



ORIGINAL ARTICLE

Testosterone levels and androgen receptor copy number variations in castration-resistant prostate cancer treated with abiraterone or enzalutamide

Cristian Lolli¹  | Delia De Lisi² | Vincenza Conteduca¹  | Giorgia Gurioli³ |
Emanuela Scarpi⁴ | Giuseppe Schepisi¹ | Giorgia Ravaglia⁴ | Cecilia Menna¹ |
Alberto Farolfi¹ | Amelia Altavilla¹ | Salvatore Luca Burgio¹ | Giuseppe Tonini² |
Daniele Santini² | Ugo De Giorgi¹

¹Department of Medical Oncology, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy

²Department of Medical Oncology, Campus Bio-Medico University of Rome, Rome, Italy

³Biosciences Laboratory Division, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy

⁴Biostatistics and Clinical Trials Division, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy

Correspondence

Cristian Lolli, Biosciences Laboratory, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), IRCCS, via Maroncelli 40-42, 47014 Meldola, Italy.
Email: cristian.lolli@irst.emr.it

Abstract

Purpose: Our study aims to investigate the association between copy number of the androgen receptor (AR) and testosterone levels in metastatic castration-resistant prostate cancer (mCRPC) treated with second-generation antiandrogen therapies.

Materials and Methods: We retrospectively collected data from mCRPC treated with abiraterone acetate and enzalutamide. Serum testosterone levels were collected at baseline, at 3 months since the start of therapy and at disease progression. A cohort of cases treated with docetaxel was also used to evaluate the impact of testosterone levels.

Results: Patients treated with abiraterone with AR copy number aberrations and basal testosterone levels below 0.09 nmol/L had worse progression-free survival (PFS) compared to patients with no AR copy number abnormalities (8.5 vs 2.9 months, $P = 0.005$). No relevant differences were observed in the enzalutamide group with a PFS of 3.9 months (no AR gain) vs 2.7 months (AR gain, $P = 0.004$) for patients with below 0.09 nmol/L testosterone levels. Similar results are obtained for univariate analysis for overall survival (OS). The negative prognostic role of AR copy number gain in OS for both treatment groups (25.5 vs 10.6 months, $P = 0.0002$ for abiraterone and 14.1 vs 8.3 months, $P = 0.031$ for enzalutamide) was confirmed, and it was recognized the negative prognostic impact of testosterone below 0.09 only for patients treated with enzalutamide (8.8 vs 42.8 months, $P = 0.016$). On multivariate analysis for patients treated with abiraterone, low testosterone levels below 0.09 and plasma AR gain were significantly associated with worse PFS and OS. These data are confirmed in the enzalutamide group for PFS.

Conclusions: Testosterone levels and the AR copy number alterations were considered as independent prognostic factors. The results of this study show that serum testosterone associated with changes in copy number of AR gene could represent a noninvasive

biomarker useful to identify a subgroup of patients with worse prognosis that can benefit less from second-generation antiandrogen therapies in the mCRPC setting.

KEYWORDS

abiraterone, androgen receptor, enzalutamide, prostate cancer, testosterone

1 | INTRODUCTION

Prostate cancer is the most common cancer in men and the second cause of death for cancer.¹ When disease becomes metastatic, after a phase of androgen dependence that responds to androgen deprivation therapy (ADT), the tumor moves to a castration-resistant phase where prostate cells employ multiple mechanisms to survive in a low androgen environment. This stage of disease was usually improperly called “androgen-independent” or “hormone refractory”, but evidence suggests that both residual androgens and androgen receptor (AR) play a central role to disease progression.² The aberrant AR activity despite castrated levels of testosterone includes AR gene amplification, AR protein overexpression, AR splice variants, overexpression of AR coactivators and mutation on AR gene ligand-binding domain.^{3–6} All mechanisms above-mentioned provide AR hypersensitivity to low levels of androgen or to nonandrogenic steroidal ligands, such as glucocorticoids or independent ligand AR transcriptional activity. Apart from AR aberration, androgens present a key role in castration resistance mechanisms due to an intratumoral androgen biosynthesis, generating a low, but sufficient level of androgen to support AR transcriptional activity.⁷ Considering the role of both AR variants and testosterone levels in the CRPC pathogenesis, many studies suggest their potential role as prognostic and predictive biomarkers in mCRPC patients treated with new hormonal therapies, targeting AR or testosterone expression such as abiraterone and enzalutamide. Considering testosterone, Hashimoto et al,^{8,9} on a retrospective analysis of 115 patients treated with abiraterone and enzalutamide, showed that testosterone above 5 ng/dL is an independent predictive factor for the prostate-specific antigen's progression-free survival (PFS), suggesting a possible role as a biomarker in deciding treatment selection. Recently, several studies showed how AR copy number variation (CNV) on serum DNA is associated with outcome in patients treated with abiraterone¹⁰ while AR-V7 splice variants in circulating tumor cell, lacking the ligand-binding domain, which is the target of both enzalutamide and abiraterone, are associated with resistance to both drugs.¹¹ As no data are available about the correlation between plasma AR status and testosterone levels in mCRPC treated with novel hormonal therapies such as abiraterone and enzalutamide, our study aims to investigate their association in these settings to find possible prognostic biomarkers useful in clinical practice.

2 | MATERIALS AND METHODS

We retrospectively collected data from mCRPC patients consecutively treated with abiraterone acetate and enzalutamide after docetaxel

progression. Inclusion criteria were as follows: baseline serum testosterone at castration level 1.74 nmol/L or below; Eastern Cooperative Oncology Group (ECOG) performance status 2 or lower; adequate cardiac, hepatic, renal, and bone marrow function and ongoing ADT. Blood sample for CNV of AR detection was collected before starting treatment with abiraterone acetate or enzalutamide. The serum testosterone levels were collected at baseline, at 3 months since the start of therapy and at disease progression. A consecutive series of patients treated with docetaxel was used as another cohort of patients to evaluate the role of basal testosterone levels in patients treated with chemotherapy. The study protocol was approved by the Ethical Committee of Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST). Written informed consent was obtained from all patients.

2.1 | Copy number gain of AR and testosterone analysis in a blood sample from mCRPC

Peripheral blood samples were collected within 7 days before each treatment initiation, drawn into 10-mL tubes without anticoagulant, maintained at room temperature, processed within 30 minutes, and stored at -80°C .

Circulating DNA was extracted from 1 to 2 mL of plasma with the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) and quantified with the Quant-iT high sensitivity PicoGreen double-stranded DNA Assay Kit (Invitrogen, Carlsbad, CA) or by spectrophotometric evaluation (NanoDrop ND-1000; Celbio, Milan, Italy).

In addition, we performed AR copy number with QuantStudio3D digital PCR system (Life Technologies, Carlsbad, CA) using EIF2C1 and RNase^{10,12} or a digital droplet polymerase chain reaction (ddPCR) assay^{13–15} was optimized for the evaluation of AR copy number, 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 to 2 ng DNA and partitioned into ~20 000 droplets per sample. Digital PCR analysis was performed with QuantaSoft v1.3.2.0 software (Bio-Rad, Hercules, CA) 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.

Testosterone levels were detected through the electrochemiluminescent assay (Roche Elecsys 170, Roche Diagnostics, Risch, Switzerland).

This assay is based on a competitive test principle using a high-affinity monoclonal antibody (sheep) specifically directed against testosterone. Endogenous testosterone released from the sample by 2-bromo estradiol competes with the added testosterone derivative

labeled with a ruthenium complex; finally, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by two-point calibration and a master curve provided via the reagent barcode.

2.2 | Statistical analysis

The aim of this retrospective analysis was to evaluate a possible prognostic and predictive role of basal testosterone levels and AR gene copy number alterations in patients treated with abiraterone or enzalutamide after progression to docetaxel.

Association between testosterone levels and AR Copy Number alterations was performed using the χ^2 or Fisher test, as appropriate.

PFS was defined as the time interval between the start of abiraterone or enzalutamide and the time of radiological/clinical or biochemical progression or last tumor evaluation. Overall survival (OS) was defined as the time between the first day of abiraterone or enzalutamide and the date of death from any cause or the date of the last follow-up. Survival curves were estimated using the Kaplan-Meier method and were compared by log-rank test. A Cox regression model was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for PFS and OS. The multivariable Cox models included all factors that were significantly associated in the univariate models. 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).

3 | RESULTS

3.1 | Patient characteristics

Our analysis included 128 mCRPC patients, 54 patients treated with abiraterone and 74 treated with enzalutamide after docetaxel progression. In the whole group, the median age was 74 years (42-90). Ninety-three percent of patients had a good performance status (0-1) according to ECOG evaluation. Regarding AR status, 64.8% of the patient-presented normal AR copy number.

In the group of patient treated with abiraterone, 33 patients (61.1%) had normal AR gene and 21 (38.9%) patients presented AR copy number gain, while in the enzalutamide group 50 (67.6%) of patients showed normal AR gene while in 24 patients (32.4%) AR copy number variation was detected. More details for patients' characteristics both for docetaxel group and patients treated with enzalutamide and abiraterone acetate are showed in Tables 1,2.

3.2 | Association between testosterone levels and AR copy number alterations

There was a statistically significant association between baseline testosterone levels and copy number alterations of AR only in the group treated with enzalutamide and not in abiraterone group (the χ^2 test, *P* = 0.028 and *P* = 0.458, respectively). In the enzalutamide group, the majority of patients (23; 95.8% of the total) with AR gain exhibited testosterone levels below the calculated median value.

TABLE 1 Patient characteristics

	ABI postchemo (n = 54), N (%)	ENZA postchemo (n = 74), N (%)	Total (n = 128), N (%)
Age, median (range), y	73 (56-90)	74 (42-90)	74 (42-90)
PSA at baseline, median (range)	49.81 (0.35-1249.0)	43.53 (0.07-4351.0)	45.59 (0.07-4351.0)
Testosterone level at baseline, median (range), nmol/L	<0.09 (<0.09-10.48)	<0.09 (<0.09-0.78)	<0.09 (<0.09-10.48)
ECOG performance status			
0-1	50 (92.6)	69 (93.2)	119 (93.0)
2	4 (7.4)	5 (6.8)	9 (7.0)
Gleason score			
6-7	15 (31.2)	26 (41.3)	41 (36.9)
≥8	33 (68.8)	37 (58.7)	70 (63.1)
Unknown	6	11	17
AR CN			
Normal	33 (61.1)	50 (67.6)	83 (64.8)
Gain	21 (38.9)	24 (32.4)	45 (35.2)
Visceral metastasis			
No	46 (85.2)	64 (86.5)	110 (85.9)
Yes	8 (14.8)	10 (13.5)	18 (14.1)

Abbreviations: ABI, abiraterone; AR, androgen receptor; CN, copy number; ECOG, Eastern Cooperative Oncology Group; ENZA enzalutamide; PSA, prostate-specific antigen.

TABLE 2 Patient characteristics for docetaxel group

	Docetaxel (n = 44), N (%)
Age, median (range), y	72 (43-82)
PSA at baseline, median (range)	44.04 (2.21-523.0)
Testosterone level at baseline, median (range), nmol/L	<0.09 (<0.09-6.75)
ECOG performance status	
0-1	39 (88.6)
2	5 (11.4)
Gleason score	
6-7	16 (39.0)
≥8	25 (61.0)
Unknown	3
Visceral metastasis	
No	35 (79.6)
Yes	9 (20.4)

Abbreviations: ABL, abiraterone; ECOG, Eastern Cooperative Oncology Group; ENZA enzalutamide; PSA, prostate-specific antigen.

3.3 | Correlation between testosterone and AR CNV levels with outcome: univariate and multivariate analysis

The median follow-up at the time of statistical analysis was 35 months (range, 1-68). From the univariate analysis, a statistically significant correlation emerges between AR copy number alterations and PFS for both treatment groups. AR-gained patients had worse PFS compared to AR-normal patients; in particular, the median PFS in patients treated with abiraterone with normal AR gene was 9 months (95% CI: 6.7-11) compared to 5.3 months (95% CI: 2.6-6.5) of patients with AR gain ($P = 0.0001$).

A better PFS was found also in AR-normal patients treated with enzalutamide (4.9 vs 2.8 in patients with normal vs amplified AR, respectively, $P = 0.002$).

Patients with testosterone levels above the median value (<0.09) and treated with enzalutamide, had a statistically significant advantage in PFS compared to patients with testosterone levels below 0.09, while statistically significant better PFS was not obtained in patients treated with abiraterone and testosterone levels 0.09 or above (3.3 vs 7.3 months, $P = 0.006$ in the enzalutamide group and 5 vs 9.2 months, $P = 0.134$ in the abiraterone group).

At the analysis based on both testosterone levels and AR expression, patients with AR copy number abnormalities and testosterone levels below the median value (<0.09) had worse PFS compared to patients with no copy number abnormalities for both treatment groups. In the group of patients treated with abiraterone, the median PFS of those with AR-normal was 8.5 vs 2.9 months ($P = 0.0005$) in AR gained patients. No relevant differences were observed in the enzalutamide group 3.9 months (no AR gain) vs 2.7 months (AR gain) ($P = 0.004$).

No significant difference in PFS was shown in both treatment groups with testosterone levels 0.09 or above, but a slight, albeit not significant, benefit in PFS was observed in patients without copy number in the group treated with abiraterone acetate (10.7 months for normal AR vs 6.7 months for amplified AR, $P = 0.05$) (Table 3).

Similar results were found in the univariate analysis for OS (Table 4). Our data confirmed the negative prognostic role of AR CNVs for both treatment groups (10.6 vs 25.5 months, $P = 0.0002$ for abiraterone and 8.3 vs 14.1 months, $P = 0.031$ for enzalutamide) and the negative prognostic impact of testosterone below 0.09 only for patients treated with enzalutamide (8.8 vs 42.8 months, $P = 0.016$).

On the basis of testosterone levels and AR copy number, it was confirmed that the group with the worst prognosis was observed in AR-gained patients with testosterone below 0.09. This evidence was significant for the group of patients treated with abiraterone with a median OS of 25.5 months for patients with normal AR (95% CI: 17.7-30.6) and 10 months for patients with AR copy number alterations (95% CI: 2.9-12.3, $P < 0.0001$).

For patients with low testosterone levels and treated with enzalutamide, OS for AR-normal patients was longer than in patients with AR gain (11 vs 8 months) but the difference is not statistically significant, with only a trend in favor of the group without AR alterations ($P = 0.061$).

Figures 1 and 2 show the Kaplan-Meier curves of OS, based on testosterone levels and the presence or absence of CNVs. A worse prognosis was reported in patients with AR copy number alterations and testosterone levels below the median, for both experimental arms (8.3 vs 17.7 months of AR-normal and low testosterone levels, $P = 0.0002$).

In the cohort of patients treated with docetaxel, there is a trend of better OS in the group of patient with basal testosterone levels 0.09 or above vs below 0.09 (32.6 months for testosterone levels ≥0.09 vs 14.8 months for testosterone below 0.09, $P = 0.081$), while the correlation with PFS was not significant ($P = 0.220$) (Tables 5,6).

On multivariate analysis (Table 7) for patients treated with abiraterone, low testosterone levels below 0.09 and plasma AR gain or mutant were significantly associated with shorter PFS and OS. These data were confirmed only for PFS in the enzalutamide group. A trend for a significantly better OS was obtained in a patient treated with enzalutamide and testosterone levels 0.09 or above (HR 0.47, $P = 0.073$). The presence of visceral metastases was significantly correlated with a worse outcome in enzalutamide group ($P = 0.009$ for PFS and $P = 0.016$ for OS).

4 | DISCUSSION

Prostate cancer in the castration-resistant phase presents a remarkable sensitivity to second-generation hormone therapies such as abiraterone and enzalutamide. However, nowadays there are no

TABLE 3 Univariate analysis of progression free survival (PFS)

	ABI postchemo				ENZA postchemo				Total			
	No. of patients	No. of events	Median (95% CI), mo	P	No. of patients	No. of events	Median (95% CI), mo	P	No. of patients	No. of events	Median (95% CI), mo	P
Total	54	52	7.0 (5.4-8.6)	-	74	66	3.8 (3.0-4.9)	-	128	118	5.1 (4.0-6.5)	-
AR CN												
Normal	33	31	9.0 (6.7-11.0)	-	50	42	4.9 (3.5-7.2)	-	83	73	7.2 (4.9-8.5)	-
Gain	21	21	5.3 (2.6-6.5)	0.0001	24	24	2.8 (2.0-3.8)	0.002	45	45	3.2 (2.5-4.6)	<0.0001
Testosterone												
<0.09	30	29	5.0 (3.3-7.4)	-	59	54	3.3 (2.8-3.9)	-	89	83	3.8 (3.0-4.6)	-
≥0.09	24	23	9.2 (6.7-11.0)	0.134	15	12	7.3 (3.7-21.7)	0.006	39	35	9.0 (6.7-10.9)	0.0002
Testosterone (<0.09)												
AR CN	17	16	8.5 (4.4-15.0)	-	36	31	3.9 (2.8-6.5)	-	53	47	4.6 (3.7-7.4)	-
normal												
AR CN	13	13	2.9 (1.2-5.6)	0.0005	23	23	2.7 (2.0-3.7)	0.004	36	36	2.8 (2.0-3.7)	<0.0001
gain												
Testosterone (≥0.09)												
AR CN	16	15	10.7 (6.7-13.3)	-	14	11	7.2 (3.7-11.0)	-	30	26	9.9 (6.7-11.0)	-
normal												
AR CN	8	8	6.7 (1.6-9.3)	0.050	1	1	21.7 (-)	0.709	9	9	6.8 (1.6-16.1)	0.240
gain												

Abbreviations: ABI, abiraterone; AR, androgen receptor; CI, confidence interval; CN, copy number; ENZA, enzalutamide.

TABLE 4 Univariate analysis of overall survival (OS)

	ABI postchemo				ENZA postchemo				Total			
	No. of patients	No. of events	Median (95% CI), mo	P	No. of patients	No. of events	Median (95% CI), mo	P	No. of patients	No. of events	Median (95% CI), mo	P
Total	54	45	20.5 (14.7-25.5)	-	74	56	11.0 (8.2-16.1)	-	128	101	15.5 (11.0-18.6)	-
AR CN												
Normal	33	27	25.5 (20.5-30.6)	-	50	35	14.1 (8.8-20.5)	-	83	62	20.5 (15.3-26.4)	-
Gain	21	18	10.6 (6.4-15.5)	0.0002	24	21	8.3 (4.5-10.8)	0.031	45	39	9.8 (6.4-12.3)	0.0002
Testosterone												
<0.09	30	27	17.7 (10.6-23.1)	-	59	47	8.8 (8.0-11.7)	-	89	74	10.6 (8.8-16.1)	-
≥0.09	24	18	26.4 (15.3-35.1)	0.098	15	9	42.8 (11.0-48.3)	0.016	39	27	26.4 (15.5-35.1)	0.002
Testosterone (<0.09)												
AR CN	17	14	25.5 (17.7-30.6)	-	36	26	11.0 (8.0-19.6)	-	53	40	17.7 (10.6-22.4)	-
normal												
AR CN gain	13	13	10.0 (2.9-12.3)	<0.0001	23	21	8.0 (4.5-9.9)	0.061	36	34	8.3 (4.5-10.5)	0.0002
Testosterone (≥0.09)												
AR CN	16	13	26.4 (14.7-35.1)	-	14	9	31.7 (11.0-48.3)	-	30	22	26.4 (15.3-39.8)	-
normal												
AR CN gain	8	5	21.0 (6.4-NR)	0.403	1	0	NE	0.417	9	5	26.5 (6.4-NR)	0.641

Abbreviations: ABI, abiraterone; AR, androgen receptor; CI, confidence interval; CN, copy number; ENZA, enzalutamide; NR, not reached; NE, not estimable.

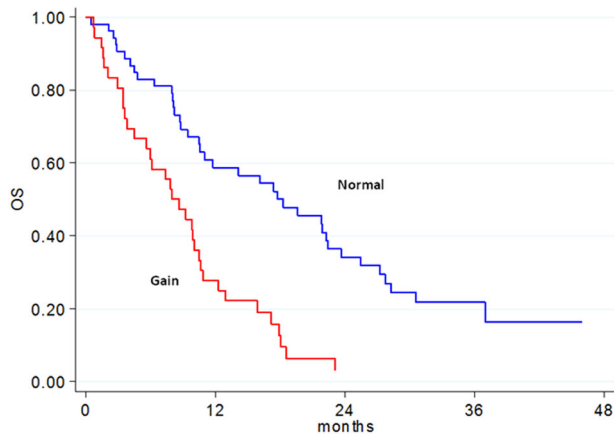


FIGURE 1 Kaplan-Meier curve for overall survival (OS) in patients with testosterone levels below 0.09 nmol/L on the basis of AR status. AR, androgen receptor [Color figure can be viewed at wileyonlinelibrary.com]

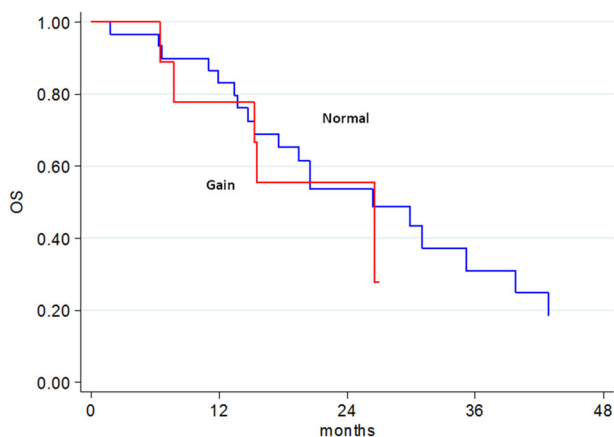


FIGURE 2 Kaplan-Meier curve for overall survival (OS) in patients with testosterone levels 0.09 nmol/L or above on the basis of AR status. AR, androgen receptor [Color figure can be viewed at wileyonlinelibrary.com]

clinical trials that suggest the best choice and therapeutic sequence allow personalized therapy. The use of both clinical prognostic and predictive response biomarkers could be a useful parameter for stratifying patients in different risk classes and guiding the clinician toward the most appropriate therapeutic choice.

The aim of this retrospective analysis was to evaluate a possible prognostic and predictive role of basal testosterone levels and AR

TABLE 5 Univariate analysis of progression-free survival (PFS)

	Docetaxel			
	No. of patients	No. of events	Median (95% CI), mo	P
Total	44	44	6.6 (5.3-9.7)	-
Testosterone				
<0.09	26	26	6.2 (3.7-7.2)	
≥0.09	18	18	9.7 (5.5-10.3)	0.220

Abbreviation: CI, confidence interval.

TABLE 6 Univariate analysis of overall survival (OS)

	Docetaxel			
	No. of patients	No. of events	Median (95% CI), mo	P
Total	44	33	26.4 (13.7-34.5)	-
Testosterone				
<0.09	26	20	14.8 (12.9-30.2)	
≥0.09	18	13	32.6 (17.5-40.4)	0.081

Abbreviation: CI, confidence interval.

gene copy number alterations in patients treated with abiraterone or enzalutamide after progression to docetaxel, to identify patients that may or may not benefit from second-generation hormonal therapies.

Our data analysis confirmed the negative prognostic and predictive impact of copy number alterations of the AR gene for both abiraterone and enzalutamide groups of patients, as already described in literature.^{10,13-16}

Patients treated with enzalutamide with testosterone below the median value have a worse PFS and OS, while the difference was not significant for patients treated with abiraterone. The mechanism of action of enzalutamide could explain these results. Enzalutamide blocks the link between androgens and their receptor in prostatic cells; when levels of serum-circulating testosterone are reduced, the role of enzalutamide as an antagonist of ARs could be lost, and the development of further acquired mechanisms of resistance based on alternative pathways could cause enzalutamide inefficacy.

In the group of patients treated with abiraterone, worse PFS and OS associated with baseline testosterone levels below the median have been observed, confirming the data described by Ryan et al.⁹ However, in our analysis, the result is not significant probably due to a lower number of patients compared to enzalutamide group. Another limitation was due to the use of enzalutamide later in the sequence of therapies compared to abiraterone. For this reason, patients treated with abiraterone have received a smaller number of previous treatment lines probably with less impact on the development of androgen-independent resistance mechanisms. As already suggested by Ryan et al, we hypothesized that a progression with suppressed levels of testosterone could represent a more aggressive disease completely independent from the hormonal axis, while a progression with residual testosterone levels may be an event potentially actionable by the use of drugs (eg, abiraterone and enzalutamide) that act on the residual activity of circulating androgens.⁹

In the cohort of patients treated with docetaxel, the prognostic impact of testosterone would show a trend for a better outcome in favor of the group of patients with higher testosterone levels, as previously described by de Liaño et al.¹⁷

It has been described that part of the antitumor activity of docetaxel in CRPC is expressed through the blockade of the internalization of the activated AR; therefore, the activity of docetaxel could be partially facilitated in presence of residual activity of the androgenic signal.¹⁸

TABLE 7 Multivariate analysis of progression-free survival (PFS) and overall survival (OS)

	PFS		OS	
	HR (95% CI)	P	HR (95% CI)	P
<i>ABI postchemo</i>				
Testosterone				
<0.09	1.00		1.00	
≥0.09	0.49 (0.26-0.94)	0.030	0.46 (0.23-0.92)	0.028
AR CN				
Normal	1.00		1.00	
Gain	4.98 (2.26-10.94)	<0.0001	5.61 (2.46-12.79)	<0.0001
Gleason score				
6-7	1.00		1.00	
≥8	0.81 (0.40-1.62)	0.547	1.03 (0.51-2.08)	0.929
Visceral metastasis				
No	1.00		1.00	
Yes	1.63 (0.68-3.91)	0.275	1.35 (0.57-3.23)	0.496
Age	0.99 (0.94-1.04)	0.643	0.99 (0.94-1.03)	0.575
<i>ENZA postchemo</i>				
Testosterone				
<0.09	1.00		1.00	
≥0.09	0.38 (0.18-0.82)	0.014	0.47 (0.20-1.07)	0.073
AR CN				
Normal	1.00		1.00	
Gain	2.11 (1.13-3.97)	0.020	1.53 (0.80-2.94)	0.202
Gleason score				
6-7	1.00		1.00	
≥8	1.29 (0.72-2.30)	0.395	0.92 (0.50-1.70)	0.794
Visceral metastasis				
No	1.00		1.00	
Yes	2.91 (1.31-6.48)	0.009	2.67 (1.20-5.93)	0.016
Age	0.99 (0.96-1.03)	0.645	1.00 (0.96-1.04)	0.873
<i>Total</i>				
Testosterone				
<0.09	1.00		1.00	
≥0.09	0.41 (0.26-0.66)	0.0002	0.53 (0.33-0.86)	0.010
AR CN				
Normal	1.00		1.00	
Gain	2.82 (1.76-4.51)	<0.0001	2.11 (1.32-3.35)	0.002
Gleason score				
6-7	1.00		1.00	
≥8	1.01 (0.65-1.56)	0.974	0.90 (0.58-1.42)	0.905
Visceral metastasis				
No	1.00		1.00	
Yes	2.12 (1.19-3.77)	0.010	2.26 (1.27-4.03)	0.005
Age	0.99 (0.97-1.02)	0.717	1.00 (0.97-1.03)	0.963

Abbreviations: ABI, abiraterone; AR, androgen receptor; CI, confidence interval; CN, copy number; ENZA, enzalutamide; HR, hazard ratio.

In this study, data on the impact of AR on outcomes of patients treated with taxanes were not available at the time of the analysis; however, a recent retrospective analysis did not show a prognostic role of AR aberrations in patients treated with docetaxel, reinforcing the importance of these aberrations only for abiraterone and enzalutamide.¹⁴

Patients with testosterone levels below the median value and AR gain have a significantly reduced PFS in comparison to patients with normal AR, for both groups of treatment; a significantly better OS was obtained in patients treated with abiraterone but only a significant trend for better OS was noticed in the enzalutamide arm.

In the multivariate analysis, AR alterations and testosterone levels were two independent predictive and prognostic factors, and another independent prognostic factor was the presence of visceral metastases, as previously shown.¹⁹

To our knowledge, this is the first study that has identified copy number alterations of the AR gene and testosterone levels below 0.09 nmol/L as factors related to poor prognosis and poor response to new hormone therapies.

The limits of this study are represented by its retrospective nature and by the reduced number of samples analyzed. In addition,

as previously highlighted, patients treated with enzalutamide have a shorter follow-up because in Italy this drug was introduced after the approval of abiraterone. The reduced sample size of patients is at the basis of the unbalanced characteristics among the two treatment groups concerning testosterone levels and AR aberrations, and in the enzalutamide group, only one patient with testosterone levels above the median and copy number alterations was detected. Finally, we focused on AR axis mechanisms of resistance, but other mechanisms could play a role as neuroendocrine differentiation.^{20,21}

Despite the above-reported limits of the study, this study must be considered an exploratory and preliminary analysis that could represent the basis for assessing the role of testosterone and plasma AR in a prospective study with larger sample size and with balanced characteristics between the patient groups.

5 | CONCLUSIONS

In the era of precision medicine, in prostate cancer treatment there is a very urgent need to define biomarkers that can orientate the clinicians towards the best therapeutic choice.²²⁻²⁶ The results of this study show that negative serum testosterone levels associated with AR gain could represent a noninvasive biomarker useful to identify a subgroup of patients with a worse prognosis that can benefit less from hormonal therapies in the CRPC setting. Further prospective studies to validate these parameters as possible prognostic and predictive biomarkers of response to second-generation abiraterone and enzalutamide are needed.

CONFLICT OF INTERESTS

Vincenza Conteduca and Ugo De Giorgi received speaker honoraria or travel support from Astellas, Janssen-Cilag, Bayer, and Sanofi Aventis. Giuseppe Tonini received honoraria for Advisory Board from Novartis, Pfizer, Italfarmaco, and Roche. Cristian Lolli received travel support from Janssen-Cilag. The other authors declare no conflict of interests.

ORCID

Cristian Lolli  <http://orcid.org/0000-0003-0347-9179>

Vincenza Conteduca  <http://orcid.org/0000-0002-6921-714X>

REFERENCES

- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;68:7-30.
- Devlin HL, Mudryj M. Progression of prostate cancer: multiple pathways to androgen independence. *Cancer Lett*. 2009;274:177-186.
- Linja MJ, Savinainen KJ, Saramäki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. *Cancer Res*. 2001;61:3550-3555.
- Antonarakis ES. Predicting treatment response in castration-resistant prostate cancer: could androgen receptor variant-7 hold the key? *Expert Rev Anticancer Ther*. 2015;15:143-145.
- Chmelar R, Buchanan G, Need EF, Tilley W, Greenberg NM. Androgen receptor coregulators and their involvement in the development and progression of prostate cancer. *Int J Cancer*. 2007;120:719-733.
- Taplin ME, Bubley GJ, Shuster TD, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. *N Engl J Med*. 1995;332:1393-1398.
- Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. *Nat Rev Cancer*. 2001;1:34-45.
- Hashimoto K, Shindo T, Tabata H, et al. Serum testosterone level is a useful biomarker to aid optimal treatment selection in men with castration-resistant prostate cancer. *J Urol*. 2017;197(4S):e713.
- Ryan CJ, Molina A, Li J, et al. Serum androgens as prognostic biomarkers in castration-resistant prostate cancer: results from an analysis of a randomized phase III trial. *J Clin Oncol*. 2013;31:2791-2798.
- Salvi S, Casadio V, Conteduca V, et al. Circulating cell-free AR and CYP17A1 copy number variations may associate with outcome of metastatic castration-resistant prostate cancer patients treated with abiraterone. *Br J Cancer*. 2015;112:1717-1724.
- Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med*. 2014;371:1028-1038.
- Salvi S, Casadio V, Conteduca V, et al. Circulating AR copy number and outcome to enzalutamide in docetaxel-treated metastatic castration-resistant prostate cancer. *Oncotarget*. 2016;7:37839-37845.
- Conteduca V, Jayaram A, Romero-Laorden N, et al. Plasma androgen receptor and docetaxel for metastatic castration-resistant prostate cancer. *Eur Urol*. 2019;75:368-373.
- Conteduca V, Scarpi E, Salvi S, et al. Plasma androgen receptor and serum chromogranin A in advanced prostate cancer. *Sci Rep*. 2018;8:15442.
- Salvi S, Gurioli G, De Giorgi U, et al. Cell-free DNA as a diagnostic marker for cancer: current insights. *Onco Targets Ther*. 2016;9:6549-6559.
- Conteduca V, Wetterskog D, Sharabiani MTA, et al. Androgen receptor gene status in plasma DNA associates with worse outcome on enzalutamide or abiraterone for castration-resistant prostate cancer: a multi-institution correlative biomarker study. *Ann Oncol*. 2017;28:1508-1516.
- de Líaño AG, Reig O, Mellado B, Martin C, Rull EU, Maroto JP. Prognostic and predictive value of plasma testosterone levels in patients receiving first-line chemotherapy for metastatic castrate-resistant prostate cancer. *Br J Cancer*. 2014;110:2201-2208.
- Darshan MS, Loftus MS, Thadani-Mulero M, et al. Taxane-induced blockade to nuclear accumulation of the androgen receptor predicts clinical responses in metastatic prostate cancer. *Cancer Res*. 2011;71:6019-6029.
- Conteduca V, Caffo O, Fratino L, et al. Impact of visceral metastases on outcome to abiraterone after docetaxel in castration-resistant prostate cancer patients. *Future Oncol*. 2015;11:2881-2891.
- Burgio SL, Conteduca V, Menna C, et al. Chromogranin A predicts outcome in prostate cancer patients treated with abiraterone. *Endocr Relat Cancer*. 2014;21:487-493.
- Conteduca V, Aieta M, Amadori D, De Giorgi U. Neuroendocrine differentiation in prostate cancer: current and emerging therapy strategies. *Crit Rev Oncol Hematol*. 2014;92:11-24.
- Logothetis CJ, Gallick GE, Maity SN, et al. Molecular classification of prostate cancer progression: foundation for marker-driven treatment of prostate cancer. *Cancer Discov*. 2013;3:849-861.
- De Giorgi U, Caroli P, Burgio SL, et al. Early outcome prediction on 18F-fluorocholine PET/CT in metastatic castration-resistant prostate cancer patients treated with abiraterone. *Oncotarget*. 2014;5:12448-12458.
- De Giorgi U, Caroli P, Scarpi E, et al. 18F-fluorocholine PET/CT for early response assessment in patients with metastatic castration-resistant prostate cancer treated with enzalutamide. *Eur J Nucl Med Mol I*. 2015;42:1276-1283.

25. Azad AA, Volik SV, Wyatt AW, et al. Androgen receptor gene aberrations in circulating cell-free DNA: biomarkers of therapeutic resistance in castration-resistant prostate cancer. *Clin Cancer Res*. 2015;21:2315-2324.
26. Antonarakis ES, Lu C, Luber B, et al. Clinical significance of androgen receptor splice variant-7 mRNA detection in circulating tumor cells of men with metastatic castration-resistant prostate cancer treated with first- and second-line abiraterone and enzalutamide. *J Clin Oncol*. 2017;35:2149-2156.

How to cite this article: Lolli C, De Lisi D, Conteduca V, et al. Testosterone levels and androgen receptor copy number variations in castration-resistant prostate cancer treated with abiraterone or enzalutamide. *The Prostate*. 2019;79: 1211-1220. <https://doi.org/10.1002/pros.23804>