Population pharmacokinetic modelling and evaluation of different dosage regimens for darunavir and ritonavir in HIV-infected individuals

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Abstract: OBJECTIVES Darunavir is a protease inhibitor that is administered with low-dose ritonavir to enhance its bioavailability. It is prescribed at standard dosage regimens of 600/100 mg twice daily in treatment-experienced patients and 800/100 mg once daily in naive patients. A population pharmacokinetic approach was used to characterize the pharmacokinetics of both drugs and their interaction in a cohort of unselected patients and to compare darunavir exposure expected under alternative dosage regimens. METHODS The study population included 105 HIV-infected individuals who provided darunavir and ritonavir plasma concentrations. Firstly, a population pharmacokinetic analysis for darunavir and ritonavir was conducted, with inclusion of patients' demographic, clinical and genetic characteristics as potential covariates (NONMEM(®)). Then, the interaction between darunavir and ritonavir was studied while incorporating levels of both drugs into different inhibitory models. Finally, model-based simulations were performed to compare trough concentrations (Cmin) between the recommended dosage regimen and alternative combinations of darunavir and ritonavir. RESULTS A one-compartment model with first-order absorption adequately characterized darunavir and ritonavir pharmacokinetics. The between-subject variability in both compounds was important [coefficient of variation (CV%) 34% and 47% for darunavir and ritonavir clearance, respectively]. Lopinavir and ritonavir exposure (AUC) affected darunavir clearance, while body weight and darunavir AUC influenced ritonavir elimination. None of the tested genetic variants showed any influence on darunavir or ritonavir pharmacokinetics. The simulations predicted darunavir Cmin much higher than the IC50 thresholds for wild-type and protease inhibitor-resistant HIV-1 strains (55 and 550 ng/mL, respectively) under standard dosing in >98% of experienced and naive patients. Alternative regimens of darunavir/ritonavir 1200/100 or 1200/200 mg once daily also had predicted adequate Cmin (>550 ng/mL) in 84% and 93% of patients, respectively. Reduction of darunavir/ritonavir dosage to 600/50 mg twice daily led to a 23% reduction in average Cmin, still with only 3.8% of patients having concentrations below the IC50 for resistant strains. CONCLUSIONS The important variability in darunavir and ritonavir pharmacokinetics is poorly explained by clinical covariates and genetic influences. In experienced patients, treatment simplification strategies guided by drug level measurements and adherence monitoring could be proposed.

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Objectives: Darunavir is a protease inhibitor that is administered with low-dose ritonavir to enhance its bioavailability. It is prescribed at standard dosage regimens of 600/100 mg twice daily in treatment-experienced patients and 800/100 mg once daily in naive patients. A population pharmacokinetic approach was used to characterize the pharmacokinetics of both drugs and their interaction in a cohort of unselected patients and to compare darunavir exposure expected under alternative dosage regimens.

Methods: The study population included 105 HIV-infected individuals who provided darunavir and ritonavir plasma concentrations. Firstly, a population pharmacokinetic analysis for darunavir and ritonavir was conducted, with inclusion of patients' demographic, clinical and genetic characteristics as potential covariates (NONMEM®). Then, the interaction between darunavir and ritonavir was studied while incorporating levels of both drugs into different inhibitory models. Finally, model-based simulations were performed to compare trough concentrations (C_{min}) between the recommended dosage regimen and alternative combinations of darunavir and ritonavir.

Results: A one-compartment model with first-order absorption adequately characterized darunavir and ritonavir pharmacokinetics. The between-subject variability in both compounds was important [coefficient of variation (CV%) 34% and 47% for darunavir and ritonavir clearance, respectively]. Lopinavir and ritonavir exposure (AUC) affected darunavir clearance, while body weight and darunavir AUC influenced ritonavir elimination. None of the tested genetic variants showed any influence on darunavir or ritonavir pharmacokinetics. The simulations predicted darunavir C_{min} much higher than the IC_{50} thresholds for wild-type and protease inhibitor-resistant HIV-1 strains (55 and 550 ng/mL, respectively) under standard dosing in >98% of experienced and naive patients. Alternative regimens of darunavir/ritonavir 1200/100 or 1200/200 mg once daily also had predicted adequate C_{min} (>550 ng/mL) in 84% and 93% of patients, respectively. Reduction of darunavir/ritonavir dosage to 600/50 mg twice daily led to a 23% reduction in average C_{min}, still with only 3.8% of patients having concentrations below the IC_{50} for resistant strains.

Conclusions: The important variability in darunavir and ritonavir pharmacokinetics is poorly explained by clinical covariates and genetic influences. In experienced patients, treatment simplification strategies guided by drug level measurements and adherence monitoring could be proposed.

Keywords: NONMEM, pharmacogenetics, simulations

Introduction

Darunavir is a non-peptidic HIV-1 protease inhibitor indicated for the treatment of HIV infection in antiretroviral treatment-naive and treatment-experienced adults and paediatric patients aged ≥6 years.[1,2] Darunavir is extensively metabolized by cytochrome P450 (CYP) 3A4 in the liver and intestinal lumen. *In vitro* studies indicate that it is a CYP3A4 inhibitor and a substrate and inhibitor of P-glycoprotein as well.[3,4] In order to improve its pharmacokinetic profile, darunavir must be co-administered with low doses of ritonavir, which increase its oral bioavailability and exposure through CYP3A and P-glycoprotein inhibition.[5] However, an important variability in darunavir levels has been reported that could lead to sub-optimal drug levels in certain patients. Some factors, such as demographics, concomitant medication, treatment adherence and protein concentrations, have been shown to affect protease inhibitor levels. In addition, polymorphisms in the genes encoding metabolizing enzymes and transporters could explain some of this variability.[6,7] Yet darunavir/ritonavir is administered at the standard dosage regimen of 800/100 mg once daily for treatment-naive patients and 600/100 mg twice daily as a rescue treatment in experienced patients.[3,8] In treatment-experienced patients with no mutations associated with darunavir resistance at 48 weeks of therapy, the dosage regimen of 800/100 mg once daily appears non-inferior to the standard 600/100 mg twice daily. This suggests that this regimen might be an alternative option in these experienced patients.[9,10] A better characterization of darunavir variability in relation to ritonavir-induced inhibition and other genetic and non-genetic factors would be useful for clinical practice. The objective of the present study was to develop a model for darunavir and ritonavir administered simultaneously in a cohort of HIV-1-infected patients. The aims were to identify patient characteristics influencing variability in drug disposition and to characterize the interaction between the two compounds. The model was then used to simulate and compare different combinations of darunavir/ritonavir doses administered once or twice daily.

Methods

Study population

Plasma darunavir and ritonavir levels were obtained from 99 HIV-infected individuals and measured during routine therapeutic drug monitoring according to local treatment guidelines. A median of 2 concentration samples per individual (range 1–12) were collected, drawn between 0.5 and 43.25 h after the last dose intake under steady-state conditions. In addition, darunavir and ritonavir concentrations from six patients participating in a study on raltegravir disposition were available.[11] For these patients, five samples were drawn at 1, 3, 6, 8 and 12 h post-drug intake. This study was conducted within the framework of the Swiss HIV Cohort Study (http://www.shcs.ch). The ethics committees of all participating centres approved the project and all participants gave written informed consent for genetic testing. A summary of the population characteristics is presented in Table 1.

Table 1. Demographic characteristics of the study population

Characteristic	Value	Percentage of study population
Men/women, *n*/*n*	81/25	77/23
Age (years), median (range)	49 (19–72)	
Body weight (kg), median (range)	70 (37–126)	
Height (cm), median (range)	175 (153–191)	
White/black, *n*/*n*	98/7	93/7
Protease inhibitors (*n*)		
ritonavir	105	100
lopinavir	8	8
atazanavir	7	7
saquinavir	2	2
amprenavir	1	1
Nucleoside/nucleotide reverse transcriptase inhibitors (*n*)		
didanosine	5	5
tenofovir	61	58
lamivudine	32	30
emtricitabine	41	39
Non-nucleoside/nucleotide reverse transcriptase inhibitors (*n*)		
efavirenz	8	8
nevirapine	1	0.9
etravirine	48	46
Integrase inhibitors (*n*)		
raltegravir	43	41
Others (*n*)		
maraviroc	5	5
enfuvirtide	14	13
AST (U/L), median (range)	27 (11–172)	
ALT (U/L), median (range)	26 (8–169)	
Total bilirubin (μmol/L), median (range)	8 (5–14)	
CD4+ cell count (cell/mm^3), median (range)	332 (19–1496)	
HIV RNA level (copies/mL), median (range)	0 (0–59 700)	

Analytical method

Plasma samples were isolated by centrifugation and stored at −20°C until batch analysis. On the day of analysis, samples were inactivated for virus at 60°C for 60 min. Plasma darunavir and ritonavir levels were determined by HPLC coupled with tandem mass spectrometry (LC-MS/MS) after protein precipitation with acetonitrile according to our previously reported analytical method.[12] The calibration curves are linear, with lower limits of quantification of 25 ng/mL for darunavir and 5 ng/mL for ritonavir. The laboratory participates in an international external quality assurance programme for antiretroviral drug analysis [KKGT, Stichting Kwaliteitsbewaking Klinische Geneesmiddelanalyse en Toxicologie (Association for Quality Assessment in TDM and clinical Toxicology), The Hague, The Netherlands].

Genotyping

DNA was extracted from peripheral blood mononuclear cells using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Genotyping was performed with a customized Veracode microarray (Illumina, Eindhoven, the Netherlands) designed to cover genetic variation in metabolic enzymes and nuclear receptors.[13,14] Thirty-six single-nucleotide polymorphisms (SNPs) with proven functional effect in 15 genes possibly

relevant to darunavir and ritonavir metabolism in Caucasians were available for 60 individuals. Four genes encode enzymes involved in drug metabolism and 13 genes encode nuclear receptors (Table 2). Nuclear receptors of the orphan nuclear receptor superfamily have been shown to regulate the genes that encode CYP enzymes involved in metabolism of endogenous and exogenous compounds. In addition, three polymorphisms associated with two transporter genes (*ABCB1*, rs1128503; *ABCB1*, rs1045642; and *ABCC2*, rs717620) were available for a subset of 25 individuals.[6]

Model-based pharmacokinetic modelling

The model-building process included three steps: (i) a population pharmacokinetic model was built for darunavir; (ii) a population pharmacokinetic model was built for ritonavir; and (iii) a model describing the interaction between the two drugs was developed considering either competitive or non-competitive inhibition, while taking into account variables potentially influencing the pharmacokinetics of each drug.

Structural models

A stepwise procedure was used to find models that fitted the darunavir and ritonavir data best, comparing one- and two-compartment models with first-order absorption from the gastrointestinal tract. The final pharmacokinetic model was a one-compartment model with first-order absorption and elimination for both drugs. The estimated parameters were clearance (CL), volume of distribution (V) and absorption rate constant (k_a). Since darunavir and ritonavir were only administered orally, CL and V represent apparent values (CL/F and V/F, respectively, where F is oral bioavailability). Exponential errors following a log-normal distribution were assumed for the description of between-subject variability of the

Table 2. Summary characteristics and influence of the genetic variants on darunavir log CL in the GAM analysis

Gene group	Gene	dbSNP_rs	Chromosome	Chromosome position	Allele change	ΔAIC
CYPs	*CYP3A5*	rs776746	7	99108475	T→C	1.392
CYPs	*CYP3A7*	rs10211	7	99140930	T→C	1.403
CYPs	*CYP3A7*	rs2257401	7	99144621	G→C	−0.195
CYPs	*CYP3AP5*	rs6945984	7	99186264	T→C	1.781
CYPs	*CYP3A4*	rs4646437	7	99203019	G→A	0.5
CYPs	*CYP3A4*	rs4987161	7	99204017	A→G	0
CYPs	*CYP3A4*	rs2740574	7	99220032	T→C	1.615
NRs	*NR1I2*	rs3814055	3	120982725	C→T	0
NRs	*NR1I2*	rs2276706	3	120983997	G→A	0
NRs	*NR1I2*	rs12721607	3	121008893	G→A	1.896
NRs	*NR1I2*	rs12721608	3	121013109	G→A	0
NRs	*NR1I2*	rs6785049	3	121016423	A→G	1.077
NRs	*NR1I2*	rs1054190	3	121019408	C→T	1.792
NRs	*NR1I2*	rs6438550	3	121019507	A→G	−0.346
NRs	*NR1I2*	rs3814058	3	121019981	T→C	1.289
NRs	*PPARG*	rs1801282	3	12368125	C→G	0.182
NRs	*PPARG*	rs1899951	3	12369840	G→A	1.205
NRs	*PPARGC1A*	rs8192678	4	23424760	C→T	1.953
NRs	*NR3C1*	rs41423247	5	142758768	G→C	1.997
NRs	*NR3C1*	rs6198	5	142637814	T→C	1.714
NRs	*ESR1*	rs2234693	6	152205028	T→C	−0.089
NRs	*AHR*	rs2066853	7	17345635	G→A	2
NRs	*AHR*	rs2074113	7	17340296	G→T	2
NRs	*NR1H4*	rs56163822	12	99411232	G→T	1.608
NRs	*VDR*	rs2228570	12	46559162	A→G	0.394
NRs	*VDR*	rs1544410	12	46526102	C→A	1.166
NRs	*VDR*	rs11568820	12	46588812	C→T	1.767
NRs	*HNF1A*	rs1169288	12	119901033	A→C	0.443
NRs	*HNF1A*	rs2244608	12	119901371	A→G	1.97
NRs	*HNF1A*	rs2650000	12	119873345	C→T	1.621
NRs	*HNF4A*	rs1800961	20	42475778	C→T	0
NRs	*PPARA*	rs4253778	22	45009298	G→C	−0.304
NRs	*PPARA*	rs1800206	22	44992938	C→G	−1.021
NRs	*NR1I2*	rs6785049	3	121016423	A→G	−0.852
NRs	*HNF1A*	rs1169288	12	119901033	A→C	−0.5813
NRs	*HNF1A*	rs2650000	12	119873345	C→T	−2.505

NRs, nuclear receptors.
$\Delta AIC = AIC_i - AIC_{tot}$ or $AIC_i - AIC_{min}$ in relation to the best model.

pharmacokinetic parameters and a proportional error model was used to model the residual variability.

Covariate models

Analyses of covariate effects were performed in two main steps: (i) the influence of demographic characteristics, concomitant medications and markers of hepatic function was assessed by incorporating them directly in the model; and (ii) the impact of candidate SNPs with functional effect was tested using graphical plots and a generalized additive model (GAM) using the stepwise method in R (R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/). This GAM analysis was performed for 36 SNPs of metabolizing enzyme and nuclear receptors available for 60 patients using log-transformed Bayesian individual estimates of CL. The analysis first included all SNPs, then SNPs were dropped one by one with replacement, and the difference in the Akaike criterion (ΔAIC) was computed for each SNP. Only significant SNPs were tested for significance in the models. In addition, the three SNPs in genes encoding transporters available for a subset of 25 patients were directly tested for significance in the model building.

Covariates (continuous variables centred on the mean and categorical covariates being coded as indicator variables, as 0 or 1) were incorporated in the models, testing linear and non-linear relationships. The demographic covariates available were sex, race, age, body weight and height; other covariates were aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in addition to antiretroviral co-medications. Genetic variants were classified according to the number of allelic variants into three groups (reference allele, heterozygote and homozygote rare allele groups) coded as indicator variables 0, 1 and 2, respectively.

Darunavir/ritonavir interaction models

Different models were tested to describe the interaction between ritonavir and darunavir. First, non-competitive models based on ritonavir AUC (AUC_{RTV}) were explored using linear, power and exponential functions, as follows:

$$CL = CL_0 - \theta_1 \cdot (AUC_{RTV} - \text{mean } AUC_{RTV}) \quad (1)$$

$$CL = CL_0 \cdot \left(\frac{AUC_{RTV}}{\text{mean } AUC_{RTV}}\right)^{-\theta_1} \quad (2)$$

$$CL = CL_0 \cdot \frac{1}{EXP(\theta_1 \cdot AUC_{RTV})} \quad (3)$$

where CL_0 is the mean population CL, θ_1 is the factor associated with the effect of ritonavir on darunavir CL and mean AUC_{RTV} was 4.56 mg·h/L in the study population. AUC_{RTV} (from 0 to 12 or 24 h) was derived from the final model incorporating covariates that best described ritonavir plasma levels, using $Dose_{RTV}/CL_{ind.}$

In the next step, the inhibition of darunavir clearance by ritonavir was tested assuming competitive inhibition and modelled using a direct concentration-dependent relationship of the form $CL = CL_0 \cdot I(t)$, where CL_0 is the mean darunavir clearance and I(t) describes a linear, exponential or Emax-type inhibitory time-dependent model induced by ritonavir concentrations (C_{RTV}), as follows:

$$I = 1 - (\theta_1 \times C_{RTV}) \quad (4)$$

$$I = \frac{1}{EXP(\theta_1 \times C_{RTV})} \quad (5)$$

$$I = 1 - \frac{I_{max} \times C_{RTV}}{(IC_{50} + C_{RTV})} \quad (6)$$

where θ_1 is the parameter associated with the influence of C_{RTV}, I_{max} is the maximum inhibitory effect of ritonavir and IC_{50} is the C_{RTV} producing 50% of the I_{max}. The same approach was applied to test the influence of darunavir on ritonavir clearance, using AUC_{DRV}, derived as $Dose_{DRV}/CL_{ind}$ (mean $AUC_{DRV} = 51.8$ mg h/L) or instantaneous darunavir concentration (C_{DRV}) in direct competitive inhibition models.

Parameter estimation and selection

NONMEM®[15] (version VII, NM-TRAN, version II) was used with the FOCE INTERACTION method to fit the data. The minimum objective function value (OFV) provided by NONMEM® (−2 log likelihood, approximate χ^2 distribution) was used to discriminate between models using the likelihood ratio test. A model was considered superior to another nested model when the OFV value was reduced by ≥3.84 points ($P < 0.05$). Covariate analysis comprised forward selection of influential factors followed by backward deletion and influential factors were retained in the final model at the statistical level of $P < 0.01$. Model assessment was based on diagnostic plots (goodness-of-fit plots and visual predictive checks) along with the standard errors, the correlation matrix of parameter estimates and the size of residual errors.

Model validation

The stability and performance of the final population pharmacokinetic model were validated by the bootstrap method using 2000 resamplings with replacement. The final population pharmacokinetic model was fitted repeatedly to the 2000 bootstrapped samples and the pharmacokinetic parameters were calculated for each dataset. The median and 95% CI of each parameter obtained with the bootstrapped data were then compared with the corresponding parameters obtained with the original dataset. The statistical analysis was performed using Perl-speaks-NONMEM (version 3.2.4: http://psn.sourceforge.net/). The final model was also validated using a visual predictive check (VPC). Using the parameter values of the final population pharmacokinetic model, we simulated data for 1000 individuals and generated 2.5th, 50th and 97.5th percentiles. The observed concentrations were plotted against the 95% prediction interval (PI) of the simulated dataset at each timepoint and visually compared. The figures were generated using GraphPad Prism (Version 6.0 for Windows, GraphPad Software, San Diego, CA, USA; http://www.graphpad.com).

Simulations

Monte Carlo simulations of darunavir and ritonavir were performed for 1000 individuals based on the final models for darunavir (including the effect of simulated AUC_{RTV} and assuming no lopinavir intake) and ritonavir (for a 70 kg individual with average AUC_{DRV}). They were used to predict darunavir C_{min} for various combinations of darunavir and ritonavir in once-daily or twice-daily dosage regimens (800/100 mg once daily and 600/100 mg twice daily, 1200/100 mg once daily, 1200/200 mg once daily, 800/50 mg once daily and 600/50 mg twice daily). Simulated darunavir average C_{min} and 95% PI at steady state were compared between the different dosage regimens. The percentage of patients with a C_{min} less than the 50% effective concentration (EC_{50}) for darunavir corrected for protein binding for both wild-type (55 ng/mL) and protease inhibitor-resistant HIV-1 strains (550 ng/mL) served for dosage regimen comparisons as well.[16–18]

Results

A total of 289 observations of both darunavir and ritonavir concentrations were obtained from 105 HIV-positive individuals and were included in the population pharmacokinetic analysis.

Population pharmacokinetic analysis of darunavir

Darunavir concentration measurements ranged between 17 and 14635 ng/mL at doses between 300 and 2400 mg, given once daily or twice daily A one-compartment model with first-order absorption from the gastrointestinal tract fitted the data appropriately and the addition of a second compartment did not significantly improve the fit (ΔOFV = −2). No lag time in absorption was observed (ΔOFV = 0.0). Assignment of interindividual variability to V and k_a in addition to CL did not improve the model fit (ΔOFV = 0.0).

Among the demographic covariates tested, body weight showed a linear influence on darunavir pharmacokinetics (ΔOFV = −4.3, P = 0.03), suggesting a 43% increase in darunavir clearance on body weight doubling. The inclusion of lopinavir in CL improved the fit (ΔOFV = −4.18, P = 0.04); darunavir CL was increased by 48% in the presence of this drug.

The GAM regression analysis testing for the influence of genetic variants on darunavir elimination is presented in Table 2. Two SNPs (rs1801282 and rs2650000 of the *PPARG* and *HNF1A* genes, respectively) provided the minimum AIC (AIC_{min} = 26.78). However, only rs2650000 (ΔAIC = −2.505, P = 0.039) showed a significant influence on darunavir log CL. Both SNPs were tested in NONMEM but neither showed any significant improvement of the fit (rs1801282, ΔOFV = −1.84, P = 0.175; rs2650000, ΔOFV = −5.69, P = 0.017). The influence of *ABCB1* rs1128503, *ABCB1* rs1045642 and *ABCC2* rs717620 tested on darunavir pharmacokinetics in a subset of 25 patients was insignificant.

Population pharmacokinetic analysis of ritonavir

Ritonavir was administered at the dose of 100 mg once daily, except in five patients who received 200 mg once daily; resulting concentrations ranged between 0 and 5195 ng/mL. A one-compartment model with first-order absorption from the gastrointestinal tract fitted the data appropriately, whereas a two-compartment did not improve the description of the data (ΔOFV = +5.0). In addition to CL, assignment of interindividual variability to V (ΔOFV = −30.1, P < 0.001) and a covariance term between CL and V (ΔOFV = −4.6, P < 0.05) improved the model. The data did not support the addition of an absorption lag time (ΔOFV = −1.0) or the assignment of variability to k_a (ΔOFV = 0.0).

Among the demographic covariates tested, body weight influenced ritonavir pharmacokinetics (ΔOFV = −16.9, P < 0.001); we estimated a 100% increase in CL upon body weight doubling. Gender, height and age also had an impact on ritonavir exposure (ΔOFV = −11.6, −17.03 and −6.9, respectively) but were all correlated to body weight. No influence of any of the available covariates was observed on V.

The results of the stepwise GAM analysis are presented in Table 3. For ritonavir, three SNPs (rs6785049, rs1169288 and rs2650000, of *NR1I2*, *HNF1A* and *HNF1A*, respectively) were associated with the lowest AIC. Only two SNPs, rs6785049 (ΔAIC = −5.513, P = 0.0085) and rs2650000 (ΔAIC = −3.881, P = 0.019), showed a significant influence on ritonavir log CL. These SNPs were included in the modelling steps but none showed any significant improvement of model fit (rs1169288 snp28, ΔOFV = −0.24 for 3 df, P = 0.970; rs6785049, ΔOFV = −4.07 for 3 df, P = 0.254; rs2650000, ΔOFV = −2.50 for 3 df, P = 0.475.) The effects of rs1128503, rs1045642 and rs717620 tested in a subset of patients did not significantly affect ritonavir pharmacokinetics either (ΔOFV < 2.06, P = 0.15).

Darunavir/ritonavir interaction models

The influence of ritonavir on darunavir CL was best described using a power function of AUC_{RTV} (equation 2) (ΔOFVV = −9.4, P = 0.001 compared with the model without integration of AUC_{RTV}), whereas the linear and exponential model fitted the data slightly worse (ΔOFVV > −5.7). A 16% reduction in darunavir CL was expected upon AUC_{RTV} doubling; this model explained 7% of interindividual variability in darunavir clearance. The analysis of darunavir and ritonavir incorporating both drug plasma concentrations using competitive models based on either linear (equation 4) or exponential (equation 5) components did not improve the goodness of fit (ΔOFVV > −2.5). The I_{max} model (equation 6) better described the data, with an estimated EC_{50} of 0.19 mg/L and an I_{max} of 55% (ΔOFVV = −4.4, P < 0.05). Since the assignment of AUC_{RTV} provided the best fit and largest drop in OFV, this effect was kept in the final model.

The combination of statistically relevant covariates with respect to darunavir CL revealed that only AUC_{RTV} and lopinavir remained significant (ΔOFVV = −14.6, P < 0.001) and explained only 9% of the interindividual variability in darunavir levels.

For ritonavir, the inclusion of AUC_{DRV} for CL improved the fit. The use of a power function (equation 2) best described this interaction (ΔOFVV = −17.0, P < 0.0001). Doubling AUC_{DRV} resulted in a 24% lower ritonavir CL. Similarly to darunavir, the use of inhibitory competitive models did not describe the data better. The multivariate analysis showed that both body weight and AUC_{DRV} remained significant with respect to CL (ΔOFVV = −11.6, P < 0.001) and explained 16% of its variability. The final population pharmacokinetic parameters for darunavir and ritonavir are presented in Tables 4 and 5, respectively, and goodness-of-fit plots of population and individual predictions for both drugs are given in Figure S1 (available as Supplementary data at *JAC* Online).

Model validation

The median parameter estimates obtained by bootstrapping with the 90% CI are presented in Tables 4 and 5. Median parameters differed by <10% from those obtained with the original dataset. The parameter estimates of the final population pharmacokinetic model lay within the 95% CI of the bootstrap results, suggesting that the model was acceptable. The VPC of the observed darunavir concentrations versus time with the 90% PI for the standardized dosing regimen of 600/100 mg twice daily and 800/100 mg once daily are shown in Figure 1.

Simulations

Simulations based on the final model for darunavir/ritonavir 600/100 mg twice daily yielded a median C_{min} of 2863 ng/mL (95% PI 725–9726 ng/mL), whereas the median C_{min} was 981 ng/mL (95% PI 110–4449 ng/mL) for 800/100 mg once daily. Although average C_{min} would be above the targets of 550 and 55 ng/mL for naive and experienced patients, respectively, 28% of the patients on the 800 mg once-daily regimen would present levels under the 550 ng/mL threshold due to the large interpatient variability. Increasing the once-daily regimen to 1200/100 mg or 1200/200 mg would result

Table 3. Summary characteristics and influence of the genetic variants on ritonavir log CL in the GAM analysis

Gene group	Gene	Chromosome	dbSNP_rs	Chromosome position (build 36)	Allele change	ΔAIC
CYPs	*CYP3A5*	7	rs776746	99108475	T→C	−4.39
CYPs	*CYP3A7*	7	rs10211	99140930	T→C	−0.34
CYPs	*CYP3A7*	7	rs2257401	99144621	G→C	2
CYPs	*CYP3AP5*	7	rs6945984	99186264	T→C	1.75
CYPs	*CYP3A4*	7	rs4646437	99203019	G→A	1.89
CYPs	*CYP3A4*	7	rs4987161	99204017	A→G	0
CYPs	*CYP3A4*	7	rs2740574	99220032	T→C	1.96
NRs	*NR1I2*	3	rs3814055	120982725	C→T	0
NRs	*NR1I2*	3	rs2276706	120983997	G→A	0
NRs	*NR1I2*	3	rs12721607	121008893	G→A	−0.35
NRs	*NR1I2*	3	rs12721608	121013109	G→A	0
NRs	*NR1I2*	3	rs6785049	121016423	A→G	−0.49
NRs	*NR1I2*	3	rs1054190	121019408	C→T	0.49
NRs	*NR1I2*	3	rs6438550	121019507	A→G	2
NRs	*NR1I2*	3	rs3814058	121019981	T→C	1.33
NRs	*PPARG*	3	rs1801282	12368125	C→G	0.45
NRs	*PPARG*	3	rs1899951	12369840	G→A	0.1
NRs	*PPARGC1A*	4	rs8192678	23424760	C→T	1.12
NRs	*NR3C1*	5	rs41423247	142758768	G→C	1
NRs	*NR3C1*	5	rs6198	142637814	T→C	0.2
NRs	*ESR1*	6	rs2234693	152205028	T→C	1.23
NRs	*AHR*	7	rs2066853	17345635	G→A	0.11
NRs	*AHR*	7	rs2074113	17340296	G→T	0.14
NRs	*NR1H4*	12	rs56163822	99411232	G→T	1.98
NRs	*VDR*	12	rs2228570	46559162	A→G	1.53
NRs	*VDR*	12	rs1544410	46526102	C→A	1.45
NRs	*VDR*	12	rs11568820	46588812	C→T	0.55
NRs	*HNF1A*	12	rs1169288	119901033	A→C	1.98
NRs	*HNF1A*	12	rs2244608	119901371	A→G	1.04
NRs	*HNF1A*	12	rs2650000	119873345	C→T	−1.96
NRs	*HNF4A*	20	rs1800961	42475778	C→T	0
NRs	*PPARA*	22	rs4253778	45009298	G→C	2
NRs	*PPARA*	22	rs1800206	44992938	C→G	−0.54
NRs	*NR1I2*	3	rs6785049	121016423	A→G	−5.513
NRs	*HNF1A*	12	rs1169288	119901033	A→C	−1.524
NRs	*HNF1A*	12	rs2650000	119873345	C→T	−3.881

NRs, nuclear receptors.
$\Delta AIC = AIC_i - AIC_{tot}$ or $AIC_i - AIC_{min}$ in relation to the best model.

in an average C_{min} of 1472 ng/mL (95% PI 166–6673 ng/mL) and 2150 ng/mL (95% PI 327–8814 ng/mL), respectively. The regimen of 1200/100 mg darunavir once daily would decrease the number of patients below the cut-off of 550 ng/mL to 16% while increasing ritonavir boosting to 200 mg once daily would reduce it to <7%. Reduction of the ritonavir dose from 100 to 50 mg in the 600 mg twice-daily regimen would lead to an average C_{min} of 2174 ng/mL (95% PI 490–7754 ng/mL). Figure 2 represents darunavir C_{min} predicted after simulations of the different dosage regimens.

Discussion

A population pharmacokinetic analysis of both darunavir and ritonavir incorporating the relationship between ritonavir exposure and darunavir elimination was developed. Darunavir and ritonavir pharmacokinetic parameters are in line with previously reported values of population pharmacokinetic studies.[7,19] The current analysis supports the limited influence of demographic covariates on darunavir pharmacokinetics. The small influence of age previously reported was not confirmed in the present study.[20] The reported influence of α-1-acid glycoprotein on the darunavir volume of distribution could not be tested because this information was not available.[7] Despite the small number of individuals on lopinavir ($n=8$), we found that this drug increased darunavir CL by 48%, as previously reported.[5,21] Due to their mutual interaction, co-administration of the two drugs is not recommended.

Darunavir is administered with low doses of ritonavir as a booster to enhance its pharmacokinetic profile. The exact mechanism of CYP3A inhibition by ritonavir is not clearly established,

Table 4. Final population pharmacokinetic parameters for darunavir with median and 90% CI of the pharmacokinetic parameters obtained from the 200 bootstrapped samples

	Final population pharmacokinetic parameters		Bootstrap ($n=2000$ samples)			
Parameters	estimate	SE (%)[a]	median	SE (%)[a]	90% CI	
CL/*F* (L/h)	10.9	4.8	10.85	5.1	9.6	12.3
V/*F* (L)	121.2	11.2	121.3	11.9	80.2	156.9
k_a (h^{-1})	1.04	32.7	1.09	35.4	0.4	2.4
$\theta_{AUC\ RTV}$[b]	−0.25	27.4	−0.24	30.2	−0.48	−0.06
θ_{LPV}[c]	0.48	46.3	0.49	51.3	0.06	1.64
ω (CL/*F*) (CV%)[d]	34.0	47.5[e]	33.09	49.1[e]	24.8[e]	39.5[e]
σ (CV%)	44.0	25.8[e]	43.8	27.1[e]	35.4[e]	53.7[e]

CL/*F*, mean apparent clearance; *V*/*F*, mean apparent volume of distribution.
Final model: $CL/F_i = CL/F \times ((AUC_{RTV}/4.52)^{\theta}AUC_{RTV}) \times (1+\theta_{LPV} \times LPV)$.
[a]Standard errors of the estimates (SE), defined as SE/estimate and expressed as percentages.
[b]Relative influence of the AUC of ritonavir on darunavir clearance (see text).
[c]Relative influence of the prescription of lopinavir on darunavir clearance (see text).
[d]Estimate of between-subject variability, expressed as CV (%).
[e]Standard errors of the coefficient of variation, taken as SE/(estimate×2) and expressed as a percentage.

Table 5. Final population pharmacokinetic parameters for ritonavir with median and 90% CI of the pharmacokinetic parameters obtained from the 200 bootstrapped samples

	Final population pharmacokinetic parameters		Bootstrap ($n=2000$ samples)			
Parameters	estimate	SE (%)[a]	median	SE (%)[a]	90% CI	
CL/*F* (L/h)	20.5	6.8	20.2	7.3	17.0	23.7
V/*F* (L)	50.6	36.9	48.3	37.2	17.1	85.7
k_a (h^{-1})	0.14	13.6	0.14	13.6	0.10	0.19
θ_{BW}[b]	1.02	23.4	0.99	25.7	0.58	1.51
$\theta_{AUC\ DRV}$[c]	−0.38	28.1	−0.39	30.6	−0.69	−0.18
ω (CL/*F*) (CV%)[d]	46.8	46.9[e]	46.2	46.2[e]	31.3	57.7
Correlation CL/*V*	70.9	59.4	71.8	64.4	38.1	105.6
ω (*V*) (CV%)[d]	156.7	66.2[e]	164.4	69.3[e]	92.3	369.9
σ (CV%)[d]	50.8	28.3[e]	50.6	31.5[e]	41.1	61.5

CL/*F*, mean apparent clearance; *V*/*F*, mean apparent volume of distribution.
Final model: $CL/F_i = CL/F \times ((AUC_{DRV})/52)^{\theta}AUC_{DRV} \times (1+\theta_{BW} \times FBW)$ with $FBW = (BW - 70)/70$.
[a]Standard errors of the estimates (SE), defined as SE/estimate and expressed as percentages.
[b]Relative influence of body weight on ritonavir clearance (see text).
[c]Relative influence of the AUC of darunavir on ritonavir clearance (see text).
[d]Estimate of variability (between-subject ω and residual σ), expressed as CV (%).
[e]Standard errors of the coefficient of variation, estimated as SE/(estimate×2) and expressed as a percentage.

with reports of reversible competitive and mechanism-based inhibition.[22,23] We found that the impact of ritonavir on darunavir pharmacokinetics was governed by the overall ritonavir exposure, which is in agreement with previous studies reporting that ritonavir inactivates CYP3A through a mechanism-based inhibition.[23] Competitive inhibitory models have also been proposed,[21,24] suggesting that ritonavir may act via both competitive and non-competitive inhibition. The results of this study indicate that a doubling of ritonavir dose and thus the AUC_{RTV} would be associated with an average 16% decrease in darunavir elimination, a value far below the important variability observed in ritonavir pharmacokinetics. Yet this effect explained no more than 3% of the interpatient variability in darunavir CL, which remains largely unexplained.

The population pharmacokinetics analysis of ritonavir produced parameters comparable to those reported in previous studies.[6,19,21,24] The effect of body weight on ritonavir elimination was responsible for 6% of the between-subject variability in

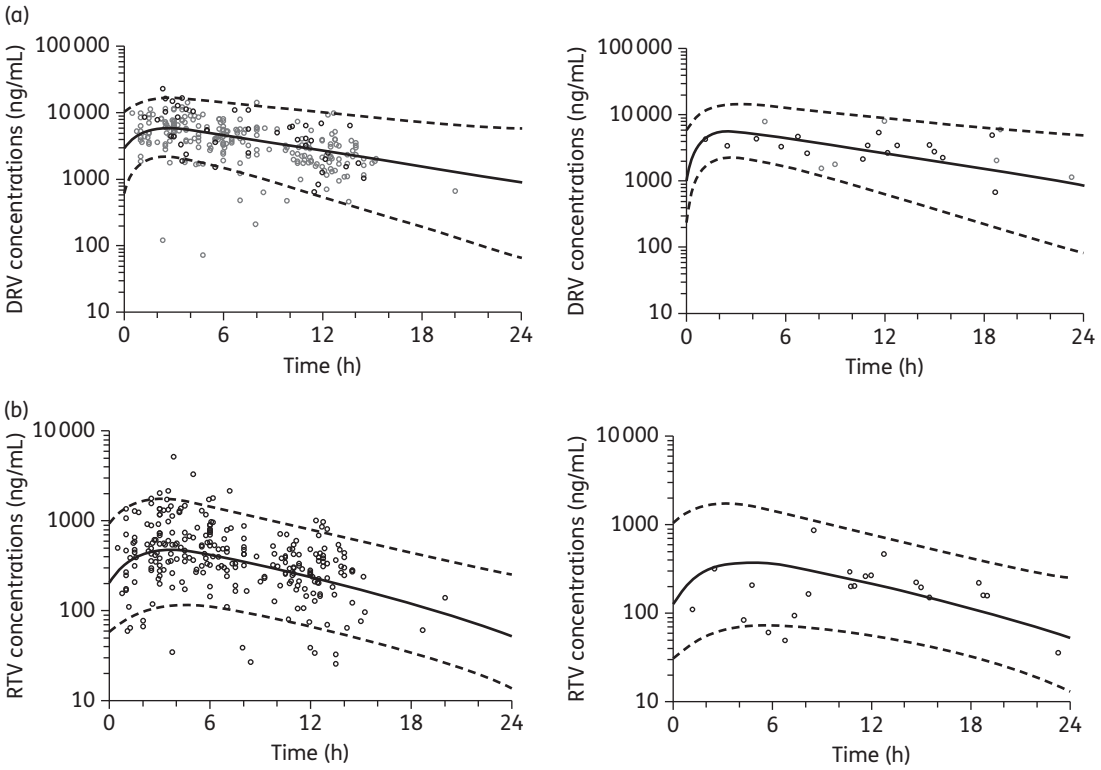

Figure 1. (a) Darunavir (DRV) concentrations versus time standardized for 600/100 mg twice-daily (left panel) and 800/100 mg once-daily (right panel) dosing. (b) Ritonavir (RTV) concentrations versus time standardized after the darunavir/ritonavir 600/100 mg twice-daily (left panel) and 800/100 mg once-daily (right panel) regimens. The continuous line represents the population prediction from the final model and the broken lines represent the 95% PI.

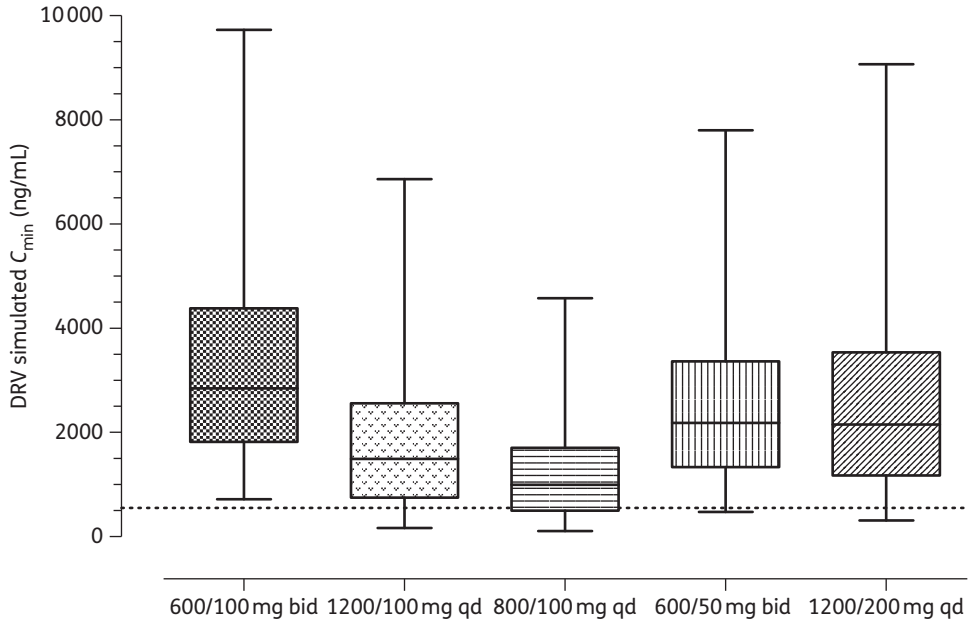

Figure 2. Simulated darunavir (DRV) minimal concentrations (C_{min}) for various combinations of darunavir and ritonavir doses. Each box represents the median and IQR and the bar represents the 95% PI. The horizontal broken line represents the average EC_{50} for protease inhibitor-resistant HIV strains (550 ng/mL). bid, twice daily; qd, once daily.

ritonavir disposition and confirmed a previous finding.[7] Darunavir is a potent inhibitor of CYP3A4[4] and an influence of this drug was found on ritonavir CL as well. The mutual inhibition of the two drugs' elimination might add to the synergistic effect of this association.

Genetic variants have been shown to affect the pharmacokinetics of many protease inhibitors.[25] In the current study, the pharmacogenetic discovery analysis did not reveal any genetic variant that could possibly explain some of the variability in the pharmacokinetics of darunavir or ritonavir. The study was, however, only powered to detect strong genetic effects and, except for a small subset of participants, we did not have genetic information on drug transporters. So far, no genetic influences on darunavir pharmacokinetics have been reported in the literature, except for a recent report on the influence on darunavir CL and central *V* of two SNPs (rs8027174 and rs4294800) located in the *SLCO3A1* locus.[7] These findings need validation because, among the 148 SNPs investigated, only two intronic SNPs with unknown functional effect on SLCO3A1 expression or transport activity show a nominally significant effect, which is not retained after multiple comparisons correction.[7] There is no *in vitro* or *in vivo* evidence that darunavir can interact with the SLCO3A1 transporter as either substrate or inhibitor. No influence of genetic variability associated with genes encoding blood–brain barrier transporters has been observed either.[26]

So far, target C_{min} values for darunavir associated with virological response have not been formally determined. In addition, there is limited value in considering drug exposure data separately from information on resistance for the prediction of the virological response to salvage antiretroviral therapy. Nevertheless, the protein-adjusted EC_{50} for both wild-type (55 ng/mL) and protease inhibitor-resistant (550 ng/mL) HIV-1 strains can serve as useful targets to evaluate the likely efficacy of a given regimen for a given virus isolate.[16,18,27] In this study, the simulation of darunavir/ritonavir 600 mg twice daily and 800 mg once daily provided an average darunavir C_{min} substantially above the putative targets of 550 and 55 ng/mL for naive and experienced patients, respectively. However, taking into account the variability of darunavir pharmacokinetics, about one-third of the patients under the 800 mg once-daily regimen would present levels under the 550 ng/mL threshold, thus justifying the idea that this regimen can be considered sub-optimal in patients with resistant HIV-1 strains. The shorter elimination half-life estimated in this study (7 h) compared with the 15 h reported by the manufacturer[2] could be the consequence of a change in patients' adherence patterns over time, leading to lower levels of darunavir, and thus a shorter apparent elimination half-life. It has been reported that darunavir concentrations are reduced by nearly half compared with baseline after 48 weeks of therapy.[17] Although a large proportion of treatment-experienced patients might still benefit from a once-daily darunavir/ritonavir dosing schedule, this strategy is less forgiving against the possibility of missed doses than twice-daily administration and should only be considered in highly adherent patients with a limited number of mutations in their HIV strains.

Considering the growing interest in once-daily administration of darunavir, we tested alternative regimens that would be useful in treatment-experienced patients in particular. The 1200 mg darunavir once-daily schedule boosted with 100 or 200 mg of ritonavir would decrease the probability of having concentrations below the cut-off of 550 ng/mL. Although losing the benefit of halving the daily dose of ritonavir, the once-daily dosing regimen would offer the possibility of improving convenience and potentially patient compliance while maintaining higher plasma concentrations over the dosing interval in the presence of drug resistance. Reduction of the booster could be beneficial in some patients, as previously suggested by the potential improvements in adverse events or lipid profiles for tipranavir[28] and atazanavir.[29] Although the darunavir/ritonavir 600/50 mg twice-daily regimen resulted in 23% lower average C_{min}, only a few patients would present concentrations below the EC_{50} of 550 ng/mL. This result is in good agreement with a 30% decrease in darunavir exposure predicted after reducing ritonavir from 100 to 50 mg once daily.[7]

In conclusion, a population analysis was used to predict the pharmacokinetic parameters of darunavir in the presence of ritonavir. Darunavir variability was large and only to a small extent explained by ritonavir exposure, which exhibited important between-subject variability as well. No influence of other covariates or genetic polymorphism was found, potentially due to the relatively small population size and the low allelic frequency of some of the polymorphisms. The concentrations predicted under the standard 600/100 mg twice-daily regimen for treatment-experienced patients and 800/100 mg once daily for naive or experienced patients with limited resistance-associated mutations lay within a safe margin with regard to the EC_{50} for wild-type and resistant HIV-1 strains. This suggests that alternative dosing regimens reducing pill burden, improving convenience and adherence or potentially improving adverse events could be prospectively evaluated. The use of once-daily dosing for treatment-experienced patients, however, requires close monitoring of treatment adherence and darunavir concentration measurements, which should be interpreted in the light of resistance data.

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Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Thompson MA, Aberg JA, Hoy JF *et al*. Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society-USA panel. *JAMA* 2012; **308**: 387–402.

2 US FDA. *Prezista® Drug Information*. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm (14 March 2014, date last accessed).

3 McKeage K, Perry CM, Keam SJ. Darunavir: a review of its use in the management of HIV infection in adults. *Drugs* 2009; **69**: 477–503.

4 Rittweger M, Arasteh K. Clinical pharmacokinetics of darunavir. *Clin Pharmacokinet* 2007; **46**: 739–56.

5 Brown KC, Paul S, Kashuba AD. Drug interactions with new and investigational antiretrovirals. *Clin Pharmacokinet* 2009; **48**: 211–41.

6 Lubomirov R, di Iulio J, Fayet A *et al*. ADME pharmacogenetics: investigation of the pharmacokinetics of the antiretroviral agent lopinavir coformulated with ritonavir. *Pharmacogenet Genomics* 2010; **20**: 217–30.

7 Molto J, Xinarianos G, Miranda C *et al*. Simultaneous pharmacogenetics-based population pharmacokinetic analysis of darunavir and ritonavir in HIV-infected patients. *Clin Pharmacokinet* 2013; **52**: 543–53.

8 Llibre JM. First-line boosted protease inhibitor-based regimens in treatment-naive HIV-1-infected patients--making a good thing better. *AIDS Rev* 2009; **11**: 215–22.

9 Cahn P, Fourie J, Grinsztejn B *et al*. Week 48 analysis of once-daily vs. twice-daily darunavir/ritonavir in treatment-experienced HIV-1-infected patients. *AIDS* 2011; **25**: 929–39.

10 De Meyer SM, Spinosa-Guzman S, Vangeneugden TJ *et al*. Efficacy of once-daily darunavir/ritonavir 800/100 mg in HIV-infected, treatment-experienced patients with no baseline resistance-associated mutations to darunavir. *J Acquir Immune Defic Syndr* 2008; **49**: 179–82.

11 Fayet Mello A, Buclin T, Franc C *et al*. Cell disposition of raltegravir and newer antiretrovirals in HIV-infected patients: high inter-individual variability in raltegravir cellular penetration. *J Antimicrob Chemother* 2011; **66**: 1573–81.

12 Fayet A, Beguin A, Zanolari B *et al*. A LC-tandem MS assay for the simultaneous measurement of new antiretroviral agents: raltegravir, maraviroc, darunavir, and etravirine. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009; **877**: 1057–69.

13 Lubomirov R, Arab-Alameddine M, Rotger M *et al*. Pharmacogenetics-based population pharmacokinetic analysis of etravirine in HIV-1 infected individuals. *Pharmacogenet Genomics* 2013; **23**: 9–18.

14 Arab-Alameddine M, Fayet-Mello A, Lubomirov R *et al*. Population pharmacokinetic analysis and pharmacogenetics of raltegravir in HIV-positive and healthy individuals. *Antimicrob Agents Chemother* 2012; **56**: 2959–66.

15 Beal SL, Sheiner LB, Boeckmann A. *NONMEM User's Guides (1989–2006)*. Ellicot City, MD, USA: Icon Development Solutions, 2006.

16 Boffito M, Miralles D, Hill A. Pharmacokinetics, efficacy, and safety of darunavir/ritonavir 800/100 mg once-daily in treatment-naive and -experienced patients. *HIV Clin Trials* 2008; **9**: 418–27.

17 Molto J, Santos JR, Perez-Alvarez N *et al*. Darunavir inhibitory quotient predicts the 48-week virological response to darunavir-based salvage therapy in human immunodeficiency virus-infected protease inhibitor-experienced patients. *Antimicrob Agents Chemother* 2008; **52**: 3928–32.

18 Soon GH, Shen P, Yong EL *et al*. Pharmacokinetics of darunavir at 900 milligrams and ritonavir at 100 milligrams once daily when coadministered with efavirenz at 600 milligrams once daily in healthy volunteers. *Antimicrob Agents Chemother* 2010; **54**: 2775–80.

19 Dickinson L, Boffito M, Back D *et al*. Sequential population pharmacokinetic modeling of lopinavir and ritonavir in healthy volunteers and assessment of different dosing strategies. *Antimicrob Agents Chemother* 2011; **55**: 2775–82.

20 Dickinson L, Jackson A, Garvey L *et al*. Population pharmacokinetic modelling of once daily ritonavir-boosted darunavir in HIV-infected patients. *J Int AIDS Soc* 2010; **13**: Suppl 4: P184.

21 Dickinson L, Khoo S, Back D. Pharmacokinetics and drug-drug interactions of antiretrovirals: an update. *Antiviral Res* 2010; **85**: 176–89.

22 Zalma A, von Moltke LL, Granda BW *et al*. In vitro metabolism of trazodone by CYP3A: inhibition by ketoconazole and human immunodeficiency viral protease inhibitors. *Biol Psychiatry* 2000; **47**: 655–61.

23 Zhou SF, Xue CC, Yu XQ *et al*. Clinically important drug interactions potentially involving mechanism-based inhibition of cytochrome P450 3A4 and the role of therapeutic drug monitoring. *Ther Drug Monit* 2007; **29**: 687–710.

24 Molto J, Barbanoj MJ, Miranda C *et al*. Simultaneous population pharmacokinetic model for lopinavir and ritonavir in HIV-infected adults. *Clin Pharmacokinet* 2008; **47**: 681–92.

25 Arab-Alameddine M, Decosterd LA, Buclin T *et al*. Antiretroviral drug toxicity in relation to pharmacokinetics, metabolic profile and pharmacogenetics. *Expert Opin Drug Metab Toxicol* 2011; **7**: 609–22.

26 Calcagno A, Yilmaz A, Cusato J *et al*. Determinants of darunavir cerebrospinal fluid concentrations: impact of once-daily dosing and pharmacogenetics. *AIDS* 2012; **26**: 1529–33.

27 Sekar V, Vanden Abeele C, Van Baelen B *et al*. Effect of extrinsic and intrinsic factors on the pharmacokinetics of darunavir/ritonavir in HIV-1-infected patients: results of a randomized, controlled, Phase III study (ARTEMIS). In: *Proceedings of the Ninth International Workshop on Pharmacology of HIV Therapy, New Orleans, April 2008*. Poster P41.

28 Molto J, Valle M, Santos JR *et al*. Efficacy and safety of ritonavir dose reduction based on the tipranavir inhibitory quotient in HIV-infected patients on salvage antiretroviral therapy with tipranavir/ritonavir. *AIDS Res Hum Retroviruses* 2010; **26**: 1191–6.

29 Estevez JA, Molto J, Tuneu L *et al*. Ritonavir boosting dose reduction from 100 to 50 mg does not change the atazanavir steady-state exposure in healthy volunteers. *J Antimicrob Chemother* 2012; **67**: 2013–9.