

# Role of free testosterone levels in patients with metastatic castration-resistant prostate cancer receiving second-line therapy

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**Abstract.** A range of new treatment options has recently become available for patients with advanced metastatic castration-resistant prostate cancer (mCRPC). Androgen deprivation therapy (ADT) with luteinizing hormone-releasing hormone is continued when performing chemotherapy or androgen deprivation with new second-generation therapeutic agents such as enzalutamide or abiraterone acetate. Despite the fact that free testosterone (FT) is the biologically active form, it is common practice that androgen suppression is monitored via total testosterone levels only. The aim of the present study was to evaluate the role of FT as a prognostic biomarker for cancer-specific survival (CSS) and its feasibility as an ADT monitoring biomarker in patients with mCRPC for the first time. The requirement for continued ADT in mCRPC patients is discussed within the basis of the current literature. A total of 34 patients with continuous measurements of FT levels and mCRPC status underwent therapy with docetaxel, abiraterone acetate, enzalutamide, cabozantinib, carboplatin or cabazitaxel. Data were obtained from the Departments of Urology and Urological Oncology, Hannover Medical School (Hannover, Germany) between March 2009 and April 2014. A cutoff point of 0.5 pg/ml was used to discriminate between patients according to FT levels. Statistical evaluation of CSS was performed by applying Kaplan Meier survival estimates, multivariate Cox regression analyses and log-rank tests. The median age of all 34 patients was 72 years (range, 51-86 years). The mean follow-up interval was 16.1 months (range, 0.7-55.6 months). Despite the fact

that all patients were undergoing androgen deprivation, the mean serum FT levels for each patient varied; the mean FT concentration in the cohort was 0.328 pg/ml, ranging from 0.01-9.1 pg/ml. A notable difference with regard to CSS was observed for patients with regard to serum FT concentration; CSS was significantly longer for patients with a serum FT level below the cutoff level (43.6 vs. 17.3 months, respectively,  $P=0.0063$ ). Upon multivariate Cox regression analysis, the mean FT concentration during treatment remained a significant prognostic factor for CSS (hazard ratio, 1.22; 95% confidence interval, 1.03-1.43;  $P=0.0182$ ). In conclusion, in patients with mCRPC, the serum FT level is a strong predictor of CSS in patients under therapy with second-line anti-hormonal therapeutic medication and chemotherapy. It may be concluded that FT levels should be included into the routine control of androgen suppression while under treatment with ADT and second-generation hormonal therapy.

## Introduction

Despite various established and curatively intended therapeutic regimens, new options for chemotherapy and novel modes of androgen deprivation, >70,000 patients succumb to prostate cancer each year in Europe (1). Huggins *et al* (2) described the dependence of prostate cancer on androgen levels in 1941. This finding marked the beginning of systemic and targeted treatment for advanced and metastasized adenocarcinoma of the prostate (3). To date, the backbone for the initial systemic treatment of prostate cancer is androgen deprivation therapy (ADT) (4). Androgen suppression, however, is associated with adverse clinical effects for the patient (5) and invariably leads to the resistance to androgen deprivation and the progression of the disease over time (6). The term 'hormone-refractory' or '-resistant' prostate cancer was used to describe progressing prostate cancer under ADT, which appeared to grow independently from androgen manipulation. With today's understanding of prostate tumor biology, the term has eventually been adapted to 'castration-resistant prostate cancer' (CRPC), indicating that progression remains driven by androgen signaling at the castration-resistant stage. CRPC is the current and recommended term established by

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the Prostate Cancer Working Group 2 (PCWG 2) (7). The new understanding of castration-resistant disease has led to the development and implementation of second-generation androgen ablative regimens, the most established of which thus far are the two orally administered substances abiraterone acetate and enzalutamide (8,9). Aside from advances in hormone ablative therapy, chemotherapeutic options have also expanded, including the introduction of cabazitaxel for the treatment of docetaxel-resistant prostate cancer, which showed a survival benefit in the preceding TROPIC trial (10). Prostate cancer at the metastatic CRPC (mCRPC) stage progresses apparently independent of conventional ADT. However, it is common practice that ADT is continued when switching to chemotherapy or second-line hormone manipulation with abiraterone acetate or enzalutamide. The monitoring of androgen suppression is normally achieved by measuring total testosterone levels, however, the biologically active androgen is free testosterone (FT), which only comprises 1-2% of total testosterone (11,12).

Discontinuation of luteinizing hormone-releasing hormone (LHRH) therapy would reduce treatment costs, as well as the incidence of adverse events attributed to LHRH therapy (5). The question of whether conventional ADT may be omitted in progressive prostate cancer remains under debate. This question will be addressed for abiraterone acetate in the ongoing SPARE trial (13). To date, there is no reliable clinical data on patients with second-generation ADT and discontinuation of LHRH-analogue therapy. The present study analyzed a series of patients with advanced mCRPC receiving second-line chemotherapy and/or second generation ADT with regard to FT serum levels and evaluated the effect of FT on cancer-specific survival (CSS).

## Patients and methods

**Patient selection.** Patients were followed up between March 2009 and April 2014. Patients were deemed eligible for this retrospective study if they had histologically confirmed mCRPC. All patients were androgen ablated with an LHRH agonist, with the exception of 2 patients who underwent a bilateral subcapsular orchiectomy. ADT was continued throughout the follow-up. FT represents the biologically active fraction of total testosterone (11). Out of 4,642 patients from the Departments of Urology and Urological Oncology, Hannover Medical School (Hannover, Germany) database, only 34 exhibited CRPC and were monitored with the inclusion of FT level. Levels of FT were measured in the morning. Patients receiving 1,000 mg/day abiraterone acetate received concomitant steroid medication with 10 mg prednisolone per day. The Eastern Cooperative Oncology Group (ECOG) status at the beginning of the follow-up was 0 for all patients (14), with the exception of 2 (1 patient with an ECOG score of 1 and 1 patient with an ECOG score of 2). Patients received abiraterone acetate during the compassionate use program, which was approved by the Hannover Medical School Ethics Committee (Hannover, Germany). Carboplatin AUC5 plus docetaxel at a dose of 35 mg/m<sup>2</sup> was used as a salvage chemotherapy option after failure of docetaxel chemotherapy (15). Cabozantinib was administered to patients participating in the COMET-1 trial (phase III, cabozantinib vs. prednisone).

Prostate-specific antigen (PSA) measurements and testing of FT concentration was performed at Hannover Medical School exclusively.

**Laboratory measurements.** For the measurement of FT concentration, an enzyme immunoassay was applied for the quantitative determination of FT (IBL International GmbH, Hamburg, Germany). To determine the serum PSA concentration, an Electrochemiluminescence Immunoassay (Cobas® 6000, Roche Diagnostics, Rotkreuz, Switzerland) was used. The PCWG-2 criteria were applied to define the progression of the cancer (7). Change of therapy under follow-up was allowed on progression of the disease while continuing constant androgen ablative therapy (7).

**Statistical analysis.** Survival rates were estimated using the Kaplan-Meier method. The log-rank test was applied for comparing survival between patients with different mean FT concentrations. Hazard ratios for the prediction of CSS were calculated using multivariate Cox regression with Efron's approximation. Likelihood ratio-, Wald- and score (log-rank) tests were applied to test the effect of covariates of the Cox regression model. Proportionality of all predictor variables were tested using Pearson's product-moment correlation between the scaled Schoenfeld residuals and time for each covariate. All mortalities during observation were attributable to the underlying prostate cancer disease, hence no patient had to be censored for competing causes of mortality.  $P < 0.05$  was considered to indicate a statistically significant difference. Statistical analyses and graphical illustrations were performed using R statistical software (R version 3.0.3; R Foundation for Statistical Computing, Vienna, Austria). Patient characteristics are depicted in Table I.

**Ethics and approval.** The present study was performed in accordance with all ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Ethics board approval was obtained for this observational retrospective study. All patient data was anonymized prior to statistical analysis. No additional data was created nor used aside from the retrospective evaluation of our database.

## Results

In total, 34 patients with mCRPC were eligible and had sufficient follow-up of serum FT values. The median follow-up time was 16.1 months (range, 0.7-55.6 months) and the median patient age was 72 years (range, 51-86 years). The mean FT concentration in the cohort was 0.328 pg/ml. Despite the fact that all patients were under continuous ADT, mean FT levels for each patient varied, ranging from 0.01-9.1 pg/ml, with a variance of 0.4 pg/ml. A total of 17 patients succumbed during follow-up. Median survival over all patients according to Kaplan-Meier survival estimation was 32.8 months [95% confidence interval (CI), 17-not available (NA)], as shown in Fig. 1. Mean PSA correlated with CSS (log-rank test,  $P = 0.0063$ ), which was expected since all patients succumbed during the progression of the disease. Mean PSA was 264.2 ng/ml (range, 1.9-1,486.2 ng/ml). All mortalities were associated with a rising PSA at the end of follow-up. The mean PSA value was associated with mortality

Table I. Patient characteristics: Pathology and previous treatment.

| Characteristic             | Value              |
|----------------------------|--------------------|
| Patients, n (%)            | 34 (100.0)         |
| Median age (range), years  | 72 (51-86)         |
| Primary therapy, n (%)     |                    |
| Retropubic prostatectomy   | 19 (55.9)          |
| Laparoscopic prostatectomy | 2 (5.9)            |
| External beam radiation    | 3 (8.8)            |
| LDR brachytherapy          | 1 (2.9)            |
| No primary therapy         | 8 (23.5)           |
| NA                         | 1 (2.9)            |
| TNM stage, n (%)           |                    |
| T2a                        | 1 (2.9)            |
| T2b                        | 1 (2.9)            |
| T2c                        | 2 (5.9)            |
| T3a                        | 4 (11.8)           |
| T3b                        | 13 (38.2)          |
| T4a,b                      | 4 (11.8)           |
| NA                         | 9 (26.5)           |
| Gleason score, n (%)       |                    |
| ≤6                         | 1 (2.9)            |
| 7                          | 10 (29.4)          |
| 8                          | 12 (35.3)          |
| 9                          | 5 (14.7)           |
| 10                         | 3 (8.8)            |
| NA                         | 3 (8.8)            |
| Hormonal therapy, n (%)    |                    |
| Orchiectomy                | 2 (5.9)            |
| ADT                        | 1 (2.9)            |
| CAB                        | 27 (79.4)          |
| Abiraterone                | 25 (73.5)          |
| Enzalutamide               | 24 (70.6)          |
| Chemotherapy, n (%)        |                    |
| Docetaxel                  | 31 (91.2)          |
| Carboplatin+docetaxel      | 19 (55.9)          |
| Cabazitaxel                | 8 (23.5)           |
| Cabozantinib               | 3 (8.8)            |
| Mean PSA (range), pg/ml    | 182.8 (1.9-1486.2) |

NA, data not available; ADT, androgen deprivation therapy; CAB, complete androgen blockade; PSA, prostate-specific antigen; TNM, tumor-node-metastasis.

in the multivariate Cox model for patients with a mean PSA of  $\geq 200$  ng/ml [hazard ratio (HR), 4.6; 95% CI, 1.1-19.3;  $P=0.0354$ ]. A notable difference with regard to CSS was observed for patients with FT concentrations below and above 0.5 pg/ml: Median survival for patients with a FT level below and above the cutoff were 43.6 months (range, 31.5-NA months) and 17.3 months (range, 12.7-NA months), respectively (log-rank test,  $P=0.0063$ ). The Kaplan-Meier estimation of survival according to FT serum concentration is depicted in Fig. 2. When

Table II. HRs, 95% CIs and P-values for the multivariate Cox regression analysis on cancer-specific survival for patients with metastatic castration-resistant prostate cancer.

| Variable      | HR   | 95% CI     | P-value             |
|---------------|------|------------|---------------------|
| FT            | 1.22 | 1.03-1.43  | 0.0182 <sup>a</sup> |
| PSA           | 4.63 | 1.11-19.28 | 0.0354 <sup>a</sup> |
| Age           | 1.03 | 0.95-1.12  | 0.4293              |
| Gleason score | 1.35 | 0.69-2.66  | 0.3854              |
| ECOG          | 1.08 | 0.15-7.6   | 0.9391              |

<sup>a</sup> $P<0.05$ . FT, free testosterone; PSA, prostate-specific antigen; ECOG, Eastern Cooperative Oncology Group; CI, confidence interval; HR, hazard ratio.

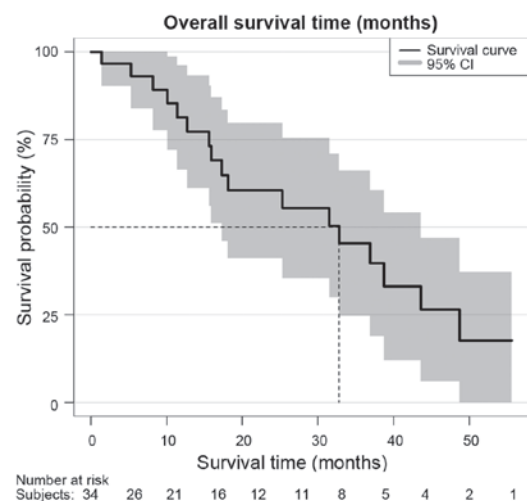


Figure 1. Kaplan Meier estimation of survival based on cancer-specific survival data of all 34 patients with metastatic castration-resistant prostate cancer, showing a limited median life expectancy of 32.8 months (range, 17-not available). CI, confidence interval.

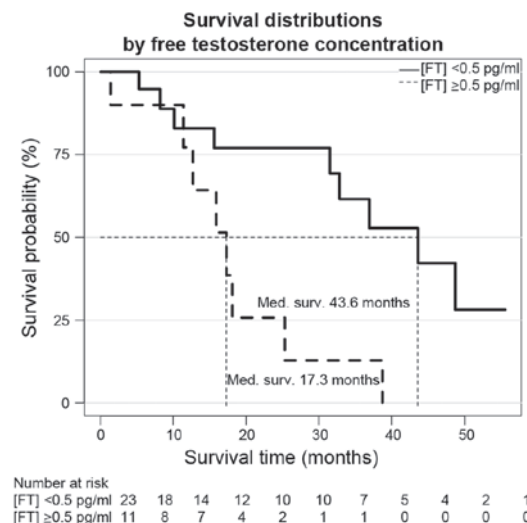


Figure 2. Kaplan Meier survival estimation of all patients according to FT level; dichotomization at a cutoff at 0.5 pg/ml was applied. The median FT concentration was 0.33 pg/ml. Median survival time for patients with FT levels below and above the cutoff were 43.6 and 17.3 months, respectively (log-rank test,  $P=0.0063$ ). FT, free testosterone.

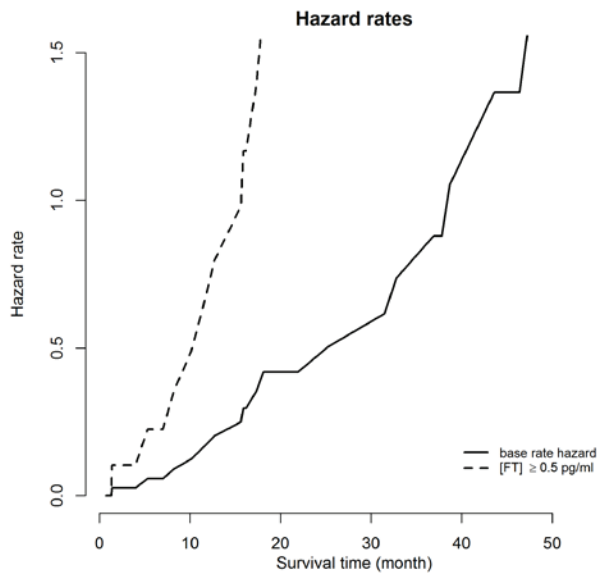


Figure 3. Base rate hazard and hazard rate for patients with a mean FT concentration. Cutoff,  $\geq 0.5$  pg/ml. FT, free testosterone.

applying multivariate Cox regression analysis, the HR for the risk of mortality for patients with FT concentrations  $\geq 0.5$  pg/ml was 1.2 (95% CI, 1-1.4;  $P=0.0182$ ); hazard rates and HRs are depicted in Figs. 3 and 4, respectively. For the covariates of patient age, primary Gleason score and ECOG performance status (14,16,17), there was no significant statistical association for the risk of mortality ( $P=0.4293$ ,  $P=0.3854$  and  $P=0.9391$ , respectively), as shown in Table II.

## Discussion

When prostate cancer progresses apparently independent of conventional hormonal manipulation, the question arises as to whether a conventional ADT regimen should be continued during second-line ADT. Abiraterone acetate and enzalutamide exert their effects on intracellular signaling by more substantial and firm effects on androgen biosynthesis and androgen receptor binding than their predecessors: LHRH analogues cause a downregulation of LHRH receptors in the pituitary gland, thereby decreasing the release of gonadotropins and consecutively the production of testosterone; however, this effect on prostate neoplastic cells is confined to the hypothalamic-pituitary-gonadal axis (18). Abiraterone decreases serum testosterone and androgen levels by inhibiting  $17\alpha$ -hydroxylase/C17,20-lyase in steroid biosynthesis, and is not only limited to the testicular Leydig cells, but is also exerting its effect in the adrenal gland and in prostate cancer cells (19). First-generation non-steroidal anti-androgens, including flutamide and bicalutamide, block the androgen receptor, thereby inhibiting intracellular signaling. Enzalutamide has a higher binding affinity to the androgen-receptor, and it not only acts competitively at the receptor level, but also blocks the activation of androgen-responsive genes and inhibits the preceding translocation of the homodimerized receptor-ligand (20). Applying LHRH analogues or non-steroidal anti-androgens while administering abiraterone acetate or enzalutamide may therefore appear redundant when considering modes of action for these substances. The

concept of continued conventional ADT and serum testosterone level monitoring with regard to clinical parameters and overall survival (OS), originates from a notable study by Perachino *et al*, showing a clear association between OS and serum testosterone levels measured 6 months after initiation of ADT (21). The study was based on previous results of a study by Morote *et al*, in which it was deduced that from a cohort of 73 patients,  $\sim 25\%$  of all men being treated with LHRH-depot injection exhibited testosterone levels higher than the formerly recommended serum level of 0.5 ng/ml. The study found a direct correlation between 'androgen-independent' (originally used expression) progression and serum testosterone levels. It was also able to show that breakthrough increases of testosterone levels during LHRH agonist therapy exhibited a markedly negative effect on 'androgen-independent' progression. The mean survival time, free from 'androgen-independent' progression, was 137 months for the subgroup of patients without breakthrough increases of testosterone and it decreased to 88 months for patients with breakthrough increases of  $>32$  ng/dl (22).

The study by Byar was also able to show a contributing effect of insufficient androgen suppression on overall mortality, however, this effect was observed with the administration of diethylstilbestrol (23), and unlike our current study, not with LHRH analogues in conjunction with abiraterone acetate or enzalutamide.

Other retrospective studies evaluating the positive effects of continued ADT therapy in patients with CRPC have also shown survival advantages for patients who sustained LHRH analogue therapy (24,25). These findings clearly emphasize the requirement for laboratory monitoring of ADT therapy, however, the aforementioned studies were undertaken a long time prior to the advent of second-line anti-androgens. Also, the previously mentioned studies by Morote *et al* (22) and others, used total testosterone, which is easier to measure than FT. In the current study, an emphasis was placed on FT, which is the active fraction responsible for biological activity (11). Notably, there is not yet much data on FT with regard to prostate cancer.

A more recent finding that does potentially support the continuation of LHRH therapy, including serum testosterone measurements on a regular schedule, was derived from the COU-AA-301 study itself (26). Data from the trial, initially comparing the efficacy of abiraterone acetate plus low-dose prednisone versus prednisone only, was subsequently analyzed with regard to androgen dynamics in correlation with serum PSA: In an ultra-sensitive assay, PSA measurements showed a reduction to undetectable levels in 47% of patients in the abiraterone arm, while none of the patients continuing regular androgen deprivation exhibited serum testosterone levels below the detection threshold. The study compared androgen levels with radiographic progression-free survival and time to PSA progression, but found no significant correlation. However, unlike the present study, the measurements were timed exclusively 12 weeks after the initiation of therapy. Additionally, the focus was on total testosterone concentration and not FT levels. Nonetheless, these findings show that inadequate androgen suppression may and does occur in patients with inhibition of the hypothalamic-pituitary-gonadal axis, even when combined with

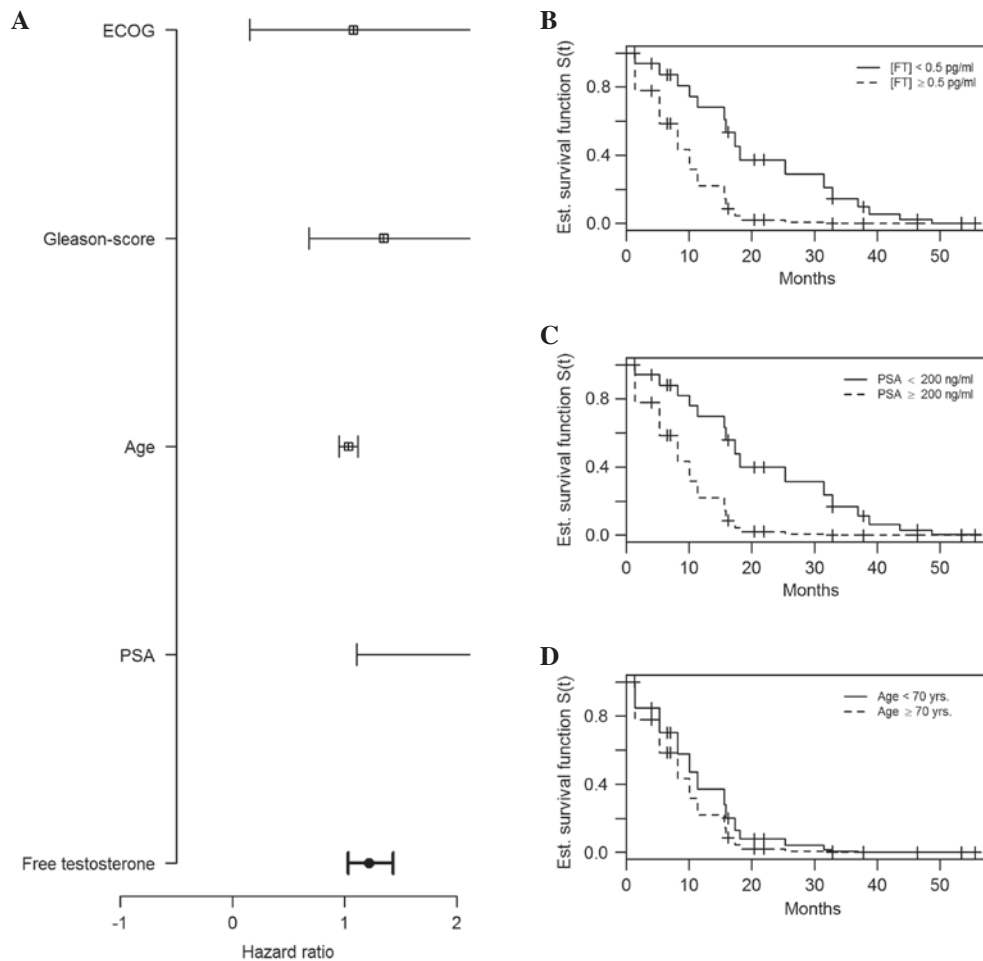


Figure 4. (A) Forest plot of hazard ratios for cancer-specific survival and corresponding 95% confidence intervals. Estimated survival function from multivariate Cox regression model for (B) FT concentration, (C) PSA  $\geq 200$  ng/ml and (D) patient age. FT, free testosterone; PSA, prostate-specific antigen; ECOG, Eastern Cooperative Oncology Group.

inhibitors of precursor steroid biosynthesis (27). Notably, 13-42% of patients under therapy with LHRH analogues fail to reach serum testosterone levels of  $< 0.5$  ng/ml (28). These involuntary elevations of serum testosterone may provide an insight as to what extent circulating androgens play a role in the advanced mCRPC setting when next-generation ADT is in place. The reason for the significant increases of androgen levels under therapy is not fully understood. Certain men may experience a surge in serum testosterone concentration while under long-term therapy with LHRH analogues. This phenomenon was previously described as the 'acute-on-chronic response' (29). Also, obese patients tend to have higher testosterone concentration levels under LHRH therapy than men with a normal body mass index (30). Other reasons for insufficient androgen suppression are a faulty preparation of the LHRH depot injection or inadvertent discontinuation of therapy. While FT and total testosterone concentrations can frequently be assessed, the aforementioned reasons for insufficient androgen suppression cannot adequately be identified or monitored. The present study showed a variance in FT serum concentration. However, it did not provide a clear explanation for these surges in FT levels. One possible incentive for the continuation of conventional ADT may lay in the assumption of a broader mode of

therapy in the castration-resistant state. The mechanisms that lead to the castration-resistant state are numerous and have been subjected to extensive research in the past. Intracellular cell signaling promoting growth and tumor progression may continue by means of 'bypass' or 'outlaw' pathways, even without the binding of the androgen receptor ligand. One example for these mechanisms is the expression of B-cell lymphoma 2, which is a critical anti-apoptotic protein in CRPC and prostate cancer in general (31). Another example is the Akt signaling cascade (32) or the overexpression of human epidermal growth factor receptor-2/neu tyrosine kinase. The latter is able to boost prostate cancer growth and androgen receptor signaling independently of the androgen ligand binding to the receptor (33). These models for the alternate activation of prostate cancer cells appear to be independent of androgen signaling and do not give a clear justification for the continuation of LHRH therapy in the mCRPC state. Androgen receptor splice variant-7 (AR-V7), presented at the 2014 Genitourinary Cancer Symposium annual meeting, lacks the ligand-binding domain for enzalutamide, but it remains active as a transcription factor. PSA response rates were 0% for abiraterone and enzalutamide in patients with the AR-V7 splice variant, which directly translated into shorter progression-free survival times (34).

However, these data do neither support nor negate the beneficial effect of an ongoing conventional ADT while starting with enzalutamide or abiraterone acetate. Free androgen levels appear to significantly affect CSS, as shown in the current study. This indicates that progression in a cohort of patients with mCRPC is not merely driven by escape mechanisms and resistance completely independent of androgen signaling, but is dependent on serum FT levels, even with the combination of conventional ADT and second-generation hormone manipulation. This finding can be explained through clonal heterogeneity or by resistance mechanisms that rely on FT, such as androgen receptor overexpression (35,36). We hypothesize that one possible argument in favor for continuing conventional ADT, while administering enzalutamide or abiraterone acetate, may be an overlap in treatment that could potentially reduce the risk of FT surges due to accidental pauses of treatment. One of the most notable studies with regard to LHRH therapy during second-line hormonal manipulation was conducted by Pinski *et al*, which showed that LH receptors exist on prostate cancer cells and stimulate cancer growth by increasing intrinsic steroidogenesis (37). This finding would be an argument in favor for the continuation of conventional LHRH analogue therapy. The inhibition of steroid biosynthesis in addition to LHRH analogue therapy is not new; it was used even prior to the advent of abiraterone acetate, when ketoconazole was combined with complete androgen blockade resulting in a markedly lower testosterone concentration when compared to complete androgen blockade alone (38). Probably the most important argument in favor of the continued use of LHRH analogue therapy in the mCRPC state is, however, the lack of clinical studies with regard to survival. The current study, therefore, may represent one of the first pieces of clinical evidence on the topic.

The current study was limited by its retrospective design and the heterogeneity of treatments that, however, reflect the therapeutic reality of patients with mCRPC today.

In conclusion, patients with advanced mCRPC who have progressed under conservative ADT have FT as a significant predictor of CSS, even in the sequence of second-generation ADT (abiraterone acetate or enzalutamide) and chemotherapy. The present findings support the recommendation that LHRH-analogue therapy and measurements of androgen suppression on a regular basis should not be omitted in this setting.

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