Title

Exploration of reduced doses and short cycle therapy for darunavir/cobicistat in HIV patients using population pharmacokinetic modeling and simulations

Running title

Exploration of reduced doses for darunavir using population pharmacokinetics

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DECLARATIONS

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Conflicts of interest/Competing interests

All authors declare that they have no conflict of interest.

Ethics approval

The study protocol was approved by the local ethics committee (Comité d'Éthique Hospitalo-Facultaire des Cliniques Saint-Luc, approval number B403201731249). This study was conducted in accordance with the Declaration of Helsinki.

Consent to participate

Written informed consent was obtained from all participants before any study-related procedure.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

ABSTRACT

Background

Protease inhibitors such as darunavir are an important therapeutic option in the anti-HIV arsenal. Current dosage guidelines recommend using cobicistat- or ritonavir-boosted darunavir 800 mg q24h in protease inhibitor-naïve patients, or ritonavir-boosted darunavir 600 mg q12h in experienced patients. However, darunavir displays a large, poorly characterized inter-individual pharmacokinetic variability.

Objectives

To study the pharmacokinetics of darunavir and to elucidate the sources of its inter-individual variability using population pharmacokinetic modeling. Then, to determine the appropriateness of current treatment guidelines and the feasibility of alternative dosing regimens in a representative cohort of adult patients using simulations.

Patients and methods

Sparse pharmacokinetic samples were collected in 127 HIV-1 patients, then supplemented with rich sampling data from a subset of 12 individuals. Data were analyzed using the nonlinear mixed effects modeling software NONMEM. The effect of reduced doses (600 mg q24h and 400 mg q24h) or reduced frequency of administration (800 mg q24h for 5 days followed by 2 days of treatment interruption) was simulated.

Results

Our model adequately described the pharmacokinetics of darunavir. Predictors of individual exposure were *CYP3A5*3* and *SLCO3A1* rs8027174 genotypes, sex and alpha-1 acid glycoprotein level. No relationship was apparent between DRV AUC and treatment efficacy or safety. For reduced doses regimens, darunavir concentrations remained above the protein-binding-corrected EC_{50} in the majority of subjects. More stringent pharmacokinetic targets were not reached in a significant proportion of patients.

Conclusions

These results add to the growing body of evidence that darunavir-based therapy could be simplified in order to reduce costs and toxicity, as well as to improve patient compliance. However, the heterogeneity in PK response should be considered when assessing whether individual patients could benefit from a particular regimen, for instance through the use of population PK models.

This study is registered at ClinicalTrials.gov (identifier: NCT03101644, date of registration: April 5, 2017).

KEY POINTS

- A population pharmacokinetic model of darunavir was developed and validated. *CYP3A5* and *SLCO3A1* genotypes, alpha-acid glycoprotein level and sex were predictors of darunavir pharmacokinetics.
- Reduced dosages (600 mg q24h and 400 mg q24h) were predicted to be safe in the majority of patients. Our model could be used to assess changes in exposure for various alternative regimens in specific individuals.

1 INTRODUCTION

According to international guidelines, protease inhibitors (PIs), in combination with a background regimen of two nucleoside reverse transcriptase inhibitors (NRTIs), are a first-line option for the management of HIV-1 infection [1,2]. Darunavir (DRV) is the preferred PI, but must be coadministered with a pharmacokinetic booster – either ritonavir (RTV) or cobicistat (COB) – in order to maximize exposure. Standard dosages include DRV/RTV 800/100 mg q24h or DRV/COB 800/150 mg q24h for PI-naïve patients [3] (or those pretreated with PIs but with no known resistance mutations) [4], and DRV/RTV 600/100 mg q12h for patients presenting PI resistance mutations [5–8]. One of the main advantages of DRV is its high genetic barrier to resistance as it binds to a highly conserved domain of the viral protease [9–11]. This makes it useful in patients with incomplete adherence, since subtherapeutic exposure is unlikely to result in the emergence of resistance mutations, making re-challenge with the same drug possible.

However, treatment with DRV is rendered delicate by its complex spectrum of drug-drug interactions (DDIs). Not only must certain drugs be avoided to ensure adequate exposure to antiretrovirals (ARVs), but DRV and its boosters can also inhibit (and sometimes induce) various cytochromes and transporters, thereby affecting other therapies [12–15]. Indeed, DRV is a substrate for cytochrome P450 3A (CYP3A); a substrate for efflux transports of the ABC family ABCB1 (P-glycoprotein) and ABCC2; a substrate for influx transporters of the OATP family OATP1A2, OATP1B1 and OATP1B3; and has the potential to inhibit CYP3A4, OATP1B1 and OATP1B3. Meanwhile, RTV and COB exert slightly different effects: both are capable of inhibiting CYP3A, OATP1B1 and OATP1B3. RTV may also induce other CYP isoforms and pregnane X receptors (PXR), while COB is more selective in this regard [16].

DRV pharmacokinetics (PK) are highly variable between individuals, although the sources of this heterogeneity remain poorly understood. Single nucleotide polymorphisms (SNPs) in genes coding for biotransformation enzymes and transporters, DDIs and other patient-specific covariates may all contribute to this phenomenon [17]. Previous studies have already highlighted the role played by certain individual factors such as alpha-glycoprotein concentration [18,19], sex [20], weight [21], age [20], SNPs in *SLCO3A1* [19] and RTV exposure [21–23]. Our first aim was to use population PK to model this variability in a representative cohort of adult HIV patients and to identify the best set of predictors. In addition, we evaluated whether changes in DRV PK were correlated with biomarkers of treatment efficacy (viral load and lymphocyte count) and toxicity (occurrence of adverse events).

Our second aim was to simulate the effect of alternative dose regimens. Simplified antiretroviral therapies (ART) involving fewer drugs or lower doses have gained attention as they could help to reduce toxicity, reduce costs (therefore increasing access to the drug in developing countries) and improve convenience and patient compliance. A reduction of the daily dose of DRV/RTV from 800/100 to 600/100 mg q24h was demonstrated to be safe in virologically suppressed patients [24,25], as was a further reduction to 400/100 mg q24h [26,27]. Additionally, DRV 400 mg plus NRTI backbone was found to be non-inferior to standard dose lopinavir plus NRTI backbone [27]. RTV- or COB-boosted DRV monotherapy has also seen some success [28–34], even with the lower 600 mg q24h DRV dosage [35]. Although this type of regimen may expose patients to a higher risk of viral blips, they may easily be re-suppressed by restoring their initial treatment due to the aforementioned pharmacological properties of DRV. Short cycle therapy (SCT) is a different approach

consisting of alternating on- and off-treatment phases for a few days each, which would significantly reduce the drug burden for the patient. Moreover, lower pill burden has been correlated with better adherence in HIV patients, which could help with long-term viral suppression [36]. Based on what little data is available, SCT for DRV-containing triple combination ART appears to be a viable option, despite low plasma levels of DRV during the off-treatment phase [37]. The effect of both reduced doses and SCT were investigated in the present study using simulations, based on the population PK model we developed.

2 MATERIALS AND METHODS

2.1 Study design

This was a prospective study conducted at the Cliniques universitaires Saint-Luc (Brussels, Belgium). Blood samples were obtained during routine hospital visits, once every three to six months on average. One sample for quantification of ARVs was drawn in a heparinized tube at each patient visit (dataset A). Self-reported drug intake time as well as blood sampling time were recorded. One additional sample for DNA extraction was drawn in an EDTA tube at the patient's first study visit.

Intensive sampling was performed in a subset of 12 individuals selected for their high treatment compliance based on available PK data and anamnesis by the physician. In those individuals, a dose journal over the past four days was requested, dose intake on the day of sampling was witnessed by a nurse, and then eight samples were collected at pre-specified time points: pre-dose, 0.5, 1, 2, 3, 4, 5 and 6 hours post-intake (dataset B).

2.2 Drug quantification

DRV, RTV and COB were quantified using a validated UPLC-diode array detection method [38]. Briefly, samples were spiked with the internal standard (clomipramine), buffered to alkaline pH, then a liquid-liquid extraction was performed using methyl-ter-butyl-ether. Samples were centrifuged and the supernatant was evaporated to dryness by heating at 40°C under a stream of nitrogen. The residue was reconstituted in mobile phase and 5 µl were injected on an Acquity UPLC BEH C18 column. The mobile phase was a gradient of triethylammonium phosphate (pH 3) and acetonitrile. All reagents were of analytical grade.

The lower limit of quantification (LLOQ) was 0.1 $mg.l^{-1}$ for DRV and RTV. For COB, no LLOQ was formally defined but a threshold of 0.2 $mg.l^{-1}$ was used based on the signal-to-noise ratio. The method was linear over the range of 0.1 to 10 $mg.l^{-1}$ for all three analytes. Samples were analyzed in batches along with external quality controls (containing DRV and RTV) and internal controls (blank plasma spiked with known amounts of all analytes, as well as previously determined patient samples). For this analytical method, our laboratory successfully participates in an external quality assessment scheme organized by the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML, Nijmegen, The Netherlands); the trueness and precision of the method were within 10% for DRV and RTV. The stability of DRV in various conditions (autosampler, room temperature, freeze-thaw and long-term storage) has been determined by other authors [39,40].

2.3 Genotyping

DNA was isolated using the QIAamp® DNA mini-kit (QIAGEN, CA, USA) according to the manufacturer's instructions. Allelic discrimination was performed on a Step One Plus™ real time PCR system using Taqman® genotyping assays (Applied Biosystems, CA,USA) for

ABCB1 rs2229109 and rs1045642; *CYP3A4* rs35599367; *CYP3A5* rs776746; *SLCO3A1* rs8027174 and rs4294800. These SNPs were selected based on expected clinical relevance and results from previously published studies. Haldane's exact test (as implemented in the HardyWeinberg package for R) [41] was used to check for deviation from Hardy-Weinberg equilibrium.

2.4 Modeling software

PK data was analyzed using the nonlinear mixed effects modeling software NONMEM (version 7.4.3) [42]. Additional functionality was provided by PsN (version 4.7.0) [43]. NONMEM output was processed in R (version 3.5.2) [44] and with the npde package (version 2.0) [45].

2.5 Structural model

Dataset B was analyzed on its own then pooled with dataset A. For the pooled analysis, the PRIOR routine in NONMEM was used to specify a prior distribution of all fixed and random effects (except residual variability terms) based on final estimates of the model built on dataset B [46]. The reason we thus constrained parameter estimates was because it was assumed that dataset B would be more informative, especially regarding the absorption phase. Separate structural models were first developed for DRV and COB (development of a RTV model was not possible due to a lack of data). For each drug, a one- or two-compartment disposition model was fitted to the data. Models with first-order and delayed first-order absorption were evaluated. The interaction between COB and DRV was modeled according to a linear or I_{max} (Michaelis-Menten) function. To account for the nonlinear dose-exposure relationship of DRV [23,47], dose-dependent and saturable clearance models were tested.

2.6 Covariate model

The following covariates were investigated: age, body weight, race (subjects were classified as Caucasian or African, other races were lumped with Caucasians), food intake with last DRV dose, plasma albumin and α1-acid-glycoprotein (AAG), genotypes and DDIs. Genetic covariates have already been described in section 2.3. For each SNP, subjects were stratified according to their number of functional alleles, or simply as expressors versus non-expressors in the case of *CYP3A5*3*. For DDIs, concomitant drugs (ARVs and non-ARVs) were classified as CYP3A inhibitors, CYP3A inducers or P-glycoprotein inhibitors based on published lists of interactions [48,49]. Missing covariate values were substituted by the most recent measurement, the population median or the most frequent category, as appropriate.

Covariates were first screened for univariate association with the empirical Bayesian estimates of PK parameters. The process of covariate stepwise selection was then partly automated using PsN, based on the decrease of the objective function value (OFV). α thresholds for statistical significance were set to 0.05 for forward inclusion and 0.01 for backward elimination. The following equations were used to describe covariate relationships (for continuous covariates, linear and exponential models with normalization to the median were both considered):

Exponential: $P = \theta \times e^{\theta cov \times (cov - median)}$

Linear: $P = \theta \times (1 + \theta_{cov} \times (cov - median))$

Categorical: $P = \theta \times (1 + \theta_{cov})$

Cov: Covariate value, θ: Typical value, θ_{cov}: Covariate scale factor (0 for reference group), P: Model parameter.

Association between model-derived AUCs and pharmacodynamic biomarkers was assessed using Wilcoxon tests or by calculating Spearman's rho, as appropriate. The viral load (> or < 40 copies/ml, i.e., undetectable), CD4+ and total lymphocyte counts were used as markers of efficacy, and the occurrence of adverse events at any point during the study, stratified by type of symptom (neurological or lipodystrophy), as markers of toxicity.

2.7 Goodness of fit and internal validation

Goodness of fit was assessed using standard graphical methods (individual and population predictions versus observations, residuals versus observations and time). Reduction of unexplained variability, shrinkage or standard errors of estimates were additional criteria for model selection. The model was validated by verifying the normality of the NPDE distribution. Parameter uncertainty was assessed by constructing 95% CIs from 1000 nonparametric bootstraps.

2.8 Target attainment

Target attainment probabilities were computed by simulating DRV steady-state PK profiles based on the original dataset (1000 simulations in the absence of residual error). Evaluated strategies were DRV 800 mg q24h, 600 mg q24h, 400 mg q24h, and SCT 800 mg q24h for 5 days. Trough concentration (C_0) targets were set to 0.055 mg/l (protein-binding adjusted EC_{50} for wild-type HIV-1 protease, serving as the therapeutic target in PI-naïve patients with no resistance-associated mutations) [11], 0.55 mg/l (10 times the wild-type EC_{50}, serving as the target in PI-experienced patients) and 2 mg/l (recommended trough level in PI-experienced patients) [50].

3 RESULTS

3.1 Study population

127 patients were enrolled, 12 of which underwent intensive sampling. A total of 309 sparse samples (dataset A) and 96 rich samples (dataset B) were collected. For sparse samples, the post-intake time ranged from 1 to 29 hours (median: 11.9 hours). Concentrations were censored if the post-intake time was unknown (1.9%), if the patient reported a lack of treatment compliance (0.3%) or due to analytical reasons (1%). DRV concentrations below the LLOQ (3.6%) were set to half of the LLOQ.

Summary characteristics of the cohort can be found in Table 1. At baseline, median DRV treatment duration was 4.2 years, 85.8% of patients were using COB-boosted DRV, and DRV 800 mg q24h was the most frequent dosage (91.3%), followed by 600 mg q12h (7.9%) and 1200 mg q24h (0.8%). The three most common ARTs in this cohort were: DRV/tenofovir/emtricitabine (22.0%), DRV/dolutegravir (18.1%) and DRV/dolutegravir/lamivudine (17.3%). Two patients (1.6%) were using DRV 800 mg q24h monotherapy. Frequent complaints possibly related to ART that occurred at least once during follow-up for a given subject included lipodystrophy (29.1%) and neurological symptoms (insomnia, fatigue or headaches) (26.0%). Genotypes for pharmacogenes of interest are sumarized in Table 2. All SNPs were in Hardy-Weinberg equilibrium with the exception of rs776746 (this deviation was no longer significant when analyzing Africans and Caucasians separately, suggesting population stratification). *CYP3A5* genotypes within each racial group matched their expected distribution (from, e.g., the 1000 Genome project) [51].

3.2 Structural model

DRV kinetics were best described by a one-compartment model with first-order absorption and elimination. Inter-individual variability was described by an exponential error term, while a mixed (additive plus exponential) residual error was used. For dataset B only, the model fit was improved by the addition of a lag term to absorption (ΔOFV = –36), and by the addition of a block diagonal matrix between random effects (ΔOFV = –12). On the other hand, treating the daily dose of DRV as a covariate on CL or modeling a saturable clearance did not or prevented the model from converging. Neither the lag time nor the full random effect matrices were retained in the model developed on the combined dataset.

Similarly, COB kinetics were described by a simple one-compartment model. There appeared to be a positive correlation between DRV CL and COB CL (dataset B: rho = 0.33, p-value = 0.3, see Online Resource 1). Since inclusion of this relationship in a joint model on dataset B did not improve the fit, the COB portion of the model was dropped at this stage.

3.3 Covariate model

For dataset B alone, two covariates were retained in the final model: all other things being equal, V was decreased by 53% in subjects harboring the *CYP3A5*3* allele and by 70% in Caucasian individuals compared to Africans. In the combined dataset, the *CYP3A5* phenotype was associated with CL instead, with CL being 19% higher in expressors compared to non-expressors. Additionally, for the combined dataset, CL and V were both decreased in subjects with higher AAG, CL was 21% lower in females compared to males, and V was increased by 81% in individuals harboring *SLCO3A1* rs8027174. Overall, AAG was the most relevant covariate based on the total reduction of the OFV (ΔOFV = –45), although its inclusion only decreased unexplained residual variability by 7% and 10% for CL and V, respectively. Final

model parameters are displayed in Table 3. Details of the covariate selection process can be found in Online Resource 2.

There was no apparent correlation between DRV AUC and viral load (W = 582, p-value = 0.45), CD4 lymphocyte count (rho = 0.09, p-value = 0.3) or occurrence of adverse events at any point during follow-up (W = 1687, p-value = 0.97 for lipodystrophy; W = 1613.5, p-value = 0.42 for CNS adverse events).

3.4 Goodness of fit and internal validation

There was good adequation between observed and individual predicted concentrations (rho = 0.87), except for two outlier individuals with very high levels of AAG (2.66 and 2.2 $g.l^{-1}$), thus low model-predicted clearance, while their observed concentrations were actually low (Online Resource 3). A total of 5 further observations identified as outliers based on their weighted residuals were discarded, then the model was re-run. Figure 1 shows the goodness of fit after removal of these outliers. NPDE were normally distributed, with mean 0 and variance 1 (Figure 2); p-values for the Shapiro-Wilk, Wilcoxon signed rank and Fisher variance tests were 0.56, 0.84 and 0.79. Final parameter estimates were contained in the 95% CIs generated through nonparametric bootstrapping (Table 3).

3.5 Target attainment

Table 4 shows the probability of target attainment (PTA) for each scenario, based on a representative population. DRV C_0 remained above the recommended cutoff of 0.055 $mg.l^{-1}$ in the majority of subjects for the 600 mg q24h and 400 mg q24h strategies, while only 84.0% and 69.8% of individuals remained above 0.55 $mg.l^{-1}$ for these two respective strategies. The 2 $mg.l^{-1}$ target was reached in less than a third of patients, even with the standard once daily

dosage. Weekends-off SCT resulted in subtherapeutic levels of DRV in a significant portion of individuals: 39.3% failed to reach even the lowest C_0 target.

4 DISCUSSION

Using population PK, we leveraged rich and sparse data to estimate individual PK parameters of DRV. A one-compartment model described the data well, with CL and V being in the range of those previously estimated with one-compartment models in adult populations [22,52]. The estimate of k_a was less precise, probably due to a lack of data in the absorption phase, with its CI encompassing lower values such as those found in [19,53] all the way up to values closer to those calculated in [21,22]. Earlier publications have also applied two-compartment models [18,19,21,53,54] and more complex absorption kinetics such as transition compartment models [55], but these were not supported by our relatively sparse PK data. Among the investigated covariates, AAG and sex, along with *CYP3A5* and *SLCO3A1* genotypes, were found to be predictive of DRV PK.

DRV CL and V both decreased with increasing AAG levels, which is consistent with previous reports of AAG affecting DRV PK [18,19]. The clinical relevance of this association is unclear since total concentrations may be altered without any impact on unbound concentrations. In addition to AAG concentration, AAG phenotype is known to modulate the plasma PK of some PIs, which may also play a role [56].

Regarding pharmacogenetics, both the loss-of-function variant *CYP3A5*3* and Caucasian race were associated with smaller V based on the rich dataset. However, the latter association was no longer apparent in the whole cohort and was not retained in the final model. *CYP3A5*3* is

commonly found in Caucasians, which may result in CYP3A4 being the major isoform responsible for DRV metabolism for that ethnicity, while it is far less frequent in Africans, in whom both isoforms (CYP3A4 and CYP3A5) may contribute to some extent. Indeed, in subjects receiving etravirine (a known CYP inducer), plasma exposure to DRV was previously found to be lower in carriers of the functional *CYP3A5*1* allele compared to *CYP3A5*3* carriers, suggesting that CYP3A5 is also involved in DRV biotransformation [57]. It is also worth noting that race correlated with the presence of the *CYP3A4*22*, *CYP3A5*3* and *ABCB1 3435C>T* alleles in this population. The fact that race remained a significant predictor in a model already including *CYP3A5* genotype suggests there may be other race-specific factors that we did not identify. Additionally, the *SLCO3A1* rs8027174 variant was correlated with larger V in our dataset. This intronic polymorphism was previously associated with smaller CL; however, it is unknown if DRV is a substrate of SLCO3A1 [19].

Lastly, DDIs were not identified as a significant covariate, most likely due to the small number of clinically relevant interactions found in our patients (mostly limited to a few etravirine users) and due to RTV- and COB-boosting blunting any inductive effects or exceeding the inhibitory effects of concomitantly administered drugs.

Next, we used our model to carry out simulations of alternative dosing schemes. There is growing interest in reducing the dose of DRV in virally suppressed patients: it was recently demonstrated that the daily dose of DRV/RTV could be reduced from 800/100 mg to 400/100 mg q24h while maintaining adequate viral suppression in plasma and semen, although bioequivalence between the dosages could not be assessed [26,54]. So far, it is unknown if a similar regimen could be used in patients using DRV/COB, especially since COB may not be quite as powerful as RTV. Moreover, dose reductions are typically performed in virally

suppressed subjects. It would be important to assess their feasibility in more diverse groups of patients. With regards to ART simplification, one option would be “weekends-off” therapy (i.e., patients take ART for five days then interrupt for two days), although longer off-treatment phases may also be worth looking into.

In our simulations, for an unchanged COB dose, DRV 600 mg q24h and 400 mg q24h resulted in decreased exposure, but concentrations remained well above the target of 0.055 $mg.l^{-1}$ in the majority of subjects. Weekends-off therapy appeared unsafe from a purely PK point of view. Although concentrations fell below the cutoff of 0.55 $mg.l^{-1}$ in a significant portion of subjects, this value can be considered fairly conservative since it is already ten times higher than the typical wild-type EC_{50}, and consequently, does not apply to PI-naïve individuals. Finally, when targeting a C_0 of 2 $mg.l^{-1}$, the PTA was extremely low, even for the standard dosage. Interestingly, target achievement rates observed in our cohort were lower than the model-predicted ones: for q24h dosing, 7.1% and 25.7% of measured C_0 fell below 0.055 $mg.l^{-1}$ and below 0.55 $mg.l^{-1}$, respectively (compared to the model-predicted probabilities of 0.7% and 10.4%). Among individuals with available C_0, only four had detectable viremia (comprised between 40 and 63 $copies.ml^{-1}$), and only one of these had what could be considered as a low DRV C_0 (0.246 $mg.l^{-1}$). It is possible that in individuals with low DRV concentrations, concomitant ARVs ensured adequate anti-HIV activity, which would suggest that DRV is not a necessary component of the ARV regimen for those individuals, or it may be that the PK target itself is too stringent and that lower plasma levels are acceptable. Of course, plasma exposure is only a surrogate for intracellular accumulation and target-bound DRV. These results should be contrasted with those from a four days a week intermittent ART study, in which virological failure was rare despite low DRV exposure [37]. Additionally, the simulations used to derive PTA in the present paper were performed on a dataset that is

representative of a certain patient population; it may not necessarily reflect what would be observed in populations with completely different genotypes, for instance. Further, PTA for groups such as children and pregnant women would have to be calculated based on data obtained in those groups specifically because of how much they differ from a standard adult population. For example, dosing recommendations for DRV/RTV in children aged 3 to 12 years have been derived using a PK model that incorporated not only AAG, but also body weight, as is often the case for pediatric models [58]. For pregnant women, in whom changes in CYP activity and protein binding can alter PK processes, a recent population PK study showed that administering DRV/RTV q12h instead of the standard q24h dosing could compensate for the reduction in exposure caused by pregnancy, thereby ensuring therapeutic drug levels, although systematic dose adjustments might not be warranted [23].

There were a few limitations to our study, the first of which was the sparseness of PK data in most subjects, for whom one sample per dose interval was obtained at a random post-intake time. This resulted in high levels of shrinkage, especially on V and k_a, which could have prevented us from detecting all relevant covariate associations. Despite the addition of richer data and the use of a prior distribution, only a marginal improvement in model fit could be achieved and there was little change in individual parameter estimates. As a result, covariate associations should be regarded with caution. Further, there were few subjects using the 600 mg q12h dosage in our cohort. The limited amount of information on this dose level could be problematic for simulations, as DRV exposure (namely, the AUC) is known to increase less than dose proportionally [23,47]. Consequently, extrapolation to lower doses using simulations may yield more pessimistic results than expected (i.e., predicted AUC will be lower than its true value), although this would not change our general conclusions. While the relationship between dose and total exposure is nonlinear, that between dose and unbound

exposure may be linear [23], which brings us to another limitation of our study: only total concentrations were measured. DRV being highly protein bound, it has been argued that unbound concentrations could be a useful biomarker for therapeutic drug monitoring (TDM) [59]. If DRV is assumed to have a low extraction ratio, displacement of its binding to AAG could affect the total concentration without influencing the unbound concentration, rendering the interpretation of TDM more delicate. Ultimately, since TDM for this drug traditionally relies on total concentrations and since PTA targets are defined based on protein-binding adjusted EC_{50}, it was more rational to use total concentration for the present work, but further studies are needed to establish the relationship between changes in total and unbound PK parameters, and how this could affect TDM of DRV. Lastly, most of the data used to build our model was collected in COB-boosted patients. While RTV and COB provide similar boosting effects, they are not identical and some minute differences have been demonstrated [16]; for instance, the boosting effect of COB was found to be insufficient in pregnant women [60]. Thus, model predictions could potentially be biased if applied to RTV-boosted patients.

5 CONCLUSION

In conclusion, some individual factors were highlighted as potential predictors of DRV PK. Despite an important inter-individual variability, the probability of achieving commonly defined PK targets with reduced dose regimens was high, suggesting that this type of strategy could contribute to treatment simplification in a wide range of patients. Population PK models such as the one developed in this work could be used to predict changes in individual exposure based on a combination of TDM and covariate data.

ABBREVIATIONS

3TC: Lamivudine

AAG: α-1 acid glycoprotein

ABC: ATP-binding cassette

ART: Antiretroviral therapy

ARV: Antiretroviral

C_0: Trough concentration

COB: Cobicistat

CWRES: Conditional weighted residual

CYP: Cytochrome P450

DDI: Drug-drug interaction

DRV: Darunavir

DTG: Dolutegravir

IQ: Inhibitory quotient

NPDE: Normalized prediction distribution error

NRTI: Nucleoside reverse transcriptase inhibitor

OATP: Organic anion transporting polypeptide

OFV: Objective function value

PI: Protease inhibitor

PK: Pharmacokinetic

PTA: Probability of target attainment

PXR: Pregnane X receptor

QC: Quality control

RTV: Ritonavir

SCT: Short cycle therapy

SNP: Single nucleotide polymorphism

TDM: Therapeutic drug monitoring

FIGURE CAPTIONS AND LEGENDS

Fig. 1 Goodness of fit plots

(**a**) Population predicted concentrations (PRED) versus observations. (**b**) Individual predicted concentrations (IPRED) versus observations. (**c**) Conditional weighted residuals (CWRES) versus PRED. (**d**) CWRES versus time after dose.

Fig. 2 Normalized distribution prediction error (NPDE)

(**a**) Q-Q plot of NPDE. (**b**) Histogram of NPDE. Shaded area represents theoretical distribution. (**c**) NPDE versus time after dose. Shaded areas represent the prediction intervals associated with the 5^{th}, 50^{th} and 95^{th} percentiles. (**d**) NPDE versus predicted concentrations.

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