Electronic Supplementary Material

Targeted Oncology

Evaluation of Clinically Relevant Drug–Drug Interactions with Darolutamide in the Phase 3 ARAMIS Trial for Patients with Nonmetastatic Castration-Resistant Prostate Cancer: Results of Pre-Specified and Post-Hoc Analyses of the Phase III ARAMIS Trial

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Supplementary materials

Supplementary methods

Pharmacokinetic assessments

PK samples were taken on Day 15, Day 29, and Week 16 at the following timepoints: 10–14 hours after the previous dose ('trough'), 1–4 hours after the previous dose ('early') and 4–8 hours after the previous dose ('late'). A quantitative liquid chromatography–tandem mass spectrometry (LC-MS/MS) method was established for the determination of (*S,R*)-darolutamide, (*S,S*)-darolutamide and keto-darolutamide in human plasma and urine (darolutamide diastereomers only). The plasma method utilised solid phase extraction followed by chiral high-performance liquid chromatography with tandem mass spectrometric detection, quantitation being achieved by weighted linear regression using ^{13}C-labelled internal standards. Method validation and study sample analysis were performed in accordance with pertinent guidelines [1, 2]. The validated plasma assay range was 5 to 5000 ng/mL for the darolutamide diastereomers and keto-darolutamide. The determined analyte concentrations in study samples were verified by assaying quality control samples of blank matrix spiked with known concentrations of the respective analytes. Concentrations below the lower limit of quantification (LLOQ) were omitted. Concentrations above the LLOQ were determined with a precision better than 15% and an accuracy within 85–115%, with concentrations at the LLOQ being determined with a precision of 20% and accuracy within 80–120%, in accordance with standard operating procedures and pertinent method validation guidelines. Bioanalytical results are summarized in the table below.

	Mean inter-assay accuracy and precision of back-calculated concentrations[a]		Accuracy and precision of lowest calibrator (LLOQ)			QC samples	
	Accuracy (bias), %	**Precision, %**	**Accuracy, %**	**Precision, %**	**Concentration range, ng/mL**	**Accuracy (bias), %**	**Precision, %**
(*S,R*)-darolutamide	–3.2 to 2.4	≤4.5	–0.4	2.0	15–4000	–4.3 to –1.3	≤5.1
(*S,S*)-darolutamide	–3.4 to 2.4	≤5.1	–0.8	2.2	15–4000	–4.0 to –0.4	≤5.
Keto-darolutamide	–1.6 to 1.0	≤3.8	–0.2	2.0	15–4000	–1.3 to 0	≤5.5

LLOQ lower limit of quantification; *QC* quality control.

Individual exposure values at steady state for all patients were calculated after 15 days of darolutamide dosing using numerical integration of the population pharmacokinetic (PK) model.

Population PK analysis

Models were built so that all structural parameters of the three analytes were estimated simultaneously, and the standard NONMEM first-order conditional estimation method with interaction was used. The starting point for the base model was taken from preclinically identified metabolic pathways, specifically incorporating the conversion of the metabolite, keto-darolutamide, back to (*S,S*)-darolutamide and to a lesser extent also to (*S,R*)-darolutamide. The base model was parameterized using distribution volumes and clearances. The difference of individual parameters P_i from the population mean e was parameterized as $'i$ and described using one of the following equations:

$P_i = e + \eta$ Constant (additive) variance model

$P_i = e \cdot \exp(\eta)$ Exponential (lognormal) variance model

The residual variability, which is the result of assay error, data error and model misspecification was modelled using one of the following equations:

$C_{ij} = C_{pred,ij} + \varepsilon_{ij}$ Additive error model

$C_{ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij})$ Proportional error model

Where C_{ij} is the j^{th} observation of the i^{th} individual, $C_{pred,i,j}$ is the model-predicted value and ε_{ij} is the residual error for the current observation. All variability parameters were characterized by assuming normal distributions with a mean of 0 and an estimated variance of w^2 for IIV and a^2 or residual error.

In the multivariate analysis, the covariate effects were implemented in the base model as follows:

- Allometric scaling was done for bodyweight:

  $P_i = e_{TV} \cdot (BW_i/BW_{med})^{e_{cov}} \cdot \exp(i7)$

- Continuous covariates:

  $P_i = e_{TV} \cdot \exp(e_{cov} \cdot (COV_i - COV_{med})) \cdot \exp(i7)$

- Categorical covariates:

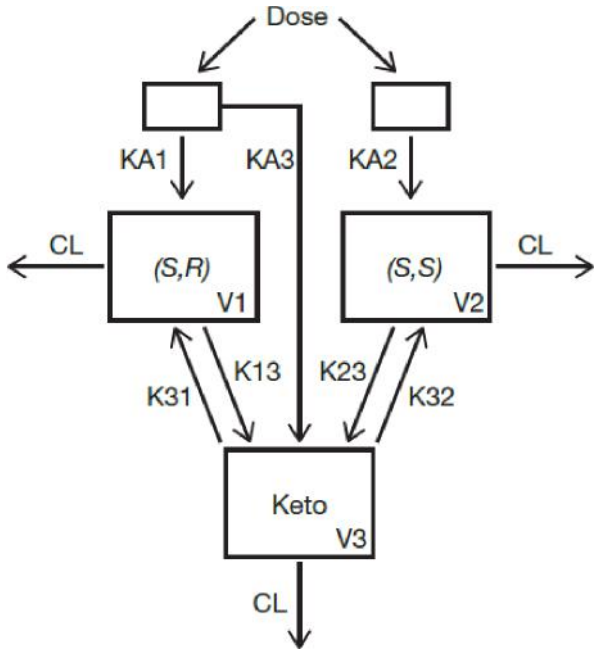
  $P_i = e_{TV} \cdot (1 + N1 \cdot e_{cov1} + N2 \cdot e_{cov2} + \cdots) \cdot \exp(i7)$

- Where P_i denoted the individual structural parameter value; e_{TV} the estimated typical parameter value; BW_i the individual's bodyweight; BW_{med} the population median bodyweight; COV_i and COV_{med} are the individual and population median covariate values, respectively; e_{cov} the estimated parameter for the covariate effect; N1 = N2 = 0 for the most prevalent covariate category; N1 = 1 and N2 = 0 for the next most prevalent category; and N1 = 0 and N2 = 1 for the next category etc. The number of terms in the equation depends on the number of covariate categories.

The covariate analysis was performed with a full stepwise forward inclusion/backward elimination procedure. During forward inclusion, a reduction in the objective function corresponding to $p < 0.001$ ($\Delta OFV < -10.828$ for one estimated parameter, with adjustment for greater than one parameter according to Chi-squared distribution) was required for the declaration of a significant covariate effect. In backward elimination, the requirement was increased to $p > 0.0001$ ($\Delta OFV > +15.137$ for one estimated parameter). The covariate model after the forward inclusion and backward elimination was called the selected model. Prediction-corrected visual predictive check was used for model qualification. These

were always generated from 1000 simulations of the entire dataset with all random variables (inter-individual and residual) being sampled from the estimated variance-covariance matrix.

Supplementary Figure 1 Structure of the population PK covariate model



$_{13}$ = ,

$_{31}$ = (exp ()⁄) • • , 1 + exp ()

$_{23}$ = ,

$_{32}$ = [1 − (exp ()⁄ exp ())] • • , 1 +

= • (1 + B • (− 1)) • exp(• (− 75) + • (− 81.35)) •

where B=1 when geographical region = JPN, else B=0.

1 = 2 = 3 = ,

= + • (− 21) + 2.

CL clearance, *KA1* absorption rate constant for (*S,R*)-darolutamide, *KA2* absorption rate constant for (S,S)-darolutamide, *KA3* absorption rate constant for keto-darolutamide, *Knm* rate constants for conversion between keto-darolutamide and (*S,R*)- and (*S,S*)-darolutamide, *CLpop* estimated CL for a patient with median value of AGE, SCRE (serum creatinine), and geographic region not equal to JPN, parameter describing factor by which CL is multiplied if geographical region = JPN, parameter describing influence of AGE on CL, parameter describing the influence of SCRE on CL, *V1* volume of distribution for (*S,R)*-darolutamide, *V2* volume of distribution for (*S,S*)-darolutamide, *V3* volume of distribution for keto-darolutamide, *RR* parameter describing the conversion ratio of keto-darolutamide to either (*S,R*)-darolutamide or (*S,S*)-darolutamide, estimated value of parameter describing the conversion ratio of keto-darolutamide to either (*S,R*)-darolutamide or (*S,S*)-darolutamide for a patient with AST = 21, parameter describing influence of AST on RR.

Supplementary Table 1 Baseline covariates included in the population PK analysis

Covariate	Unit	Type	Description
Age	years	Continuous	Age at start of treatment
Age	years	Categorical	<65, 65–74, 75–84, ≥85
Age	years	Categorical	>65, and ≥65
Bodyweight	kg	Continuous	Bodyweight at start of treatment
Height	cm	Continuous	Height at start of treatment
Ethnicity	–	Categorical	1-White; 2-Asian; 3-Other
Geographical region Japan	–	Categorical (dichotomous)	0-Rest of world; 1-Japan
Serum creatinine	µmol/L	Continuous	Serum creatinine at start of treatment
eGFR	mL/min/1.73m^2	Continuous	eGFR at start of treatment
Renal function	–	Categorical (ordered)	1-Normal (eGFR ≥90); 2-Mild (eGFR <90 and ≥60); 3-Moderate or worse (eGFR <60)
Serum albumin	g/L	Continuous	Serum albumin at start of treatment
Total protein	g/L	Continuous	Total protein at start of treatment
Total bilirubin	µmol/L	Continuous	Total bilirubin at start of treatment
AST	U/L	Continuous	AST at start of treatment
ALT	U/L	Continuous	ALT at start of treatment
Hepatic function	–	Categorical (ordered)	Hepatic function based on NCI-ODWG criteria: 1-Normal; 2-mild or worse
Comedication	–	Categorical	Comedication given during treatment (Y/N) including: BCRP inhibitor CYP3A4 inducer CYP3A4 inhibitor P-gp inducer P-gp inhibitor Protein pump inhibitor

	UGT1A9 inducer
	UGT1A9 inhibitor

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *BCRP* breast cancer resistance protein, *CYP* cytochrome P450, *eGFR* estimated glomerular filtration rate, *NCI-ODWG* National Cancer Institute Organ Dysfunction Working Group, *P-gp* P-glycoprotein, *PK* pharmacokinetic, *UGT* UDP-glucuronosyltransferase.

Supplementary Table 2 Summary of concomitant statins that are BCRP substrates used by ARAMIS patients (safety population)

	Patients using concomitant statin, *n* (%)	
Standardized medication name	**Darolutamide (*N* = 954)**	**Placebo (*N* = 554)**
Any concomitant statin	306 (32.1)	202 (36.5)
Any concomitant statin known to be a BCRP substrate	280 (29.4)	171 (30.9)
Atorvastatin	75 (7.9)	51 (9.2)
Atorvastatin calcium	61 (6.4)	40 (7.2)
Simvastatin	92 (9.6)	64 (11.6)
Rosuvastatin	23 (2.4)	7 (1.3)
Rosuvastatin calcium	38 (4.0)	12 (2.2)
Fluvastatin	2 (0.2)	1 (0.2)
Fluvastatin sodium	1 (0.1)	0

BCRP breast cancer resistance protein.

This analysis used data from the January 17, 2019 datacut.

References

1. Food and Drug Administration. Bioanalytical method validation: guidance for industry. 2018. https://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf. Accessed February 21, 2019.

2. European Medicines Agency. Guideline on bioanalytical method validation. 2011. https://www.ema.europa.eu/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. Accessed February 21, 2019.