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Darolutamide does not interfere with OATP-mediated uptake of docetaxel

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Abstract

The addition of darolutamide, an androgen receptor signalling inhibitor, to therapy with docetaxel has recently been approved as a strategy to treat metastatic prostate cancer. OATP1B3 is an SLC transporter that is highly expressed in prostate cancer and is responsible for the accumulation of substrates, including docetaxel, into tumours. Given that darolutamide inhibits OATP1B3 in vitro, we sought to characterise the impact of darolutamide on docetaxel pharmacokinetics. We investigated the influence of darolutamide on OATP1B3 transport using in vitro and in vivo models. We assessed the impact of darolutamide on the tumour accumulation of docetaxel in a patient-derived xenograft (PDX) model and on an OATP1B biomarker in patients. Darolutamide inhibited OATP1B3 in vitro at concentrations higher than the reported C_{max}. Consistent with these findings, in vivo studies revealed that darolutamide does not influence the pharmacokinetics of Oatp1b substrates, including docetaxel. Docetaxel accumulation in PDX tumours was not decreased in the presence of darolutamide. Metastatic prostate cancer patients had similar levels of OATP1B biomarkers, regardless of treatment with darolutamide. Consistent with a low potential to inhibit OATP1B3-mediated transport in vitro, darolutamide does not significantly impede the transport of Oatp1b substrates in vivo or in patients. Our findings support combined treatment with docetaxel and darolutamide, as no OATP1B3 transporter based drug–drug interaction was identified.

KEYWORDS

cabazitaxel, darolutamide, docetaxel, OATP, prostate cancer

What's new?

The combination of docetaxel and the androgen receptor inhibitor darolutamide can improve survival in metastatic hormone-sensitive prostate cancer patients. To enter tumor cells, docetaxel uses the drug transporter OATP1B3, which may be inhibited by darolutamide. Here, using *in vitro* and *in vivo* models and clinical samples, the authors investigated the impact of darolutamide on

docetaxel pharmacokinetics. Darolutamide inhibited OATP1B3 but only above maximum serum concentrations. The drug had no impact on docetaxel accumulation in patient-derived xenograft tumors or OATP1B biomarkers in patient samples. An absence of drug trans-porter-related pharmacokinetic interactions supports the combined use of docetaxel and darolu-tamide.

1 | INTRODUCTION

A recent development in the treatment of metastatic prostate cancer is the addition of an androgen receptor signalling inhibitor (ARSi) to androgen deprivation therapy (ADT) and docetaxel chemotherapy. The ARASENS phase III trial demonstrated increased overall survival in metastatic hormone-sensitive prostate cancer patients when the ARSi darolutamide was added to ADT and docetaxel, in comparison to placebo.[1] These data led to a second registration of darolutamide, following its previous approval for non-metastatic castration-resistant prostate cancer.[2] Furthermore, a phase II trial (NCT05762536) is currently investigating the efficacy of darolutamide added to ADT and docetaxel or cabazitaxel in metastatic castration-resistant prostate cancer (mCRPC) patients after progression on an ARSi, in comparison to only ADT and docetaxel or cabazitaxel.

Despite the potential for increased efficacy with synergistic drug combinations, adding drugs to a chemotherapy regimen could have unintended consequences that impact patient outcomes. One of these consequences is potential drug–drug interactions (DDI) that impair the delivery of a substrate drug to the tumour. Membrane transport proteins are responsible for drug transport into cells. One of the largest families of drug transporters is the solute carrier (SLC) superfamily.[3] Of these, the organic anion transporting polypeptides (OATPs) are predominantly known for transporting substrates from the blood into hepatocytes; however, the subtype OATP1B3 is also highly expressed in prostate cancer.[4–7] Previously, we identified docetaxel as a substrate of OATP1B3.[8,9] Subsequently, we demonstrated in patient-derived xenograft (PDX) prostate cancer tumours that intratumoural docetaxel concentrations are directly correlated to OATP1B3 expression on the tumour cells.[10] Thus, the cellular influx of docetaxel – and consequent delivery to the tumour – may be obstructed by drug-induced inhibition of OATP function. Darolutamide and its major metabolite keto-darolutamide have been described to inhibit OATPs in vitro, but no in vivo studies have been performed to date.[11] Considering known discrepancies between in vitro and in vivo results[12] and the potential impact of this DDI on patient outcomes, we sought to assess this DDI using in vivo and clinical approaches. The aim of the current study was to assess the influence of darolutamide on OATP-mediated transport using in vitro and in vivo models and clinical samples.

2 | MATERIALS AND METHODS

2.1 | Cell lines and chemicals

Human embryonic kidney 293T (HEK 293T; RRID: CVCL_0063, ATCC, Manassas, VA, USA) cells stably expressing *SLCO1B3* (coding for OATP1B3) or an empty vector were maintained as described previously.[13] All human cell lines have been authenticated using STR profiling within the last 3 years. All experiments were performed with mycoplasma-free cells. Darolutamide was purchased from Selleckchem (Houston, TX, USA, cat. no. S7559) and keto-darolutamide was purchased from MedChemExpress (Monmouth Junction, NJ, USA, cat. no. HY-19337). Pravastatin was obtained from Toronto Research Chemicals (Toronto, Canada, cat. no. P702000) and rifampin from MedChemExpress (cat. no. HY-B0272). Docetaxel and cabazitaxel were purchased from LC-Labs (Woburn, MA, USA, cat. no. D-1000 and C-2581). Estradiol-17β-D-glucuronide (EβG) was obtained from Sigma-Aldrich (Saint Louis, MO, USA, cat. no. E1127) and 3H-EβG was purchased from American Radiolabeled Chemicals (Saint Louis, cat no. ART-1320-250). Radiolabelling of 3H-darolutamide, 3H-docetaxel and 14C-cabazitaxel was performed by Moravek (Brea, CA, USA).

2.2 | Transport inhibition assays

Cells were seeded in 12-well plates, coated with poly-d-lysine, at a density of 2×10^6 cells per well in phenol-red free DMEM supplemented with 10% FBS for 24 h prior to transport assays. Substrates and/or inhibitors were added in phenol-red free and serum-free DMEM for the desired time. Cells were lysed in 1 N NaOH for at least 4 h and neutralised in HCl before liquid scintillation counting using a Tri-Carb 4810TR (PerkinElmer, Waltham, MA, USA). Counts were normalised to total protein level, as assessed by a Pierce BCA Protein Assay (Thermo Fisher Scientific, Waltham, MA, USA, cat. no. 23225). According to the FDA Guidelines for In Vitro Metabolism and Transporter Mediated Drug–Drug Interaction Studies, a drug was considered a substrate for OATP1B3 if the uptake of the drug was ≥2-fold higher in OATP1B3 overexpressing cells, as compared to cells containing an empty vector.[14]

Separation of cell membranes and intracellular fractions was completed as previously described.[15] In short, HEK 293 T cells were seeded in poly-d-lysin coated 6-well plates at a density of 4×10^6 cells per well and incubated as described above. Alternatively, cells were detached with TrypLE and processed using the ProteoExtract Native Membrane Protein Extraction Kit (EMD Millipore, Burlington, MA, USA, cat. no. 444810), according to the manufacturer's protocol, before liquid scintillation counting.

2.3 | Cell viability assays

Cells were seeded in 96-well plates at a density of 5×10^3 cells per well and incubated with drugs for the desired time in phenol-red free

and serum-free DMEM. 5 mg/mL 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) was added for quantification of cell viability, which was normalised to vehicle treated cells.

2.4 | In vivo pharmacokinetic studies

Age and sex-matched Oatp1a/b(−/−) and wild-type (WT) mice on an FVB background (Taconic, Germantown, NY, USA) were housed in a temperature- and light-controlled environment. Mice within the same experiment were of similar age (±7 weeks) and weight (±6 g). Mice were randomly assigned to treatment groups ($n = 4–5$ per group[16]). Pravastatin was administered orally at a dose of 20 mg/kg in saline. Docetaxel and cabazitaxel were administered intravenously at a dose of 10 mg/kg in tween 80/EtOH/Saline (20:13:67, v/v/v). Darolutamide was administered orally at a dose of 200 mg/kg in PEG 400/propylene glycol/5% glucose (5:3:2, v/v/v). Drug dosages were previously established and found to be safe.[17] When assessing darolutamide as a DDI perpetrator, darolutamide or vehicle was administered 15 min before pravastatin, docetaxel, or cabazitaxel. Blood samples for pharmacokinetic analysis were obtained according to a previously described sample strategy.[16]

For assessment of intratumoural docetaxel concentrations, 8 NMRI nu/nu male mice (Janvier, Le Genest-Saint-Isle, France) underwent subcutaneous implantation of human prostate cancer PC346C tumours.[18] At a tumour volume of 300 mm^3, 200 mg/kg darolutamide was administered daily for 7 days. Docetaxel (33 mg/kg i.v.) was administered once at day 5. Tumours and plasma were harvested at day 8.

2.5 | Clinical samples

For analysis of the endogenous OATP1B biomarker glycochenodeoxycholate-3-sulfate (GCDCA-S), blood samples were collected from 18 patients with mCRPC, participating in a clinical trial (MEC-20-0394), treated with cabazitaxel, sequentially without and with darolutamide.[19] Blood samples were collected before and during darolutamide treatment (600 mg bid for 6 weeks) simultaneously with cabazitaxel administrations until 24 h after the start of infusion.

2.6 | LC–MS/MS conditions

Plasma and/or tissue concentrations of GCDCA-S, docetaxel, cabazitaxel and darolutamide were measured using validated liquid chromatography–tandem mass spectrometry methods, as described previously.[20–23] An LC–MS method was developed for the measurement of pravastatin concentrations. In brief, a Vanquish UHPLC coupled with a Altis Plus triple quadrupole mass spectrometer from Thermo Fisher Scientific was used for LC–MS analysis. An Accucore aQ column (50 × 2.1 mm, dp = 2.6 µm, Thermo Fisher Scientific) was protected by a C18 AQUASIL guard cartridge (2.1 mm × 10 mm, dp = 3 µm, Thermo Fisher Scientific). The temperature of the autosampler rack was 4°C, and the temperature of the column was maintained at 40°C. The mobile phase was composed of solvent A (0.1% formic acid in H_2O) and solvent B (0.1% formic acid in acetonitrile–methanol, 1:3 v:v). The total run time was 5.0 min. The gradient conditions were as follows: 0–0.5 min, 5% B; 0.5–4.0 min, 5 to 95%, 4.0–4.5 min, 95% B; 4.5–5.0 min, 5% B with a flow rate of 0.4 mL/min. The MS assay setting with the negative voltage applied to the ESI capillary was set at 3000 V, and the capillary temperatures was 350°C with a vaporiser temperature of 380°C. Argon was used as the collision gas at a pressure of 1.5 mTorr. Precursor molecular ions and product ions were recorded for confirmation and detection of pravastatin (423.383 > 321.217), using [$^2H_3$]-pravastatin as an internal standard (426.467 > 321.217). Results from assay validation studies revealed that the within-day precision and between-day precision of all QCs including lower limit of quantification (LLOQ) ranged from 1.9% to 6.8%, and the accuracy of all QCs ranged from 91.5% to 104%. The lower limit of quantification was 0.1 µg/mL in mouse plasma and the acceptable stability of pravastatin in matrix had already been fully evaluated by Sparidans et al.[24] A single one-step protein precipitation method was used to extract pravastatin from mouse plasma samples. Prior to analysis, frozen samples were thawed at room temperature, 5-µL aliquots of plasma were transferred into a 0.5-mL Eppendorf tube, followed by the addition of 5 µL of internal standard working solution at 20 µg/mL and 90 µL of neat methanol. The samples were vortex-mixed for 30 s and centrifuged at 13,000 rpm for 10 min at 4°C. Next, 75-µL aliquots of the supernatant were added to autosampler vials (Agilent Technologies, Palo Alto, CA), and a 5-µL volume was injected into the LC–MS/MS system.

Pharmacokinetic parameters were estimated using non-compartmental analysis in Phoenix WinNonLin version 8.3 (Certara, Princeton, NJ, USA).

2.7 | Statistical analysis

All data are presented as mean ± standard deviation (SD), except for Area Under the Curve (AUC) values and maximum observed plasma concentrations (C_{max}), which are presented as geometric mean with coefficient of variation (CV) and plasma concentration time curves, which are presented as geometric mean ± 95% confidence interval. Differences between groups were tested for statistical significance by an unpaired Student's *t*-test or a two-way ANOVA in case of multiple categorical variables. For AUC and C_{max} data, a log-transformation was performed first, as these data are assumed to follow a log-normal distribution. Differences between samples collected from the same patient were tested by a paired Student's *t*-test. Dose–response curves were interpolated by log-logistic regression. IC_{50} values were compared using the function EDcomp of the package *drc* in R version 4.1.1 (R Core Team, Vienna, Austria).[25] A two-sided alpha of 0.05 was considered statistically significant.

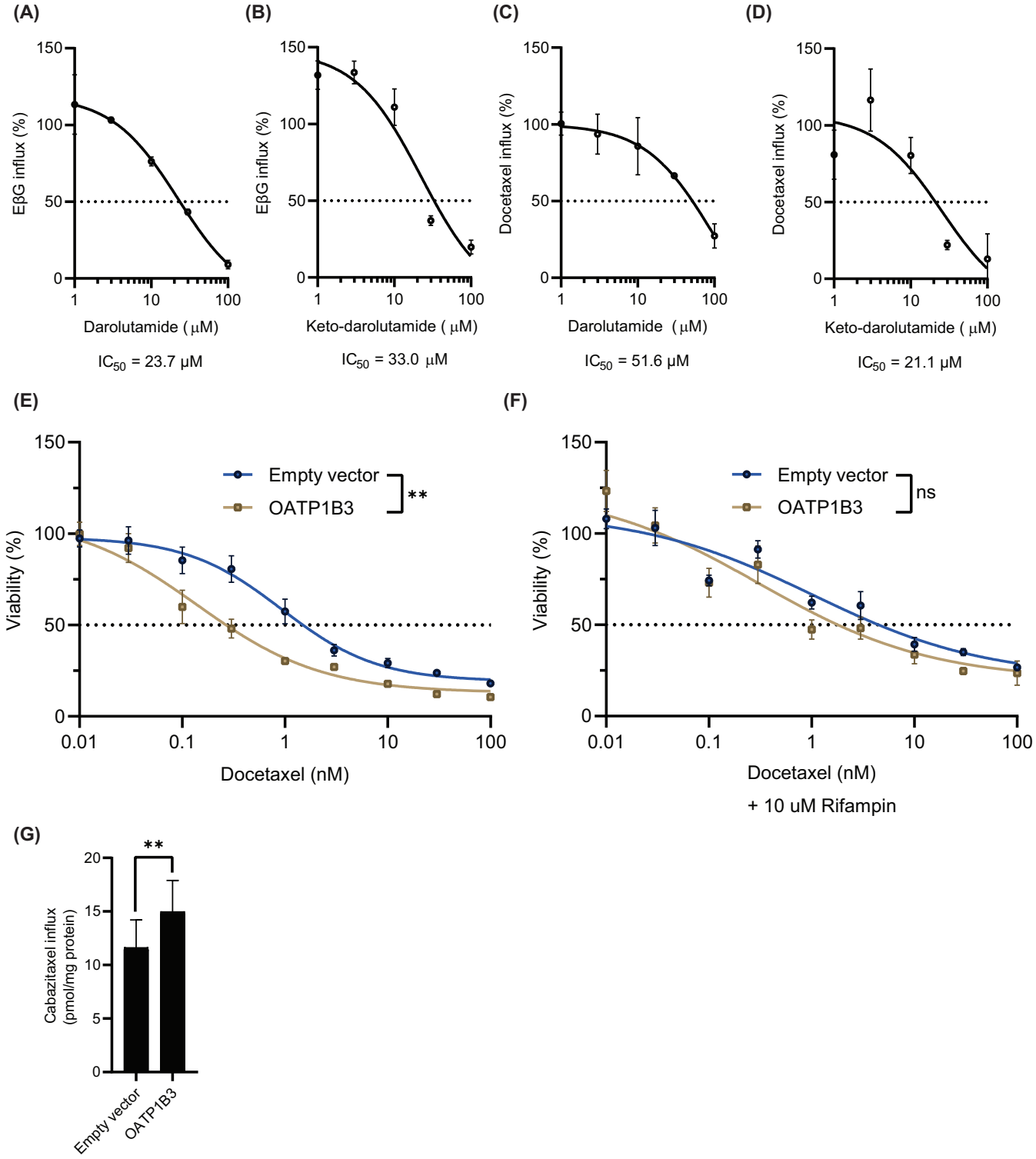

FIGURE 1 Darolutamide inhibits OATP1B3 mediated transport in vitro. (A–D) Cells were pre-incubated with (keto-)darolutamide for 1 h, after which 0.2 μM 3H-EβG (A-B) or 1 μM 3H-docetaxel (C, D) and (keto-)darolutamide were added for 5 min and liquid scintillation was counted. Values are counts of OATP1B3 overexpressing cells minus counts of empty vector containing cells, normalised to 0 μM (keto-)darolutamide ($n = 3$ representative of 2 (A, B) or 3 (C, D) biological replicates). (E, F) Cells were incubated with docetaxel (and rifampin [F]) for 72 h ($n = 6$ across 1 biological replicate). **: $p < .01$; ns: not significant. (G) Cells were incubated with 1 μM 14C-cabazitaxel for 5 min before membrane separation and liquid scintillation counting ($n = 3$ across 3 biological replicates). **: $p < .01$.

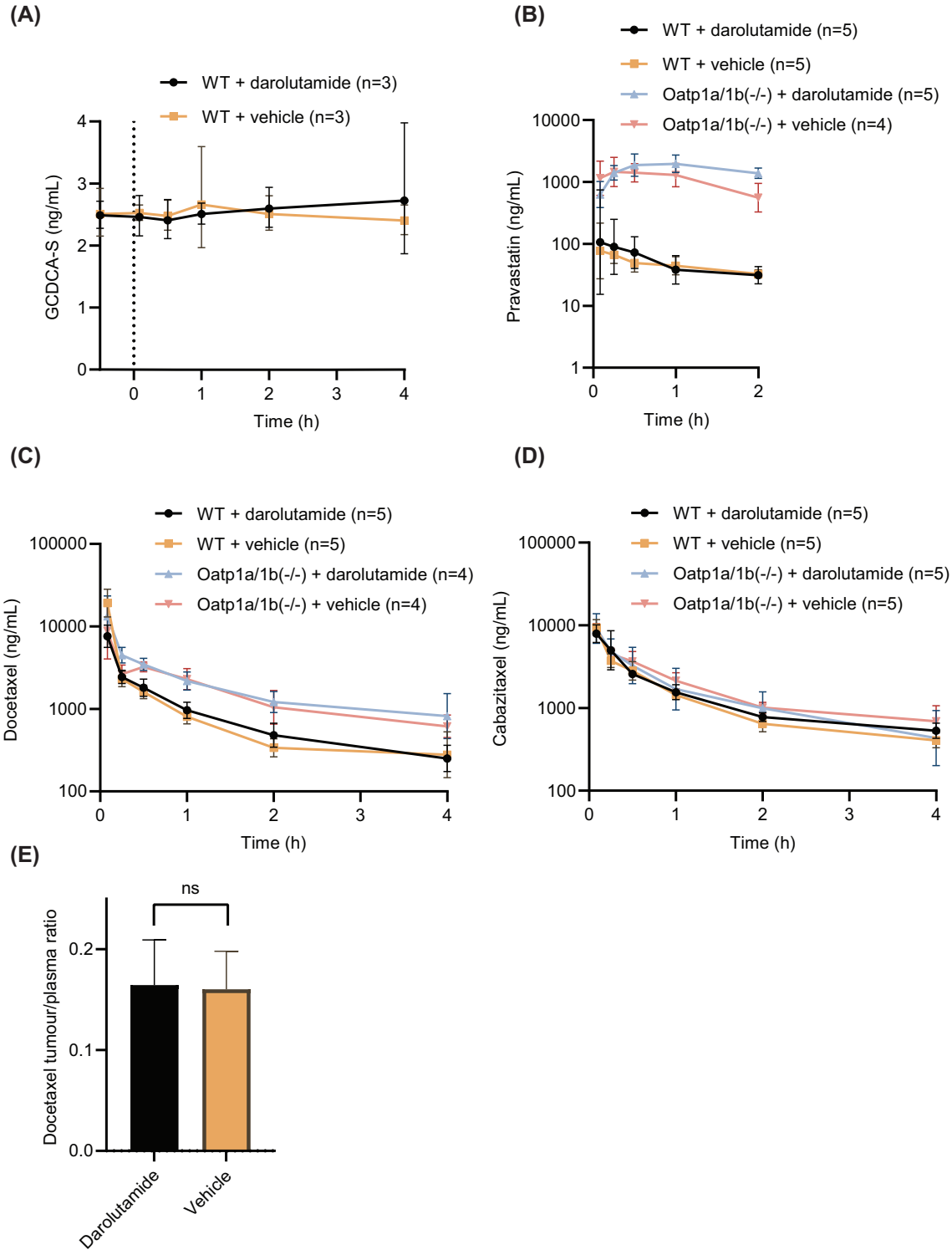

FIGURE 2 Darolutamide does not inhibit Oatp mediated transport in vivo. (A) GCDCA-S concentrations were measured in plasma samples of female wild type mice receiving darolutamide or vehicle at $t = 0$ h. (B–D) Pravastatin (B), docetaxel (C) and cabazitaxel (D) concentrations were measured in plasma samples of male wild type and Oatp1a/1b(−/−) mice receiving darolutamide or vehicle at $t = -0.25$ h. Pravastatin, docetaxel and cabazitaxel were administered at $t = 0$ h. (E) Docetaxel concentrations were measured in tumour and plasma samples of male wild type mice ($n = 4$ per group) subcutaneously bearing a PC346C tumour, after treatment with darolutamide or vehicle for 7 days and docetaxel once at day 5. The ratio between tumour and plasma concentrations of docetaxel is depicted.

TABLE 1 Murine biomarker and pharmacokinetic parameters.

Measurement	Genotype	Sex	*n*	Co-treatment	C_{max} (ng/mL) (CV%)	*p*-Value	T_{last} (h)	$AUC_{0\text{-last}}$ (ng*h/mL) (CV%)	*p*-Value
GCDCA-S	WT	F	3	Darolutamide	2.8 (11)	.66	4	10.0 (5)	.71
		F	3	Vehicle	2.7 (8)			9.9 (5)	
Pravastatin	WT	M	5	Darolutamide	143 (190)	.39	2	112 (74)	.59
		M	5	Vehicle	96 (60)			95 (14)	
	Oatp1a/1b (−/−)	M	5	Darolutamide	2322 (17)	<.001 vs. WT		3275 (14)	<.001 vs. WT
		M	4	Vehicle	1555 (20)	<.001 vs. WT		2179 (28)	<.001 vs. WT
					C_{max} (μg/mL) (CV%)			$AUC_{0\text{-last}}$ (μg*h/mL) (CV%)	
Docetaxel	WT	M	5	Darolutamide	7.6 (23)	<.001	4	4.3 (15)	.02
		M	5	Vehicle	19.2 (28)			6.4 (28)	
	Oatp1a/1b (−/−)	M	4	Darolutamide	13.2 (32)	.03 vs. WT		9.0 (16)	<.001 vs. WT
		M	4	Vehicle	8.8 (45)	.02 vs. WT		7.3 (24)	.48 vs. WT
Cabazitaxel	WT	M	5	Darolutamide	7.9 (18)	.19	4	6.2 (12)	.50
		M	5	Vehicle	9.4 (16)			5.9 (12)	
	Oatp1a/1b (−/−)	M	5	Darolutamide	9.1 (31)	.43 vs. WT		6.9 (39)	.56 vs. WT
		M	5	Vehicle	9.3 (8)	.94 vs. WT		7.7 (14)	.01 vs. WT
Darolutamide	WT	M	5	Docetaxel	56.4 (18)		4	147.8 (13)	
	Oatp1a/1b (−/−)	M	4		58.3 (23)			144.4 (23)	
	WT	M	5	Cabazitaxel	42.9 (28)			124.2 (28)	
	Oatp1a/1b (−/−)	M	5		49.6 (46)			124.5 (46)	

3 | RESULTS

3.1 | Concentration-dependent inhibition of OATP1B3 by darolutamide in vitro

To determine whether darolutamide inhibits OATP1B3, cells overexpressing OATP1B3 or an empty vector were incubated with various concentrations of darolutamide or its major active metabolite keto-darolutamide, after which EβG, a prototypical OATP1B3 substrate, was added and its influx was quantified. Darolutamide and keto-darolutamide concentration-dependently inhibited EβG influx (IC_{50}: 23.7 μM and 33.0 μM, respectively; Figure 1A,B). However, complete inhibition of EβG influx was only observed at concentrations beyond the reported C_{max} in patients of 12.0 μM.[26] This inhibition of OATP1B3 by darolutamide was noncompetitive, as determined by a Dixon plot (Figure S1A). In agreement with this, darolutamide was not a substrate of OATP1B3, as its uptake in OATP1B3 overexpressing cells was only 1.2 to 1.4-fold higher than in empty vector containing cells (Figure S1B). Furthermore, pre-incubation with darolutamide substantially augmented OATP1B3 inhibition (1.8 to 2.2-fold) in comparison to no pre-incubation (Figure S1C).

Next, we investigated the impact of darolutamide on OATP1B3-mediated uptake of docetaxel. Docetaxel transport was highly dependent on OATP1B3. OATP1B3 overexpressing cells transported more than twice the amount of docetaxel as compared to empty vector containing cells (113.8 vs. 55.3 pmol/mg protein; $p < .001$; Figure S2A). Similar to EβG, docetaxel influx was reduced by darolutamide and keto-darolutamide, but only at high concentrations (IC_{50}: 51.6 μM and 21.1 μM, respectively; Figure 1C,D). The reliance of docetaxel on transport by OATP1B3 was further substantiated by viability assays. OATP1B3 overexpressing cells were significantly more sensitive to docetaxel than empty vector containing cells (IC_{50}: 0.3 nM vs. 1.5 nM; $p = .002$; Figure 1E). Importantly, this discrepancy was reversible by adding the prototypical OATP inhibitor rifampin (IC_{50}: 1.8 nM vs. 4.5 nM; $p = .87$ for OATP1B3 overexpressing cells, compared to empty vector containing cells; Figure 1F).

Initial experiments demonstrated that overall influx of cabazitaxel was not significantly increased in cells overexpressing OATP1B3 (85.0 vs. 88.0 pmol/mg protein, $p = .37$; Figure S3A). However, these data were confounded by extensive membrane binding by cabazitaxel (22.8 vs. 22.9 pmol/mg protein for membrane fractions; $p = .95$; Figure S3B). After correction by removal of membrane fractions, the intracellular fractions of OATP1B3 overexpressing cells were significantly more enriched with cabazitaxel than empty vector containing cells (15.0 vs. 11.6 pmol/mg protein, $p < .01$; Figure 1G). Nonetheless, this was only a 29% increase in the overexpressing cells. For

docetaxel, on the other hand, intracellular fractions were marked by a substantial increase of 66% ($p = .03$; Figure S2B,C). Consistent with these findings, darolutamide did not significantly alter cabazitaxel uptake between OATP1B3 overexpressing cells and empty vector containing cells in both total and intracellular fractions (182 vs. 173 pmol/mg protein, $p = .22$ and 16.7 vs. 22.4 pmol/mg protein, $p = .57$; Figure S3C).

3.2 | Darolutamide does not impact OATP1B transport in vivo

Despite high IC_{50} values of darolutamide in vitro, we conducted preclinical in vivo experiments to exclude a potential clinically relevant DDI. We first assessed the influence of darolutamide on bile acid metabolite GCDCA-S, which is a validated endogenous biomarker of Oatp1b transport function.[27] No differences in GCDCA-S concentrations were observed between WT mice after administration of darolutamide or vehicle ($AUC_{0\text{-}4h}$ (CV): 10.0 (5%) vs. 9.9 ng*h/mL (5%), $p = .71$ Figure 2A and Table 1). Plasma concentrations of pravastatin—a prototypical exogenous biomarker of Oatp1b transport—were not significantly different between WT mice receiving darolutamide or vehicle ($AUC_{0\text{-}2h}$ 112 (74%) vs. 95 ng*h/mL (14%), $p = .59$), while mice with genetic deficiency of the Oatp1a/1b cluster (Oatp1a/1b(−/−) mice) had a 29 and 23 fold higher plasma exposure than WT mice, respectively, for darolutamide and vehicle treatment ($AUC_{0\text{-}2h}$ 3275 (14%), $p < .001$ and 2179 ng*h/mL (28%), $p < .001$; Figure 2B and Table 1).

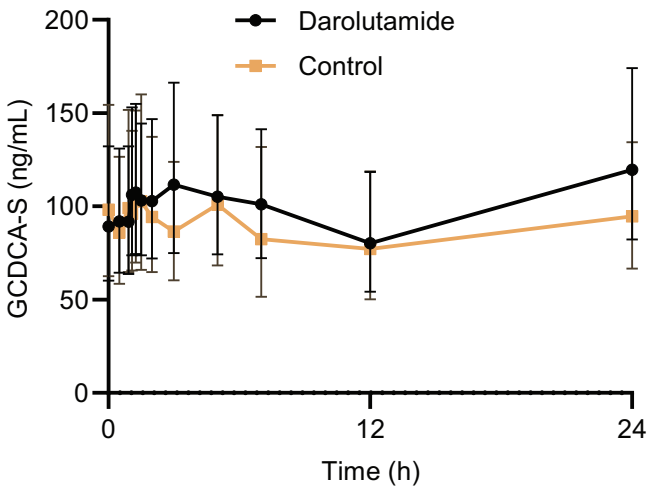

FIGURE 3 Darolutamide does not influence GCDCA-S concentrations in patients. GCDCA-S concentrations were measured in plasma samples of patients with mCRPC ($n = 18$) before and during treatment with darolutamide.

Subsequently, we tested if darolutamide would impact plasma concentrations of docetaxel or cabazitaxel. First, we sought to confirm the importance of OATP transporters for the hepatic uptake of docetaxel using mice with genetic deficiency of the Oatp1a/1b cluster. Docetaxel concentrations were not significantly different in Oatp1a/1b(−/−) mice compared to WT mice treated with vehicle (7.3 (24%) vs. 6.4 µg*h/mL (28%), $p = .48$; Figure 2C and Table 1). Surprisingly, docetaxel concentrations were lower in WT mice receiving darolutamide, compared to WT mice receiving vehicle ($AUC_{0\text{-}4h}$: 4.3 (15%) vs. 6.4 µg*h/mL (28%), $p = .02$; Figure 2C and Table 1). Nonetheless, levels were significantly higher in Oatp1a/1b(−/−) mice treated with darolutamide ($AUC_{0\text{-}4h}$: 9.0 (16%) vs. 4.3 µg*h/mL (15%), $p < .001$; Figure 2C and Table 1). Consistent with some OATP1B3-mediated influx of cabazitaxel (Figure 1G), cabazitaxel exposure was significantly higher in Oatp1a/1b(−/−) mice treated with vehicle versus WT mice ($AUC_{0\text{-}4h}$: 7.7 (14%) vs. 5.9 µg*h/mL (12%), $p = .01$; Figure 2D and Table 1). Nonetheless, consistent with expectations, darolutamide did not impact cabazitaxel concentrations in WT mice ($AUC_{0\text{-}4h}$: 6.2 (12%) vs. 5.9 µg*h/mL (12%), $p = .50$; Figure 2D and Table 1). Importantly, darolutamide plasma exposure in mice treated with docetaxel and cabazitaxel exceeded IC_{50} values observed in vitro with an $AUC_{0\text{-}4h}$ of 146.3 (17%) and 124.3 µg*h/mL (36%), respectively (Figure S4A,B and Table 1), and a darolutamide C_{max} of 57.2 (19%) and 46.2 µg/mL (26%), respectively (Figure S4C,D and Table 1).

Although darolutamide did not impact hepatic Oatp function in vivo, it is also essential to determine if tumoural OATP1B3 function was inhibited. OATP-mediated transport of docetaxel into tumours relies fully on OATP1B3 without potential compensatory transport mediated by other OATP subtypes.[5,8] In the PC346C PDX, which highly expresses *SLCO1B3*,[10] we assessed the ratio between intratumoural and systemic docetaxel concentrations after 7 days of darolutamide treatment. The ratio was not significantly different between mice treated with docetaxel and vehicle and mice treated with docetaxel and darolutamide (0.16 (20%) vs. 0.16 (22%), $p = .90$; Figure 2E and Figure S4E). Darolutamide concentrations were 7.5 µg/mL (63%) in plasma and 0.5 µg/mL (62%) in tumours (Figure S4F).

3.3 | Darolutamide does not alter OATP1B function in patients

While we did not observe an OATP-mediated DDI between darolutamide and docetaxel in our murine model nor in our patient-derived

TABLE 2 Clinical biomarker and pharmacokinetic parameters.

Measurement	Subject	Sex	*n*	Co-treatment	C_{max} (µg/mL) (CV%)	*p*-Value	T_{last} (h)	$AUC_{0\text{-}last}$ (µg*h/mL) (CV%)	*p*-Value
GCDCA-S	mCRPC patients	M	18	Darolutamide	0.17 (70)	.61	24	2.4 (75)	.13
				None	0.15 (80)			2.1 (84)	

tumour model, underscoring the lack of effect was not caused by species-specific differences, it is possible that other transporters (e.g., OATP1A) may be involved. Thus, we assessed GCDCA-S concentrations in blood of 18 mCRPC patients before and during treatment with darolutamide. Patients had steady state darolutamide plasma concentrations ($AUC_{0\text{-}12h}$: 39.2 μg*h/mL (41%)).[19] GCDCA-S concentrations were not significantly different during darolutamide treatment (dose administration at $t = 0$ h) in comparison to before darolutamide treatment ($AUC_{0\text{-}24h}$ 2.4 (75%) vs. 2.1 μg*h/mL (84%), $p = .13$; Figure 3 and Table 2).

4 | DISCUSSION

In an era of combination regimens for oncologic diseases, exclusion of DDIs is pivotal to achieve maximal therapeutic benefit.[28] While such investigations in vitro are enforced by regulatory agencies,[14] in vivo investigations are not performed regularly before a combination of drugs is marketed.[29] Thus, warnings are often presented in the label based exclusively on in vitro experiments, as is the case for darolutamide and OATP1B1/3. Darolutamide's label cautions against co-administration of substrates of OATP1B1 or OATP1B3, as their plasma concentrations may increase. However, prior to our study, this was not assessed in vivo or in patients. Beyond in vitro studies, the only additional evidence was an observed DDI between darolutamide and rosuvastatin, which could be due to transporters other than OATP1B1 or OATP1B3.[11] The current study suggests that this warning may be inappropriate, given that we observed no interaction between darolutamide and OATP1B3 substrates in vivo and in patients.

Here, we show that darolutamide does not inhibit OATP1B3 at clinically-relevant concentrations in vitro. Nonetheless, given that in vitro experiments are not always predictive of in vivo observations[12] and especially considering our previous incongruent negative in vitro results[15] we pursued in vivo experiments. A major strength of the in vivo studies conducted here, is the investigation of transport of taxanes in an Oatp1a/1b knockout mouse model, in line with a previous study investigating transport of docetaxel.[30] Other previous studies on docetaxel and cabazitaxel transport by Oatps were predominantly performed in Oatp1b2 knockout mice, not accounting for the fact that members of the murine Oatp1a subfamily could maintain transport function and compensate for the loss of Oatp1b2.[8,31,32] Maximum observed concentrations of darolutamide in mice receiving docetaxel were equal to nearly three times the IC_{50} for inhibition of docetaxel uptake as established in vitro. Yet, no inhibition of Oatp1b was observed in vivo. Lack of inhibition may be the result of extensive protein binding by darolutamide and keto-darolutamide in vivo, which is 92% and 99% in human plasma, respectively, possibly hampering their inhibitory potential.[33] Another explanation for the lack of inhibition could be that additional hepatic uptake transporters in vivo that are not inhibited by darolutamide, compensate for the transport of substrates. Regardless, our in vivo findings are consistent with our in vitro findings suggesting that there is no DDI between darolutamide and docetaxel.

We did not observe any influence of darolutamide on OATP1B biomarkers in patients. Our findings in vivo and in patients support combined treatment with docetaxel and darolutamide, as no SLC transporter based drug–drug interaction was identified. In accordance with this, recent pharmacokinetic analyses of the ARASENS phase III trial revealed no clinically relevant effect of darolutamide on docetaxel systemic exposure.[34] In patients treated with darolutamide, docetaxel systemic exposure was only 6% higher when compared with patients receiving placebo. In contrast, concentrations of the prototypical Breast Cancer Resistance Protein (BCRP) and OATP substrate rosuvastatin were fivefold higher in healthy subjects when darolutamide was administered concomitantly.[11] With the present knowledge, this is likely due to inhibition of BCRP by darolutamide, rather than an effect on OATP1B1 or OATP1B3.

In vivo experiments excluded inhibition of hepatic Oatps by darolutamide, as well as inhibition of tumour uptake of docetaxel in an OATP1B3 expressing PDX tumour. Likewise, darolutamide did not influence hepatic OATP1B function in patients, as assessed by a validated endogenous biomarker.[27] Based on these results, there is no clinically relevant DDI between darolutamide and OATP1B3 substrates including docetaxel. Our findings support the use of combination treatment of metastatic prostate cancer with docetaxel and darolutamide in its current mode, as no drug transporter-related pharmacokinetic interaction was identified in vivo and drug delivery to the tumour was not compromised.

AUTHOR CONTRIBUTIONS

S.A.J.B., A.S. and R.H.J.M. conceived this study. S.A.J.B., Z.T., T.D., E.D.E, C.M.A.R. and D.S. performed the in vitro and/or in vivo studies. Y.J. developed the analytical methods. P.H and P.B. performed pharmacokinetic sample analyses. S.A.J.B. and E.D.E. wrote the manuscript. A.A.G., S.H., A.S. and R.H.J.M. performed supervision. All authors reviewed the manuscript. All authors have read and agreed to the published version of the manuscript. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

CONFLICT OF INTEREST STATEMENT

Stefan A.J. Buck has received travel support and speakers fee from Bayer. Wytske M. van Weerden has received research funding (Institutional) from Bayer and AstraZeneca. Ronald de Wit has acted in a consulting or advisory role for Merck, Astellas, Bayer; and has received research funding from Bayer. Ron H.J. Mathijssen has received research funding (institutional) from Astellas, Bayer, Boehringer-Ingelheim, Cristal Therapeutics, Novartis, Nordic Pharma, Pamgene, Pfizer, Roche, Sanofi, Deuter Oncology and Servier. The other authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ETHICS STATEMENT

After approval by the independent ethics board BEBO Foundation for the Assessment of Ethics of Biomedical Research and the local ethics board under MEC-20-0394, written informed consent was obtained from all patients participating in the clinical trial investigating the DDI between cabazitaxel and darolutamide. The trial was conducted in accordance with the Declaration of Helsinki and was registered in the Netherlands Trial Register under NL8611. All animal studies were conducted in accordance with the ARRIVE 2.0 guidelines.[35] Animal studies conducted at The Ohio State University were approved under IACUC protocol # 201500000101-R2. Animal studies conducted at The Erasmus Medical Centre were approved under the Dutch experiments on Animal Act, with reference number AVD101002017867.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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