Population pharmacokinetics and pharmacogenetics of ritonavir-boosted darunavir in the presence of raltegravir or tenofovir disoproxil fumarate/emtricitabine in HIV-infected adults and the relationship with virological response: a substudy of NEAT001/ANRS143 randomised trial

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Short Title: Darunavir/ritonavir pharmacokinetics and pharmacogenetics in NEAT001/ANRS143
SYNOPSIS

Objectives: NEAT001/ANRS143 demonstrated non-inferiority of once daily darunavir/ritonavir (800/100 mg) + twice daily raltegravir (400 mg) versus darunavir/ritonavir + tenofovir disoproxil fumarate/emtricitabine (245/200 mg once daily) in treatment-naïve patients. We investigated the population pharmacokinetics of darunavir, ritonavir, tenofovir and emtricitabine and relationships with demographics, genetic polymorphisms and virological failure.

Methods: Nonlinear mixed effect models (NONMEM v. 7.3) were applied to determine pharmacokinetic parameters and assess demographic covariates and relationships with SNPs (SLCO3A1, SLCO1B1, NRII2, NRII3, CYP3A5*3, CYP3A4*22, ABCC2, ABCC10, ABCG2 and SCL47A1). The relationship between model-predicted darunavir AUC\textsubscript{0-24} and C\textsubscript{24} with time to virological failure was evaluated by Cox regression.

Results: Of 805 enrolled, 716, 720, 347 and 347 were included in the darunavir, ritonavir, tenofovir and emtricitabine models, respectively (11% female, 83% Caucasian). No significant effect of patient demographics or SNPs was observed for darunavir or tenofovir apparent oral clearance (CL/F); co-administration of raltegravir did not influence darunavir or ritonavir CL/F. Ritonavir CL/F decreased 23% in NRII2 63396C>T carriers and emtricitabine CL/F was linearly associated with creatinine clearance \((p<0.001)\). No significant relationship was demonstrated between darunavir AUC\textsubscript{0-24} or C\textsubscript{24} and time to virological failure [HR (95% CI): 2.41 (0.59-9.77), \(p=0.219\); 1.87 (0.66-5.32), \(p=0.239\)].

Conclusions: darunavir concentrations were unaltered in the presence of raltegravir and not associated with virological failure. Polymorphisms investigated had little impact on study drug pharmacokinetics. Darunavir/ritonavir+raltegravir may be an appropriate option for patients experiencing NRTI-associated toxicity.
Introduction

HIV therapy commonly consists of two NRTIs combined with an integrase inhibitor, NNRTI or boosted-protease inhibitor. However, renal and bone-associated adverse events particularly with tenofovir and concerns regarding cardiovascular risk with abacavir, have led to exploration of NRTI-sparing regimens as alternatives for treatment-naïve patients.

NEAT001/ANRS143, a phase 3, randomised, open-label trial, demonstrated non-inferiority of raltegravir (400 mg twice daily) + darunavir/ritonavir (800/100 mg once daily) compared to tenofovir disoproxil fumarate/emtricitabine (245/200 mg once daily) + darunavir/ritonavir (800/100 mg once daily) in a large group of European treatment-naïve patients [Kaplan-Meier estimated treatment failure from the primary intent-to-treat analysis at 96 weeks was 17.8% (NRTI-sparing) versus 13.8% (standard regimen). Adjusted difference in treatment failure between study arms was 4.0% (95% CI -0.8 to 8.8) and HR for attaining the primary endpoint with the NRTI-sparing regimen was 1.34 (0.96-1.88)]. The NRTI-sparing regimen was well tolerated but was not recommended in patients with CD4 counts <200 cells/mm$^3$ due to increased risk of virological failure.

This analysis investigated the interplay between patient characteristics, SNPs, pharmacokinetics and pharmacodynamics (efficacy and renal adverse events) in the large NEAT001/ANRS143 trial, with a focus on darunavir, ritonavir, tenofovir and emtricitabine.
Methods

Patients and pharmacokinetic sampling

NEAT001/ANRS143 has previously been described. In summary, HIV-infected, treatment-naïve patients were recruited between August 2010 and September 2011 from 15 European countries (78 sites). Individuals were eligible if plasma HIV-1 viral load was >1000 copies/mL, CD4 count <500 cells/mm³ (except patients with symptomatic HIV infection) and there was no previous or current evidence of major IAS-USA resistance mutations. Patients suffering from or requiring treatment for active opportunistic infections (e.g. tuberculosis, hepatitis B/C), pregnant women, those with abnormal laboratory parameters or hepatic/renal impairment were excluded.

Patients were randomised (1:1) to receive ritonavir-boosted darunavir with either tenofovir disoproxil fumarate/emtricitabine (standard regimen) or raltegravir (NRTI-sparing regimen). Timed, single blood samples were drawn at week 4 and 24 and plasma drug concentrations quantified by fully validated HPLC-MS and LC-MS methods with lower limits of quantification (LLQ) of 0.0391, 0.0098, 0.0156 and 0.0117 mg/L for darunavir, ritonavir, tenofovir and emtricitabine, respectively.

Ethics

Ethical approval was obtained from all study sites and the study conducted in accordance with the Declaration of Helsinki. All participant provided written informed consent.

Genotyping

Total genomic DNA was extracted from patient blood using the QI Amp DNA mini kit (Qiagen, West Sussex, UK) according to manufacturer’s instructions. The following SNPs,
associated with metabolism and transport, were genotyped for *darunavir* and *ritonavir*: *SLCO3A1* G>A (rs4294800), *SLCO3A1* G>T (rs8027174), *SLCO1B1* 521T>C (rs4149056), *NR1I2* (*PXR*) 63396C>T (rs2472677), *NR1I3* (*CAR*) 540G>A (rs2307424), *CYP3A5* 6986A>G (rs776746), *CYP3A4* 522-191C>T (rs35599367); for *tenofovir*: *ABCC2* (MRP2) 24C>T (rs717620), *ABCC2* 1249G>A (rs2273697), *ABCC10* (MRP7) 526G>A (rs9349256), *ABCC10* 2843T>C (rs2125739), *ABCG2* 421C>A (rs2231142) and for *emtricitabine*: *SLC47A1* (*MATE1*) G>A (rs2289669) using real-time PCR allelic discrimination assays (Applied Biosystems, Foster City, CA, USA; Table S1) essentially as described previously.\(^7\)

**Population pharmacokinetic modelling**

Nonlinear mixed effects modelling (NONMEM v. 7.3, ICON Development Solutions, Ellicott City, MD, USA) implementing FOCE-I was applied to concentration-time data of each drug.\(^8\)

With 1 sample per patient on each sampling occasion (week 4 and 24), parameter estimates from the literature were used as priors for *darunavir*, *ritonavir* and *emtricitabine*\(^9,10\) (SPRIOR subroutine of NONMEM); *tenofovir* did not require priors, but parameter estimates from the literature were used initially.\(^11\)

The impact of covariates including bodyweight, age, sex, ethnicity, treatment backbone (*i.e.* *tenofovir* disoproxil fumarate/emtricitabine versus raltegravir; for *darunavir/ritonavir*), creatinine clearance (CrCL, estimated using the Cockcroft-Gault equation; for *tenofovir* and *emtricitabine*) and the polymorphisms described above were evaluated on apparent oral clearance (CL/F). Genotypes were parameterised in the models to compare heterozygotes and homozygotes for the rare alleles to homozygotes for the common alleles as reference populations. If the proportion of homozygotes for the rare allele was <10% they were combined
with the heterozygotes. Likewise, hetero and homozygotes for the rare alleles were combined into one category if changes in CL/F were similar when compared to homozygotes for the common allele. Initially, univariable associations were assessed followed by multivariable if more than one covariate was found to be significant (see below for statistical criteria).

A decrease in the minimal objective function value (OFV; -2 log likelihood) of at least 3.84 units was required to accept a model with an extra parameter ($p=0.05$, $\chi^2$ distribution, 1d.f.). Once significant covariates were incorporated, backwards elimination was performed and biologically plausible covariates generating an increase in OFV of at least 10.83 units ($p=0.001$, $\chi^2$ distribution, 1d.f.) were retained. This threshold was chosen in order to robustly test the relationships observed, given the large sample size but sparseness of the pharmacokinetic data per individual.

Model evaluation was performed by means of prediction-corrected visual predictive checks (pcVPC)\textsuperscript{12} constructed from 1000 simulations of each dataset implemented through Perl-speaks-NONMEM (PsN; version 3.4.2)\textsuperscript{13} and plots developed using Xpose\textsuperscript{14} in RStudio (version 1.1.383). pcVPC correct for the inclusion of significant covariates and/or varying dosages per drug.

For each drug secondary pharmacokinetic parameters, AUC\textsubscript{0-24}, C\textsubscript{max} and C\textsubscript{24}, were derived for each patient and applied to the analyses incorporating virological response (outlined below). Ritonavir parameters were calculated using standard 1 compartment pharmacokinetic equations for multiple oral dosing (Table S4). For the two compartment drugs (darunavir, tenofovir and emtricitabine) full pharmacokinetic profiles were simulated for each patient per
drug using their individual predicted model parameters. $C_{\text{max}}$ and $C_{24}$ were determined directly from the profiles and $\text{AUC}_{0-24}$ as outlined (Table S4).

Pharmacokinetic-pharmacodynamic analysis

The primary pharmacodynamic endpoint was protocol-defined virological failure that included change of any component of the randomised regimen before week 32 because of insufficient virological response (reductions of $<1 \log_{10}$ copies/mL in HIV-1 RNA by week 18 or HIV-1 RNA $\geq 400$ copies/mL at week 24); failure to achieve virological response by week 32 (HIV-1 RNA $\geq 50$ copies/mL); HIV-1 RNA $\geq 50$ copies/mL at any time after week 32. All virological components of the primary endpoint had to be confirmed by a second measurement. The association between model predicted log$_{10}(C_{24})$ or log$_{10}(\text{AUC}_{0-24})$ and time to virological failure by week 96 was evaluated using multivariable Cox regression, adjusting for sex, age, mode of HIV infection, ethnicity, country, baseline CD4 count, baseline HIV-1 RNA, and drug regimen. Similarly, we also investigated the association of pharmacokinetic parameters with the primary endpoint of the NEAT001/ANRS143 trial which was time to virological or clinical failure.

The primary analyses were as randomised and based on available data. We also performed sensitivity analyses: a) censoring analysis time when any component of the initial randomised treatment was stopped; b) multiple imputation of missing pharmacokinetic parameters (using the same factors as described above plus the event indicator and the Nelson–Aalen estimator).

Additionally, we examined the association of CD4 count change from baseline to week 96 with $C_{24}$ or $\text{AUC}_{0-24}$ using multivariable regression models adjusting for baseline CD4 cell count and other factors as above.
Renal adverse events

For tenofovir, we examined the association between model predicted C_{max} or AUC_{0-24} (mean of week 4 and 24) and the tenofovir SNPs with reduced glomerular function defined as at least 25% reduction from baseline in CrCL sustained in two measurements at least 4 weeks apart. Multivariable Cox models were used adjusting for sex, age, ethnicity, baseline CD4 count, baseline HIV-1 RNA and baseline CrCL.
Results

Patients and sampling

Of 805 patients enrolled, data were available from 770 patients (n=386 raltegravir arm; n=384 tenofovir disoproxil fumarate/emtricitabine arm) totalling 1460 samples (n=726 raltegravir arm; n=734 tenofovir disoproxil fumarate/emtricitabine arm). Between 10-25% of samples were excluded: lack of recorded time post-dose, missing concentration, time post-dose >30 hours, sample below assay LLQ or a combination thereof. Overall 1317 and 1283 concentrations were used to develop darunavir and ritonavir models in a total of 716 and 720 patients, respectively. The majority of patients received 800/100 mg once daily (n=698, 97%); alternative doses were recorded for a small proportion (n=18; Table S2). For tenofovir and emtricitabine, 347 (588 concentrations) and 361 patients (656 concentrations) were included, respectively. Patient demographics and clinical characteristics are summarised (Table 1). Patients excluded from pharmacokinetic modelling had similar characteristics to patients included apart from ethnicity and country.

Genotyping

Of the patients with complete pharmacokinetic data for darunavir, ritonavir, tenofovir and emtricitabine, 618/716, 621/720, 302/347 and 314/361 (86-87%) had a blood sample for genotyping, respectively. Genotyping assays failed in one and three patients for ABCC2 24C>T and ABCC10 526G>A, therefore 301 and 299 patients had both pharmacokinetic and genetic data for these particular SNPs. All genotypes were in Hardy-Weinberg equilibrium with the exception of SLCO3A1 G>T (rs8027174) and CYP3A5*3 (rs776746), and could not be evaluated in the covariate model; allele frequencies are summarised (Table 2).

Darunavir/ritonavir population pharmacokinetic modelling
Darunavir and ritonavir plasma concentrations are presented (Figure 1a, 1b) and ranged between 0.06-16.4 and 0.01-2.76 mg/L, respectively over 0.17-30.1 hours post-dose. Due to extensive model run times, darunavir and ritonavir were ultimately modelled sequentially.\textsuperscript{16} Firstly, ritonavir was modelled, followed by darunavir with ritonavir concentrations calculated within the darunavir model using the individual posterior parameter estimates from the final ritonavir model (see below).

A one-compartment model with first-order absorption best described ritonavir, parameterised by CL/F, apparent volume of distribution (V/F) and absorption rate constant (k\textsubscript{a}); a literature prior was included for CL/F.\textsuperscript{9} Interindividual variability (IIV) was estimated on CL/F but interoccasion variability (IOV) was not supported; a proportional model best described residual error. Darunavir was described by a 2-compartment model parameterised by CL/F, volume of distribution of the central and peripheral compartment (V\textsubscript{c}/F, V\textsubscript{p}/F), intercompartmental clearance (Q/F) and k\textsubscript{a}. The interaction between ritonavir and darunavir was via a direct response model with ritonavir concentrations inhibiting darunavir CL/F parameterised by IC\textsubscript{50} (ritonavir concentration associated with 50% maximum inhibition) and I\textsubscript{MAX} (maximum inhibitory effect, fixed to 1). IIV was included on darunavir CL/F and a proportional residual error was used.

Univariable analysis identified antiretroviral backbone as a significant covariate on darunavir CL/F. Compared to tenofovir disoproxil fumarate/emtricitabine, raltegravir increased darunavir CL/F by 11% (ΔOFV -10.47). Furthermore, NR1I2 63396C>T was significantly associated with darunavir CL/F (ΔOFV -6.82). Following multivariable analysis none of the covariates remained in the model. Weight (allometrically scaled and centred on 70 kg), NR1I2 63396C>T, NR1I3 540G>A, CYP3A5*3, SLC03A1 rs8027174 G>T were significantly
associated with ritonavir CL/F with weight and NR1I2 63396C>T retained in the model at the $p<0.001$ significance level ($\chi^2$ distribution) following forwards inclusion, backwards elimination. Ritonavir CL/F was increased by 23% in NR1I2 63396 T allele carriers compared to C allele homozygotes. Model parameters and peVPC for darunavir and ritonavir are presented (Table 3 and Figure 1a, 1b). Goodness-of-fit plots are also shown (Figure S1 and S2).

**Tenofovir and emtricitabine population pharmacokinetic modelling**

Tenofovir and emtricitabine plasma concentrations are shown (Figure 1c, 1d). Tenofovir ranged between 0.016-0.42 mg/L and emtricitabine between 0.013-4.67 mg/L (0.17-29.8 hours post-dose).

Tenofovir and emtricitabine were described by 2-compartment models with first order absorption. Tenofovir concentrations were lower than those previously reported in the literature and therefore priors were unlikely to be informative; adjustment of starting estimates appeared sufficient. Literature priors were used for emtricitabine fixed effects with the exception of $k_a$. IIV was included for tenofovir CL/F and IIV on emtricitabine CL/F and $V_c/F$; a proportional error was applied for both models.

Black patients had 31% higher tenofovir CL/F compared to Caucasian, Asian and Other ethnicity patients combined ($\Delta$OFV -11.39; CL/F values similar for Asian/Other versus Caucasian) and CrCL was also significantly associated with tenofovir CL/F ($\Delta$OFV -6.47). Tenofovir CL/F was decreased by 18% in ABCG2 421 A allele carriers compared to C homozygotes ($\Delta$OFV -11.26); none of the other SNPs showed significant relationships with tenofovir CL/F. Following multivariable analysis ethnicity, CrCL and ABCG2 421C>A did not
remain in the model. Significant univariable associations were observed between several covariates and *emtricitabine* CL/F: CrCL (linear), ethnicity [Asian versus Black, Caucasian, Other (reference)], weight, age (linear) and *SCL47A1* rs2289669 G>A [GG/GA (reference) versus AA]. Only CrCL was retained in the *emtricitabine* model. Tenofovir and *emtricitabine* final model parameters are summarised (Table 3) and pcVPC shown (Figure 1c, 1d). Goodness-of-fit plots are also displayed (Figure S3 and S4, respectively).

**Secondary pharmacokinetic parameters**

Predicted AUC$_{0-24}$, C$_{max}$, C$_{24}$ for *darunavir/ritonavir* (stratified by antiretroviral backbone), tenofovir and *emtricitabine* are summarised (Table 4); *darunavir/ritonavir* doses other than 800/100 mg once daily are displayed separately (n=18; Table S2).

All patients had a predicted *darunavir* C$_{24}$ well above the protein binding-adjusted EC$_{50}$ for wild-type HIV-1 of 0.055 mg/L$^{17}$ with C$_{24}$ between 0.38-5.79 mg/L. Mean (± s.d.) predicted *darunavir* pharmacokinetic parameters were generally in agreement with those reported from the phase III ARTEMIS trial$^{17}$ and predicted *emtricitabine* AUC$_{0-24}$, C$_{max}$ and C$_{24}$ were also consistent with previously reported values$^{18}$ (Table S3). Mean *tenofovir* pharmacokinetic parameters were approximately 40-60% lower than those reported for HIV patients when administered with a meal following multiple dosing$^{19}$ (Table S3).

**Pharmacokinetic-pharmacodynamic analysis**

The analysis of *darunavir* pharmacokinetic parameters and virological failure included 716 patients with 94 virological failures (13.9%). We found no significant association of *darunavir* C$_{24}$ or AUC$_{0-24}$ with time to virological failure overall [multivariable HR: 1.82 per log$_{10}$ mg/L (95% CI 0.61-5.41), $p$=0.279; and 2.28 per log$_{10}$ mg.h/L (95% CI 0.53-9.80), $p$=0.269,
respectively] nor evidence that this was different in the two arms (interaction p-values: arm*C24
p=0.679; arm*AUC0-24 p=0.380). Results were similar when censoring after switch from
allocated regimen, after multiple imputation of missing pharmacokinetic parameters or when
analysing time to trial primary endpoint (results not shown).

Adding the corresponding pharmacokinetic parameters for tenofovir and emtricitabine to the
model with participants of the darunavir/tenofovir disoproxil fumarate/emtricitabine arm did
not reveal any significant associations (for example, HR per additional log10 mg/L emtricitabine
C24 or tenofovir C24: 1.63 (95% CI 0.50-5.37), p=0.421; and 1.46 (95% CI 0.27-8.00), p=0.661,
respectively).

There was no association between darunavir pharmacokinetic parameters and change in CD4
cell count from randomisation to week 96 for either C24 [26.6 (95% CI -66.8 to 119.9)
cells/mm3 per log10 mg/L increase, p=0.522] or AUC0-24 [53.2 (95% CI -66.7 to 173.0)
cells/mm3 per log10 mg.h/L increase, p=0.329]. CD4 cell count post randomisation was also not
associated with pharmacokinetic parameters of emtricitabine or tenofovir (results not shown).

Renal adverse events

Of 347 participants with tenofovir pharmacokinetic estimates, 10 (2.9%) experienced a
decrease in glomerular function. Both higher AUC0-24 and Cmax were significantly associated
with a higher risk, with HR 1.92 per additional mg.h/L (95% 1.20-3.05), p=0.006 and HR 4.65
per additional 0.1 mg/L (95% CI 1.54-14.08), p=0.007, respectively. No relationships were
observed with polymorphisms in ABCC2, ABCC10 or ABCG2.
Discussion

Based on the pharmacokinetic analysis of NEAT001/ANRS143, no significant difference in once daily darunavir/ritonavir CL/F were observed when co-administered with twice daily raltegravir as an NRTI-sparing regimen compared to the standard regimen containing tenofovir disoproxil fumarate/emtricitabine. Furthermore, no associations of virological failure or CD4 cell count with darunavir concentrations were detected.

Due to non-overlapping metabolic pathways between darunavir and raltegravir (CYP3A4 versus UGT1A1) the potential for predictable drug-drug interactions of clinical consequence is low. However, previous studies have demonstrated a moderate influence of raltegravir on darunavir pharmacokinetics, with one observing significantly lower C\text{max} and AUC\text{0-24} \((n=17\) with raltegravir, \(n=8\) without raltegravir) but no change in C\text{trough} \((n=31\) with raltegravir, \(n=22\) without raltegravir),\(^{20}\) and another reporting 40% lower darunavir in patients receiving darunavir+raltegravir compared to those without \((n=55)\), but no impact on virological efficacy.\(^{21}\) In contrast, a small phase I study did not observe any change in boosted darunavir when raltegravir was added to a regimen containing tenofovir disoproxil fumarate/emtricitabine, however, following removal of the NRTI-backbone, darunavir C\text{trough} decreased by 36%.\(^{22}\) NEAT001/ANRS143 was performed in a larger patient population and although darunavir CL/F was 11% higher in the presence of raltegravir, it did not reach statistical significance in the final model; moreover, model predicted C\text{24} in all patients were well above protein binding-adjusted EC\text{50} for wild-type HIV-1 \((0.055 \text{ mg/L})\).

In addition to demographic descriptors, we investigated the effect of polymorphisms governing expression and/or function of specific metabolic pathways and transporters. The SLCO3A1 gene encodes expression for the influx transporter OATP3A1. Although darunavir is not a
confirmed substrate, Moltó and colleagues observed 12% lower CL/F in carriers of the 
*SCLO3A1* rs4294800 A allele and a 2.5-fold higher Vd/F in *SCLO3A1* rs8027174 T allele 
homozygotes, although probably of more mechanistic than clinical relevance.\(^9\) We were unable 
to confirm these findings given that *SLCO3A1* rs4294800 G>A was not in Hardy-Weinberg 
equilibrium. Prevalence of *SCLO1B1* 521T>C is high in Caucasians and carriers of the C allele 
exhibit higher plasma lopinavir concentrations.\(^23\) However, a relationship with darunavir in the 
present study was not established. *CYP3A4*\(^{*22}\) (522-191C>T) and *CYP3A5*\(^{*3}\) (6986A>G) are 
linked to low CYP3A4 expression and activity and loss of CYP3A5 function.\(^24\)-\(^26\) HIV-infected 
patients homozygous for *CYP3A4*\(^{*22}\) have previously been associated with reduced 
lopinavir/ritonavir CL/F (\(\downarrow53\%\)) and increased trough compared to homozygotes for the 
common allele,\(^27\) whereas a small study in healthy volunteers determined significantly higher 
maraviroc CL/F and lower AUC\(_{0-\infty}\) in those with fully functional CYP3A5 (*CYP3A5*\(^{*1/1}\); 
n=8) compared to homozygote dysfunctional (*CYP3A5*\(^{*3/3}\) or \(*3/6\) or \(*6/7\); n=8).\(^28\) Similar 
associations with darunavir pharmacokinetics and *CYP3A4*\(^{*22}\) were not replicated in 
NEAT001/ANRS143 and *CYP3A5*\(^{*3}\) could not be evaluated due to lack of Hardy-Weinberg 
equilibrium. Moreover, no significant relationships with patient characteristics were evident, 
however derived pharmacokinetic parameters were generally consistent with those reported for 
a small group of treatment-naïve patients from the ARTEMIS trial.\(^17\)

Ritonavir CL/F was not influenced by the evaluated SNPs with the exception of *NR1I2* 
63396C>T. Carriers of the rare allele (CT/TT) exhibited an increased ritonavir CL/F of 23%, 
which is in agreement to the impact reported for unboosted atazanavir concentrations.\(^29\) 
Bodyweight was significantly associated with ritonavir CL/F which is consistent with previous 
population pharmacokinetic analyses.\(^9\),\(^30\)
Model predicted emtricitabine pharmacokinetic parameters were in agreement with literature values, however observed tenofovir concentrations and hence predicted tenofovir secondary pharmacokinetic parameters were lower than previous studies. Differences could be the result of additional covariates not captured as part of the study, for example a food effect based on meal composition (consumption of a high fat meal has been associated with enhanced tenofovir AUC and C_max compared to the fasted state). The bioanalytical laboratory participates in an external quality assurance program with excellent performance, therefore assay or analytical equipment error are unlikely to be a contributing factor.

Both tenofovir and emtricitabine are excreted relatively unchanged by the kidneys. Tenofovir is transported in the proximal tubule by ABCC4 (MRP4), ABCC10 (MRP7), ABCC11 (MRP8), OAT1 and OAT3 and has also been associated with renal toxicity. ABCC10 526G>A and ABCC10 2843T>C have previously been associated with kidney toxicity in vitro using HEK-293-ABCC10 cell lines. Tenofovir is not a proven substrate of ABCC2, however ABCC2 24C>T and ABCC2 1249G>A were found to have protective properties against kidney toxicity in Japanese populations. It has been postulated that endogenous substrates of ABCC2 compete with or exacerbate tenofovir transport by ABCC4, furthermore ABCC2 may be in linkage disequilibrium with other polymorphisms that increase toxicity. No significant relationships were evident between tenofovir CL/F and ABCC10 526G>A, ABCC10 2843T>C, ABCC2 24C>T and ABCC2 1249G>A in the present study. Impact of ABCG2 421C>A on tenofovir has produced conflicting results with one study in HIV-infected women demonstrating a significant increase in AUC_{0-24} in carriers of the rare allele whereas another observed lower tenofovir concentrations in plasma and urine of HIV-infected patients of ABCG2 421CA genotype compared to homozygotes for the common allele (CC). Our investigations found that ABCG2 421C>A was significantly associated with 18% lower
tenofovir CL/F (increased AUC$_{0-24}$ in CA/AA carriers), however it did not meet criteria to remain in the final model. Previous population pharmacokinetic analyses have demonstrated a significant relationship between tenofovir CL/F and CrCL,$^{11,40-42}$ but this was not replicated here. Although exposure to tenofovir was lower than previously reported, higher AUC$_{0-24}$ and $C_{\text{max}}$ were associated with decreased glomerular function, but the proportion of patients with reduced function was small. Previous associations between renal function parameters and relevant tenofovir transporter polymorphisms were not replicated in this study.

Emtricitabine is a substrate of the MATE1 transporter in the kidney$^{43}$ and potentially $SCL47A1$ rs2289669 G>A could reduce function or expression of MATE1.$^{44}$ The polymorphism has been linked to response to metformin in patients with type-2 diabetes.$^{45} SCL47A1$ rs2289669 G>A did not significantly impact emtricitabine CL/F, although, similar to other population pharmacokinetic studies a relationship between emtricitabine CL/F and CrCL was demonstrated.$^{10,40,46}$

Study limitations included the use of 1 sample per patient on week 4 and 24 as this is insufficient to allow adequate partition of random effects (i.e. distinguishing between interindividual variability in parameters and residual variability).$^{47}$ Therefore priors from the literature were used,$^{48}$ and this can be problematic as they may not be informative for the study population and could impact individual parameter estimates. Indeed, model misspecification was noted at the lower concentrations for ritonavir, tenofovir and emtricitabine or during time periods where data were particularly sparse however the central tendency of all drugs was well described and darunavir, ritonavir and emtricitabine were within previously reported concentration ranges. Secondly, measurements of intracellular tenofovir-diphosphate and emtricitabine-triphosphate, the pharmacologically active metabolites of tenofovir and
emtricitabine or tenofovir in urine were not performed in this study. Potentially, these would be more closely related to efficacy or renal impairment assessment, respectively.

In conclusion, within a large cohort of European HIV-infected patients we did not observe a clinically relevant drug-drug interaction between darunavir/ritonavir and raltegravir as part of an NRTI-sparing regimen, furthermore darunavir pharmacokinetic parameters were not associated with virological failure. Overall, genetic polymorphisms related to drug metabolism and transport had little impact on darunavir, ritonavir, tenofovir or emtricitabine concentrations. Within the context of the NEAT001/ANRS143 non-inferiority analysis, these data appear to confirm the potential utility of darunavir/ritonavir once daily + raltegravir twice daily as an additional option for treatment-naïve patients without protease inhibitor-associated viral mutations.

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• **Italy**: Andrea Antinori, Raffaella Bucciardini and Stefano Vella (Rome)

• **Poland**: Andrzej Horban (Warsaw)

• **Spain**: Jose Arribas (Madrid)

• **UK**: Abdel G Babiker, Marta Boffito, Deenan Pillay and Anton Pozniak (London)

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Sida-HIV Hepatites (Inserm-ANRS) is the sponsor and a funder of the trial.

Transparency Declarations
SB and GDP have received research grants, travel grants, and consultancy fees from Abbvie,
Boehringer-Ingelheim, Bristol-Myers Squibb, Merck Sharp & Dohme, Gilead Sciences,
Janssen-Cilag and ViiV Healthcare.
AO has received research funding income from ViiV Healthcare, Merck, and Janssen, as well
as consultancies from ViiV Healthcare and Merck. He is also a co-inventor of patents relating
to the use of nanotechnology in drug delivery, and is a director of Tandem Nano Ltd.
J-MM and FR have received advisory or invited speaker honoraria and have received research
grants from Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, Merck
Laboratories, Merck Sharp & Dohme, Tobira and ViiV Healthcare.
AP has received research funding income from ViiV Healthcare, Merck, Gilead and Janssen,
was NEAT co-chair and has participated in advisory boards and symposia for ViiV Healthcare,
Gilead, Janssen and Merck.
LR is involved in IMI-2 funded Ebovac2 and Ebovac3 consortia on Ebola vaccine development, in which Janssen is the industrial partner, and in a publicly funded and sponsored Ebola vaccine trial (Prevac trial) for which Janssen and Merck provide the investigational products (vaccines).

MB has received travel and research grants from and has been advisor for Janssen, Roche, ViiV, Bristol-Myers Squibb, Merck Sharp & Dohme, Gilead Sciences, Mylan, Cipla and Teva.

All other authors have none to declare.

**Author Contributions**

LD: population pharmacokinetic modelling strategy and analysis, manuscript preparation

RG: DNA extraction, genotyping, manuscript preparation

WS: statistical analysis, manuscript preparation

SB: pharmacokinetic bioanalysis, manuscript review

AO: funding, strategy and supervision of pharmacogenetic analysis, genetic data review, manuscript review

AD: pharmacokinetic bioanalysis, pharmacokinetic data review, manuscript review

AC: study management, statistical review, manuscript review

J-MM: patient enrolment, manuscript review

GF: patient enrolment, manuscript review

LV: patient enrolment, manuscript review

GD: study design, protocol review, patient enrolment, manuscript review

AP: study design, protocol review, patient enrolment, manuscript review

LR: study design, protocol review, manuscript review

FR: study design, protocol review, patient enrolment, manuscript review
MB: pharmacokinetic substudy lead, study design, protocol review, patient enrolment.

Manuscript review
References


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43. Reznicek J, Ceckova M, Cerveny L et al. Emtricitabine is a substrate of MATE1 but not of OCT1, OCT2, P-gp, BCRP or MRP2 transporters. *Xenobiotica* 2017; **47**: 77-85.


Table 1 Clinical characteristics and demographics of patients included in the population pharmacokinetic models for the NEAT001/ANRS143 pharmacokinetic substudy stratified by study drug [data expressed as median (range) unless stated otherwise].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Darunavir</th>
<th>Ritonavir</th>
<th>Tenofovir</th>
<th>Emtricitabine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Included for modelling (n)</td>
<td>716</td>
<td>720</td>
<td>347</td>
<td>361</td>
</tr>
<tr>
<td>Sex [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>634 (88.5)</td>
<td>637 (88.5)</td>
<td>309 (89.0)</td>
<td>321 (88.9)</td>
</tr>
<tr>
<td>Female</td>
<td>81 (11.3)</td>
<td>82 (11.4)</td>
<td>37 (10.7)</td>
<td>39 (10.8)</td>
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<tr>
<td>Transgender</td>
<td>1 (0.1)</td>
<td>1 (0.1)</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38 (18-76)</td>
<td>37 (18-76)</td>
<td>39 (18-76)</td>
<td>38 (18-76)</td>
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<tr>
<td>Weight (kg)</td>
<td>72 (41-135)</td>
<td>72 (41-135)</td>
<td>73 (44-125)</td>
<td>73 (44-125)</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>115 (48-222)</td>
<td>115 (48-222)</td>
<td>116 (48-198)</td>
<td>116 (48-198)</td>
</tr>
<tr>
<td>CD4+ T cell count (cells/mm³)</td>
<td>334 (4-780)</td>
<td>334 (4-780)</td>
<td>328 (4-685)</td>
<td>331 (4-685)</td>
</tr>
<tr>
<td>HIV-RNA (log₁₀ copies/mL)</td>
<td>4.79 (3.11-6.53)</td>
<td>4.79 (3.11-6.53)</td>
<td>4.79 (3.15-6.53)</td>
<td>4.77 (3.13-6.53)</td>
</tr>
<tr>
<td>Randomisation arm [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenofovir disoproxil fumarate/emtricitabine</td>
<td>359 (50.1)</td>
<td>361 (50.1)</td>
<td>347 (100%)</td>
<td>361 (100%)</td>
</tr>
<tr>
<td>Raltegravir</td>
<td>357 (49.9)</td>
<td>359 (49.9)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mode of HIV infection [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homosexual/bisexual</td>
<td>499 (69.7%)</td>
<td>502 (69.7%)</td>
<td>246 (70.9%)</td>
<td>259 (71.7%)</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>165 (23.0%)</td>
<td>166 (23.1%)</td>
<td>80 (23.1%)</td>
<td>80 (22.2%)</td>
</tr>
<tr>
<td>Other</td>
<td>52 (7.3%)</td>
<td>52 (7.2%)</td>
<td>21 (6.1%)</td>
<td>22 (6.1%)</td>
</tr>
<tr>
<td>Ethnicity [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>596 (83.2)</td>
<td>600 (83.3)</td>
<td>290 (83.6)</td>
<td>302 (83.7)</td>
</tr>
<tr>
<td>Black</td>
<td>78 (10.9)</td>
<td>78 (10.8)</td>
<td>34 (9.8)</td>
<td>34 (9.4)</td>
</tr>
<tr>
<td>Asian</td>
<td>18 (2.5)</td>
<td>18 (2.5)</td>
<td>8 (2.3)</td>
<td>10 (2.8)</td>
</tr>
<tr>
<td>Other</td>
<td>24 (3.4)</td>
<td>24 (3.3)</td>
<td>15 (4.3)</td>
<td>15 (4.2)</td>
</tr>
</tbody>
</table>
Table 2 Allele frequencies for the single nucleotide polymorphisms investigated for the NEAT001/ANRS143 pharmacokinetic substudy associated with metabolism and transport of the study drugs.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Darunavir</th>
<th>Ritonavir</th>
<th>Tenofovir</th>
<th>Emtricitabine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (n)</td>
<td>716</td>
<td>720</td>
<td>347</td>
<td>361</td>
</tr>
<tr>
<td><strong>SLCO3A1 G&gt;A (rs4294800)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GG</td>
<td>302 (42.2)</td>
<td>303 (42.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>255 (35.6)</td>
<td>257 (35.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>61 (8.5)</td>
<td>61 (8.5)</td>
<td></td>
<td></td>
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<tr>
<td>Missing</td>
<td>98 (13.7)</td>
<td>99 (13.8)</td>
<td></td>
<td></td>
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<tr>
<td><strong>SLCO3A1 G&gt;T (rs8027174)</strong></td>
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<tr>
<td>GG</td>
<td>520 (72.6)</td>
<td>522 (72.5)</td>
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<tr>
<td>GT</td>
<td>98 (13.7)</td>
<td>99 (13.8)</td>
<td></td>
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<tr>
<td>TT</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>99 (13.8)</td>
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<td>TT</td>
<td>445 (62.2)</td>
<td>446 (61.9)</td>
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<tr>
<td>CT</td>
<td>162 (22.6)</td>
<td>164 (22.8)</td>
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<tr>
<td>CC</td>
<td>11 (1.5)</td>
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<td>CC</td>
<td>125 (17.5)</td>
<td>125 (17.4)</td>
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<tr>
<td>CT</td>
<td>296 (41.3)</td>
<td>299 (41.5)</td>
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<td>TT</td>
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<tr>
<td>Gene</td>
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<td>Frequency 2</td>
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<td>294 (41.1)</td>
<td>296 (41.1)</td>
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<td>258 (36.0)</td>
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<td>99 (13.8)</td>
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<tr>
<td><em>CYP3A5</em></td>
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<td>450 (62.5)</td>
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<td>127 (17.6)</td>
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<td>AA</td>
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<td></td>
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</tr>
<tr>
<td>Missing</td>
<td>45 (13.0)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gene</td>
<td>SNP</td>
<td>Allele</td>
<td>Count (Percentage)</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
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<td>--------</td>
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<tr>
<td><strong>ABCC10</strong></td>
<td>526G&gt;A (rs9349256)</td>
<td>GG</td>
<td>110 (31.7)</td>
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</tr>
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<td>GA</td>
<td>138 (39.8)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>51 (14.7)</td>
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<td></td>
<td></td>
<td>Missing</td>
<td>48 (13.8)</td>
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<tr>
<td><strong>ABCC10</strong></td>
<td>2843T&gt;C (rs2125739)</td>
<td>TT</td>
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<tr>
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<td></td>
<td>CT</td>
<td>113 (32.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>19 (5.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Missing</td>
<td>45 (13.0)</td>
<td></td>
</tr>
<tr>
<td><strong>ABCG2</strong></td>
<td>421C&gt;A (rs2231142)</td>
<td>CC</td>
<td>251 (72.3)</td>
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<td></td>
<td></td>
<td>CA</td>
<td>47 (13.5)</td>
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<tr>
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<td></td>
<td>AA</td>
<td>1 (0.3)</td>
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<td><strong>SCL47A1</strong></td>
<td>922-158G&gt;A (rs2289669)</td>
<td>GG</td>
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<td>GA</td>
<td>163 (45.2)</td>
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<tr>
<td></td>
<td></td>
<td>AA</td>
<td>43 (11.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Missing</td>
<td>47 (13.0)</td>
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</tr>
</tbody>
</table>
Table 3  Population pharmacokinetic parameter estimates and relative standard errors (RSE) derived from the final models for darunavir, ritonavir, tenofovir and emtricitabine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Darunavir</th>
<th>Ritonavir</th>
<th>Tenofovir</th>
<th>Emtricitabine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (n)</td>
<td>716</td>
<td>720</td>
<td>347</td>
<td>361</td>
</tr>
<tr>
<td><strong>Fixed effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>14.6 (2.3)</td>
<td>20.7 (2.4)</td>
<td>101 (3.3)</td>
<td>17.0 (2.7)</td>
</tr>
<tr>
<td>V/F or V_c/F (L)</td>
<td>41.4 (5.7)</td>
<td>278 (13.7)</td>
<td>402 (67.7)</td>
<td>36.8 (3.2)</td>
</tr>
<tr>
<td>Q/F (L/h)</td>
<td>30.4 (2.4)</td>
<td>-</td>
<td>700 (21.1)</td>
<td>5.6 (14.3)</td>
</tr>
<tr>
<td>V_p/F (L)</td>
<td>1130 (0.2)</td>
<td>-</td>
<td>2910 (18.7)</td>
<td>58.8 (2.3)</td>
</tr>
<tr>
<td>k_a (h^-1)</td>
<td>0.30 (5.4)</td>
<td>0.95 (17.5)</td>
<td>1.18 (64.2)</td>
<td>0.35 (15.4)</td>
</tr>
<tr>
<td><strong>Ritonavir-darunavir interaction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC_50 (mg/L)</td>
<td>0.42 (10.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I_MAX</td>
<td>1.00 fixed</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Random effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIV CL/F (%)</td>
<td>37.4 (8.5)</td>
<td>47.7 (17.2)</td>
<td>37.8 (16.6)</td>
<td>27.5 (28.1)</td>
</tr>
<tr>
<td>IIV V_c/F (%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>84.1 (32.5)</td>
</tr>
<tr>
<td><strong>Residual error</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Proportional (%)</td>
<td>48.5 (4.4)</td>
<td>49.9 (5.3)</td>
<td>37.1 (7.8)</td>
<td>41.8 (8.4)</td>
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<tr>
<td><strong>Covariates</strong></td>
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<td></td>
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<tr>
<td>_weight CL/F</td>
<td>-</td>
<td>0.75 fixed</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>_weight V/F</td>
<td>-</td>
<td>1.00 fixed</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\theta_{CT/TT}$ CL/F</td>
<td>1.23 (5.6)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>$\theta_{MISS}$ CL/F</td>
<td>1.24 (7.5)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>$\theta_{CrCL}$ CL/F</td>
<td>-</td>
<td>-</td>
<td>0.0037 (21.9)</td>
<td></td>
</tr>
</tbody>
</table>

RSE = ($SE_{ESTIMATE}/ESTIMATE$) x 100

CL/F: apparent oral clearance; V/F: apparent volume of distribution; Vc/F: apparent volume of distribution of the central compartment; Q/F: intercompartmental clearance; Vp/F: volume of the peripheral compartment; $k_a$: absorption rate constant; IC$_{50}$: ritonavir concentration associated with 50% maximum inhibition of darunavir CL/F; I$_{MAX}$: maximum inhibitory effect of ritonavir; IIV: interindividual variability; $\theta_{\text{weight}}$: allometric scaling factors associated with changes in ritonavir CL/F and V/F with bodyweight; $\theta_{CT/TT}$, $\theta_{MISS}$: relative changes in ritonavir CL/F for NR1I2 63396CT/TT (heterozygote and homozygote mutant) and missing NR1I2 63396C>T genotype compared to the reference, NR1I2 63396CC (wild-type); $\theta_{CrCL}$: factor associated with the linear relationship between emtricitabine CL/F and creatinine clearance.
Table 4 Mean (± s.d.) individual model predicted secondary pharmacokinetic parameters for darunavir, ritonavir (800/100 mg once daily), tenofovir [245 mg once daily; dosed as disoproxil fumarate (DF)] and emtricitabine (200 mg once daily). Darunavir and ritonavir parameters are stratified by randomisation arm i.e. antiretroviral backbone (Arm 1: tenofovir-DF/emtricitabine; Arm 2: raltegravir, NRTI-sparing).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Darunavir</th>
<th>Ritonavir</th>
<th>Tenofovir</th>
<th>Emtricitabine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arm 1</td>
<td>Arm 2</td>
<td>Arm 1</td>
<td>Arm 2</td>
</tr>
<tr>
<td>Number of patients (n)</td>
<td>345</td>
<td>353</td>
<td>345</td>
<td>353</td>
</tr>
<tr>
<td>AUC(_{0-24}) (mg.h/L)</td>
<td>57.42 (17.84)</td>
<td>55.48 (19.74)</td>
<td>4.24 (1.97)</td>
<td>4.32 (3.35)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>31</td>
<td>36</td>
<td>46</td>
<td>78</td>
</tr>
<tr>
<td>C(_{max}) (mg/L)</td>
<td>5.35 (0.88)</td>
<td>5.25 (0.97)</td>
<td>0.28 (0.10)</td>
<td>0.28 (0.15)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>16</td>
<td>18</td>
<td>35</td>
<td>55</td>
</tr>
<tr>
<td>C(_{24}) (mg/L)</td>
<td>1.75 (0.73)</td>
<td>1.68 (0.80)</td>
<td>0.07 (0.07)</td>
<td>0.07 (0.12)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>41</td>
<td>48</td>
<td>98</td>
<td>166</td>
</tr>
</tbody>
</table>

AUC\(_{0-24}\): area under the curve over the 24 hour dosing interval; C\(_{max}\): maximum concentration; C\(_{24}\): concentration 24 hours post-dose (trough)
Figure Legends

Figure 1. Visual predictive check (VPC) for (a) darunavir, (b) ritonavir, (c) tenofovir and (d) emtricitabine. Plots for darunavir, ritonavir and emtricitabine are prediction-corrected (pcVPC). The lines represent the percentiles of the observed data (P5, P50, P95) and the shaded areas the 95% CI of the simulated data. Observed concentration-time data for darunavir (n=716 patients, 1317 concentrations), ritonavir (n=720 patients, 1283 concentrations), tenofovir (n=347 patients, 588 concentrations) and emtricitabine (n=361 patients, 656 concentrations) are superimposed (open circles).
Figure 1

(a) Prediction-corrected darunavir (mg/L)

(b) Prediction-corrected ritonavir (mg/L)

(c) Tenofovir (mg/L)

(d) Prediction-corrected emtricitabine (mg/L)