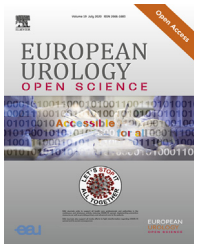
EUROPEAN UROLOGY OPEN SCIENCE 29 (2021) 59–67

available at www.sciencedirect.com
journal homepage: www.eu-openscience.europeanurology.com



European Association of Urology

Prostate Cancer

A Prospective Study of the Relationship Between Clinical Outcomes After Enzalutamide and Serum Androgen Levels Measured via Liquid Chromatography-tandem Mass Spectrometry in Patients with Castration-resistant Prostate Cancer

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Article info

Article history:
Accepted May 21, 2021

Associate Editor:
Jochen Walz

Keywords:
Prostate cancer
Enzalutamide
Androgen
Liquid chromatography-tandem mass spectrometry

Abstract

Background: Enzalutamide (ENZ) is used to treat patients with castration-resistant prostate cancer (CRPC). However, the kinetics of serum androgens before and after ENZ treatment are unknown.
Objective: To elucidate the kinetics of serum androgens and explore the possibility of identifying a useful marker for predicting the effects of ENZ.
Design, setting, and participants: We conducted a prospective study from 2014 to 2018 at Gunma University Hospital and related facilities. Data were analyzed for 104 patients with CRPC treated with ENZ.
Outcome measurements and statistical analysis: We measured serum androgen levels using liquid chromatography-tandem mass spectrometry. Relationships with outcomes were assessed using multivariable Cox regression and log-rank analyses.
Results and limitations: The median age of the patients was 73 yr. Median serum testosterone, dihydrotestosterone (DHT), androstenedione, and dehydroepiandrosterone sulfate levels were 49.0, 5.8, 222.2, and 326.3 pg/ml, respectively. We performed multivariate analysis using Cox regression to predict prostate-specific antigen progression–free survival (PSA-PFS) and overall survival (OS). Hemoglobin level ($\geq$12.5 vs $<$12.5 g/dl), docetaxel treatment history (no vs yes), and DHT level ($\geq$5.9 vs $<$5.9 pg/ml) were significant predictors of PSA-PFS ($p <$ 0.05). Eastern Cooperative Oncology Group performance status (0 vs. 1–2), hemoglobin level ($\geq$12.5 vs $<$12.5 g/dl), presence of visceral metastasis (no vs yes), amount of bone metastasis (extent of disease 0–2 vs 3–4), and docetaxel treatment history

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http://dx.doi.org/10.1016/j.euros.2021.05.003

(no vs yes) were significant predictors of OS ($p < 0.05$). Binomial logistic analysis of the predictors of any grade of anorexia, malaise, and fatigue showed that the presence of visceral metastasis and a low DHT level (<5.9 pg/ml) were significant.
Conclusions: Our results suggest that serum androgen levels before ENZ treatment may be useful for predicting efficacy, prognosis, and the incidence of adverse events.
Patient summary: We measured blood levels of testosterone and other male hormones before treatment with enzalutamide among men with prostate cancer resistant to castration. We found that the levels of these hormones may be useful for predicting the efficacy of enzalutamide treatment, prognosis, and the occurrence of adverse side effects.

1. Introduction

Prostate cancer has become one of the most prevalent male conditions in Western countries, and its incidence has increased in Japan [1,2]. The treatment of metastatic castration-resistant prostate cancer (CRPC) has changed dramatically with the advent of novel androgen receptor (AR)-targeting agents (ARTAs) and a new class of taxane agents [3]. Enzalutamide (ENZ) and abiraterone acetate are ARTAs with proven survival benefits both before and after docetaxel (DOC) treatment [4–7]. ENZ is a second-generation anti-androgen with multiple inhibitory effects on androgen signal transduction [8]. The pivotal PREVAIL and AFFIRM clinical trials showed that use of these drugs was associated with significant improvements in overall survival (OS), radiographic progression-free survival (rPFS), prostate-specific antigen progression–free survival (PSA-PFS), quality of life (as measured with the Functional Assessment of Cancer Therapy-Prostate questionnaire), and skeletal-related events [4,5,9,10].

ENZ has proven efficacy against CRPC and is widely used in this setting. No definitive biomarker predicts the effects of ENZ. It is well known that serum testosterone (T) levels before and during androgen deprivation therapy (ADT) are prognostic for the success of primary ADT [11,12]. De novo androgen synthesis in prostate cancer tissues promotes progression to CRPC, resulting in an increase in androgen levels [13,14]. Several retrospective studies in CRPC found that patients with higher serum T levels before ARTA treatment experienced better therapeutic effects than patients with lower T levels did [15–17]. We thus decided to elucidate the kinetics of androgen levels after ENZ administration. In addition, we prospectively examined the effects of ENZ on initial serum androgen concentrations among patients with CRPC.

2. Patients and methods

2.1. Patients and study design

We prospectively evaluated 104 patients with CRPC treated in the urology department of Gunma University Hospital and affiliated hospitals from 2014 to 2018. The primary endpoint was the PSA response rate (50% and 90%), and the secondary endpoints were OS, PSA-PFS, the objective tumor response, and the relationship between serum androgen levels and clinical outcomes. Extent of disease (EOD) was used as a semiquantitative grading system according to the extent of bone metastasis on bone scans as follows: 0, normal; 1, fewer than six bony metastases, each of which is <50% of the size of a vertebral body; 2, between six and 20 bony metastases; 3, more than 20 bony metastases but less than a “super scan”; and 4, “superscan” or bony metastases involving more than 75% of the ribs, vertebrae, and pelvic bones [18]. We collected blood samples before treatment, at 3 and 6 mo after the commencement of treatment, and at the end of treatment. The correlation coefficients for automated immunoassays are relatively poor when T concentrations are low (<4.0 nmol/l) [19]. Therefore, we measured serum androgen levels via liquid chromatography-tandem mass spectrometry (LC-MS/MS), which was carried out by Asuka Pharmaceutical (Tokyo, Japan). Serum T, dihydrotestosterone (DHT), androstenedione (A-dione), and dehydroepiandrosterone sulfate (DHEA-S) levels were measured. The lower limit of detection for T, DHT, A-dione, and DHEA-S was 0.5, 0.5, 1.0, and 1.0 pg/ml, respectively.

The eligibility criteria for the study were age >20 yr, histologically confirmed prostate cancer, surgical or medical castration, a diagnosis of CRPC, Eastern Cooperative Oncology Group performance status (ECOG PS) score of 0–2, and consent to participate. The exclusion criteria were a history of seizures (such as epilepsy); a history of brain metastasis, organic brain disease, or brain injury; severe liver dysfunction; allergy to ENZ; and any contraindication to ENZ treatment as judged by a doctor.

2.2. Disease progression and treatment efficacy

OS and PSA-PFS were assessed using Prostate Cancer Working Group 2 criteria [20]. PSA failure was defined according to the same group as a rise in PSA >2 ng/ml above the nadir that was ≥25% of the nadir, as confirmed by a second test performed at least 3 wk later, accompanied by a castration level of T (<50 ng/dl). The objective tumor response was evaluated using Response Evaluation Criteria for Solid Tumors (RECIST) version 1.1.

2.3. Statistical analysis

Student *t* test, the Welch *t* test, the Fisher test, and the Wilcoxon signed-rank test were used as appropriate to assess associations between androgen levels and other clinical variables. We calculated Pearson correlation coefficients among hormone levels. Univariate and multivariate Cox proportional-hazard models and the Kaplan–Meier method were used for statistical analyses. Patient inclusion and data collection

were prospective, but the multivariate analysis was conducted on a post hoc basis. Multivariate analysis of factors predicting OS and PFS was conducted on an exploratory basis. Factors with $p < 0.05$ on univariate analysis were included in a Cox proportional-hazards multivariate analysis. SPSS version 25.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. A p value <0.05 was considered indicative of significance.

2.4. Institutional ethics approval

We explained the nature of the study to all participants. All participants agreed in writing to participate. The study was approved by the institutional review board (IRB) of Gunma University Hospital (approval no. 1177). The IRB also approved the inclusion of patients from other facilities.

3. Results

Patient characteristics are summarized in Table 1. Although 104 patients were enrolled, there were two patients for whom measurement could not be completed owing to the poor condition of the sample before treatment and three patients who could not continue follow-up owing to relocation. Therefore, the final cohort for analysis was 99 cases, for which we explored the relationships between serum androgen levels and prognosis. Table 2 shows the blood test data and serum androgen levels before ENZ treatment. The median patient age was 73 yr and the median PSA level was 27.6 ng/ml. Seventeen patients (16.3%) had a history of curative treatment and 44 (42.3%) a history of DOC treatment. The proportion of patients with a 50% and 90% PSA decline was 62.5% and 25.0%, respectively (Fig. 1).

On analysis of 91 cases for whom objective tumor responses could be evaluated, the response rate (complete response [CR] + partial response [PR]) was 35.2% and the clinical benefit (CR + PR + stable disease) was 81.3%, as assessed using the RECIST criteria (Table 3). Pre-DOC PSA-PFS and OS were significantly higher than the post-DOC results (PSA-PFS: 12.3 vs 2.8 mo; $p < 0.001$; OS: not yet reached vs 17.2 mo; $p < 0.001$; Fig. 2). Mean serum T, DHT, A-dione, and DHEA-S levels were 49.0, 5.8, 222.2, and 326.3 pg/ml, respectively. The median time to treatment discontinuation was 40.0 wk (range 3–203 wk, standard deviation 58.0). Of the 99 cases, ENZ was discontinued in 12 by 12 wk because of progression and in a further 41 by 24 wk. For 58 cases, ENZ treatment continued for ≥24 wk. Mean serum levels of T, DHT, and A-dione were significantly higher at 12 and 24 wk after treatment compared to pretreatment levels (all $p < 0.05$; Fig. 3). There was a positive correlation between pretreatment T, DHT, A-dione, and DHEA-S levels. Pearson's correlation coefficients revealed significant positive correlation of pretreatment levels for each pair of hormones (Supplementary Fig. 1).

The study was conducted using previously reported prognostic factors such as baseline PSA, Gleason score, presence or absence of visceral metastasis, and extent of metastasis. Patients were divided into two groups according to the median value for each blood test and hormone, and we assessed whether these were significant factors. Of the 99 patients, 51 died during the observation period. PSA-PFS and OS were significantly longer for patients with androgen levels greater than the median (log-rank test, $p < 0.05$). We performed multivariate Cox regression analysis for predic-

Table 1 – Patient characteristics

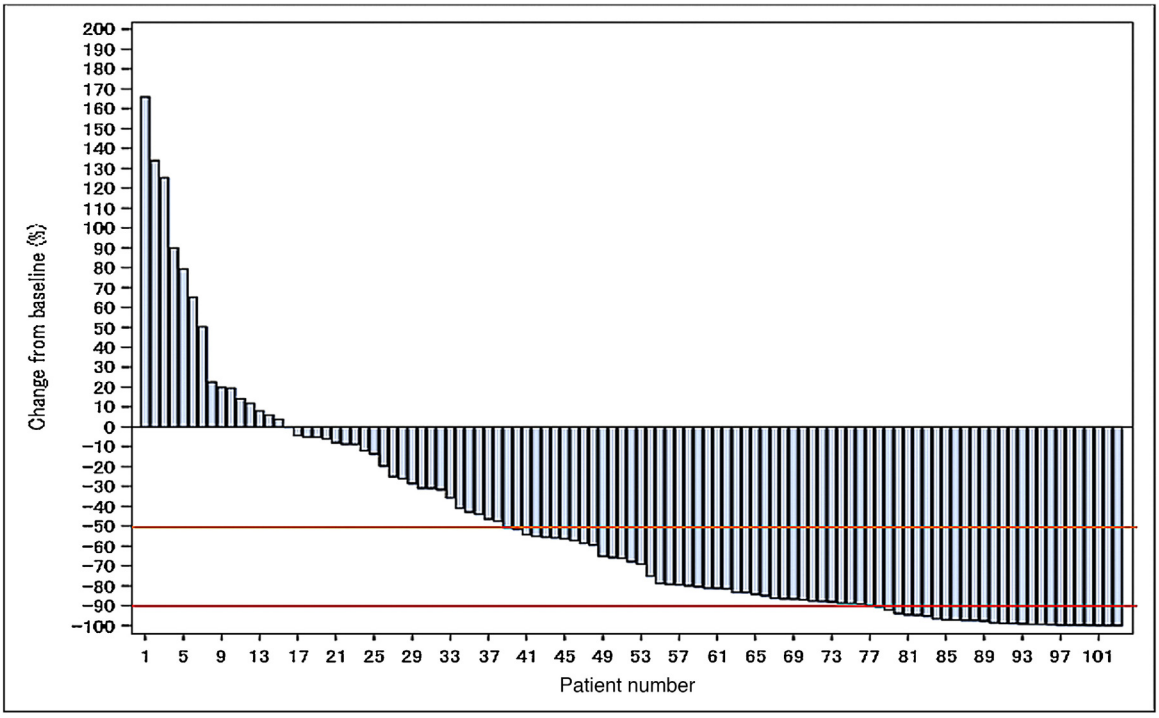
Variable	*N*	%
TNM classification at diagnosis		
T stage		
T1	2	1.9
T2	11	10.6
T3	46	44.2
T4	40	38.5
Tx	5	4.8
N stage		
N0	44	42.3
N1	53	51.0
Nx	7	6.7
M stage		
M0	31	29.8
M1a	7	6.7
M1b	52	50.0
M1c	10	9.6
Mx	4	3.8
Eastern Cooperative Oncology Group performance status		
0	73	70.2
1	22	21.2
2	6	5.8
Unknown	3	2.9
Gleason score		
6	2	1.9
7	13	12.5
8	17	16.3
9	48	46.2
10	19	18.3
Unknown	5	4.8
Metastasis at study baseline		
Metastasis negative	3	2.9
Metastasis positive	101	97.1
Metastasis site		
Regional lymph nodes	35	33.7
Distant lymph nodes	31	29.8
Bone	80	79.6
Lung	10	9.6
Liver	1	1.0
Other	2	1.9
Extent of disease at study baseline		
1	17	16.3
2	13	12.5
3	14	13.5
4	5	4.8
Unknown	31	29.8
Previous radical treatment		
Yes	17	16.3
Surgery	12	11.5
Radiation	5	4.8
No	87	83.7
Previous docetaxel treatment		
No	60	57.7
Yes	44	42.3
Time to castration-resistant prostate cancer from initial ADT		
>12 mo	74	71.2
<12 mo	30	28.8

ADT = androgen deprivation therapy.

Table 2 – Age, blood test results, and androgen levels at study baseline before enzalutamide treatment

Variable	Median	Mean	SD
Age (yr)	73.0	72.6	8.18
Prostate-specific antigen (ng/ml)	23.1	69.3	126.8
Hemoglobin (g/dl)	12.3	12.0	1.78
Alkaline phosphatase (IU/l)	267.0	344.4	257.9
Lactate dehydrogenase (IU/l)	222.0	254.8	153.9
Calcium (mg/dl)	9.4	9.3	0.55
Albumin (g/dl)	3.9	3.9	0.49
Testosterone (pg/ml)	49.0	59.7	67.0
Dihydrotestosterone (pg/ml)	5.80	8.37	7.80
Androstendione (pg/ml)	326.3	482.6	501.1
Dehydroepiandrostendione sulfate (pg/ml)	222.2	236.4	201.9

SD = standard deviation.



Best response	*N* (total 104 cases)	%
PSA decline ≧ 50 %	65	62.5
PSA decline ≧ 90 %	26	25.0

Fig. 1 – Prostate-specific antigen (PSA) response. The 50% and 90% PSA decline rates were 62.5% and 25.0%, respectively.

tion of OS and PSA-PFS. We found that hemoglobin (≥11.4 vs <11.4 g/dl), a history of DOC treatment (no vs yes), and DHT (≥5.8 vs <5.8 pg/ml) were significant predictors of PSA-PFS (all $p < 0.05$; Table 4). In addition, ECOG PS (0 vs 1–2), hemoglobin (≥11.4 vs <11.4 g/dl), visceral metastasis status (no vs yes), extent of bone metastasis (0–2 vs 3–4), and history of DOC treatment (no vs yes) were significant predictors of OS (all $p <$ 0.05; Table 5).

The grade 1–2 adverse events observed were anorexia in 22 patients (21.1%), malaise in 22 (21.1%), fatigue in 13 (12.5%), nausea in seven (6.7%), dysgeusia in five (4.8%), and hot flushes in three (2.9%). The grade 3 adverse events were hypertension, anorexia, fatigue, elevated liver enzyme levels, and retinal artery occlusion. No adverse events of grade ≥4 were observed. The most common adverse events (>20% of cases; 33/99) were anorexia, malaise, and fatigue;

Table 3 – Objective tumor response rate according to Response Evaluation Criteria in Solid Tumors version 1.1 among the 91 patients

Objective tumor response	*N*	%
Complete response	4	4.4
Partial response	28	30.8
Stable disease	42	46.2
Progressive disease	17	18.7
Overall response (complete response + partial response)	32	35.2
Clinical benefit (complete response + partial response + stable disease)	74	81.3

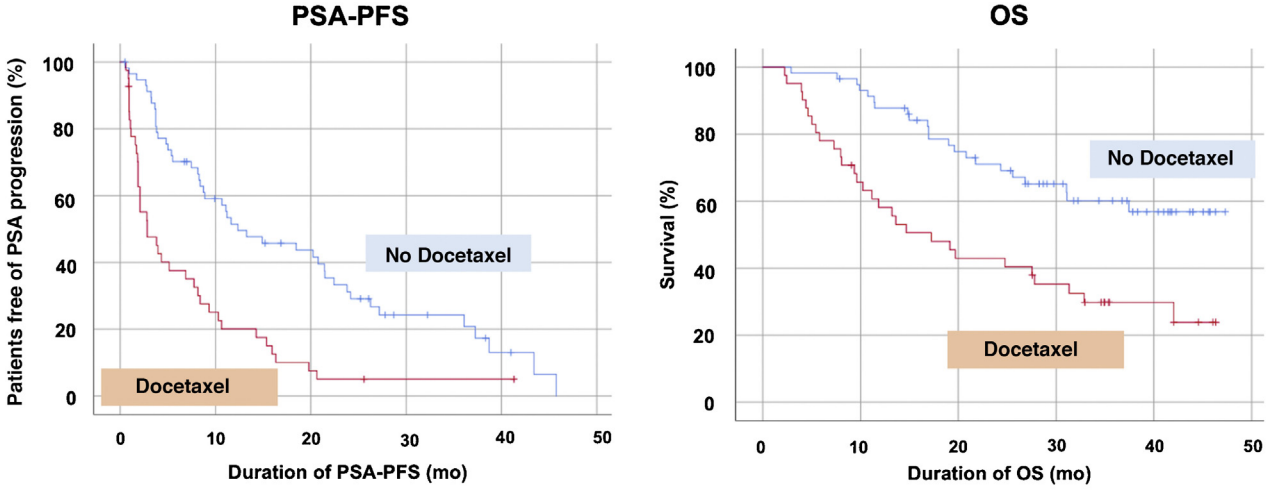
we sought factors predictive of these events using binomial logistic analysis. Visceral metastasis and a DHT level below the median (<5.8 pg/ml) significantly predicted any grade of these three events (Table 6).

4. Discussion

We explored serum androgen kinetics in patients with metastatic CRPC receiving ENZ. The therapeutic effects of ENZ were significantly better in the group with higher androgen levels. On univariate analysis, OS was also better, but significance was lacking for the relationship with androgen levels on multivariate analysis. The incidence of common side effects (anorexia, malaise, and fatigue) was significantly lower for patients with high androgen levels. To the best of our knowledge, this is the first prospective examination of the relationship between ENZ therapy and blood androgen levels in Japanese patients with metastatic CRPC.

Several earlier retrospective studies reported that higher T levels before treatment with AR-targeting agents such as ENZ and abiraterone were beneficial [15–17]. Lolli et al [21] reported that changes in (circulatory) AR gene copy number and T levels <0.09 nmol/l were associated with poor prognosis and poor response to ENZ and abiraterone. Our prospective study confirmed these findings. We found that high blood DHT levels predicted PSA-PFS, but not OS, on multivariate analysis. Shiota et al [15] found that PFS for patients with serum T >0.05 ng/ml was significantly inferior to that for patients with serum T <0.05 ng/ml. DOC chemotherapy for these patients was associated with poorer OS in the former group [15]. Ando et al [22] reported that higher serum T levels were predictive of poor prognosis for patients with CRPC treated with DOC. As cross-resistance to abiraterone treatment may develop after ENZ treatment [23,24], DOC is often selected if the drug has not previously been prescribed, and cabazitaxel otherwise. The effects of taxane-based drugs are poorer for patients with higher T levels, and the drugs did not significantly enhance OS in the present study.

Efstathiou et al [25,26] reported on the relationships between changes in blood and bone-marrow androgen levels after treatment with ENZ or abiraterone, as well as the therapeutic effects. T concentrations declined to less than picogram-per-milliliter levels, and remained at that level, during treatment with abiraterone [25]. In contrast to the inhibition of androgen biosynthesis by abiraterone, T levels in blood and bone marrow increased, accompanied by a nuclear-to-cytoplasmic AR shift, following 8 wk of ENZ therapy [26]. T levels increased after 8 wk of treatment in most patients for whom paired samples of both blood (40/51, 78%) and bone marrow aspirate (34/44, 77%) were available [26]. Similarly, in the present study, T, DHT, DHEA-S, and A-dione levels tended to increase after ENZ administration, and T, DHT, and A-dione levels were significantly higher by 12 and 24 wk after treatment



	All patients	No docetaxel	Docetaxel	*p* value
PSA-PFS	8.4 mo	12.3 mo	2.8 mo	<0.001
OS	31.1 mo	NYR	17.2 mo	<0.001

Fig. 2 – Kaplan-Meier curve of PSA-PFS and OS for patients with and without docetaxel treatment. A log rank test was performed to determine the significance of differences.
PSA = prostate-specific antigen; PFS = progression free survival; OS = overall survival; NYR = not yet reached.

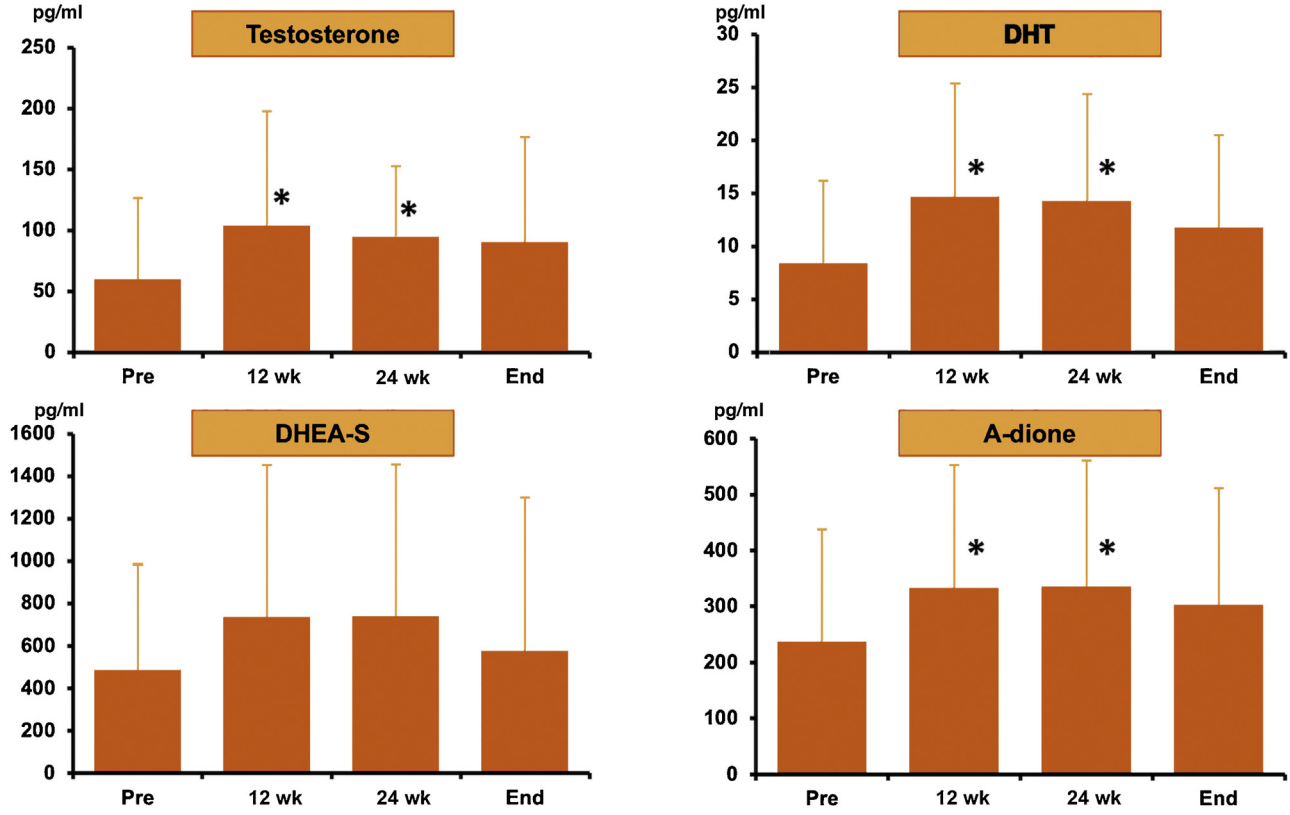


Fig. 3 – Kinetics of serum androgen levels during enzalutamide treatment. Mean serum levels of testosterone, dihydrotestosterone (DHT), and androstenedione (A-dione) were significantly higher at 12 and 24 wk after treatment compared to before treatment (Pre; * $p < 0.05$). DHEA-S = dehydroepiandrosterone sulfate.

initiation. Efstathiou et al [26] suggested that such increases reflect physiological feedback. It is unclear whether such androgen signaling contributes to treatment resistance and, if so, to what extent. A future study should explore whether androgen kinetics affect prognosis and ENZ efficacy.

Fatigue, anorexia, and malaise are relatively common with ENZ treatment [4,5]. The incidence of these adverse events was higher in the group of patients with higher DHT levels before ENZ treatment. Fatigue is common during prostate cancer treatment and may persist even when

Table 4 – Cox regression analysis of PSA progression–free survival

Variable	Univariate analysis		Multivariate analysis	
	p value	HR (95% CI)	*p* value	HR (95% CI)
Age (≤73.0 vs >73.0 yr)	0.062			
Baseline PSA (≤23.0 vs >23.0 ng/ml)	0.028	1.647 (1.055–2.572)	0.557	
Curative treatment history (yes vs no)	0.282			
Gleason ≤7 vs Gleason ≥8	0.161			
Gleason ≤8 vs Gleason ≥9	0.036	1.677 (1.035–2.718)	0.081	
ECOG performance status (0 vs 1–2)	0.105			
Initial ADT response duration (>12 vs ≤12 mo)	0.807			
Docetaxel treatment (no vs yes)	<0.001	2.615 (1.668–4.099)	0.003	2.046 (1.268–3.302)
Visceral metastasis at baseline (no vs yes)	0.660			
Baseline extent of disease (0–2 vs 3–4)	0.580			
Hemoglobin (≥11.4 vs <11.4 g/dl)	0.002	1.975 (1.273–3.063)	0.047	1.583 (1.006–2.493)
Alkaline phosphatase (<277 vs ≥277 IU/l)	0.495			
Lactate dehydrogenase (<221 vs ≥221 IU/l)	0.524			
Albumin (≥3.9 vs <3.9 g/dl)	0.075			
PTx testosterone (≥49.0 vs <49.0 pg/ml)	<0.001	2.210 (1.416–3.448)	0.485	
PTx DHT (≥5.8 vs <5.8 pg/ml)	<0.001	2.641 (1.684–4.142)	0.006	2.046 (1.268–3.302)
PTx DHEA-S (≥326.3 vs <326.3 pg/ml)	<0.001	2.362 (1.514–3.684)	0.226	
PTx A-dione (≥222.2 vs <222.2 pg/ml)	<0.001	2.362 (1.514–3.684)	0.232	

HR = hazard ratio; CI = confidence interval, PSA = prostate-specific antigen; ECOG = Eastern Cooperative Oncology Group; ADT = androgen deprivation therapy; PTx = before treatment with enzalutamide; DHT = dihydrotestosterone; A-dione = androstenedione, DHEA-S = dehydroepiandrosterone sulfate.

Table 5 – Cox regression analysis of overall survival

Variable	Univariate analysis		Multivariate analysis	
	p value	HR (95% CI)	*p* value	HR (95% CI)
Age (≤73.0 vs >73.0 yr)	0.259			
Baseline PSA (≤23.0 vs >23.0 ng/ml)	0.001	2.637 (1.472–4.725)	0.168	
Curative treatment history (yes vs no)	0.334			
Gleason ≤7 vs Gleason ≥8	0.446			
Gleason ≤8 vs Gleason ≥9	0.637			
ECOG performance status (0 vs 1–2)	<0.001	2.830 (1.607–4.981)	0.005	2.351 (1.292–4.276)
Initial ADT response duration (>12 vs ≤12 mo)	0.430			
Docetaxel treatment (no vs yes)	0.001	2.631 (1.509–4.588)	0.002	2.593 (1.427–4.715)
Visceral metastasis at baseline (no vs yes)	0.016	2.871 (1.215–6.786)	0.003	4.312 (1.652–11.257)
Baseline extent of disease (0–2 vs 3–4)	0.005	2.514 (1.329–4.753)	0.004	2.879 (1.390–5.962)
Hemoglobin (≥11.4 vs <11.4 g/dl)	<0.001	2.83 (1.607–4.981)	<0.001	3.540 (1.797–4.276)
Alkaline phosphatase (<277 vs ≥277 IU/l)	0.545			
Lactate dehydrogenase (<221 vs ≥221 IU/l)	0.059			
Albumin (≥3.9 vs <3.9 g/dl)	0.024	1.927 (1.091–3.043)	0.051	
PTx testosterone (≥49.0 vs <49.0 pg/ml)	0.004	2.314 (1.309–4.091)	0.106	
PTx DHT (≥5.8 vs <5.8 pg/ml)	0.001	2.65 (1.498–4.687)	0.597	
PTx DHEA-S (≥326.3 vs <326.3 pg/ml)	0.013	2.035 (1.158–3.577)	0.280	
PTx A-dione (≥222.2 vs <222.2 pg/ml)	0.013	2.039 (1.165–3.570)	0.061	

HR = hazard ratio; CI = confidence interval; PSA = prostate-specific antigen; ECOG = Eastern Cooperative Oncology Group; ADT = androgen deprivation therapy; PTx = before treatment with enzalutamide; DHT = dihydrotestosterone; A-dione = androstenedione; DHEA-S = dehydroepiandrosterone sulfate.

Table 6 – Binomial logistic analysis of the predictors of any grade of anorexia, malaise, and fatigue

Variable	Univariate analysis		Multivariate analysis	
	p value	HR (95% CI)	*p* value	HR (95% CI)
Age (≤73.0 vs >73.0 yr)	0.562			
Baseline PSA (≤23.0 vs >23.0 ng/ml)	0.768			
Curative treatment history (yes vs no)	0.177			
Gleason ≤7 vs Gleason ≥8	0.071			
Gleason ≤8 vs Gleason ≥9	0.447			
ECOG performance status (0 vs 1–2)	0.752			
Initial ADT response duration (>12 vs ≤12 mo)	0.744			
Docetaxel treatment (no vs yes)	0.313			
Visceral metastasis at baseline (no vs yes)	0.030	3.625 (1.132–11.609)	0.025	4.011 (1.190–13.519)
Baseline extent of disease (0–2 vs 3–4)	0.052	2.754 (0.991–7.655)	0.221	
Hemoglobin (≥11.4 vs <11.4 g/dl)	0.320			
Alkaline phosphatase (<277 vs ≥277 IU/l)	0.477			
Lactate dehydrogenase (<221 vs ≥221 IU/l)	0.887			
Albumin (≥3.9 vs <3.9 g/dl)	0.049	2.400 (1.005–5.734)	0.276	
PTx testosterone (≥49.0 vs <49.0 pg/ml)	0.017	2.889 (1.205–6.926)	0.658	
PTx DHT (≥5.8 vs <5.8 pg/ml)	0.002	4.025 (1.643–9.860)	0.002	4.302 (1.703–10.869)
PTx DHEA-S (≥326.3 vs <326.3 pg/ml)	0.002	4.103 (1.649–10.207)	0.120	
PTx A-dione (≥222.2 vs <222.2 pg/ml)	0.005	3.538 (1.451–8.630)	0.150	

HR = hazard ratio; CI = confidence interval; PSA = prostate-specific antigen; ECOG = Eastern Cooperative Oncology Group; ADT = androgen deprivation therapy; PTx = before treatment with enzalutamide; DHT = dihydrotestosterone; A-dione = androstenedione; DHEA-S = dehydroepiandrosterone sulfate.

therapy is complete. The underlying mechanism remains poorly understood. Feng et al [27] showed that combination ADT and radiation therapy worsened fatigue and was associated with anemia and mitochondrial dysfunction. By contrast, Bandara et al [28] used multivariate models with fatigue as the outcome and found that neither hemoglobin nor T levels had a significant within-patient effect on fatigue developing during ADT/radiation therapy. Further study on this issue is necessary.

Our work has several limitations. One of the main limitations of the study is the low number of patients included, so the multivariate analysis has limited meaning. The multivariable analysis in our study was considered as exploratory and hypothesis-generating. In terms of blood androgen measurements, it was not possible to collect samples from patients exhibiting early progression (within 12 or 24 wk). In addition, the inclusion of patients with and without prior DOC therapy might have affected the outcomes of ENZ treatment. ENZ was approved in Japan only as our study commenced. The number and degree of adverse events were lower than in previous reports [4,5], and may have affected of the relationship between androgen levels and fatigue, anorexia, and malaise. LC-MS/MS measurement of androgen levels is very costly, precluding routine clinical application of this approach.

5. Conclusions

In conclusion, we studied androgen kinetics during ENZ administration. Pretreatment androgen levels usefully predicted treatment efficacy, prognosis, and the incidence of adverse events for patients with mCRPC treated with ENZ.

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

Study concept and design: Miyazawa, Suzuki.
Acquisition of data: Miyazawa, Nakamura, Takezawa, Shimizu, Matsuo, Ogura, Takei.
Analysis and interpretation of data: Miyazawa, Arai, Sekine.
Drafting of the manuscript: Miyazawa.
Critical revision of the manuscript for important intellectual content: Miyazawa.
Statistical analysis: Miyazawa.
Obtaining funding: Suzuki.
Administrative, technical, or material support: Suzuki.
Supervision: Suzuki.
Other: None.

Financial disclosures: Yoshiyuki Miyazawa 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: Kazuhiro Suzuki has acted as a consultant for Takeda Pharmaceutical, Astellas Pharma, Daiichi-Sankyo, AstraZeneca, Sanofi, Janssen, and Bayer, and has received grants from Takeda Pharmaceutical, Astellas Pharma, Daiichi-Sankyo, and Ono Pharmaceutical. The remaining authors have nothing to disclose.

Funding/Support and role of the sponsor: Our research funded by Astellas Pharma (Tokyo, Japan). The sponsor played a role in approval of the manuscript.

Ethics statement: We explained the study to all participants and all agreed in writing to participate. This study was approved by the institutional review board at Gunma University Hospital (approval no. 1177). There are no administrative permissions or licenses to access the data to formally note. The study was conducted according to the International Conference on Harmonization/Good Clinical Practice (ICH/GCP) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.euros.2021.05.003.

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