PHARMACOTHERAPY

Exposure-Response Assessment of Enzalutamide and Its Major Metabolites in a Real-World Cohort of Patients with Metastatic Castration-Resistant Prostate Cancer

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Study Objective Enzalutamide is an oral agent for the treatment of metastatic castration-resistant prostate cancer (mCRPC); *N*-desmethyl enzalutamide is its active metabolite, which has clinically relevant anti-androgen capacities similar to enzalutamide, and carboxylic acid enzalutamide is an inactive metabolite. The aim of our study was to investigate the relationship between enzalutamide and *N*-desmethyl enzalutamide exposure and treatment response in a real-world cohort of patients with mCRPC.

Design Retrospective, observational, pharmacokinetic study.

Setting Outpatient clinic at a tertiary cancer center in Amsterdam, the Netherlands.

Patients Sixty-five patients with mCRPC who were treated with enzalutamide 160 mg daily and had at least one steady-state enzalutamide plasma concentration between May 2015 and June 2018; of these patients, 38 were prostate-specific antigen (PSA) responders and 27 were nonresponders.

Measurements and Main Results Plasma concentrations, determined by using liquid chromatography with tandem mass spectrometry (LC-MS/MS), were compared between PSA responders and nonresponders. Three clinical end points were evaluated separately in this study: PSA-independent progression-free survival (PFS), time to PSA progression (TTPP), and rate of PSA response (defined as ≥ 50% decrease in PSA level from baseline). Enzalutamide toxicity was defined as discontinuation due to adverse events, dose reductions due to adverse events, or temporary treatment interruption. For these analyses, plasma concentrations of enzalutamide and *N*-desmethyl enzalutamide were divided into quartiles. Mean ± SD plasma concentrations in the 65 patients were as follows: enzalutamide 11.2 ± 2.8 μg/ml, *N*-desmethyl enzalutamide 9.9 ± 2.9 μg/ml, and carboxylic acid enzalutamide 6.1 ± 4.3 μg/ml. Plasma concentrations were not significantly different in the PSA responder versus nonresponder groups for enzalutamide (11.5 vs 10.6 μg/ml, p=0.20), *N*-desmethyl enzalutamide (10.1 vs 9.6 μg/ml, p=0.48), and carboxylic acid enzalutamide (6.5 vs 5.5 μg/ml, p=0.34). Univariate and multivariate analyses did not show a relationship between plasma concentrations and PSA-independent PFS, TTPP, or toxicity.

Conflict of interest: The authors have declared no conflicts of interest for this article.

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CONCLUSION This study confirmed that enzalutamide plasma concentrations were not related to PSA-independent PFS, TTPP, or toxicity in patients with mCRPC, and demonstrated that plasma concentrations of its major metabolites were also not associated with treatment response. Based on these findings, there is no role for therapeutic drug monitoring of enzalutamide in patients with mCRPC in daily practice.

KEY WORDS enzalutamide, metabolites, prostate-specific antigen, exposure, response, toxicity.
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Introduction

Enzalutamide is a potent inhibitor of the androgen receptor, blocking multiple steps in the androgen signaling pathway; it competitively inhibits androgen binding to the androgen receptor, prevents nuclear translocation of the androgen receptor, and inhibits receptor association with DNA.[1] Enzalutamide was approved for the treatment of metastatic castration-resistant prostate cancer (mCRPC) because it improved overall survival (OS) and progression-free survival (PFS) in this patient population.[2, 3]

After oral ingestion, enzalutamide is converted into its major metabolites, *N*-desmethyl enzalutamide and carboxylic acid enzalutamide, by cytochrome P450 (CYP) 3A4/5 and CYP2C8, respectively.[1] *N*-Desmethyl enzalutamide has clinically relevant anti-androgen capacities similar to enzalutamide, whereas carboxylic acid enzalutamide is inactive. Enzalutamide is administered orally once daily in a fixed dose of 160 mg and is well tolerated in clinical practice.[4] Mean ± SD steady-state trough concentrations (C_{min}) at this approved dose are 11.4 ± 3.0 μg/ml for enzalutamide, 13.0 ± 3.8 μg/ml for *N*-desmethyl enzalutamide, and 8.4 ± 6.8 μg/ml for carboxylic acid enzalutamide.[5] In a phase III pivotal trial, no significant enzalutamide exposure-response relationship was identified for the primary efficacy endpoint of OS, as all exposure quartiles performed uniformly better relative to placebo ($p \leq 0.0001$).[1] Unfortunately, measurement of the active metabolite *N*-desmethyl enzalutamide concentration was not included in that study; however, due to its similar potency to enzalutamide and high abundance, an exposure-response analysis would be justified as well. In a phase I trial, positron emission tomography imaging with 16β-^{18}F-5α-dihydrotestosterone showed higher androgen receptor binding at enzalutamide 150 mg once daily compared to 60 mg once daily, with corresponding C_{min} concentrations of 11.4 and 5.0 μg/ml, respectively.[6] At doses above 150 mg once daily, plasma concentrations did not further increase, suggesting a concentration plateau at ~ 11 μg/ml. Based on these findings, our research group previously suggested a C_{min} of 5.0 μg/ml as a target for exposure to enzalutamide in a therapeutic drug monitoring (TDM) setting.[7]

Although the phase IIII pivotal trial suggested that there is no exposure-response relationship for enzalutamide,[1] there is lack of real-world data from daily clinical practice to underscore these findings. Obtaining real-world data is relevant in medical research because patients from clinical trials may not fully reflect the patient population, given the clear set of inclusion and exclusion criteria such as having comorbidities or the use of concomitant medications. Furthermore, the pivotal trial did not include exposure to the major metabolites in these analyses. Given the limited data from clinical practice, to our knowledge, we are the first to investigate the exposure-response relationship of enzalutamide and its major metabolites. Specifically, the objective of our study was to investigate the relationship between enzalutamide and *N*-desmethyl enzalutamide exposure and treatment response in a real-world cohort of patients with mCRPC.

Methods

Study Design, Patient Population, Sampling, and Data Collection

This retrospective, observational, pharmacokinetic study was performed at the outpatient clinic of the Antoni van Leeuwenhoek/Netherlands Cancer Institute (Amsterdam, the Netherlands). Patients with mCRPC who were treated with enzalutamide 160 mg once daily and had at least one steady-state enzalutamide plasma concentration between May 2015 and June 2018 were included in the study. As part of routine clinical care, dipotassium ethylenediaminetetraacetic acid samples were collected for pharmacokinetic monitoring at each hospital visit from these patients. The frequency of the outpatient visits and blood

sample collection were at the discretion of the treating physician. Data from routine clinical care were used retrospectively, as authorized by the Institute, and data on the following demographic and clinical characteristics were collected from medical records: demographics, medical history, enzalutamide dose, treatment duration, reason for enzalutamide discontinuation, time of prostate-specific antigen (PSA)-independent progression and time of PSA progression, concomitant medications, and PSA levels. Furthermore, testosterone and androstenedione levels were quantified by using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) assay.[8] The extent of enzalutamide adherence was not available due to the retrospective character of this analysis; however, plasma levels may serve as a potential indicator of compliance.

Determination of Enzalutamide and Metabolite Plasma Concentrations

Blood samples were collected as part of routine clinical care. The date and time of the last drug intake and the time of blood withdrawal were recorded. Due to the long half-life of enzalutamide (5.8 days), steady-state was considered to be reached after at least 1 month of enzalutamide treatment.[1] Enzalutamide and its metabolites were measured by using a validated LC-MS/MS method.[9] In short, 50 μl of plasma was prepared using protein precipitation. Analytes were quantified using a Triple Quad 6500 (Sciex, Framingham, MA). The lower limit of quantification was 5 ng/ml for enzalutamide and carboxylic acid enzalutamide, and 10 ng/ml for *N*-desmethyl enzalutamide. Intra-assay and interassay variabilities were within ± 15% for all analytes. Measured plasma concentrations, collected at random time points during a dosing interval, were used in the further analyses without correction for time after intake, as the difference between maximum and minimum concentrations during a dosing interval at steady-state is negligible due to the long half-lives of enzalutamide and its metabolites. Samples collected before steady state or more than 24 hours after the last dose were excluded from further analysis.

Outcome Measures

Three clinical end points were evaluated separately in this study: PSA-independent progression-free survival (PFS), time to PSA progression (TTPP), and rate of PSA response. PFS was defined as the time from start of treatment to the first event of progression—either radiographic progression, symptomatic progression (start of radiotherapy, samarium treatment, increase of analgesic dose, or an Eastern Cooperative Oncology Group (ECOG)/World Health Organization (WHO) Performance Status increase of at least 2, onset of next treatment, or death from any cause. Radiographic progression was evaluated according to the modified Response Evaluation Criteria in Solid Tumors (RECIST version 1.1).[10] TTPP was defined as the time from treatment start to a 25% or greater PSA level increase from the nadir, with an absolute increase in PSA level of at least 2 ng/ml.[10] PSA response was defined as ≥ 50% decrease in PSA level from baseline, according to the Prostate Cancer Working Group 2 criteria.[11, 12] Toxicity was defined as discontinuation due to adverse events, dose reductions due to adverse events, or temporary treatment interruption.

Statistical Analysis

For the exposure-response analyses, the means of all available enzalutamide and metabolite levels per patient were used as the parameter for exposure. Univariate analysis of PSA response and plasma concentrations involved two-sided *t* tests. For the PFS and TTPP analyses, plasma concentrations were divided into quartiles. PFS functions were estimated by using the Kaplan–Meier method, and predictive factors were assessed by using the univariate model (log-rank test). All statistical analyses were performed by using R statistical software, version 3.6.0, package 'survival' (R Foundation for Statistical Computing, Vienna, Austria). In the multivariate analysis, age, ECOG/WHO Performance Status, previous lines of treatment, prior treatment with docetaxel and testosterone, and androstenedione levels were included as covariates.[1] A post hoc sample size analysis was conducted comparing low versus high C_{min} with 80% power using a two-sided significance level and log-rank test with equal groups (31 patients per group). The hazard ratio we would be able to detect in this PFS analysis with a survival rate of S1 = 0.2 and an increase in PFS to S2 = 0.45 was 0.42.

Results

Evaluable Patients

A total of 65 patients were included in this study. Patient characteristics are presented in

Table 1. The median duration of treatment was 9.1 months (range 0.8–35 months). All patients received enzalutamide in combination with a goserelin 10.8 mg subcutaneous depot injection every 12 weeks. Measured testosterone levels were below the castration level of 0.50 ng/ml, with a mean ± SD value of 0.10 ± 0.08 ng/ml. The mean ± SD level of androstenedione was 0.31 ± 0.25 ng/ml. At data cut-off, eight patients were still receiving treatment with enzalutamide. Three patients stopped treatment due to adverse events. No relevant CYP-inhibiting or CYP-inducing concomitant medications were used during this treatment period.

Pharmacokinetics

In total, 235 samples were collected, with a mean of 3 (range 1–11) samples per patient. Mean ± SD plasma concentrations were 11.2 ± 2.8 μg/ml for enzalutamide, 9.9 ± 2.9 μg/ml for *N*-desmethyl enzalutamide, and 6.1 ± 4.3 μg/ml for carboxylic acid enzalutamide. Interpatient variability (coefficient of variation [CV%]) of mean plasma concentrations was of 28% for enzalutamide, 31% for *N*-desmethyl enzalutamide, and 67% for carboxylic acid enzalutamide. The mean intrapatient variability (CV%) was 18% for enzalutamide, 19% for *N*-desmethyl enzalutamide, and 44% for carboxylic acid enzalutamide.

Table 1. Baseline Characteristics of the Study Patients

Characteristic	Data
No. of patients	65
No. of samples	235
No. of samples per patient	3 (1–11)
Age (yrs)	69 (49–99)
Weight (kg)	91 (59–147)
Performance status	
0	19 (29)
1	35 (54)
2	7 (11)
Not reported	4 (6)
Previous lines of therapy	
0	32 (49)
1	20 (31)
2	9 (14)
3	2 (3)
4	2 (3)
Previous chemotherapy	30 (46)
Plasma concentrations (μg/ml)	
Enzalutamide	11 (3.3–18)
N-Desmethyl enzalutamide	9.9 (3.1–17)
Carboxylic acid enzalutamide	6.1 (1.1–22)
Patients with enzalutamide concentrations < 5 μg/ml	1 (1.5)
Testosterone level (ng/ml)	0.10 (< 0.010[a]–0.50)
Androstenedione level (ng/ml)	0.31 (< 0.010[a]–1.5)

Data are mean (range) values or no. (%) of patients unless otherwise specified.
ECOG/WHO = Eastern Cooperative Oncology Group/World Health Organization.
[a]Data points were below the lower limit of quantification of this bioanalytical method.

An overview of the distribution of mean enzalutamide, *N*-desmethyl enzalutamide, and carboxylic acid enzalutamide concentrations per patient is provided in Figure 1. One patient had an enzalutamide concentration below the proposed target of 5.0 μg/ml. This patient also had the lowest *N*-desmethyl enzalutamide concentration of 3.1 μg/ml. This patient received a reduced dose of enzalutamide 80 mg once daily due to the adverse events of fatigue and dyspnea. Two other patients received reduced doses of 80 and 120 mg once daily due to adverse events (fatigue, nausea, loss of appetite, and abdominal pain). While receiving treatment, these doses were increased back to the starting dose of 160 mg once daily since the drug became well tolerated by both patients. The mean enzalutamide plasma concentrations were 5.8 and 9.2 μg/ml at reduced doses, compared with 11.4 and 15.2 μg/ml at 160 mg once daily, respectively, in these two patients. Linear regression indicated that patients who were older had higher *N*-desmethyl enzalutamide and carboxylic acid enzalutamide concentrations (p=0.046 and p=0.00032).

Exposure-Response Analyses

At the time of analysis, the data from 62 patients were considered for calculation of PFS and TTPP. Three patients were excluded from the survival analysis since they discontinued treatment due to adverse events. Patients were divided into quartiles based on plasma concentrations of the active substances enzalutamide and *N*-desmethyl enzalutamide, and PFS analyses were performed using the data from these groups.

For PSA-independent PFS, the data from 62 patients with 54 events (87% of patients) were considered, with 36 radiographic progressions, 12 symptomatic progressions, 1 onset of next treatment, and 5 deaths. Eight patients were still receiving treatment. There was no significant difference among the four quartiles regarding PSA-independent PFS for enzalutamide (14 vs 11 vs 7.3 vs 13 months, p=0.44) and *N*-desmethyl enzalutamide (8.1 vs 13 vs 8.2 vs 13 months, p=0.33), as depicted in Figures 3

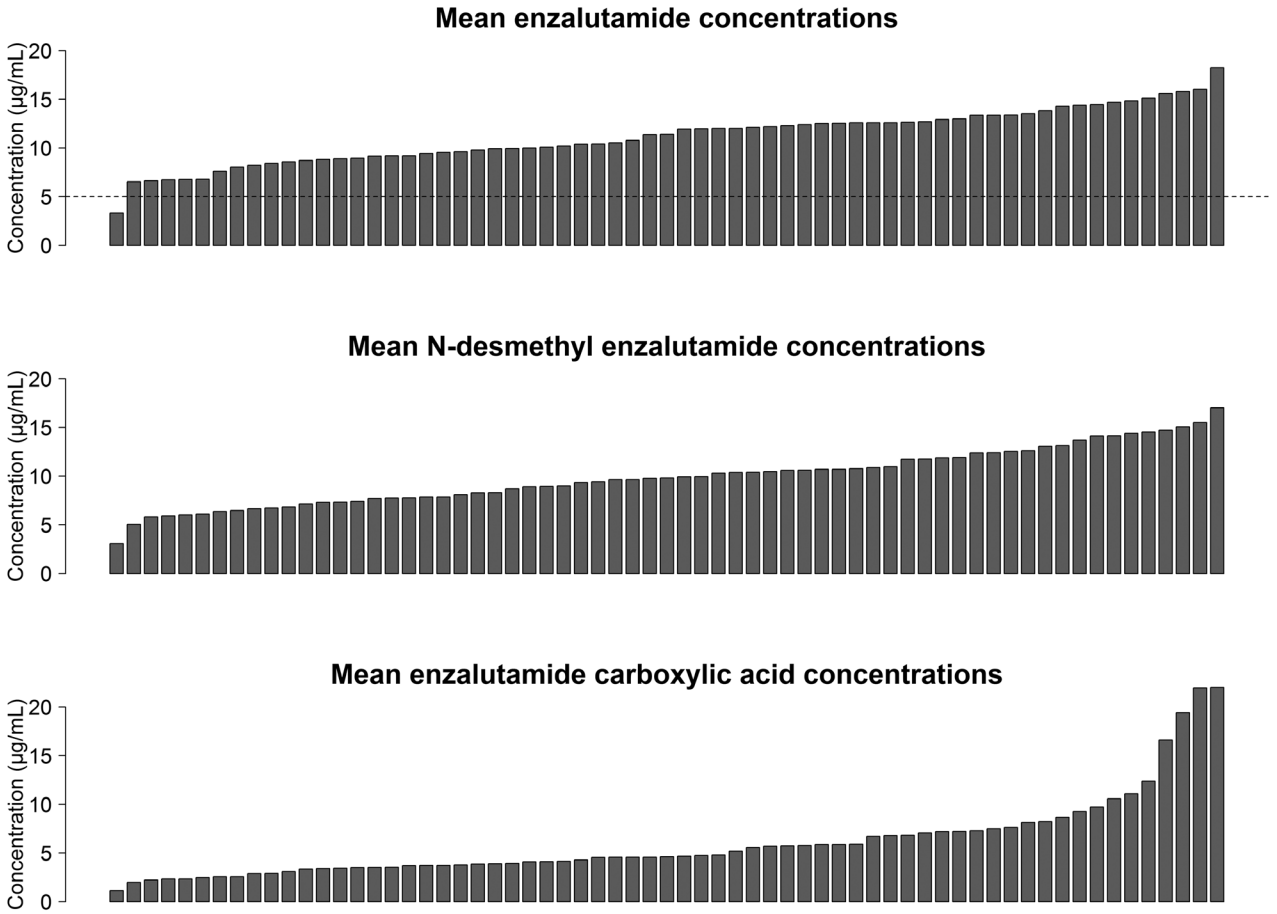

Figure 1. Distribution of plasma concentrations of enzalutamide (top panel), *N*-desmethyl enzalutamide (middle panel), and enzalutamide carboxylic acid (bottom panel) for the 65 study patients. The dotted line in the top panel represents the proposed target of 5.0 µg/ml for enzalutamide.

and 4, respectively. Furthermore, the sum of enzalutamide and *N*-desmethyl enzalutamide plasma concentrations was also not associated with PSA-independent PFS (12 vs 8.4 vs 9.2 vs 12 months, p=0.40) (Figure 5). In the univariate analysis, plasma concentrations of enzalutamide, *N*-desmethyl enzalutamide, and a combination of the two were not related to PFS. Similarly, no evidence to support a relationship between concentrations of enzalutamide, *N*-desmethyl enzalutamide, and a combination of both with PSA-independent PFS was found; the hazard ratios (HRs) for enzalutamide, *N*-desmethyl enzalutamide, and the combination of the two were 1.2 (95% confidence interval [CI] 0.90–1.5), 1.1 (95% CI 0.81–1.4), and 1.2 (95% CI 0.89–1.6) per quartile change in concentration, respectively.

For TTPP analysis, 62 patients were included with 53 events (85% of patients) of PSA progression. Six patients were still receiving treatment, 1 patient died prior to PSA progression, and 2 patients did not show PSA progression but discontinued treatment due to radiographic progression. These patients were censored in the survival analysis, as information about their survival was incomplete. Similar to PSA-independent PFS, TTPP was not significantly different among the four quartiles for enzalutamide (6.7 vs 7.2 vs 4.8 vs 8.7 months, p=0.39), *N*-desmethyl enzalutamide (4.9 vs 8.0 vs 5.6 vs 7.23 months, p=0.41), and the sum of enzalutamide and *N*-desmethyl enzalutamide plasma concentrations (6.7 vs 7.6 vs 6.6 vs 8.7 months, p=0.50), as depicted in Figures 2–4, respectively. In the multivariate analysis, plasma concentrations were not related to TTPP, with HRs for enzalutamide, *N*-desmethyl enzalutamide, and the combination of the two being 1.1 (95% CI 0.86–1.5), 1.0 (95% CI 0.76–1.4), and 1.1 (95% CI 0.85–1.5) per quartile change in concentration, respectively.

Among the 65 patients, 38 patients (58%) were PSA responders. Figure 5 shows the relationship

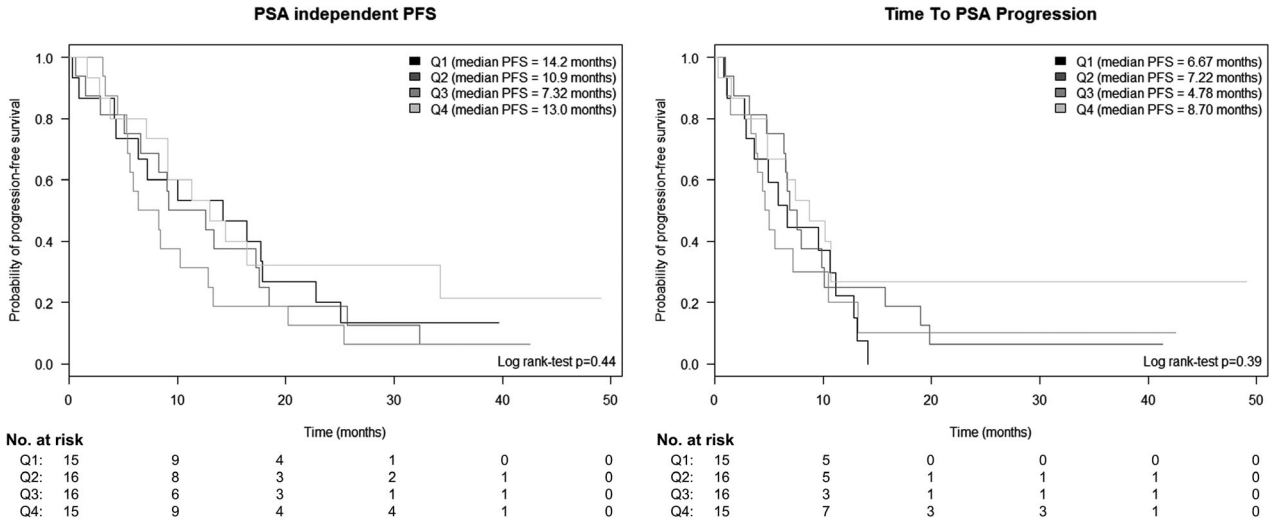

Figure 2. Kaplan–Meier plots of progression-free survival (PFS) for the 62 patients with metastatic castration-resistant prostate cancer included in the survival analysis for each enzalutamide concentration quartile (Q1–Q4). Prostate-specific antigen (PSA)-independent PFS is shown in the left panel, and time to PSA progression is shown in the right panel.

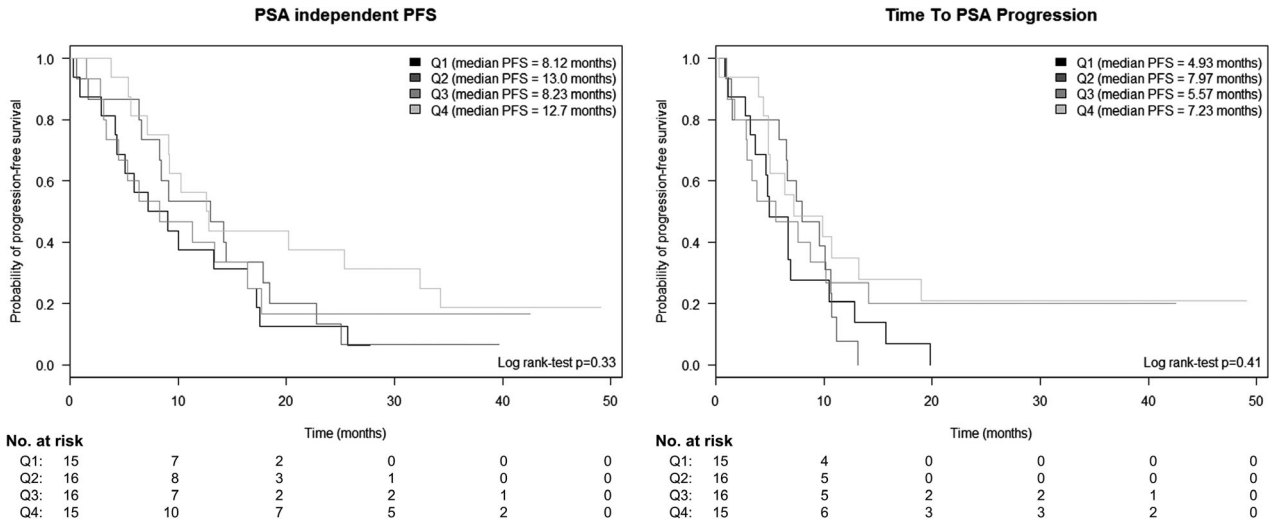

Figure 3. Kaplan–Meier plots of progression-free survival (PFS) for the 62 patients with metastatic castration-resistant prostate cancer in the survival analysis for each *N*-desmethyl enzalutamide concentration quartile (Q1–Q4). Prostate-specific antigen (PSA)-independent PFS is shown in the left panel, and time to PSA progression is shown in the right panel.

between plasma concentrations of enzalutamide, *N*-desmethyl enzalutamide, and carboxylic acid enzalutamide and PSA response. Plasma concentrations were not significantly different in the responder versus nonresponder groups for enzalutamide (11.5 vs 10.6 μg/ml, p=0.20), *N*-desmethyl enzalutamide (10.1 vs 9.6 μg/ml, p=0.48), and carboxylic acid enzalutamide (6.5 vs 5.5 μg/ml, p=0.34). In addition, the number of previous lines of treatment, prior treatment with docetaxel and testosterone, and androstenedione levels were included in the multivariate analyses, which did not show an association between plasma concentrations of enzalutamide, *N*-desmethyl enzalutamide, and carboxylic acid and PSA response (p=0.392, p=0.953 and p=0.208, respectively). Quartiles of enzalutamide and its metabolite concentrations and PSA response rates are presented in Table 2.

Of the 65 included patients, 3 patients discontinued treatment due to adverse events, 3 patients received a dose reduction due to adverse events, and 1 patient interrupted enzalutamide treatment temporarily due to adverse events. Reasons for discontinuation were fatigue, nausea, abdominal pain, loss of appetite, and dyspnea. Mean enzalutamide C_{min} was 11.2 μg/ml in those who discontinued treatment due to

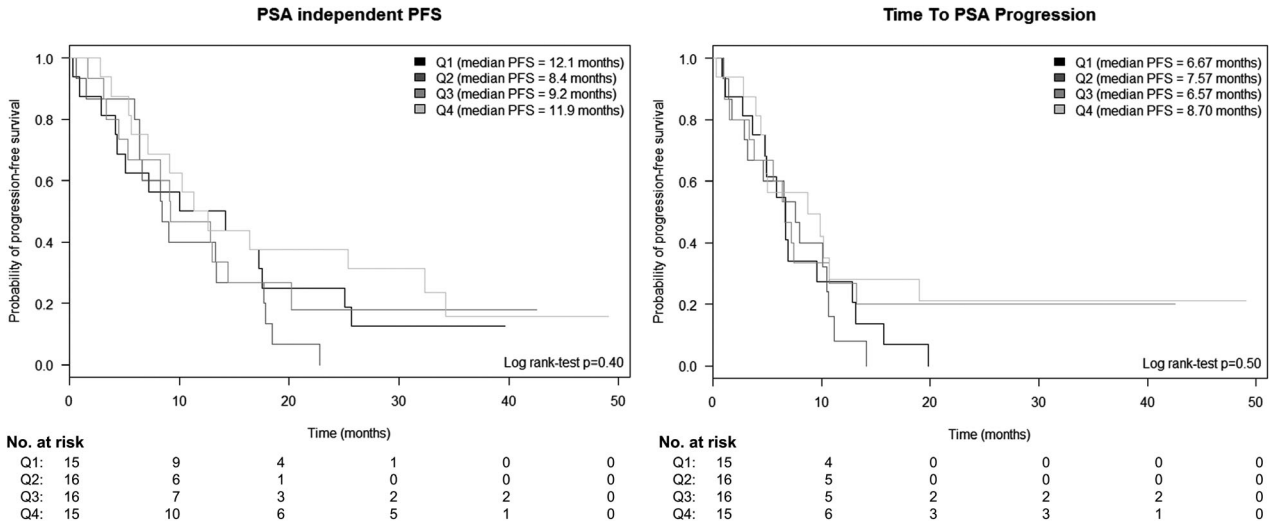

Figure 4. Kaplan–Meier plots of progression-free survival (PFS) for the 62 patients with metastatic castration-resistant prostate cancer in the survival analysis for each quartile (Q1–Q4) of the sum of enzalutamide and *N*-desmethyl enzalutamide concentrations. Prostate-specific antigen (PSA)-independent PFS is shown in the left panel, and time to PSA progression is shown in the right panel.

adverse events and was also 11.2 μg/ml in the group of patients without adverse events (p=0.99). Furthermore, *N*-desmethyl enzalutamide concentrations were 9.3 and 10.0 μg/ml, respectively, in patients who experienced adverse events compared with those who did not (p=0.67).

Discussion

In this study, we investigated the enzalutamide plasma concentrations in patients treated at our outpatient clinic to gather data from daily clinical practice. To our knowledge, this is the first study to evaluate the relationship between exposure and response to enzalutamide and its metabolites in a real-world clinical dataset. Mean plasma concentrations of enzalutamide and its metabolites were in line with those reported in pharmacokinetic studies: mean plasma concentrations, being 11.4 μg/ml for enzalutamide, 13.0 μg/ml for *N*-desmethyl enzalutamide and 8.4 μg/ml for carboxylic acid enzalutamide.[5] Exposure to enzalutamide and its metabolites was not associated with PSA-independent PFS, TTPP, or PSA response. These data confirm previous findings from the pivotal phase III study[1] and confirm our findings of no exposure-response relationship in clinical practice for enzalutamide and its metabolites. Furthermore, only one patient had a plasma concentration below the proposed target of 5.0 μg/ml, and this patient responded well to treatment, with a PSA-independent PFS of 28 months. When combining enzalutamide and *N*-desmethyl enzalutamide plasma concentrations, the difference in PFS between groups diminished, suggesting that the total level of active substance was similar in all quartiles. The PFS analyses in this study included PSA-independent PFS and TTPP, and did not focus on OS, because included patients received multiple and variable lines of treatment after cessation of enzalutamide. These different treatment regimens influence OS and, therefore, OS is not solely related to enzalutamide treatment. Both PSA-independent PFS and TTPP

Table 2. Mean Plasma Concentrations of Enzalutamide and Its Metabolites by Quartile and Prostate-Specific Antigen Response Rate in the 65 Study Patients

	Mean Plasma Concentration (μg/ml)	Range (μg/ml)	PSA Response Rate (%)
Enzalutamide			
Q1	7.6	3.3–9.2	62.5
Q2	10.0	9.2–11.4	43.8
Q3	12.3	11.4–12.7	50.0
Q4	14.5	12.9–18.2	76.5
N-desmethyl enzalutamide			
Q1	6.4	3.1–7.7	62.5
Q2	8.7	7.8–9.8	50.0
Q3	10.6	9.8–11.8	43.8
Q4	13.7	11.9–17.0	76.5
Carboxylic acid enzalutamide			
Q1	2.7	1.1–3.5	56.3
Q2	4.1	3.6–4.6	68.8
Q3	5.7	4.6–7.1	56.3
Q4	11.5	7.2–22.0	52.9

PSA = prostate-specific antigen; Q = quartile.

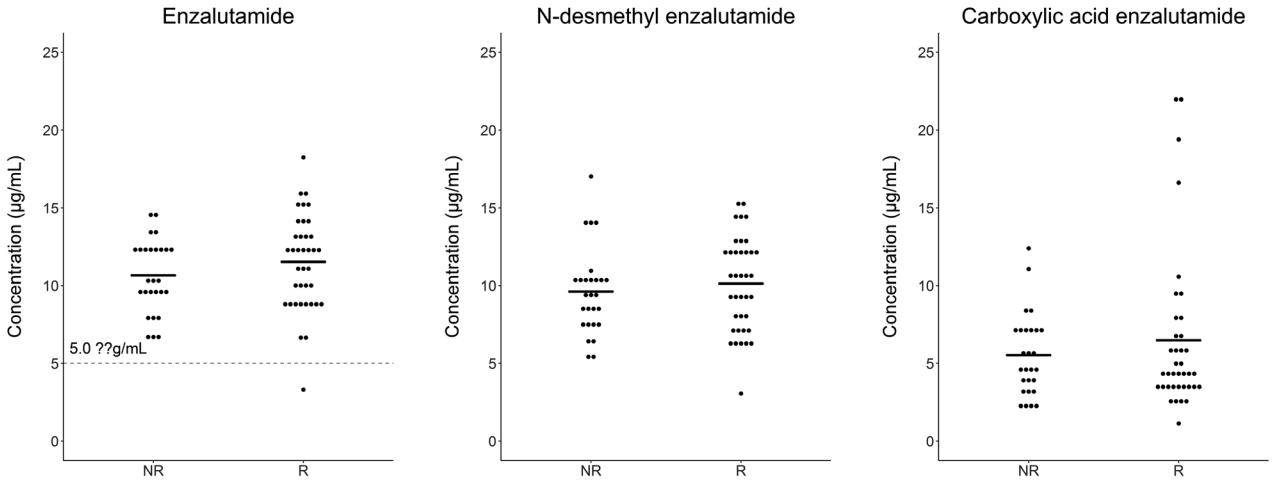

Figure 5. Relationship between prostate-specific antigen response and mean plasma concentrations of enzalutamide (left panel), *N*-desmethyl enzalutamide (middle panel), and carboxylic acid enzalutamide (right panel) in the 38 responders (R) versus the 27 nonresponders (NR). The horizontal black line represents the mean in each group, and the dotted line in the left panel represents the proposed target for enzalutamide. Mean plasma concentrations were not significantly different in the responder versus nonresponder groups for enzalutamide (11.5 vs 10.6 µg/ml, p=0.20), *N*-desmethyl enzalutamide (10.1 vs 9.6 µg/ml, p=0.48), and carboxylic acid enzalutamide (6.5 vs 5.5 µg/ml, p=0.34).

were included in our analyses because PSA as a marker for progression is still being used as an indicator of disease activity, but it does not fully reflect clinically relevant progression.

Enzalutamide and metabolite concentrations were divided into quartiles for the PSA-independent PFS and TTPP analyses instead of studying a linear relationship between concentrations and PFS. This approach was chosen because of the expected type of exposure-response relationship based on receptor occupancy. Enzalutamide and *N*-desmethyl enzalutamide show high affinity for the androgen receptor in vitro LNCaP cell lines, with inhibition concentration 50% (IC_{50}) values of 0.0098 and 0.079 µg/ml, respectively. Given that the plasma protein binding is about 98% for enzalutamide and about 95% for *N*-desmethyl enzalutamide, plasma concentrations to achieve these IC_{50} values should be 0.49 and 1.6 µg/ml, respectively. Measured plasma concentrations were far above these IC_{50} values, suggesting adequate inhibition of the androgen receptor. Based on this information, an exposure-response relationship in the measured enzalutamide concentration range was not anticipated; however, it should be noted that plasma concentrations are a surrogate marker for concentrations at the site of action.

Testosterone and androstenedione levels were measured in this study and showed no relationship with PSA-independent PFS, TTPP, or PSA response. Previous studies have shown that higher testosterone levels at baseline are associated with longer PFS in enzalutamide-treated patients with mCRPC.[13, 14] In our study, testosterone levels were determined at steady state, and no baseline values were available. All patients had adequate androgen suppression below the castration level of 0.5 ng/ml.

Our analysis has some limitations such as the Antoni van Leeuwenhoek/Netherlands Cancer Institute being a tertiary referral center. Patients visiting this hospital are referred for specialized treatment, which may influence their outcomes. Second, due to the retrospective character of this analysis, there were limitations in identifying a sufficient number of patients for this study. The fact that we did not find statistically significant differences does not imply that they would not be found with a larger number of patients. However, our results fit with previously published data and the conclusion that enzalutamide does not seem suitable for TDM. Last, measured plasma concentrations at random time points during a dosing interval were used instead of actual C_{min}. Yet, despite these limitations, to our knowledge, this is the first pharmacokinetic study that reports the results of an enzalutamide exposure-response and exposure-toxicity assessment in a real-world cohort of patients with mCRPC.

Due to the lack of a relationship between exposure and response in the measured enzalutamide concentration range, we found that there is not sufficient evidence to implement TDM in daily practice. Requirements for drugs to be

suitable for TDM include the absence of a measurable biomarker, the availability of a validated bioanalytical method, significant interpatient variability and low intrapatient variability, a narrow therapeutic range, long-term therapy, and an exposure-response relationship.[15] Although enzalutamide meets several of these requirements, such as the absence of a measurable biomarker and the availability of a validated LC-MS/MS assay for quantification, enzalutamide has only limited interpatient variability (28%), has a broad therapeutic window, and shows no exposure-response relationship at measured concentrations. Taking these data and our findings into account, we conclude that enzalutamide is not a suitable drug for TDM in daily clinical practice, with the exception of some specific situations, such as monitoring compliance, drug-drug interactions, or exposure in patients with impaired organ function, such as end-stage renal disease.[16]

Conclusion

In this observational study in a real-world population of patients with mCRPC, we found no significant relationship between exposure to enzalutamide or its major metabolites (*N*-desmethyl enzalutamide and carboxylic acid enzalutamide) and response. Furthermore, PSA-independent PFS and TTPP were not significantly different among quartiles based on plasma drug concentrations. This study suggests that enzalutamide is not a suitable drug for TDM in daily clinical practice, as plasma concentrations did not show an association with treatment response in the measured concentration range.

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