP associated with worse OS. Presence of visceral metastasis and 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 B). 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 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

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	75.65	210		31	162	
Median (range)	(0.1–5000)	(0.18–2871)	0.098	(1.01–3211)	(1.99–3150)	0.019
Serum LDH, n (%)						
<225 U/l	8 (38.1)	5 (23.8)		47 (85.5)	17 (56.7)	
≥225[a] U/l	13 (61.9)	16 (76.2)		8 (14.5)	13 (43.3)	
Unknown/missing	5	2	0.322	0	0	0.003
Haemoglobin, n (%)						
≥12.5[a] 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[a] U/l	12 (60.0)	17 (81.0)		18 (32.7)	19 (63.3)	
Unknown/missing	6	2	0.145	0	0	0.007

Abi, abiraterone; ALP, alkaline phosphatase; AR, androgen receptor; ECOG, Eastern Cooperative Oncology Group; enza, enzalutamide; LDH, lactate dehydrogenase; n, number; PSA, prostate-specific antigen.
[a] Upper normal value.

were compared (Table 2). Cabazitaxel-treated patients with plasma *AR* gain had a significantly decreased concentration of haemoglobin, 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 the 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 and B and Supplementary Table 2). Multivariable analysis including treatment type, plasma *AR* copy number and other pretreatment 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 end-point, 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 *AR*-gained patients treated with full dose cabazitaxel (Fig. 3A and B). However, in the initial reduced dose subgroup, *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

Table 3
Multivariable analysis of OS and PFS in patients with mCRPC 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
Haemoglobin, 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

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.

1.39–3.71, $P = 0.001$, respectively) (Fig. 3C and D). We performed a forest plot of survival HRs 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, pretreatment haemoglobin and 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 F).

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 in which the detection of the AR splice variant 7 (AR-V7) messenger RNA transcript in circulating tumour cells (CTCs) was associated with resistance to AR-targeted therapies but not taxanes in patients with mCRPC [16–19]. In addition, we previously observed that plasma *AR*-gained patients were likely to benefit more from docetaxel therapy than AR-directed therapies [15]. The recently presented randomised trial of cabazitaxel with AR-targeting agents [20] suggests a benefit for cabazitaxel in *AR* gain. Our results from the exploratory analysis on second-line therapies in this study support these observations, where our analysis suggests that *AR*-normal patients survived longer on AR-targeted treatments after docetaxel treatment. The lack of differences seen for *AR* gain between the treatments could be a reflection of two equally non-functional treatments in this setting and for the case of cabazitaxel could be a result of cross-resistance with the previous docetaxel treatment in this group. In addition, in our study, there was no *a priori* selection of cabazitaxel-treated patients based on poor clinical prognostic factors, as performed in the recent phase 2 trial [20]. Consequently, our biomarker study primarily underlines the importance to distinguish between treatment stage and sequencing when we evaluate the association of *AR*

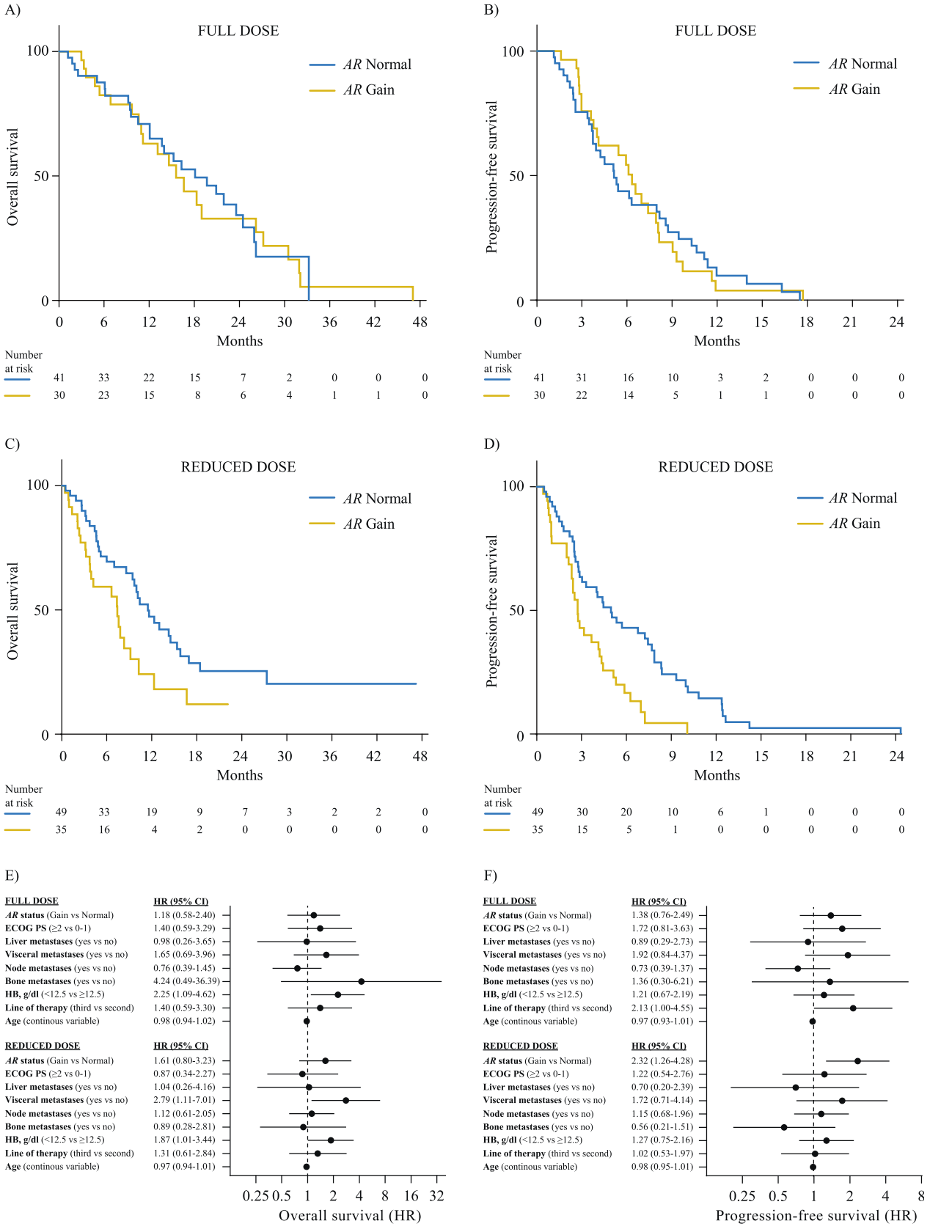

Fig. 3. The impact of plasma *AR* status on clinical outcomes in patients treated with initial standard and reduced dose of cabazitaxel. Kaplan–Meier estimates of overall survival (A) and progression-free survival (B) in *AR*-gained and *AR*-normal patients treated with initial full dose cabazitaxel. Kaplan–Meier 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. AR, androgen receptor; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HB, haemoglobin; PS, performance status; HR, hazard ratio.

status with outcome. Moreover, *in vivo* and *in vitro* studies [21–23] have demonstrated that the AR pathway can confer cross-resistance with docetaxel but not cabazitaxel in enzalutamide-treated CRPC patients. Thus, a better understanding of cross-resistance and association with *AR* status in different treatment settings should help guide treatment sequencing efforts.

Our additional exploratory analysis aimed to explore the impact of *AR* status and initial cabazitaxel dose which might be of use considering the recent non-inferiority PROSELICA trial that randomised between 20 and 25 mg/m^2 cabazitaxel [14]. We observed that patients treated with a reduced dose had worse survival and, specifically, *AR*-gained patients appeared to have a worse outcome when compared to *AR* normal. These data are hypothesis generating but could suggest that the response of *AR*-gained clones to cabazitaxel is dose dependent.

Overall, we recognise some limitations, such as the relatively modest sample size of the cohorts and its retrospective non-randomised design, of our study. In addition, as most patients included in our study were not treated under trial setting, radiological assessment was not always carried out at predetermined interval, likely influencing the evidence between *AR* status and PFS in the 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 this association seems restricted to patients receiving an initial reduced dose, and suggests 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 patients with CRPC starting new systemic treatments.

Conflict of interest statement

G.A. 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.A. 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.C., E.G.-B. and U.De G. received speaker honoraria or travel support from Astellas, Janssen-Cilag and Sanofi-Aventis. V.C. received consulting fee from Bayer. D.O. has received research funding from Janssen and Bayer. E.C. reports receiving commercial research grants from AstraZeneca, Bayer and Janssen; has received honoraria and/or travel support from the speakers' bureaus of AstraZeneca, Astellas, Bayer, Janssen, Pfizer, Bristol-Myers and Roche and is an advisory board member of Astellas, Bayer and Janssen. N.R.-L. has received honoraria and/or travel support from Bayer, Astellas, Janssen-Cilag and Sanofi-Aventis. D.O. has a compensated advisory role for Astellas, AstraZeneca, Bayer, Clovis, Genentech/Roche and Janssen and uncompensated advisory role for Bioncotech and Tokai; has received a speaker fee from Astellas, Bayer, Janssen and Sanofi and travel support from Astellas, Bayer, Janssen and Roche and has received research funding (to the institution): AstraZeneca, Bioncotech, Bayer and Janssen. M.I.S. has an advisory role with Sanofi. J.P. 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, Essa Pharma, Eisai and Sanofi. A.M. has received honoraria and/or travel support from BMS, Janssen-Cilag, Bayer healthcare, Sanofi Aventis, Astellas Medivation, Roche, Novartis and Pfizer. R.Querol.-N. has received speaker honoraria or travel support from Astellas, Janssen-Cilag and Sanofi-Aventis. M.M.-A. has received travel support from Bristol-Myers Squibb. J.A.A. has received honoraria from Novartis, MSD Oncology, Janssen-Cilag and EUSA Pharma; has a compensated consulting or advisory role from Pfizer, Astellas Pharma, Janssen-Cilag, Novartis, Bayer, Ipsen, MSD Oncology, Bristol-Myers Squibb and EUSA Pharma; has received research funding from Novartis, Pierre Fabre and Bristol-Myers Squibb and has received travel support from Bristol-Myers Squibb, Janssen-Cilag and MSD Oncology. G.F. has received speaker's fees from BMS, Janssen, Bayer, Ipsen and MSD. U.B.

has received speaker's fees from BMS, Novartis, Pfizer, Pierre Fabre, Janssen, Astellas and Sanofi Aventis and has a compensated advisory role for Novartis, Pfizer, MSD and Sanofi Aventis. B.M. reports receiving commercial research grants from Roche and Bayer; has received travel support from Pfizer and Janssen and is a consultant for/advisory board member of Roche, Sanofi, Janssen, Astellas Oncology, Pfizer, Novartis, Bristol-Myers Squibb and Ipsen. No potential conflicts of interest were disclosed by the other authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.05.007.

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