

ARTICLE

Effect of enzalutamide on PK of P-gp and BCRP substrates in cancer patients: CYP450 induction may not always predict overall effect on transporters

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Abstract

Drug-drug interaction (DDI) is an important consideration for clinical decision making in prostate cancer treatment. The objective of this study was to evaluate the effect of enzalutamide, an oral androgen receptor inhibitor, on the pharmacokinetics (PK) of digoxin (P-glycoprotein [P-gp] probe substrate) and rosuvastatin (breast cancer resistance protein [BCRP] probe substrate) in men with metastatic castration-resistant prostate cancer (mCRPC). This was a phase I, open-label, fixed-sequence, crossover study (NCT04094519). Eligible men with mCRPC received a single dose of transporter probe cocktail containing 0.25 mg digoxin and 10 mg rosuvastatin plus enzalutamide placebo-to-match on day 1. On day 8, patients started 160 mg enzalutamide once daily through day 71. On day 64, patients also received a single dose of the cocktail. The primary end points were digoxin and rosuvastatin plasma maximum concentration (C_{max}), area under the concentration-time curve from the time of dosing to the last measurable concentration (AUC_{last}), and AUC from the time of dosing extrapolated to time infinity (AUC_{inf}). Secondary end points were enzalutamide and *N*-desmethyl enzalutamide (metabolite) plasma C_{max} , AUC during a dosing interval, where tau is the length of the dosing interval (AUC_{tau}), and concentration immediately prior to dosing at multiple dosing (C_{trough}). When administered with enzalutamide, there was a 17% increase in C_{max} , 29% increase in AUC_{last} , and 33% increase in AUC_{inf} of plasma digoxin compared to digoxin alone, indicating that enzalutamide is a “mild” inhibitor of P-gp. No PK interaction was observed between enzalutamide and rosuvastatin (BCRP probe substrate). The PK of enzalutamide and *N*-desmethyl enzalutamide were in agreement with previously reported data. The potential for transporter-mediated DDI between enzalutamide and digoxin and rosuvastatin is low in men with prostate cancer. Therefore, concomitant

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administration of enzalutamide with medications that are substrates for P-gp and BCRP does not require dose adjustment in this patient population.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Enzalutamide is strong inducer of CYP3A4. Preclinical data have demonstrated that enzalutamide and its active metabolite, *N*-desmethyl enzalutamide, have the potential to inhibit the efflux transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP).

WHAT QUESTION DID THIS STUDY ADDRESS?

This clinical study evaluated the net inhibition and induction effect of enzalutamide on the pharmacokinetics (PK) of a transporter probe cocktail containing the P-gp and BCRP substrates, digoxin and rosuvastatin, in men with metastatic castration-resistant prostate cancer.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Concomitant administration with enzalutamide resulted in an increase in digoxin exposure, suggesting that enzalutamide is a “mild” inhibitor of P-gp. No PK interaction was observed between enzalutamide and rosuvastatin, suggesting that enzalutamide has “no effect” on BCRP.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Induction of CYP3A4 does not necessarily correlate with clinical effect on P-gp and BCRP transporters. These findings are beneficial to guide future treatment recommendations, whereby concomitant administration of enzalutamide with medications that are P-gp and BCRP substrates does not require dose adjustment.

INTRODUCTION

Prostate cancer was the second most frequently diagnosed cancer and the fifth leading cause of cancer death among men worldwide in 2020.¹ Advancing age is a known risk factor for prostate cancer,¹ with older men often prescribed concomitant medications for comorbid conditions, putting them at increased risk of complications due to drug-drug interactions (DDI).^{2,3} It is therefore important for prostate cancer therapies to be evaluated for potential for DDI^{4,5} in accordance with guidance from healthcare authorities, including the European Medicines Agency⁶ and US Food and Drug Administration.⁷

Enzalutamide is a potent oral androgen receptor (AR) inhibitor that targets the AR signaling pathway. It is approved for the treatment of men with metastatic (m) hormone-sensitive prostate cancer (also known as metastatic castration-sensitive prostate cancer) and castration-resistant prostate cancer (CRPC), irrespective of the presence of metastases,^{8–10} based on clinical benefit demonstrated in a number of clinical trials.^{11–17} The pharmacokinetics (PK) and DDI of enzalutamide have been evaluated in healthy subjects as well as patients with prostate cancer.^{18–22}

Enzalutamide is primarily metabolized by cytochrome P450 (CYP) 2C8 and CYP3A4/5 to form the active metabolite *N*-desmethyl enzalutamide.¹⁹ The DDI potential of enzalutamide and *N*-desmethyl enzalutamide on the PK of other medications via modulation of levels of CYPs have been assessed in vitro and in vivo.^{19,23} In vitro, enzalutamide and its metabolites were found to directly inhibit multiple CYP enzymes, including CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5.²³ Enzalutamide increased enzyme activity and mRNA levels of CYP3A4 by up to 3.88-fold and 6.90-fold (95.9% of positive control), respectively, in human hepatocytes (data on file). Previous clinical DDI studies indicated that enzalutamide was a moderate inducer of CYP2C9 and CYP2C19 and a strong inducer of CYP3A4, reducing the exposure of probe substrates by 56%, 70%, and 86%, respectively.¹⁹ Induction of these metabolizing enzymes commonly occurs via activation of shared nuclear receptors, including the constitutive androstane receptor (CAR) and pregnane xenobiotic receptor (PXR).²⁴ Whereas the primary function of PXR is to regulate the expression of drug-metabolizing enzymes (DMEs) and drug transporters, the identification of other PXR-regulated genes indicates that PXR is also involved in the elimination of compounds from the body via an

array of efflux transporters.²⁴ The transporter proteins, namely organic-anion transporter 2 (OATP2), multidrug resistance 1 (MDR1, also known as P-glycoprotein [P-gp]), multidrug resistance-associated protein 2 (MRP2), and breast cancer resistance protein (BCRP) have recently been shown to be transcriptionally regulated by PXR.^{25–28} Drug-induced PXR activation and subsequent induction of DMEs and transporters have been suggested to be a key underlying mechanism of DDI,^{19,24} with in vitro data suggesting that enzalutamide may be an inhibitor of the efflux transporters P-gp and BCRP.²³ Enzalutamide may therefore have the potential for transporter-mediated DDI when co-administered with medications that are substrates of these efflux transporters.

The use of transporter probe cocktails for investigating DDI provides a promising approach to reduce the number of clinical DDI studies performed during drug development. In our study, 0.25 mg digoxin and 10 mg rosuvastatin were assessed as clinical substrates for P-gp and BCRP, respectively, administered as an optimized and validated transporter probe cocktail where the interaction among components has been demonstrated to be unlikely.^{29,30} This cocktail combination is increasingly used as an efficient tool for the investigation of transporter-based DDI in drug development.^{29,30} The objectives of our

study were to evaluate the effect of enzalutamide on the PK of P-gp and BCRP substrates in men with mCRPC and to assess the PK, safety, and tolerability of enzalutamide when co-administered with the transporter probe cocktail.

METHODS

Study design

This was a phase I, open-label, placebo-controlled, fixed-sequence, crossover DDI study in men with mCRPC (NCT04094519) conducted between January 2020 and December 2020 at one site in Moldova. The study was conducted in accordance with the International Council for Harmonization guidelines, applicable local laws, regulations, and guidelines governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki. The study was approved by an independent ethics committee prior to initiation.

The study design is presented in Figure 1. Following patient screening, eligible men received a single oral dose of transporter probe cocktail containing 0.25 mg digoxin (P-gp probe substrate) and 10 mg rosuvastatin (BCRP probe substrate) on day 1, co-administered with a single

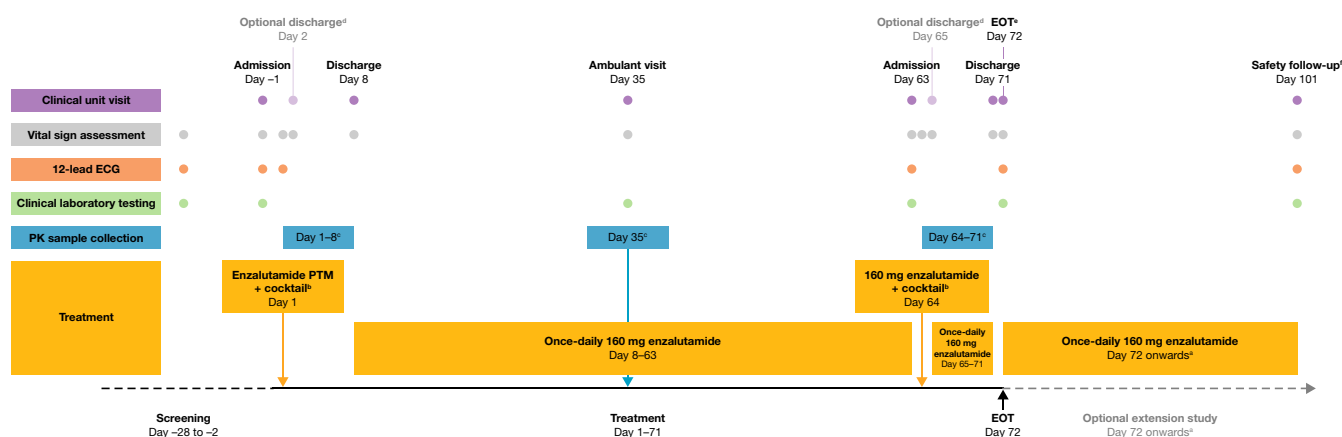


FIGURE 1 Study design. ^aPatients who experienced clinical benefit (determined by the investigator in consultation with the physician responsible for treating their prostate cancer) could roll over into an open-label, single-arm extension study. Only patients enrolled in the extension study continued to receive enzalutamide until any discontinuation criterion occurred. ^bSingle oral dose of transporter probe cocktail containing 0.25 mg digoxin (P-gp probe substrate) and 10 mg rosuvastatin (BCRP probe substrate). ^cBlood samples for digoxin PK were collected predose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 48, 72, 96, 120, 144, and 168 h postdose on days 1 and 64. Blood samples for rosuvastatin PK were collected predose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 48, 72, 96, and 120 h postdose on days 1 and 64. Blood samples for enzalutamide and *N*-desmethyl enzalutamide PK were collected predose on days 35, 64, 68, and 71 and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h postdose on day 64. Urine samples for digoxin PK were collected predose and at 0–4, 4–8, 8–12, and 12–24 h postdose on days 1 and 64. ^dPatients could be discharged after the 24-h postdose PK sample collection if all required assessments were performed and there was no medical reason requiring a longer stay in the clinical unit. Patients had to be willing to return to the clinical unit on the following 6 days for daily PK sampling. Alternatively, patients could choose to remain in the clinical unit until the 168-h postdose PK sample collection. ^eEOT visit occurred ± 3 days after the last dose of investigational product for patients who prematurely discontinued from the study or were not enrolled onto the extension study. ^fPatients who did not enter the extension study had a safety follow-up visit 30 days after their last dose of treatment or prior to initiation of another investigational product or new therapy for prostate cancer, whichever occurred first. ECG, electrocardiogram; EOT, end of treatment; PK, pharmacokinetics; PTM, placebo-to-match

oral dose of enzalutamide placebo-to-match to control for possible effects of excipients of the enzalutamide formulation. On day 8, patients started 160 mg oral enzalutamide (per the approved product label) once daily through day 71. On day 64, patients also received a single oral dose of the transporter probe cocktail containing 0.25 mg digoxin and 10 mg rosuvastatin. Androgen deprivation therapy (ADT) in the form of a luteinizing hormone-releasing hormone agonist/antagonist or bilateral orchiectomy was maintained during study treatment. PK blood samples were collected predose on days 35, 64, 68, and 71 and up to 24 h postdose on day 64 for enzalutamide and *N*-desmethyl enzalutamide, predose and up to 168 h postdose on days 1 and 64 for digoxin, and predose and up to 120 h postdose on days 1 and 64 for rosuvastatin. Urine samples for digoxin PK were collected predose and up to 24 h postdose on days 1 and 64. From day 72 onward, men experiencing clinical benefit were permitted to enter an open-label, single-arm extension study in which they continued to receive enzalutamide until discontinuation. Men who did not enter the extension study discontinued enzalutamide on day 71.

Patients were free to withdraw from treatment and/or the study at any time. Patients were required to discontinue enzalutamide if any of the following events were observed: noncompliance with the protocol; intolerable adverse event (AE), seizure, or a condition that significantly predisposed the patient to seizure; confirmed event of posterior reversible encephalopathy syndrome; initiation of another investigational agent or new therapy for prostate cancer; discontinuation of ADT; or evidence of disease progression and the patient was no longer deriving clinical benefit.

Patients

Adult men diagnosed with histologically or cytologically confirmed adenocarcinoma of the prostate without neuroendocrine differentiation, signet cell, or small cell histology were eligible for inclusion. All men were required to have newly diagnosed metastatic prostate cancer or disease progression at screening (confirmed by prostate-specific antigen [PSA] or imaging) if receiving ADT. Disease progression was considered as either PSA progression (defined as ≥ 2 rising PSA levels with an interval of ≥ 1 week; ≥ 1 PSA value was required to be ≥ 2 $\mu\text{g/L}$ [2 ng/ml] within 3 months of screening), soft tissue disease progression (defined by Response Evaluation Criteria in Solid Tumors version 1.1), or bone disease progression (defined as ≥ 2 new lesions on a bone scan). An Eastern Cooperative Oncology Group performance status of less than or equal to two, estimated life expectancy of greater

than or equal to 6 months, and serum testosterone levels less than 1.7 nmol/L (50 ng/dl) during the screening period, if receiving ADT, were also required. All eligible men were required to provide written informed consent.

Men with known liver metastases were excluded, as were those with known or suspected brain metastases or active leptomeningeal disease. Men with hepatic or gastrointestinal disorders affecting drug absorption or metabolism, history of seizures, clinically significant cardiovascular disease, active hepatitis (A [viral], B, or C), or those who were HIV positive (type 1 and/or 2) were not eligible for inclusion. Patients who had a known hypersensitivity reaction to enzalutamide, digoxin, rosuvastatin, contrast agents, or any formulation components were also not eligible. Chemotherapy less than or equal to 4 weeks prior to enrollment and prior enzalutamide treatment were not permitted, nor was ongoing treatment with digoxin or rosuvastatin (or contraindicated concomitant medications), strong CYP2C8 inhibitors, strong CYP3A4 inducers, or concomitant inducers or inhibitors of P-gp and/or BCRP. Men were excluded if any of the following laboratory parameters were measured during screening: absolute neutrophil count less than 1500/ μL ; platelet count less than 100,000/ μL ; hemoglobin less than 6.2 mmol/L (9 g/dl); total bilirubin greater than 1.5 times the upper limit of normal (ULN); alanine aminotransferase greater than 2.5 times the ULN; aspartate aminotransferase greater than 2.5 times the ULN; alkaline phosphatase (ALP) greater than 3 times the ULN (unless related to bone metastases); albumin less than 30 g/L (3.0 g/dl); or creatinine greater than 177 $\mu\text{mol/L}$ (>2 mg/dl).

End points

The primary end points were digoxin and rosuvastatin plasma C_{max} , AUC_{last} , and AUC_{inf} . C_{max} was defined as maximum concentration, AUC_{last} was defined as the area under the concentration-time curve from the time of dosing to the last measurable concentration, and AUC_{inf} was defined as the area under the concentration-time curve from the time of dosing extrapolated to time infinity.

Secondary end points were enzalutamide and *N*-desmethyl enzalutamide plasma C_{max} , AUC_{tau} , and C_{trough} . AUC_{tau} was defined as the area under the concentration-time curve during a dosing interval (where tau is the length of the dosing interval) and C_{trough} was defined as the concentration immediately prior to dosing at multiple dosing.

Exploratory end points included digoxin and rosuvastatin plasma t_{max} , $t_{1/2}$, V_z/F , and CL/F and digoxin urine Ae_{last} , $\text{Ae}_{\text{last}\%}$, and CL_R . t_{max} was defined as time of maximum concentration, $t_{1/2}$ was defined as terminal elimination half-life,

V_z/F was defined as apparent volume of distribution during the terminal elimination phase after extravascular dosing, and CL/F was defined as apparent total systemic clearance after extravascular dosing. Ae_{last} was defined as the cumulative amount of study drug excreted into the urine from the time of dosing up to the collection time of the last measurable concentration, $Ae_{last\%}$ was defined as the percentage of study drug dose excreted into the urine from the time of dosing up to the collection time of the last measurable concentration, and CL_R was defined as renal clearance.

Treatment-emergent adverse events (TEAEs) were recorded until 30 days after the final study treatment was administered, the patient entered the extension study, or another investigational therapy or new therapy for prostate cancer was initiated. All TEAEs were followed up until no longer clinically significant or considered chronic. Clinical laboratory tests (hematology, biochemistry, and urinalysis), vital signs (blood pressure and pulse), and routine 12-lead electrocardiograms (ECGs) were also monitored.

Analytical methods

Digoxin and rosuvastatin concentrations were measured using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) at Syneos Health (Quebec, Canada). Digoxin and internal standard (IS; digoxin- d_3) were extracted from 200 μ l human K_2EDTA plasma or 200 μ l human urine by liquid-liquid extraction. Samples were mixed with buffer solution and methyl-tert-butyl ether (plasma) or with buffer solution, hydrochloric acid, and methyl-tert-butyl ether (urine); residues were reconstituted with water, methanol with ammonium formate, and formic acid solution. The analyte and IS were injected onto an ACE Excel 2 C18-PFP column (50 \times 3.0 mm, 2 μ m; Canadian Life Science) and eluted with an isocratic mobile phase consisting of water, methanol with ammonium formate, and formic acid (mobile phase A), and methanol (mobile phase B). The analyte was monitored on an AB Sciex API 5000 (plasma) or API4000 (urine) using positive Turbo Ion Spray. The lower limit of quantification (LLOQ) was 10 pg/ml for plasma samples and 2 ng/ml for urine samples. The calibration curves were constructed using peak area ratios by applying a linear, 1/concentration weighted, least squares (LS) regression algorithm. The calibration curve was 10–2500 pg/ml for plasma and 2–200 ng/ml for urine. The quality control samples for plasma and urine were prepared at concentrations of 30, 185, 1250, and 1875 pg/ml and 6, 15, 100, and 150 ng/ml, respectively. Inter-accuracy was between –3.19% and 3.33% relative error (RE) and precision was less than 7.32% relative standard deviation (RSD) for plasma; the

same values for urine were between –4.00% and –3.33% RE and less than 3.60% RSD.

Rosuvastatin and IS (rosuvastatin- d_3) were extracted from 300 μ l human K_2EDTA plasma using liquid-liquid extraction. Samples were mixed with hydrochloric acid and methyl tert-butyl ether; residues were re-dissolved in water, methanol with ammonium formate, and formic acid solution. The analyte and IS were injected onto an ACE Excel 2 C18 column (100 \times 2.1 mm, 2 μ m; Canadian Life Science) and eluted with a gradient consisting of water, acetonitrile, and formic acid. The analyte was monitored on an AB Sciex API5000 using positive Turbo Ion Spray. The LLOQ was 20 pg/ml. The calibration curve was constructed using peak area ratios by applying a linear, 1/concentration weighted, LS regression algorithm and was 20–25,000 pg/ml. The quality control samples were prepared at concentrations of 60, 1875, 12,500, and 18,750 pg/ml. Inter-accuracy was between –1.06% and 2.33% RE, and precision was less than 3.90% RSD.

Enzalutamide and *N*-desmethyl enzalutamide concentrations in K_2EDTA plasma were measured using a validated LC-MS/MS at Syneos Health (Princeton, New Jersey, USA). A 50 μ l plasma sample was combined with IS (N^{13} - CD_3 -MDV3100 and MDPC0002- $^{13}CD_3$) for liquid-liquid extraction. Samples were mixed with sodium bicarbonate solution and methyl tert-butyl ether and the supernatant was evaporated to dryness. The residues were re-dissolved in formic acid, methanol, and water solution. The analytes and IS were injected onto an ACE5 C18 column (2.1 mm \times 30 mm, 5 μ m; Advanced Chromatography Technologies) and eluted with a gradient mobile phase consisting of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). The analytes were monitored on a Sciex API4000 using positive Turbo Ion Spray. The LLOQ was 0.0200 μ g/ml for both enzalutamide and *N*-desmethyl enzalutamide. The calibration curves were constructed using peak area ratios by applying a linear, 1/concentration weighted, LS regression algorithm and were 0.0200–50.0 μ g/ml for both analytes. The quality control samples were prepared at concentrations of 0.0600, 1.00, and 40.0 μ g/ml for both analytes. Inter-accuracy was between –10.0% and –4.6% RE and precision was less than 6.7% RSD for enzalutamide; the same values for *N*-desmethyl enzalutamide were between –6.8% and –0.2% RE and less than 5.9% RSD.

Pharmacokinetic and statistical analyses

A total of 24 patients were planned to be enrolled to allow for 18 patients to complete the study. Intra-patient variability for the PK parameters C_{max} and AUC_{inf} of digoxin and rosuvastatin was estimated to be 10–30%.³⁰ Assuming

a true underlying ratio of 100%, the 90% confidence intervals (CIs) were estimated to be within 82–121%, with greater than 80% probability.

The PK analysis set (PKAS) comprised all patients who received at least one dose of study treatment for whom concentration data were available to derive at least one primary PK end point. The safety analysis set (SAS) comprised all patients who received at least one dose of study treatment.

Data were summarized using descriptive statistics for continuous end points, while using frequency and percentage for categorical end points. There was no imputation for missing data, with the exception of start/stop dates for TEAEs. Noncompartmental analysis was used for the estimation of plasma and urine PK parameters using Phoenix® WinNonlin® version 8.0. To assess the effect of enzalutamide on the PK of digoxin and rosuvastatin, a separate mixed-effects analysis of variance (ANOVA) model for each treatment was fitted on natural logarithm-transformed C_{max} , AUC_{last} , and AUC_{inf} , with treatment as a fixed effect and patient as a random effect. Within the ANOVA, the LS mean differences and 90% CIs were estimated. The LS means for C_{max} , AUC_{last} , and AUC_{inf} were back transformed to provide the geometric LS means (GLSM) for each treatment. GLSM ratios and their corresponding 90% CIs for each PK parameter were provided by back transforming. TEAEs were coded using the Medical Dictionary for Regulatory Authorities version 23.0. Descriptive statistics were used to summarize laboratory assessments, vital signs, and 12-lead ECG data.

RESULTS

In total, 24 patients were enrolled in the study and comprised the PKAS and SAS. One patient discontinued treatment and withdrew from the study on day 34 and 21 patients opted to continue into the extension study.

The total population consisted of white men, with an age range of 53–82 years (Table 1). A mean study drug compliance of 95.0% was achieved for doses not taken in the clinical unit.

Pharmacokinetics

Digoxin

The mean plasma concentration-time profile of digoxin is presented in Figure 2. Following administration of the cocktail on day 1, all patients had digoxin plasma

TABLE 1 Baseline demographics and disease characteristics

Parameter	Patients (N = 24)
Male, N (%)	24 (100.0)
White, N (%)	24 (100.0)
Mean age ± SD (years)	67.1 ± 7.8
Mean BMI ± SD (kg/m ²)	29.3 ± 4.1
ECOG PS, N (%)	
1	15 (62.5)
2	9 (37.5)

Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; SD, standard deviation.

concentrations above the 10 pg/ml LLOQ from 0.5–168 h postdose.

Digoxin plasma PK parameters are summarized in Table 2. GLSM ratios indicate a 17% increase in C_{max} , 29% increase in AUC_{last} , and 33% increase in AUC_{inf} when co-administered with enzalutamide versus when taken alone. As the GLSM ratios were less than two-fold, the effect of enzalutamide on P-gp inhibition can be considered “mild.”

Digoxin urine PK parameters are summarized in Table 3. A decrease of 10% was observed in Ae_{last} when co-administered with enzalutamide, which correlates with the observed 22% decrease in CL_R .

Rosuvastatin

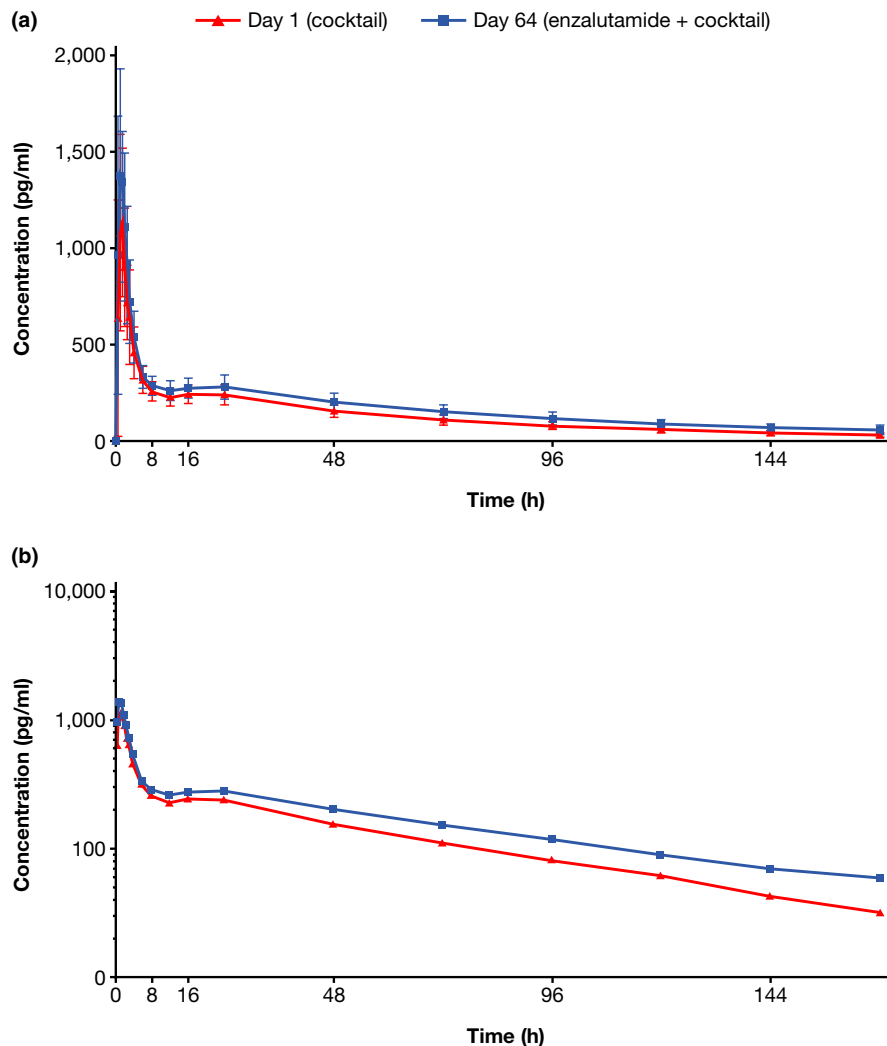
The mean plasma concentration-time profile of rosuvastatin is presented in Figure 3.

Rosuvastatin plasma PK parameters are summarized in Table 2. GLSM ratios indicate a 6% increase in C_{max} when rosuvastatin was co-administered with enzalutamide versus when taken alone; however, a 14% reduction in AUC_{last} and 10% reduction in AUC_{inf} were observed. As the 90% CI for C_{max} and AUC_{inf} GLSM ratios were within the no-effect boundary of 80–125%, it was considered that enzalutamide had “no effect” on the PK of rosuvastatin.

Enzalutamide and N-desmethyl enzalutamide

Enzalutamide and N-desmethyl enzalutamide plasma PK parameters are summarized in Table 4. Mean C_{trough} for enzalutamide was 14.1, 13.9, 14.0, and 14.0 µg/ml on days 35, 64, 68, and 71, respectively, indicating that enzalutamide steady state was achieved prior to day 64. Similarly, mean C_{trough} for N-desmethyl enzalutamide was 9.1, 11.6, 11.9, and 12.1 µg/ml on days 35, 64, 68, and 71,

FIGURE 2 Mean plasma concentration-time profile for digoxin (a) linear scale plot and (b) semi-logarithmic scale plot. Day 1 analyses include all 24 patients. Day 64 analyses include 23 patients following the discontinuation of one patient on day 34. Transporter probe cocktail contains 0.25 mg digoxin (P-gp probe substrate) and 10 mg rosuvastatin (BCRP probe substrate). BCRP, breast cancer resistance protein; P-gp, P-glycoprotein



respectively, indicating that *N*-desmethyl enzalutamide steady state was also achieved before day 64.

Safety

Overall, 14 TEAEs were reported by five patients (20.8%) during the study. Of those, 12 TEAEs were reported by five patients (20.8%) following administration of enzalutamide and two TEAEs were reported by one patient (4.3%) following administration of the cocktail in combination with enzalutamide. Of these, three TEAEs reported by three patients (12.5%) were considered by the investigator to be potentially related to enzalutamide (one TEAE of eosinophilia and two TEAEs of decreased white blood cell count); no TEAEs were considered to be related to digoxin or rosuvastatin. The only TEAEs reported by more than one patient were urinary tract infection, decreased white blood cell count, and dizziness (each reported by 2 patients [8.3%] following enzalutamide). All TEAEs were considered to be mild in severity. No serious TEAEs were reported and there were no deaths.

There were no clinically relevant changes in laboratory evaluations for urinalysis. Four patients had hematology and biochemistry evaluations that were recorded as TEAEs; however, no dose changes or treatment were required. Two patients (8.7%) had potentially clinically significant ALP values greater than 1.5 times the ULN. There were no clinically meaningful findings in vital signs or 12-lead ECG measurements.

DISCUSSION

In this phase I study, we assessed the transporter-mediated DDI potential of concomitant administration of enzalutamide with P-gp and BCRP substrates in men with mCRPC.

The observed PK parameters of enzalutamide and *N*-desmethyl enzalutamide were in agreement with previously reported data,^{18,31} with steady state achieved before day 64 when concomitant cocktail dosing occurred. Enzalutamide alone and in combination with digoxin and rosuvastatin was found to be well tolerated, with 14 TEAEs reported by five patients (20.8%) throughout the

TABLE 2 Summary of plasma PK parameters for digoxin and rosuvastatin

Parameter	Digoxin		Rosuvastatin	
	Cocktail (day 1)	Enzalutamide + cocktail (day 64)	Cocktail (day 1)	Enzalutamide + cocktail (day 64)
C_{max}, pg/ml				
<i>N</i>	24	23	24	23
Mean ± SD	1450 ± 314	1690 ± 384	4960 ± 2250	5130 ± 2530
% CV	21.7	22.7	51.4	49.4
Median (range)	1470 (888–1910)	1620 (1180–2500)	4710 (1110–11,500)	4490 (1370–12,500)
GLSM	1410	1650	4300	4570
GLSM ratio (95% CI)	117.0 (105.8–129.3)		106.3 (94.7–119.3)	
AUC_{last}, h*pg/ml				
<i>N</i>	24	23	24	23
Mean ± SD	22,100 ± 4100	28,700 ± 5790	61,800 ± 24,300	52,800 ± 21,100
% CV	18.6	20.2	39.3	39.9
Median (range)	22,700 (14,100–30,900)	27,100 (19,500–44,100)	56,100 (17,500–105,000)	48,400 (22,400–108,000)
GLSM	21,700	28,000	56,700	48,800
GLSM ratio (95% CI)	129.1 (121.0–137.7)		86.1 (77.5–95.7)	
AUC_{inf}, h*pg/ml				
<i>N</i>	24	21	17	11
Mean ± SD	24,600 ± 5020	33,200 ± 7680	69,500 ± 23,200	59,700 ± 21,400
% CV	20.4	23.2	33.4	35.9
Median (range)	25,100 (15,300–33,800)	31,900 (21,500–54,700)	68,600 (27,300–106,000)	55,500 (31,900–109,000)
GLSM	24,100	32,100	62,700	56,000
GLSM ratio (95% CI)	133.5 (125.3–142.2)		89.3 (80.0–99.8)	
t_{max}, h				
<i>N</i>	24	23	24	23
Median (range)	1.50 (0.50–3.00)	1.00 (0.50–2.50)	4.0 (1.0–6.0)	3.0 (0.5–6.0)
t_{1/2}, h				
<i>N</i>	24	21	17	11
Mean ± SD	52.3 ± 8.2	63.5 ± 9.5	22.5 ± 11.4	20.0 ± 10.0
% CV	15.6	14.9	50.5	49.7
Median (range)	50.5 (39.4–66.5)	65.2 (47.6–86.1)	17.1 (9.9–50.6)	16.1 (9.1–41.2)
V_z/F, L				
<i>N</i>	24	21	17	11
Mean ± SD	785 ± 138	710 ± 114	5400 ± 3950	5420 ± 3510
% CV	17.6	16.1	73.1	64.6
Median (range)	786 (537–1150)	727 (466–881)	4310 (2090–17,300)	3490 (2120–14,100)
CL/F, L/h				
<i>N</i>	24	21	17	11
Mean ± SD	10.6 ± 2.3	7.9 ± 1.7	163 ± 68.5	186 ± 62.4
% CV	21.7	21.7	41.9	33.5
Median (range)	9.9 (7.4–16.3)	7.8 (4.6–11.6)	146 (94.5–366)	180 (91.6–314)

Abbreviations: AUC_{inf}, area under the concentration-time curve from the time of dosing extrapolated to time infinity; AUC_{last}, area under the concentration-time curve from the time of dosing to the last measurable concentration; CI, confidence interval; CL/F, apparent total systemic clearance after extravascular dosing; C_{max}, maximum concentration; CV, coefficient of variation; GLSM, geometric least squares mean; PK, pharmacokinetics; SD, standard deviation; t_{1/2}, terminal elimination half-life; t_{max}, time of maximum concentration; V_z/F, apparent volume of distribution during the terminal elimination phase after extravascular dosing.

TABLE 3 Summary of urine PK parameters for digoxin

Parameter	Digoxin	
	Cocktail (day 1)	Enzalutamide + cocktail (day 64)
Ae_{last}, mg		
N	24	23
Mean ± SD	0.05 ± 0.01	0.05 ± 0.01
% CV	25.6	29.7
Median (range)	0.05 (0.02–0.08)	0.04 (0.02–0.07)
Ae_{last}%, %		
N	24	23
Mean ± SD	20.1 ± 5.1	18.2 ± 5.4
% CV	25.6	29.7
Median (range)	21.3 (9.2–31.5)	17.9 (6.6–26.5)
GLSM	19.4	17.3
GLSM ratio (95% CI)	89.6 (79.1–101.6)	
CL_R, L/h		
N	24	23
Mean ± SD	6.3 ± 1.9	4.9 ± 1.7
% CV	30.1	35.4
Median (range)	6.0 (2.9–9.4)	4.7 (1.6–8.9)

Abbreviations: Ae_{last}, cumulative amount of study drug excreted into urine from time of dosing up to the collection time of the last measurable concentration; Ae_{last}%, percentage of study drug dose excreted into urine from time of dosing up to the collection time of the last measurable concentration; CI, confidence interval; CL_R, renal clearance; CV, coefficient of variation; GLSM, geometric least squares mean; PK, pharmacokinetics; SD, standard deviation.

study period, all of which were mild in severity. Only two patients (8.7%) reported potentially clinically significant laboratory evaluations, with ALP values greater than 1.5 times the ULN.

There was a small increase in the exposure of plasma digoxin (P-gp probe substrate) in the presence of enzalutamide, as evidenced by a 29% increase in AUC_{last}, 33% increase in AUC_{inf}, and 17% increase in C_{max}. The observed 33% increase in AUC_{inf} and 17% increase in C_{max} of digoxin following co-administration with enzalutamide are contrary to the previously reported effect of rifampin, another PXR inducer. Co-administration with rifampin was observed to decrease AUC and C_{max} of digoxin by 30% and 58%, respectively.³² These differences in AUC and C_{max} may be attributable to the composite yet counteracting inhibition and induction effects of enzalutamide, whereby it directly inhibits intestinal P-gp while also mediating P-gp induction via PXR activation. As a result, the composite impact on P-gp was rather weak inhibition when co-administered

with enzalutamide, and, consequently, the net effect was an increase in digoxin exposure (both AUC and C_{max}) as opposed to the decrease in digoxin exposure due to P-gp induction when co-administered with rifampin.³² The interpretation of the extent of interaction in our study was conducted in accordance with European Medicines Agency⁶ and US Food and Drug Administration⁷ guidance. Per these regulatory guidelines, a drug that causes a 1.25- to 2-fold increase in plasma AUC is classed as a mild inhibitor, a moderate inhibitor results in a greater than two-fold increase in plasma AUC, and a strong inhibitor causes a greater than five-fold increase in plasma AUC.^{6,7} The observed GLSM ratios were less than two-fold in our study and thus the effect of enzalutamide on P-gp inhibition was considered “mild.”

For rosuvastatin (BCRP probe substrate), our results demonstrated that there was an increase of 6% in C_{max} when co-administered with enzalutamide compared to when taken alone. This slight increase may be attributable to interpatient variability and is contrary to the previously published 30% decrease in C_{max} of rosuvastatin when co-administered with rifampin.³³ The extent of reduction in AUC_{last} (14%) and AUC_{inf} (10%) of rosuvastatin when co-administered with enzalutamide in our study are inconsistent with the observed 63% decrease in AUC of rosuvastatin when co-administered with rifampin.³³ Because, in our study, the 90% CI for the GLSM ratios were within the “no effect” boundary, it is reasonable to conclude that no PK interaction was observed between enzalutamide and rosuvastatin (BCRP probe substrate). Similar to the composite inhibition and induction effects observed with P-gp, the net effect of enzalutamide on BCRP is nullified given the contrasting nature of the underlying effects.

Because the expression of CYP and drug transporters is regulated by shared nuclear receptors, PXR in this instance, it has long been believed that a drug that activates PXR would, in turn, induce both CYP and drug transporters, albeit with varying magnitude. However, this hypothesis may not always hold true in a clinical setting. Although it is known that enzalutamide is a moderate inducer of CYP2C9 and CYP2C19 and a strong inducer of CYP3A4,¹⁹ our results demonstrate that enzalutamide-mediated induction of CYP2C9 and CYP3A4 via PXR activation does not necessarily correlate with clinical effect on P-gp and BCRP transporters, likely due to the counteracting inhibition and induction effects of enzalutamide on these transporters. The specific inhibitory effect of enzalutamide on P-gp and BCRP cannot be ascertained from this study; future modeling analyses may help to shed light on the combined and separate inhibition and induction effects at play.

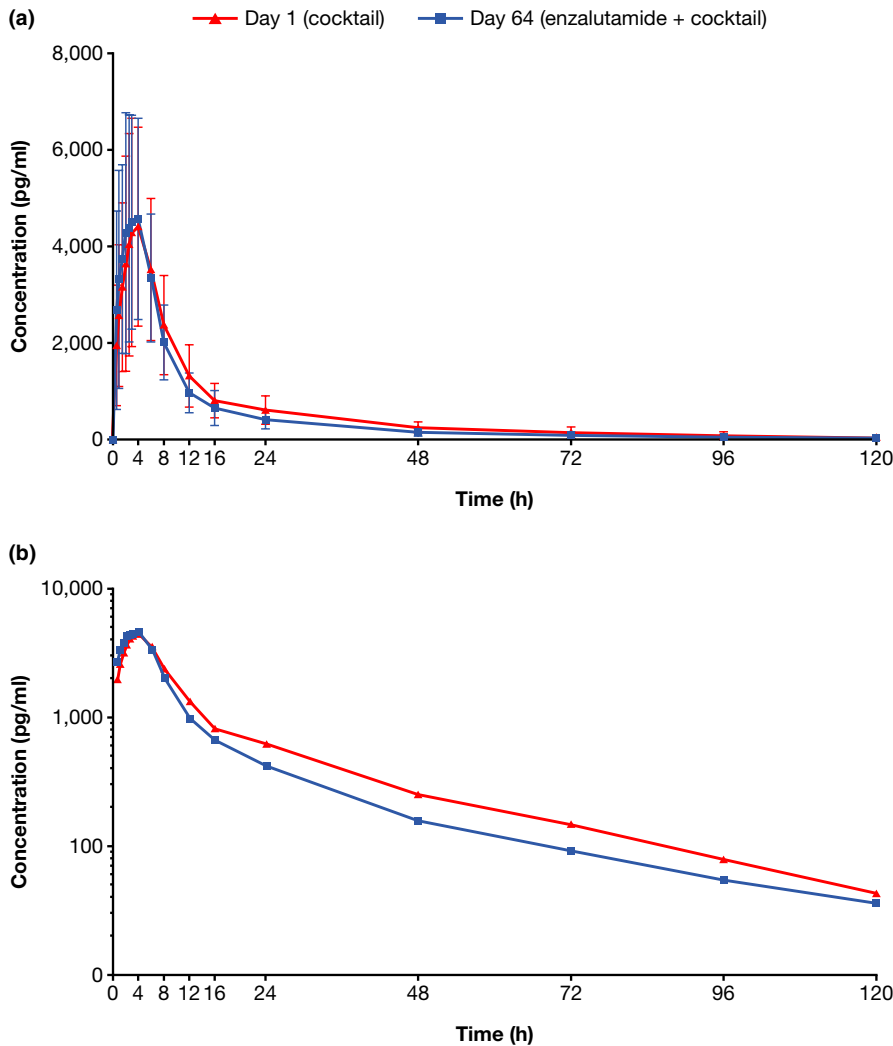


FIGURE 3 Mean plasma concentration-time profile for rosuvastatin (a) linear scale plot and (b) semi-logarithmic scale plot. Day 1 analyses include all 24 patients. Day 64 analyses include 23 patients following the discontinuation of one patient on day 34. Transporter probe cocktail contains 0.25 mg digoxin (P-gp probe substrate) and 10 mg rosuvastatin (BCRP probe substrate). BCRP, breast cancer resistance protein; P-gp, P-glycoprotein

Parameter	Enzalutamide (day 64)	<i>N</i> -desmethyl enzalutamide (day 64)
C_{max}, $\mu\text{g/ml}$		
<i>N</i>	23	23
Mean \pm SD	17.1 \pm 2.8	12.3 \pm 1.7
% CV	16.4	13.9
Median (range)	17.2 (11.2–22.2)	12.1 (8.9–15.3)
AUC_{tau}, h·$\mu\text{g/ml}$		
<i>N</i>	23	23
Mean \pm SD	333 \pm 57.8	259 \pm 37.9
% CV	17.3	14.6
Median (range)	344 (210–435)	251 (189–327)
C_{trough}, $\mu\text{g/ml}$		
<i>N</i>	23	23
Mean \pm SD	13.9 \pm 2.9	11.6 \pm 1.9
% CV	20.5	16.0
Median (range)	14.1 (8.3–19.4)	11.7 (7.7–15.0)

TABLE 4 Summary of plasma PK parameters for enzalutamide and *N*-desmethyl enzalutamide

Abbreviations: AUC_{tau} , area under the concentration-time curve during a dosing interval, where tau is the length of the dosing interval; C_{max} , maximum concentration; C_{trough} , concentration immediately prior to dosing at multiple dosing; CV, coefficient of variation; PK, pharmacokinetics; SD, standard deviation.

CONCLUSION

To our knowledge, this is the first study to evaluate the composite induction and inhibitory effects on PK of digoxin and rosuvastatin, probe substrates for P-gp and BCRP, respectively. These results suggest that enzalutamide may be a “mild” inhibitor of P-gp but indicate that enzalutamide has no impact on BCRP. Our study suggests enzalutamide-mediated induction of CYP2C9 and CYP3A4 via PXR activation does not necessarily predict overall effect on P-gp and BCRP transporters, either directionally or quantitatively.

The low potential for transporter-mediated DDI between a clinical dose of enzalutamide and P-gp and BCRP substrates suggests that concomitant administration of enzalutamide with medications that are substrates of these transporters does not require dose adjustment in patients with prostate cancer. These findings will provide additional insights for clinical dosing and will be pivotal to inform future treatment guidelines for patients with prostate cancer.

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CONFLICTS OF INTEREST

S.P., P.M., N.H., S.M., T.W., M.P., and G.P.H. report employment with Astellas Pharma Inc. V.G. reports employment with PMSI Republican Clinical Hospital “Timofei Moşneaga” and ARENSIA EM. R.K. reports employment with Pfizer Inc. and ownership in Pfizer Inc.

AUTHOR CONTRIBUTIONS

S.P., V.G., R.K., P.M., N.H., S.M., T.W., M.P., and G.P.H. wrote the manuscript. S.P., V.G., R.K., T.W., M.P., and G.P.H. designed the research. V.G., S.M., and G.P.H. performed the research. P.M., N.H., and T.W. analyzed the data.

DATA AVAILABILITY STATEMENT

Researchers may request access to anonymized participant-level data, trial-level data, and protocols from

Astellas-sponsored clinical trials at www.clinicalstudydatarequest.com. For the Astellas criteria on data sharing see: <https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Astellas.aspx>.

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