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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 sub-study of the NEAT001/ANRS143 randomized trial

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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-naive patients. We investigated the population pharmacokinetics of darunavir, ritonavir, tenofovir and emtricitabine and relationships with demographics, genetic polymorphisms and virological failure.

Methods: Non-linear mixed-effects models (NONMEM v. 7.3) were applied to determine pharmacokinetic parameters and assess demographic covariates and relationships with SNPs (*SLCO3A1*, *SLCO1B1*, *NR1I2*, *NR1I3*, *CYP3A5*3*, *CYP3A4*22*, *ABCC2*, *ABCC10*, *ABCG2* and *SCL47A1*). The relationship between model-predicted darunavir AUC_{0-24} and C_{24} with time to virological failure was evaluated by Cox regression.

Results: Of 805 enrolled, 716, 720, 347 and 361 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); coadministration of raltegravir did not influence darunavir or ritonavir CL/F. Ritonavir CL/F decreased by 23% in *NR1I2* 63396C>T carriers and emtricitabine CL/F was linearly associated with creatinine clearance ($P<0.001$). No significant relationship was demonstrated between darunavir AUC_{0-24} or C_{24} and time to virological failure [HR (95% CI): 2.28 (0.53–9.80), $P=0.269$; and 1.82 (0.61–5.41), $P=0.279$, respectively].

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 PI.[1] However, renal and bone-associated adverse events, particularly with tenofovir,[2,3] and concerns regarding cardiovascular risk with abacavir, have led to exploration of NRTI-sparing regimens as alternatives for

treatment-naive patients. NEAT001/ANRS143, a Phase III, randomized, open-label trial, demonstrated non-inferiority of raltegravir (400 mg twice daily) + darunavir/ritonavir (800/100 mg once daily) compared with tenofovir disoproxil fumarate/emtricitabine (245/200 mg once daily) + darunavir/ritonavir (800/100 mg once daily) in a large group of European treatment-naive patients [Kaplan–Meier-estimated treatment failure from the primary ITT analysis at 96 weeks was 17.8% (NRTI-sparing) versus 13.8% (standard regimen)]. The adjusted difference in treatment failure between study arms was 4.0% (95% CI −0.8 to 8.8) and the HR for attaining the primary endpoint with the NRTI-sparing regimen was 1.34 (95% CI 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.[4]

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.[4] In summary, HIV-infected, treatment-naive patients were recruited between August 2010 and September 2011 from 15 European countries (78 sites). Individuals were eligible if their plasma HIV-1 viral load was >1000 copies/mL, CD4 count was <500 cells/mm^3 (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 and those with abnormal laboratory parameters or hepatic/renal impairment were excluded.

Patients were randomized (1:1) to receive ritonavir-boosted darunavir with either tenofovir disoproxil fumarate/emtricitabine (standard regimen) or raltegravir (NRTI-sparing regimen).[4] Timed, single blood samples were drawn at Weeks 4 and 24 and plasma drug concentrations quantified by fully validated HPLC-MS and LC-MS methods[5,6] 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 participants provided written informed consent.[4]

Genotyping

Total genomic DNA was extracted from patient blood using the QIAamp DNA Mini Kit (QIAGEN, West Sussex, UK) according to the 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*3* 6986A>G (rs776746) and *CYP3A4*22* 522–191C>T (rs35599367); for tenofovir: *ABCC2* (*MRP2*) 24C>T (rs717620), *ABCC2* 1249G>A (rs2273697), *ABCC10* (*MRP7*) 526G>A (rs9349256), *ABCC10* 2843T>C (rs2125739) and *ABCG2* 421C>A (rs2231142); and for emtricitabine: *SCL47A1* (MATE1) 922–158G>A (rs2289669) using real-time PCR allelic discrimination assays (Applied Biosystems, Foster City, CA, USA; Table S1, available as Supplementary data at *JAC* Online), essentially as described previously.[7]

Population pharmacokinetic modelling

Non-linear 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 one sample per patient on each sampling occasion (Weeks 4 and 24), parameter estimates from the literature were used as priors for darunavir, ritonavir and emtricitabine[9,10] ($PRIOR 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 (CL_{CR}, 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 parameterized 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, heterozygotes and homozygotes for the rare alleles were combined into one category if changes in CL/F were similar when compared with 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, χ^2 distribution, 1 *df*). 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, χ^2 distribution, 1 *df*) 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 (pcVPCs)[12] constructed from 1000 simulations of each dataset implemented through Perl-speaks-NONMEM (PsN; v. 3.4.2)[13] and plots developed using Xpose4[14] in RStudio (v. 1.1.383). The use of pcVPC corrects for the inclusion of significant covariates and/or varying dosages per drug.

For each drug secondary pharmacokinetic parameters, AUC_{0-24}, C_{max} and C_{24}, were derived for each patient and applied to the analyses incorporating virological response (outlined below). Ritonavir parameters were calculated using standard one-compartment pharmacokinetic equations for multiple oral dosing (Table S2). 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_{max} and C_{24} were determined directly from the profiles and AUC_{0-24} as outlined (Table S2).

Pharmacokinetic–pharmacodynamic analysis

The primary pharmacodynamic endpoint was protocol-defined virological failure that included change of any component of the randomized 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 ≥400 copies/mL at Week 24); failure to achieve virological response by Week 32 (HIV-1 RNA ≥50 copies/mL); and HIV-1 RNA ≥50 copies/mL at any time after Week 32. All virological components of the primary endpoint had to be confirmed by a second measurement.[4] The association between model-predicted $\log_{10}$ (C_{24}) or $\log_{10}$ (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.[4]

The primary analyses were as randomized and based on available data. We also performed sensitivity analyses: (i) censoring analysis time when

Table 1. Clinical characteristics and demographics of patients included in the population pharmacokinetic models for the NEAT001/ANRS143 pharmacokinetic sub-study stratified by study drug

Parameter	Darunavir	Ritonavir	Tenofovir	Emtricitabine
Included for modelling (*n*)	716	720	347	361
Sex, *n* (%)				
male	634 (88.5)	637 (88.5)	309 (89.0)	321 (88.9)
female	81 (11.3)	82 (11.4)	37 (10.7)	39 (10.8)
transgender	1 (0.1)	1 (0.1)	1 (0.3)	1 (0.3)
Age (years)	38 (18–76)	37 (18–76)	39 (18–76)	38 (18–76)
Weight (kg)	72 (41–135)	72 (41–135)	73 (44–125)	73 (44–125)
CL_{CR} (mL/min)	115 (48–222)	115 (48–222)	116 (48–198)	116 (48–198)
CD4+ T cell count (cells/mm^3)	334 (4–780)	334 (4–780)	328 (4–685)	331 (4–685)
HIV-RNA (log_{10} copies/mL)	4.79 (3.11–6.53)	4.79 (3.11–6.53)	4.79 (3.15–6.53)	4.77 (3.13–6.53)
Randomization arm, *n* (%)				
tenofovir disoproxil fumarate/emtricitabine	359 (50.1)	361 (50.1)	347 (100)	361 (100)
raltegravir	357 (49.9)	359 (49.9)	—	—
Mode of HIV infection, *n* (%)				
homosexual/bisexual	499 (69.7)	502 (69.7)	246 (70.9)	259 (71.7)
heterosexual	165 (23.0)	166 (23.1)	80 (23.1)	80 (22.2)
other	52 (7.3)	52 (7.2)	21 (6.1)	22 (6.1)
Ethnicity, *n* (%)				
Caucasian	596 (83.2)	600 (83.3)	290 (83.6)	302 (83.7)
Black	78 (10.9)	78 (10.8)	34 (9.8)	34 (9.4)
Asian	18 (2.5)	18 (2.5)	8 (2.3)	10 (2.8)
other	24 (3.4)	24 (3.3)	15 (4.3)	15 (4.2)

Data expressed as median (range) unless stated otherwise.

any component of the initial randomized treatment was stopped; and (ii) multiple imputation of missing pharmacokinetic parameters (using the same factors as described above plus the event indicator and the Nelson–Aalen estimator[15]).

Additionally, we examined the association of CD4 count change from baseline to Week 96 with C_{24} or AUC_{0-24} using multivariable linear 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} and the tenofovir SNPs with reduced glomerular function defined as at least 25% reduction from baseline in CL_{CR} 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 CL_{CR}.

Results

Patients and sampling

Of 805 patients enrolled, data were available from 770 patients (*n*=386 in raltegravir arm; *n*=384 in tenofovir disoproxil fumarate/emtricitabine arm) totalling 1460 samples (*n*=726 in raltegravir arm; *n*=734 in tenofovir disoproxil fumarate/emtricitabine arm). Between 10% and 25% of samples were excluded due to the lack of recorded time post-dose, missing concentration, time post-dose >30 h, 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 darunavir/ritonavir 800/100 mg once daily (*n*=698, 97%); alternative doses were recorded for a small proportion (*n*=18; Table S3). For tenofovir and emtricitabine, 347 (588 concentrations) and 361 patients (656 concentrations) were included, respectively. Patient demographics and clinical characteristics are summarized in Table 1. Patients excluded from pharmacokinetic modelling had similar characteristics to included patients 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%), respectively, had a blood sample for genotyping. Genotyping assays failed in one and three patients, respectively, 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 summarized (Table 2).

Darunavir/ritonavir population pharmacokinetic modelling

Darunavir and ritonavir plasma concentrations are presented (Figure 1a and b) and were 0.06–16.4 and 0.01–2.76 mg/L, respectively, over 0.17–30.1 h post-dose. Due to extensive model run times, darunavir and ritonavir were ultimately modelled

Table 2. Allele frequencies for the SNPs investigated for the NEAT001/ANRS143 pharmacokinetic sub-study associated with metabolism and transport of the study drugs

SNP	Darunavir	Ritonavir	Tenofovir	Emtricitabine
Number of patients (*n*)	716	720	347	361
SLCO3A1 G>A (rs4294800)				
GG	302 (42.2)	303 (42.1)		
GA	255 (35.6)	257 (35.7)		
AA	61 (8.5)	61 (8.5)		
missing	98 (13.7)	99 (13.8)		
SLCO3A1 G>T (rs8027174)				
GG	520 (72.6)	522 (72.5)		
GT	98 (13.7)	99 (13.8)		
TT	0 (0.0)	0 (0.0)		
missing	98 (13.7)	99 (13.8)		
SLCO1B1 521T>C (rs4149056)				
TT	445 (62.2)	446 (61.9)		
CT	162 (22.6)	164 (22.8)		
CC	11 (1.5)	11 (1.5)		
missing	98 (13.7)	99 (13.8)		
NR1I2 63396C>T (rs2472677)				
CC	125 (17.5)	125 (17.4)		
CT	296 (41.3)	299 (41.5)		
TT	197 (27.5)	197 (27.4)		
missing	98 (13.7)	99 (13.8)		
NR1I3 540G>A (rs2307424)				
GG	294 (41.1)	296 (41.1)		
GA	258 (36.0)	258 (35.8)		
AA	66 (9.2)	67 (9.3)		
missing	98 (13.7)	99 (13.8)		
*CYP3A5**3 6986A>G (rs776746)				
CC	448 (62.6)	450 (62.5)		
CT	127(17.7)	127 (17.6)		
TT	43(6.0)	44 (6.1)		
missing	98 (13.7)	99 (13.8)		
*CYP3A4**22 522–191C>T (rs35599367)				
GG	574 (80.2)	577 (80.1)		
GA	44 (6.1)	44 (6.1)		
AA	0 (0.0)	0 (0.0)		
missing	98 (13.7)	99 (13.8)		
ABCC2 24C>T (rs717620)				
CC			210 (60.5)	
CT			80 (23.1)	
TT			11 (3.2)	
missing			46 (13.3)	
ABCC2 1249G>A (rs2273697)				
GG			188 (54.2)	
GA			100 (28.8)	
AA			14 (4.0)	
missing			45 (13.0)	
ABCC10 526G>A (rs9349256)				
GG			110 (31.7)	
GA			138 (39.8)	
AA			51 (14.7)	
missing			48 (13.8)	

Continued

Table 2. *Continued*

SNP	Darunavir	Ritonavir	Tenofovir	Emtricitabine
ABCC10 2843T>C (rs2125739)				
TT			170 (49.0)	
CT			113 (32.6)	
CC			19 (5.5)	
missing			45 (13.0)	
ABCG2 421C>A (rs2231142)				
CC			251 (72.3)	
CA			47 (13.5)	
AA			1 (0.3)	
missing			48 (13.8)	
SCL47A1 922–158G>A (rs2289669)				
GG				108 (29.9)
GA				163 (45.2)
AA				43 (11.9)
missing				47 (13.0)

Values are expressed as *n* (%).

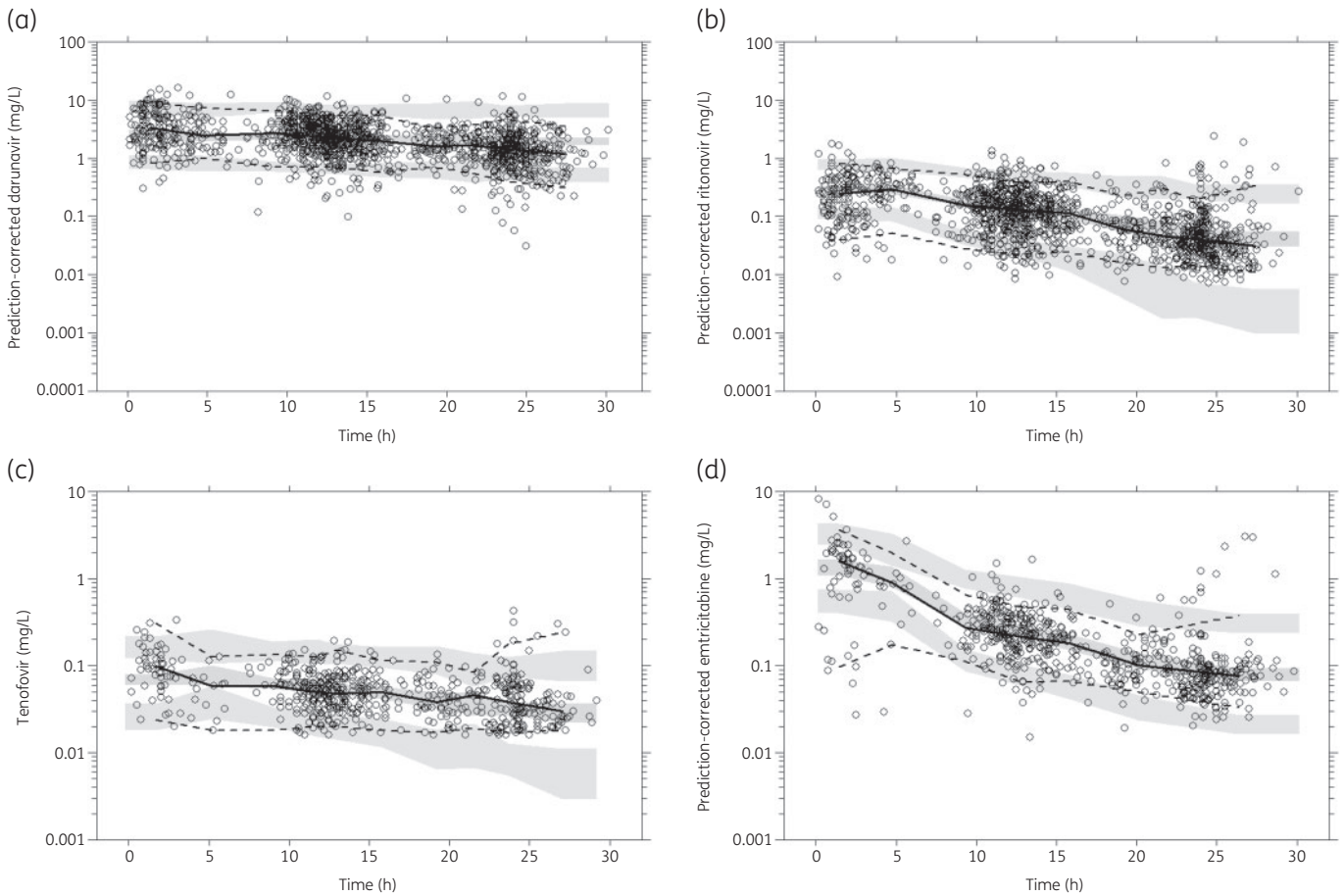

Figure 1. VPC for (a) darunavir, (b) ritonavir, (c) tenofovir and (d) emtricitabine. Plots for darunavir, ritonavir and emtricitabine are pcVPC. The lines represent the percentiles of the observed data (P5, P50, P95) and the shaded areas the 95% CI of the simulated data. Observed concentration–time data 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).

Table 3. Population pharmacokinetic parameter estimates and relative standard errors (RSEs) derived from the final models for darunavir, ritonavir, tenofovir and emtricitabine

	Parameter estimate (RSE%)			
Parameter	darunavir	ritonavir	tenofovir	emtricitabine
Number of patients (*n*)	716	720	347	361
Fixed effects				
CL/F (L/h)	14.6 (2.3)	20.7 (2.4)	101 (3.3)	17.0 (2.7)
V/F or V_c/F (L)	41.4 (5.7)	278 (13.7)	402 (67.7)	36.8 (3.2)
Q/F (L/h)	30.4 (2.4)	—	700 (21.1)	5.6 (14.3)
V_p/F (L)	1130 (0.2)	—	2910 (18.7)	58.8 (2.3)
k_a (h^{-1})	0.30 (5.4)	0.95 (17.5)	1.18 (64.2)	0.35 (15.4)
Ritonavir–darunavir interaction				
IC_{50} (mg/L)	0.42 (10.2)		—	—
I_{max}	1.00 *fixed*		—	—
Random effects				
IIV CL/F (%)	37.4 (8.5)	47.7 (17.2)	37.8 (16.6)	27.5 (28.1)
IIV V_c/F (%)	—	—	—	84.1 (32.5)
Residual error				
proportional (%)	48.5 (4.4)	49.9 (5.3)	37.1 (7.8)	41.8 (8.4)
Covariates				
θ_{weight} CL/F	—	0.75 *fixed*	—	—
θ_{weight} *V*/F	—	1.00 *fixed*	—	—
$\theta_{CT/TT}$ CL/F	—	1.23 (5.6)	—	—
θ_{MISS} CL/F	—	1.24 (7.5)	—	—
θ_{CLCR} CL/F	—	—	—	0.0037 (21.9)

RSE is calculated as ($SE_{ESTIMATE}$/ESTIMATE) × 100.
IC_{50}, ritonavir concentration associated with 50% maximum inhibition of darunavir CL/F; I_{max}, maximum inhibitory effect of ritonavir; θ_{weight}, allometric scaling factors associated with changes in ritonavir CL/F and *V*/F with bodyweight; $\theta_{CT/TT}$, θ_{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 (WT); θ_{CLCR}, factor associated with the linear relationship between emtricitabine CL/F and CL_{CR}.

sequentially.[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, parameterized by CL/F, apparent volume of distribution (*V*/F) and absorption rate constant (k_a); a literature prior was included for CL/F.[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 two-compartment model parameterized by CL/F, volume of distribution of the central and peripheral compartments (V_c/F, V_p/F), intercompartmental clearance (Q/F) and k_a. The interaction between ritonavir and darunavir was via a direct-response model with ritonavir concentrations inhibiting darunavir CL/F parameterized by IC_{50} (ritonavir concentration associated with 50% maximum inhibition) and I_{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 with 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 and *SLCO3A1* 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 (χ^2 distribution) following forwards inclusion, backwards elimination. Ritonavir CL/F was increased by 23% in *NR1I2* 63396T allele carriers compared with C allele homozygotes. Model parameters and pcVPCs for darunavir and ritonavir are presented (Table 3 and Figure 1a and b). Goodness-of-fit plots are also shown (Figures S1 and S2).

Tenofovir and emtricitabine population pharmacokinetic modelling

Tenofovir and emtricitabine plasma concentrations are shown (Figure 1c and d) and were 0.016–0.42 mg/L for tenofovir and 0.013–4.67 mg/L for emtricitabine (0.17–29.8 h post-dose).

Tenofovir and emtricitabine were described by two-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

Table 4. Mean (±SD) individual model-predicted secondary pharmacokinetic parameters for darunavir, ritonavir (800/100 mg once daily), tenofovir (245 mg once daily; dosed as disoproxil fumarate) and emtricitabine (200 mg once daily)

	Darunavir		Ritonavir		Tenofovir	Emtricitabine
Parameter	arm 1	arm 2	arm 1	arm 2		
Number of patients (*n*)	345	353	345	353	347	361
AUC_{0-24} (mg·h/L)	57.42 (17.84)	55.48 (19.74)	4.24 (1.97)	4.32 (3.35)	1.43 (0.60)	11.84 (3.54)
CV (%)	31	36	46	78	42	30
C_{max} (mg/L)	5.35 (0.88)	5.25 (0.97)	0.28 (0.10)	0.28 (0.15)	0.13 (0.03)	1.50 (0.19)
CV (%)	16	18	35	55	19	12
C_{24} (mg/L)	1.75 (0.73)	1.68 (0.80)	0.07 (0.07)	0.07 (0.12)	0.04 (0.02)	0.10 (0.13)
CV (%)	41	48	98	166	59	135

Darunavir and ritonavir parameters are stratified by randomization arm, i.e. antiretroviral backbone (arm 1: tenofovir disoproxil fumarate/emtricitabine; arm 2: raltegravir, NRTI-sparing).
CV, coefficient of variation; C_{24}, concentration 24 h post-dose (trough).

of k_a.[10] IIV was included for tenofovir CL/F and emtricitabine CL/F and V_c/F; a proportional error was applied for both models.

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

Secondary pharmacokinetic parameters

Predicted AUC_{0-24}, C_{max} and C_{24} for darunavir/ritonavir (stratified by antiretroviral backbone), tenofovir and emtricitabine are summarized (Table 4); darunavir/ritonavir doses other than 800/100 mg once daily are displayed separately (*n*=18; Table S3).

All patients had a predicted darunavir C_{24} well above the protein binding-adjusted EC_{50} for WT HIV-1 of 0.055 mg/L[17] with C_{24} values of 0.38–5.79 mg/L. Mean (±SD) 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 S4). Mean tenofovir pharmacokinetic parameters were approximately 40%–60% lower than those reported for HIV patients when administered with a meal following multiple dosing (Table S4).[19]

Pharmacokinetic–pharmacodynamic analysis

The analysis of darunavir pharmacokinetic parameters and virological failure included 716 patients with 94 virological failures (13.1%). 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*C_{24} *P*=0.679; arm*AUC_{0-24} *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 $\log_{10}$ mg/L emtricitabine C_{24} or tenofovir C_{24}: 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 randomization to Week 96 for either C_{24} [26.6 (95% CI −66.8 to 119.9) cells/mm^3 per $\log_{10}$ mg/L increase, *P*=0.522] or AUC_{0-24} [53.2 (95% CI −66.7 to 173.0) cells/mm^3 per $\log_{10}$ mg·h/L increase, *P*=0.329]. CD4 cell count post-randomization 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 AUC_{0-24} and C_{max} were significantly associated with a higher risk, with an HR of 1.92 per additional mg·h/L (95% CI 1.20–3.05), *P*=0.006 and an HR of 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 was observed when coadministered with twice-daily raltegravir as an

NRTI-sparing regimen compared with 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_{max} and AUC_{0-24} (n=17 with raltegravir, n=8 without raltegravir) but no change in C_{trough} (n=31 with raltegravir, n=22 without raltegravir),[20] and another reporting 40% lower darunavir concentrations in patients receiving darunavir + raltegravir compared with 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_{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_{24} values in all patients were well above the protein binding-adjusted EC_{50} for WT HIV-1 (0.055 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 of the influx transporter OATP3A1. Although darunavir is not a confirmed substrate, Moltó *et al.*[9] observed 12% lower CL/F in carriers of the *SCLO3A1* rs4294800 A allele and a 2.5-fold higher V_c/F in *SCLO3A1* rs8027174 T allele homozygotes, although probably of more mechanistic than clinical relevance. 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 a 53% reduction in ritonavir-boosted lopinavir CL/F and increased C_{trough} compared with 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 with dysfunctional homozygotes (*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-naive 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 with 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 with the fasted state).[19] The bioanalytical laboratory participates in an external quality assurance programme[31] with excellent performance, therefore assay or analytical equipment errors 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),[32] ABCC10 (MRP7),[33] ABCC11 (MRP8),[34] OAT1 and OAT3[35] and has also been associated with renal toxicity.[2] *ABCC10* 526G>A and *ABCC10* 2843T>C have previously been associated with kidney toxicity *in vitro* using HEK-293-ABCC10 cell lines.[34] 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.[36] 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.[37] 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. The 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[38] whereas another observed lower tenofovir concentrations in plasma and urine of HIV-infected patients of *ABCG2* 421CA genotype compared with homozygotes for the common allele (CC).[39] 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 the criteria to remain in the final model. Previous population pharmacokinetic analyses have demonstrated a significant relationship between tenofovir CL/F and CL_{CR},[11,40–42] but this was not replicated here. Although exposure to tenofovir was lower than previously reported, higher tenofovir AUC_{0-24} and C_{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* (922–158G>A) rs2289669 G>A could reduce function or expression of MATE1.[44] The polymorphism has been linked to the response to metformin in patients with type 2 diabetes.[45] *SCL47A1* rs2289669 G>A did not significantly impact emtricitabine CL/F, although a relationship between emtricitabine CL/F and CL_{CR} was demonstrated, similar to other population pharmacokinetic studies.[10,40,46]

Study limitations included the use of one sample per patient at Weeks 4 and 24 as this is insufficient to allow adequate partition of random effects (i.e. distinguishing between IIV 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,[4] these data appear to confirm the potential utility of once-daily darunavir/ritonavir + twice-daily raltegravir as an additional option for treatment-naive patients without PI-associated viral mutations.

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Supplementary data

Tables S1–S4 and Figures S1–S4 are available as Supplementary data at *JAC* Online.

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