European Journal of Drug Metabolism and Pharmacokinetics

Supplementary Materials

Supplement to: Drug–Drug Interaction Potential of Darolutamide: *In Vitro* and Clinical Studies.

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Table S1 Suppliers of materials for *in vitro* studies

Study	Material	Supplier
Substrate characteristics for drug metabolizing enzymes	Human male hepatocytes Donor 1 HH-TWT, Donor 2 HH-TZU, Donor 3 HH-4070B	Bioreclamation IVT, Baltimore, MD, USA; Triangle Research Labs, Triangle Research Park, NC, USA
	Pooled human renal microsomes (15 mixed gender donors): lot 510251	Xenotech LLC, Lenexa, KS, USA
	Pooled human liver cytosol (200 mixed gender donors): lot 1310087, 1410012	
	Pooled human liver microsomes (200 mixed gender donors): Xtreme 200 lot 1010420 and lot 1210223	
	Pooled human intestinal microsomes (15 mixed gender donors): lot 510408	
	Human liver microsomes (single donor): HH13, H023, HK25, H030, H032, H066, H088, H089, H093	BD Gentest Corp., Woburn, MA, USA

	Recombinant CYP isoforms; Supersomes™	Corning, Woburn, MA, USA
	Recombinant AKR isoforms	Bayer AG, Berlin, Germany
	UDPGA	Sigma-Aldrich GmbH, Steinheim, Germany
Substrate characteristics for drug transporters	Caco-2, CPT-B1 and CPT-P1 cells	American Type Culture Collections, Rockville, MD, USA
	BCRP-MDCK cells	Absorption Systems, Exton, PA, USA
	Atorvastatin, cladribine, cyclosporin A, digoxin, Ko143 and rifamycin; HEK cells	Sigma, St Louis, MO, USA
	Decynium 22	Sigma-Aldrich GmbH, Steinheim, Germany
	^{3}H-MPP+	American Radiolabeled Chemicals, St Louis, MO, USA
Inhibitory potential of darolutamide for CYP isoforms	Pooled human liver microsomes Xtreme 200 lot 1210223 (200 mixed gender donors) and lot TWE (25 mixed gender donors)	In Vitro Technologies, Inc, Baltimore, MD and XenoTech LLC, Lenexa, KS, USA);

	Bufuralol, bupropion, coumarin, diclofenac, paclitaxel, phenacetin, *S*-mephenytoin,	Sigma-Aldrich GmbH, Steinheim, Germany
	Chlorzoxazone, testosterone,	Acros Organics, Geel, Belgium
	Midazolam	Fagron BV, Nieuwerkerk a/d IJssel, The Netherlands
	NADPH	Sigma, Zwijndrecht, The Netherlands
Inhibitory potential of darolutamide for UGT isoforms	Pooled human liver microsomes Xtreme 200 lot 1210223 (200 mixed gender donors) and lot 1010420 (200 mixed gender donors)	BD Biosciences, Woburn, MA, USA
	Pooled human liver microsomes UltraPool™ HLM 150 lot 38290 (150 mixed gender donors)	
	Single donor: HH13, H023, HK25, H030, H032, H066, H088, H089, H093	
	UGT-selective substrates	
Induction potential of darolutamide for CYP isoforms	Primary-cultured human hepatocytes	Triangle Research Labs, LLC, NC and Bioreclamation IVT, LLC, NY, USA

	Donor 1 lot no. HUM4082A (male), Donor 2 lot no. HUM4112 (female), Donor 3 lot no. OII (female)	
	Omeprazole, penicillin, phenacetin, phenobarbital, pioglitazone, rifampicin	Sigma-Aldrich GmbH, Steinheim, Germany
	S-mephenytoin	Enzo Life Sciences GmbH, Lörrach, Germany
	Testosterone	Fluka, Munich, Germany
	Bosentan	USP, Maryland, United States
Inhibitory potential of darolutamide for drug transporters	Caco-2 cells	American Type Culture Collections, Rockville, MD, USA
	MDCK cells	Absorption Systems, Exton, PA, USA
	Sandwich-cultured human hepatocytes	Triangle Research Labs, LLC, NC, USA
	HEK cells (Cat# CRL-1573™)	American Type Culture Collections, Rockville, MD, USA

	Atorvastatin, cladribine, digoxin, imipramine, MMP$^+$, p-aminohippuric acid, probenecid	Sigma, St Louis, MO, USA
	Taurocholic acid	Matrix Scientific, Columbia, SC, USA
	5(6)-carboxy-2,′7′-dichlorofluorescein, benbromarone, cimetidine, cyclosporin A, decynium 22, estrone-3-sulfate, furosemide, gilbenclamide, metformin, pravastatin, pyrimethamine, rifamycin	Sigma Aldrich, Deisenhofen, Germany
	^{3}H-MMP+	American Radiolabeled Chemicals, St Louis, MO, USA

^{3}H-MPP+, ^{3}H-N-Methyl-4-phenylpyridinium; AKR, aldo-keto reductase; BCRP, breast cancer resistance protein; Caco-2, wildtype human colon adenocarcinoma; CPT-B1 cells, proprietary cell line with reduced expression on human BCRP; CPT-P1 cells, proprietary cell line with reduced expression on human P-gp; CYP, cytochrome P450; MDCK, Madin-Darby Canine Kidney; NADPH, nicotinamide adenine dinucleotide phosphate; P-gp, P-glycoprotein; UDPGA, uridine 5'-diphospho-glucuronic acid; UGT, uridine-diphosphate glucuronosyltransferase.

Table S2 Blood and urine sampling schedules for phase I clinical trials with (a) a strong CYP3A4/P-gp and BCRP inhibitor and a CYP3A4 / P-gp inducer, (b) CYP3A4 and P-gp substrates, and (c) a substrate for BCRP, OATP1B1, OATP1B3, and OAT3

(a)

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Plasma samples			Blood / urine samples for laboratory examination
			Darolutamide	Itraconazole	Rifampicin	Darolutamide PK	Itraconazole PK	Rifampicin PK	
Screening	Days −28 to −1								X
	Day −1	Pre-dose (morning)							X
Period 1:	Day 1	−0.5				X			X
		0	X						

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Plasma samples			Blood / urine samples for laboratory examination
			Darolutamide	Itraconazole	Rifampicin	Darolutamide PK	Itraconazole PK	Rifampicin PK	
Darolutamide reference period		0.5				X			
		1							
		1.5				X			
		2				X			
		2.5				X			
		3				X			
		4				X			X[b]
		6				X			
		8				X			
		12				X			
	Day 2	0				X			

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Darolutamide	Itraconazole	Rifampicin	Darolutamide PK	Itraconazole PK	Rifampicin PK	Blood / urine samples for laboratory examination
		12				X			
	Day 3	0				X			X
		12				X			
	Day 4	0				X			X
Period 2: darolutamide + itraconazole	Day 1	0		X					X[c]
		12		X					
	Day 2	0		X					
	Day 3	0		X					
	Day 4	0		X					X[b,c]
	Day 5	−0.5				X	X		X
		0	X	X					
		0.5				X			

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Plasma samples			Blood / urine samples for laboratory examination
			Darolutamide	Itraconazole	Rifampicin	Darolutamide PK	Itraconazole PK	Rifampicin PK	
		1				X			
		1.5				X			
		2				X			
		2.5				X			
		3				X			
		4				X			X[b]
		6				X			
		8				X			
		12				X			
	Day 6	0		X		X[c]	X[c]		X[c]
		12				X			
	Day 7	0		X		X[c]	X[c]		X[c]

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Darolutamide	Itraconazole	Rifampicin	Darolutamide PK	Itraconazole PK	Rifampicin PK	Blood / urine samples for laboratory examination
		12				X			
	Day 8	0				X	X		X
Period 3: darolutamide + rifampicin	Day 1	0			X				X[c]
	Day 2	0			X				
	Day 3	0			X				
	Day 4	0			X				X[b]
	Day 5	0			X				
	Day 6	0			X				
	Day 7	0			X				X[b]
	Day 8	−0.5				X		X	X
		0	X						
		0.5				X			

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Plasma samples			Blood / urine samples for laboratory examination
			Darolutamide	Itraconazole	Rifampicin	Darolutamide PK	Itraconazole PK	Rifampicin PK	
		1				X			
		2				X			
		2.5				X			
		3				X			
		4				X			X[b]
		6				X			
		8				X			
		12			X	X[c]			
	Day 9	0			X	X[c]		X[c]	X
		12		.		X			
	Day 10	0			X	X[c]		X[c]	X
		12				X			

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Plasma samples			Blood / urine samples for laboratory examination
			Darolutamide	Itraconazole	Rifampicin	Darolutamide PK	Itraconazole PK	Rifampicin PK	
	Day 11	0				X		X	X
Follow-up	Day 14 ± 3[d]								X

(b)

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Blood samples		Blood / urine samples for laboratory examination
			Darolutamide	Midazolam	Dabigatran	Midazolam PK	Dabigatran PK	
Screening	Days –28 to –1							X
	Day –1	Pre-dose (morning)						X
Period 1: midazolam and dabigatran reference period	Day 1	–0.5[e]				X	X	X
		0		X	X			
		0.25				X	X	
		0.5				X	X	
		1				X	X	
		1.5				X	X	

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Blood samples		Blood / urine samples for laboratory examination
			Darolutamide	Midazolam	Dabigatran	Midazolam PK	Dabigatran PK	
		2				X	X	
		2.5				X	X	
		3				X	X	
		4				X	X	
		5				X	X	
		6				X	X	
		8				X	X	
		10				X	X	
		12				X	X	
		15				X	X	
	Day 2	0				X	X	X[b]
		12					X	

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Blood samples		Blood / urine samples for laboratory examination
			Darolutamide	Midazolam	Dabigatran	Midazolam PK	Dabigatran PK	
	Day 3	0					X	
	Day 4	0					X	
Period 2: darolutamide + dabigatran + midazolam	Day 1	−1						X[f]
		0	X					
		12	X					
	Day 2	0	X					
		12	X					
	Day 3	−1.5			X		X[c]	X[c]
		−1.25					X	
		−1					X	
		−0.5					X	
		0	X				X[e]	

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Blood samples		Blood / urine samples for laboratory examination
			Darolutamide	Midazolam	Dabigatran	Midazolam PK	Dabigatran PK	
		0.5					X[c]	
		1					X	
		1.5					X	
		2						
		2.5					X	
		3.5					X	
		4.5					X	
		6.5					X	
		8.5					X	
		10.5					X	
		12	X					
		13.5					X	

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Blood samples		Blood / urine samples for laboratory examination
			Darolutamide	Midazolam	Dabigatran	Midazolam PK	Dabigatran PK	
	Day 4	−1.5					X	
		0	X					X[b,c]
		10.5					X	
		12	X					
	Day 5	−1.5					X	
		0	X					
		12	X					
	Day 6	−1.5					X	
		−0.5						X[b,e]
		0	X					
		12	X					
	Day 7	0	X					

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Blood samples		Blood / urine samples for laboratory examination
			Darolutamide	Midazolam	Dabigatran	Midazolam PK	Dabigatran PK	
	Day 8	12	X					
		0	X					
		12	X					
	Day 9	−0.5						X[e]
		0	X	X	X	X[c]	X[c]	
		0.25				X	X	
		0.5				X	X	
		1				X	X	
		1.5				X	X	
		2				X	X	
		2.5				X	X	
		3				X	X	

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Blood samples		Blood / urine samples for laboratory examination
			Darolutamide	Midazolam	Dabigatran	Midazolam PK	Dabigatran PK	
		4				X	X	
		5				X	X	
		6				X	X	
		8				X	X	
		10				X	X	
		11.5						
		12	X			X[c]	X[c]	
		15				X	X	
	Day 10	0	X			X[c]	X[c]	X[b,c]
		12	X				X[c]	
	Day 11	0	X				X[c]	
		12	X					

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]			Blood samples		Blood / urine samples for laboratory examination
			Darolutamide	Midazolam	Dabigatran	Midazolam PK	Dabigatran PK	
	Day 12	0					X	X[b]
Follow-up	Day 14 ± 3[e]							X[f]

(c)

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]		Plasma samples		Urine samples	Blood / urine samples for laboratory examination
			Darolutamide	Rosuvastatin	Darolutamide PK	Rosuvastatin PK	Rosuvastatin PK	
Screening	Days −28 to −1							X
	Day −1	Pre-dose (morning)						X
Period 1: rosuvastatin reference period	Day 1	0		X		X[c]	X[g]	
		0.5				X	→	
		1				X	→	
		1.5				X	→	
		2				X	→	

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]		Plasma samples		Urine samples	Blood / urine samples for laboratory examination
			Darolutamide	Rosuvastatin	Darolutamide PK	Rosuvastatin PK	Rosuvastatin PK	
		2.5				X	→	
		3				X	→	
		4				X	→	
		6				X	X	
		8				X	→	
		12				X	→	
	Day 2	0				X	X	
Period 2: darolutamide + rosuvastatin	Day −2	0						X[h]
	Day 1	0	X		X[c]			
		0.5			X			
		1			X			

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]		Plasma samples		Urine samples	Blood / urine samples for laboratory examination
			Darolutamide	Rosuvastatin	Darolutamide PK	Rosuvastatin PK	Rosuvastatin PK	
		1.5			X			
		2			X			
		2.5			X			
		3			X			
		4			X			
		6			X			
		8			X			
		12			X			
	Day 2	0			X			X
		12			X			
	Day 3	0			X			
		12			X			

| Study Period | Study Day(s) | Time in relation to darolutamide dosing (hours) | Study drug administration[a] | | Plasma samples | | Urine samples | Blood / urine samples for laboratory examination |
			Darolutamide	Rosuvastatin	Darolutamide PK	Rosuvastatin PK	Rosuvastatin PK	
	Day 4	0	X		X[c]			X[c]
		12	X		X[c]			
	Day 5	0	X		X[c]			
		12	X		X[c]			
	Day 6	0	X		X[c]			
		12	X		X[c]			
	Day 7	0	X		X[c]			X[h]
		0.5			X			
		1			X			
		1.5			X			
		2			X			
		2.5			X			

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]		Plasma samples		Urine samples	Blood / urine samples for laboratory examination
			Darolutamide	Rosuvastatin	Darolutamide PK	Rosuvastatin PK	Rosuvastatin PK	
	Day 8	3			X			
		4			X			
		6			X			
		8			X			
		12	X		X[c]			
		0	X	X	X[c]	X[c]	X[g]	X
		0.5			X	X	→	
		1			X	X	→	
		1.5			X	X	→	
		2			X	X	→	
		2.5			X	X	→	

Study Period	Study Day(s)	Time in relation to darolutamide dosing (hours)	Study drug administration[a]		Plasma samples		Urine samples	Blood / urine samples for laboratory examination
			Darolutamide	Rosuvastatin	Darolutamide PK	Rosuvastatin PK	Rosuvastatin PK	
		3			X	X	→	
		4			X	X	→	X[i]
		6			X	X	X	
		8			X	X	→	
		12	X		X[c]	X	→	
	Day 9	0			X	X	X	X
	Day 10	12			X			
	Day 11	0			X			
Follow-up	Day 14 ± 3[e]							X[f]

[a]Darolutamide, itraconazole, midazolam, dabigatran and rosuvastatin were administered orally 30 minutes after starting a standardized meal and with 240 mL of water, whereas rifampicin was administered 1 hour before starting a standardized meal and, on the day of co-administration with darolutamide, 12 hour after darolutamide and 1 hour prior to a standardized evening meal); [b]only blood sample for creatine kinase, liver enzymes (AST, ALT, GGT), and alkaline phosphatase; [c]before study drug administration; [d]after last dose of study medication; [e]measures/actions completed before the start of the standardized breakfast; [f]in fasted state; [g]additional urine sample before dosing of

rosuvastatin; [h]results were checked the same day (latest in the evening) to decide whether the subject could continue participating in the study; [i]blood sample for creatine kinase and liver enzymes.

→, Done continuously from the time point indicated; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase.

Table S3 *In vitro* **analysis of UGT isoform inhibition by (***S,R***)-darolutamide, (***S,S***)-darolutamide, and keto-darolutamide in human liver microsomes using standard substrates**

UGT isoform	Substrate	IC_{50} (μM)		
		(*S,R*)-darolutamide	(*S,S*)-darolutamide	Keto-darolutamide
1A1	Estradiol	26.5	31.7	6.4
1A4	Trifluoperazine	>100	>100	>50
1A6	Serotonin	>100	>100	>50
1A9	Propofol	12.5	>100	>50
2B7	AZT	>100	>100	>50

AZT, 3'-azido-3'-deoxythymidine; IC_{50}, concentration required for 50% inhibition; UGT, uridine diphosphate glucuronosyltransferase.

Table S4 Potential risk for DDI induction with darolutamide in the clinic using the RIS method

Test compound	% decrease in AUC of midazolam			Overall assessment
	Donor 1	Donor 2	Donor 3	
Darolutamide	82	84	82	Strong inducer
(*S,R*)-darolutamide	65	71	64	Moderate inducer
(*S,S*)-darolutamide	80	83	81	Moderate to strong inducer
Keto-darolutamide	58	50	51	Weak to moderate inducer

AUC, area under the curve; DDI, drug–drug interaction, RIS, relative induction score.

Table S5 Summary of PK data from the phase I studies: (a) effect of itraconazole and rifampicin on the pharmacokinetics of darolutamide, its diastereomers, and keto-darolutamide, (b) effect of rosuvastatin coadministration on the pharmacokinetics of darolutamide, its diastereomers, and keto-darolutamide, and (c) effect of darolutamide coadministration on pharmacokinetic parameters of dabigatran, midazolam, and rosuvastatin

a.

Parameter	Period 1 Reference Treatment Darolutamide 600 mg QD (Fed)	Period 2 Darolutamide 600 mg QD (Fed) + Itraconazole 200 mg BID (Fed)	Period 3 Darolutamide 600 mg QD (Fed) + Rifampicin 600 mg QD (Fasted)
Darolutamide			
$AUC_{(0-12)}$ µg·h/mL	14.1/29.5 (7.41 - 22.0)	20.7 /24.1 (12.1 - 27.7)	5.42/31.7 (2.51 - 9.23)
C_{max} µg/mL	1.86/25.1 (1.07-2.63)	2.53/19.6 (1.66-3.53)	0.883/27.8 (0.514-1.48)
(S,R)-**darolutamide**			
$AUC_{(0-12)}$ µg·h/mL	3.34/38.1 (1.77-5.83)	5.32/28.7 (3.02-7.30)	0.934/42.2 (0.360-1.76)
$C_{max,}$ µg/mL	0.573/31.0 (0.269-0.835)	0.862/26.6 (0.539-1.33)	0.210/39.3 (0.0957-0.412)
(S,S)-**darolutamide**			
$AUC_{(0-12)}$ µg·h/mL	10.7/27.8 (5.61-16.3)	15.2/24.0 (9.06-20.3)	4.45/30.4 (2.12-7.46)
C_{max} µg/mL	1.36/23.5 (0.794-1.94)	1.87/20.0 (1.30-2.64)	0.696/25.8 (0.418-1.07)
Keto-darolutamide			
$AUC_{(0-12)}$ µg·h/mL	31.4/29.1 (17.7-45.3)	47.2/24.3 (29.8-77.8)	10.8/36.5 (4.81-20.0)
C_{max} µg/mL	4.32/23.3 (2.64-6.10)	5.99/17.7 (4.33-8.85)	2.04/27.2 (1.18-3.27)

b.

	Period 2, Day 1 Darolutamide 600 mg, Single Dose	Period 2, Day 7 Darolutamide 600 mg BID	Period 2, Day 8 Rosuvastatin 5 mg QD, Single Dose + Darolutamide 600 mg BID
Darolutamide			
$AUC_{(0-12)}$ µg·h/mL	16.7/30.2 (8.90-29.7)	34.5/27.8 (20.2-56.9)	34.1/28.9 (18.6-54.3)
C_{max} µg/mL	2.10/27.7 (1.14-3.30)	3.84/25.1 (2.28-6.38)	3.72/25.3 (2.07-5.44)
(S,R)-darolutamide			
$AUC_{(0-12)}$ µg·h/mL	3.76/31.1 (1.85-7.03)	4.67/32.4 (2.07-8.01)	4.75/28.8 (2.46-7.79)
$C_{max,}$ µg/mL	0.62/0.03 (0.34-0.11)	0.73/0.03 (0.42-0.11)	0.71/0.02 (0.39-0.11)
(S,S)-darolutamide			
$AUC_{(0-12)}$ µg·h/mL	12.9/30.6 (6.60-22.6)	29.8/27.8 (17.6-48.9)	29.3/29.4 (15.7-46.9)
C_{max} µg/mL	1.61/27.4 (0.910-2.45)	3.17/25.1 (1.93-5.24)	3.09/27.0 (1.70-4.51)
Keto-darolutamide			
$AUC_{(0-12)}$ µg·h/mL	37.0/36.2 (19.0-69.2)	82.8/39.5 (29.1-152)	82.5/38.7 (38.0-152)
C_{max} µg/mL	4.89/35.6 (2.51-9.31)	9.40/35.4 (3.66-16.7)	9.20/33.3 (4.35-16.2)

c.

	Period 1, Day 1 Dabigatran 75 mg, Single Dose (Fed)	Period 2, Day 9 Dabigatran 75 mg, Single Dose (Fed) + Darolutamide 600 mg BID (Fed)	Period 1, Day 1 Midazolam 1 mg, Single Dose (Fed)	Period 2, Day 9 Midazolam 1 mg, Single Dose (Fed) + Darolutamide 600 mg BID	Period 1, Day 1 Rosuvastatin 5 mg, Single Dose	Period 2, Day 8 Rosuvastatin 5 mg, Single Dose + Darolutamide 600 mg BID
AUC µg·h/mL	502 / 27.9 (338-929)	475 /39.9 (214-818)	12.9 / 0.04 (5.90-20.1)	9.22 / 0.03 (4.75-14.0)	–	–
$AUC_{(0-24)}$ µg·h/mL	–	–	–	–	6.52/40.5 (3.35-14.7)	33.8/41.3 (14.7-74.3)
C_{max} µg/L	52.6 / 28.8 (32.2-87.3)	44.1 / 60.1 (13.1-88.8)	3.17 / 0.03 (2.22-4.68)	2.15 / 0.03 (1.22-2.99)	0.660/47.4 (0.325-1.69)	3.31/42.5 (1.49-6.70)

Data are presented as geometric means/CV% (range) for PK populations.

$AUC_{(0–12)}$, area under the plasma concentration time curve from time 0 to 12 hours; BID, twice-daily; C_{max}, peak concentration; CV%, coefficient of variation; DABE, dabigatran etexilate; ICZ, itraconazole; MDZ, midazolam; PK, pharmacokinetic; RIF, rifampicin; RSV, rosuvastatin.

Table S6 Summary of the DDI potential of darolutamide and AR inhibitors with CYP enzymes and drug transporters [1-7]

Category	Enzyme/Transporter	Apalutamide	Enzalutamide	Darolutamide
Substrates	CYP3A4	✔	✔	
Addition of an AR inhibitor may affect the concentration of other medications	CYP2C9	✔	✔	
	CYP2C19	✔	✔	
	UGT	✔	✔	
	P-gp	✔	✔	
	BCRP	✔	✔	✔†
	OATP1B1	✔	✔	
Inhibitors	CYP3A4	✔		✔‡
Other medications may increase	CYP2C8	✔	✔	

AR inhibitor concentrations			
Inducers	CYP3A4	✓	✓§
Other medications may decrease AR inhibitor concentrations			

† Avoid concomitant use with drugs that are BCRP substrates where possible. If used together, monitor patients more frequently for adverse reactions and consider dose reduction of BCRP substrate drug. ‡If P-gp and strong CYP3A4 inhibitors are used together, monitor patients more frequently for darolutamide adverse reactions. §Avoid concomitant use of P-gp and strong or moderate CPY3A4 inducers.

AR, androgen receptor; BCRP, breast cancer resistance protein; CYP, cytochrome P450; mCRPC, metastatic castration-resistant prostate cancer; P-gp, P-glycoprotein; UGT, Uridine 5'-diphospho-glucuronosyltransferase.

Fig. S1 *In vitro* effects of darolutamide on efflux transporters (a) P-gp, with digoxin as the probe substrate, and (b) BCRP, with cladribine as the probe substrate


a
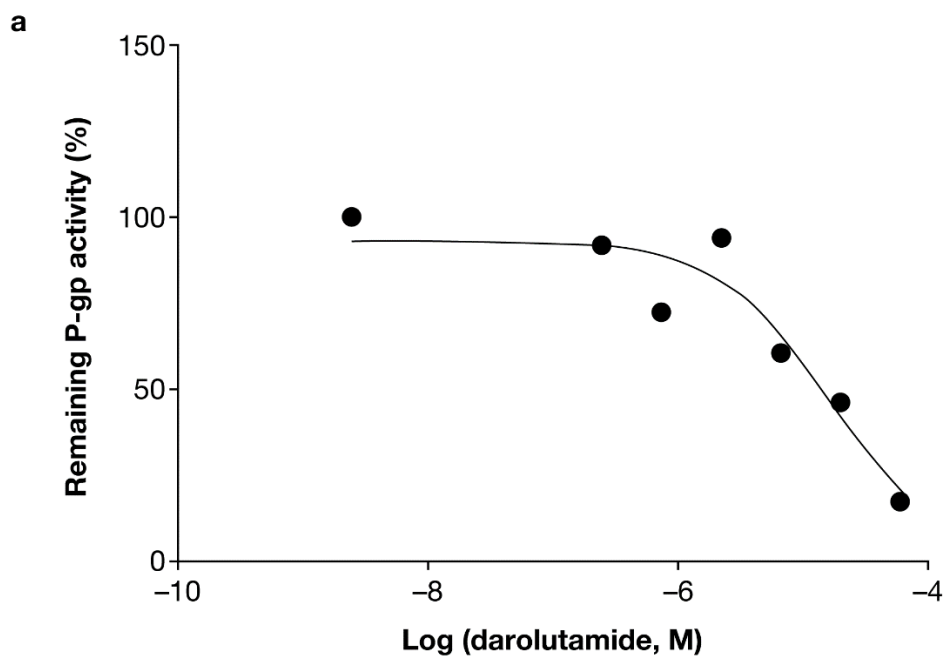
Darolutamide showed a concentration-dependent inhibition towards P-gp in Caco-2 cells, and its IC_{50} value was 16.4 µM

b
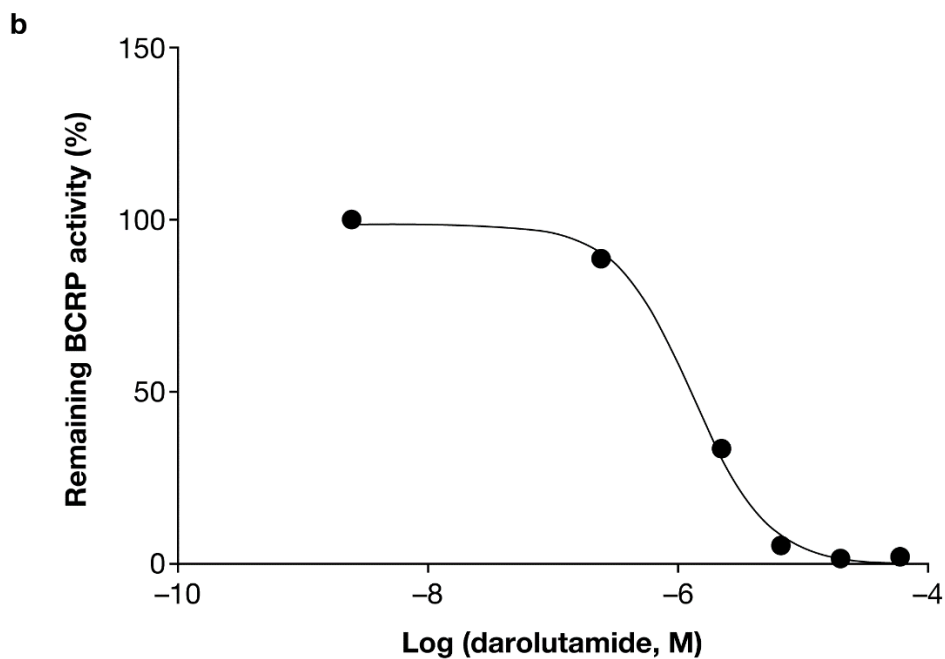
For the purpose of fitting, the data at 0.741 µM of ODM-201 was excluded in the BCRP IC_{50} assessment, and its IC_{50} value was 1.33 µM.

BCRP, breast cancer resistance protein; P-gp, P-glycoprotein.

Fig. S2 Mean plasma concentration–time profiles of (a) darolutamide and (b) keto-darolutamide after multiple oral doses of 600 mg BID (Day 7) and given in combination with a single oral dose of 5 mg rosuvastatin (Day 8) in Treatment Period 2 (N = 29)
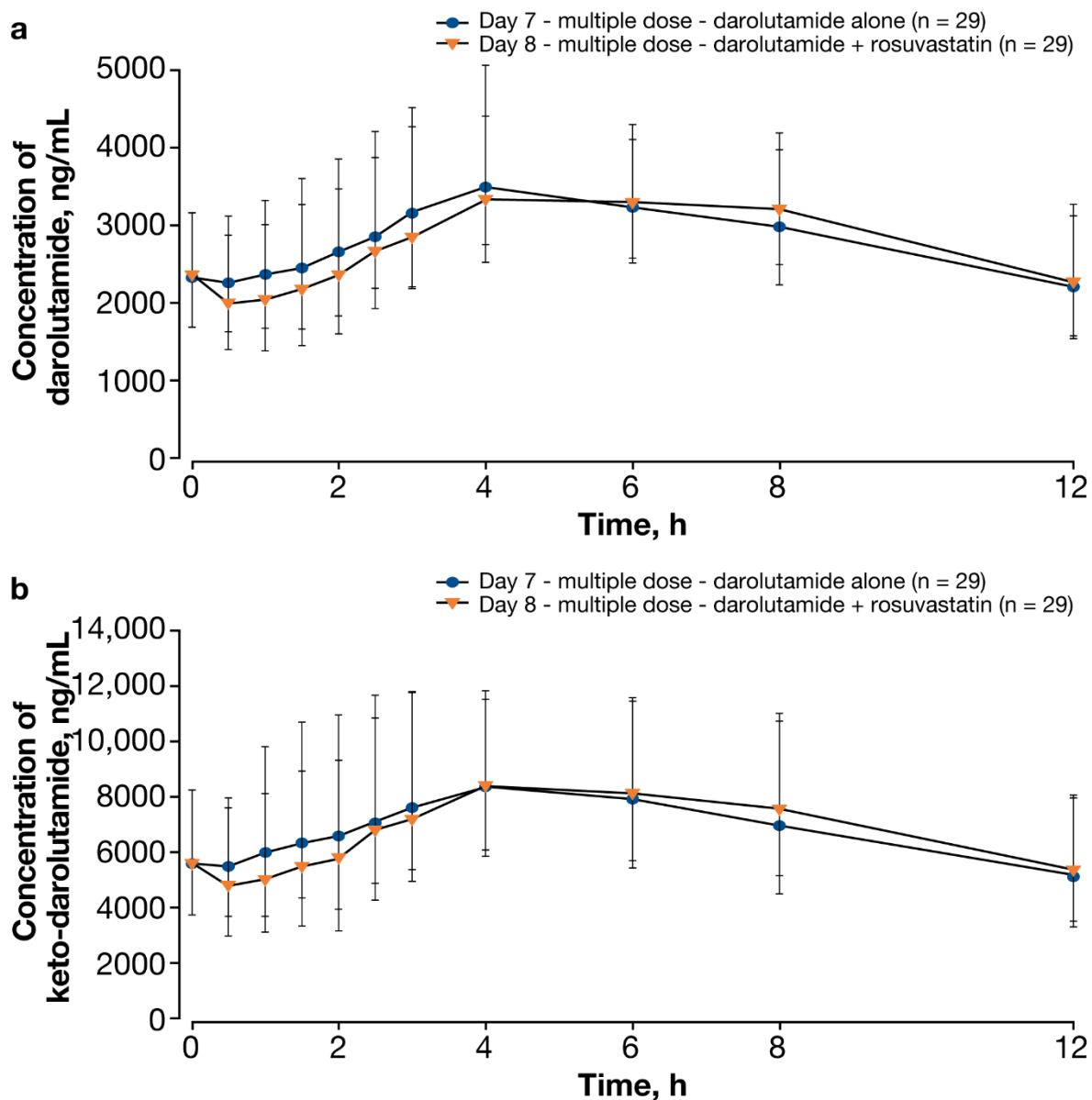
BID, twice daily.

Supplementary Methods: Bioanalytical Methods used in the Phase I Clinical Trials

Validation of bioanalytical methods and analysis of study samples were performed in compliance with internal standard operating procedures of the clinical research organizations and regulatory guidelines on bioanalytical method validation (e.g. from the FDA and EMA [8, 9]). Bioanalytical results are summarized in the table.

To determine the concentrations of the diastereomers (S,R)- and (S,S)-darolutamide and the major metabolite, keto-darolutamide, K_2EDTA plasma samples were spiked with internal standard and extracted using a solid phase extraction procedure. The extracted samples were analyzed by liquid chromatography with tandem mass spectrometry. Concentrations of darolutamide were calculated as the sum of the two diastereomers (S,R)- and (S,S)-darolutamide. All samples were stored at –20°C; stability data indicated that the analytes were stable for the storage period ($\geq$506 days and $\leq$94 days in the studies evaluating the effects of darolutamide on midazolam/dabigatran and rosuvastatin, respectively).

Itraconazole was determined in human K_3EDTA plasma samples after addition of the internal standard itraconazole-d_3 and automated liquid-liquid extraction with methyl tert-butyl ether. Rifampicin was determined in human K_2EDTA plasma samples after addition of the internal standard rifampin-d_3 and automated protein precipitation with methanol. Total dabigatran and non-conjugated dabigatran (free form) were determined in human K_2 EDTA plasma after addition of the internal standard dabigatran-d_7 and solid phase extraction. Dabigatran was found in study samples as free form and as dabigatran-acyl-β-D-glucuronide. To obtain the total dabigatran concentrations, study samples were hydrolyzed to cleave the glucuronide from

dabigatran-acyl-β-D-glucuronide and form free dabigatran. Midazolam and α-hydroxymidazolam (1-OH midazolam) were determined in human lithium heparinized plasma after addition of the internal standards midazolam-d_4 and α-hydroxymidazolam-d_4 and automated liquid-liquid extraction with methyl tert butyl ether. Rosuvastatin was determined in human EDTA K_2 plasma or urine after addition of the internal standard rosuvastatin-d_3 and automated liquid-liquid extraction with a mixture of methyl tertbutyl ether.

For agents co-administered with darolutamide, separation was achieved by means of a liquid chromatographic system. For the mass spectrometric detection, a triple quadrupole mass spectrometer in positive TurboIonSpray™ ionization mode was applied. All samples were stored at –20°C and analyzed within 50 days (itraconazole), 28 days (rifampicin), 48 days (total dabigatran), 47 days (non-conjugated dabigatran, midazolam, 1-OH midazolam), 87 days (rosuvastatin, plasma), or 90 days (rosuvastatin, urine) after sample collection; stability data indicated that the analytes were stable for these time periods.

	Calibration range (ng/mL)		Mean inter-assay accuracy and precision of back-calculated concentrations[a]		Accuracy and precision of lowest calibrator (LLOQ)		QC samples		
	LLOQ	ULOQ	Accuracy (bias), %	Precision, %	Accuracy, %	Precision, %	Concentration range, ng/mL	Accuracy (bias), %	Precision, %
Effects of itraconazole and rifampicin on darolutamide									
(S,R)-darolutamide	4.9	4940	−3.9 to 3.9	≤5.8	0.3	2.4	14.8 to 3950	0.8 to 3.0	≤8.3
(S,S)-darolutamide	5.1	5060	−3.1 to 2.8	≤6.7	0.2	3.7	15.2 to 4050	1.5 to 3.8	≤6.5
Keto-darolutamide	10.0	10 000	−3.9 to 3.4	≤5.7	1.1	3.1	30.0 to 8 000	3.2 to 3.7	≤7.9
Itraconazole	1.0	1 000	96.0 to 102.5	≤2.8	102.0	1.6	3.0 to 750	92.6 to 95.7	0.6 to 2.5
Rifampicin	5.0	5 000	97.8 to 102.0	≤7.7	100.0	9.4	15.0 to 3750	85.6 to 99.6	2.8 to 12.5
Effects of darolutamide on midazolam and dabigatran									

(S,R)-darolutamide	4.9	4940	95.9 to 105.2	≤5.9	101.4	3.1	14.8 to 3950	99.5 to 101.8	≤6.1
(S,S)-darolutamide	5.1	5060	96.2 to 103.7	≤9.0	102.0	4.5	15.2 to 4050	96.6 to 100.5	≤7.2
Keto-darolutamide	10.0	10 000	98.0 to 104.0	≤17.1	101.1	7.8	30.0 to 8 000	98.6 to 103.2	≤8.2
Total dabigatran	1.0	400	97.9 to 101.0	≤4.7	99.7	2.6	3.3 to 330	94.5 to 95.8	3.3 to 5.3
Non-conjugated dabigatran	1.0	400	97.6 to 101.8	≤5.4	100.0	6.2	3.0 to 300	97.3 to 101.7	2.6 to 4.7
Midazolam	0.002	2	98.9 to 101.5	≤4.1	99.5	3.6	0.006 to 1.5	97.3 to 101.6	1.8 to 2.9
1-OH midazolam	0.005	5	98.2 to 102.0	≤2.3	99.2	3.8	0.015 to 3.8	96.8 to 102.0	1.6 to 2.8
Effects of darolutamide on rosuvastatin									
(S,R)-darolutamide	4.9	4940	−3.6 to 3.8	≤7.0	−0.7	4.7	14.8 to 3950	−0.6 to 5.7	≤7.4
(S,S)-darolutamide	5.1	5060	−4.8 to 3.7	≤5.4	3.2	5.4	15.2 to 4050	−5.3 to 7.3	≤6.1
Keto-darolutamide	10.0	10 000	−3.3 to 3.4	≤6.6	3.4	5.4	30.0 to 8 000	−3.4 to 7.2	≤6.4
Rosuvastatin, plasma	0.05	15	98.7 to 100.7	≤3.4	100.0	2.8	0.15 to 11.3	97.4 to 102.4	3.2 to 4.7

| Rosuvastatin, urine | 5 | 2 000 | 99.4 to 101.0 | ≤2.5 | 99.6 | 3.5 | 15 to 1 500 | 94.6 to 100.7 | 0.96 to 3.16 |

[a]Except LLOQ.

1-OH midazolam, α-hydroxymidazolam; LLOQ, lower limit of quantification; QC, quality control; ULOQ, upper limit of quantification.

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