

## ARTICLE

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# Influence of *UGT1A1* and *SLC22A6* polymorphisms on the population pharmacokinetics and pharmacodynamics of raltegravir in HIV-infected adults: a NEAT001/ANRS143 sub-study

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Using concentration-time data from the NEAT001/ANRS143 study (single sample at week 4 and 24), we determined raltegravir pharmacokinetic parameters using nonlinear mixed effects modelling (NONMEM v.7.3; 602 samples from 349 patients) and investigated the influence of demographics and SNPs (*SLC22A6* and *UGT1A1*) on raltegravir pharmacokinetics and pharmacodynamics. Demographics and SNPs did not influence raltegravir pharmacokinetics and no significant pharmacokinetic/pharmacodynamic relationships were observed. At week 96, *UGT1A1\*28\*/28* was associated with lower virological failure ( $p = 0.012$ ), even after adjusting for baseline CD4 count ( $p = 0.048$ ), but not when adjusted for baseline HIV-1 viral load ( $p = 0.082$ ) or both ( $p = 0.089$ ). This is the first study to our knowledge to assess the influence of SNPs on raltegravir pharmacodynamics. The lack of a pharmacokinetic/pharmacodynamic relationship is potentially an artefact of raltegravir's characteristic high inter and intra-patient variability and also suggesting single time point sampling schedules are inadequate to thoroughly assess the influence of SNPs on raltegravir pharmacokinetics.

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## INTRODUCTION

Raltegravir was the first integrase inhibitor approved for the treatment of HIV-1. Safety and efficacy of raltegravir have been demonstrated in treatment-experienced (BENCHMARK 1 and 2) [1] and treatment-naïve (STARTMRK) [2] patients and is recommended for initial therapy in numerous guidelines [3, 4]. Initially dosed at 400 mg twice daily, it is also available as a new 600 mg formulation; 2 pills once daily for first-line therapy [5].

Raltegravir is unique since it is metabolised through glucuronidation by uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) with no involvement of cytochrome P450 (CYP450) enzymes [6]. Additionally, raltegravir does not alter CYP450 activity [7] making it less prone to drug-drug interactions and safe to co-administer with CYP450 substrates [8]. Furthermore, raltegravir is generally well tolerated and has a low incidence of adverse events causing treatment discontinuation [9]. Notwithstanding its advantages, raltegravir displays a broad inter-subject and intra-subject variability [10] and has a low genetic barrier to drug resistance [11]. This has complicated pharmacokinetic/

pharmacodynamics (PK/PD) analysis and made it difficult to estimate PK thresholds for efficacy and toxicity [12].

The influence of *UGT1A1* polymorphisms on the PK and efficacy of raltegravir has been a matter of dispute, owing to numerous studies producing variable results [10, 13–17]. Additionally, raltegravir is a substrate of *SLC22A6* (OAT1) [18] and polymorphisms in the *SLC22A6* gene [19] could influence raltegravir disposition. We investigated the population pharmacokinetics (popPK) of raltegravir 400 mg twice daily and the influence of demographic covariates and polymorphisms in the *UGT1A1* and *SLC22A6* genes on raltegravir treatment response in patients randomised to the ritonavir-boosted darunavir (800/100 mg once daily) plus raltegravir arm in the Phase III NEAT 001/ANRS 143 study [20].

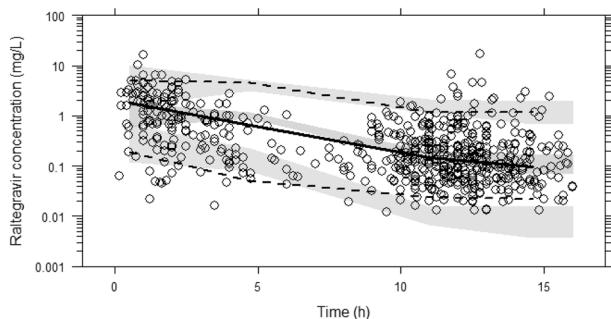
## RESULTS

### Patients and pharmacokinetic sampling

Of 401 patients randomised to the raltegravir arm, 386 ( $n = 726$  samples) provided data for this analysis. In total

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**Fig. 1 Raltegravir visual predictive check (VPC).** The lines represent the percentiles of the observed data (P5, P50, P95) and the shaded areas the 95% CI of the simulated data. Observed raltegravir concentration-time data ( $n=349$  patients, 602 concentrations) are superimposed (open circles).

602 samples ( $n=313$  week 4,  $n=289$  week 24) from 349 patients receiving raltegravir 400 mg twice daily were used for model development. A total of 124 samples (17.1%) were excluded due to missing time post-dose, missing concentration, time post-dose greater than 16 h, plasma raltegravir below the bioanalytical assay lower limit of quantification (LLQ) or a mixture of the above. Raltegravir concentrations ranged between 0.012 and 17.3 mg/L sampled 0.17–16.0 h post-dose (Fig. 1). Patient characteristics are described (Table 1). Patients excluded from the PK modelling ( $n=52$ ) had similar characteristics apart from country and HIV-RNA (4.55  $\log_{10}$  copies/mL in those excluded).

### Genotyping

Fifty-six patients did not have a blood sample drawn for genotyping; 84.0% (293/349) had both PK and genetic data for *SLC22A6* 453G>A and *SLC22A6* 728C>T whereas genotype was not available in an additional 28 patients for *UGT1A1*\*28 (265/349, 75.9% with data). One patient possessing *UGT1A1*\*36/\*36 was excluded from the pharmacogenetic analysis due to the unknown impact of this allele [21]. Genotypes did not deviate from Hardy–Weinberg equilibrium and allele frequencies are summarised in Table 1.

### Population pharmacokinetic modelling

Raltegravir was described by a two-compartment model with first-order absorption parameterised by apparent oral clearance (CL/F), apparent volume of distribution of the central and peripheral compartments ( $V_c/F$  and  $V_p/F$ , respectively), intercompartmental clearance (Q/F) and absorption rate constant ( $k_a$ ); priors from the literature were used for all fixed effects with the exception of raltegravir CL/F [22]. Interindividual variability was included on CL/F and a proportional error model described residual variability.

None of the covariates evaluated (weight, age, sex, ethnicity, and polymorphisms in *UGT1A1* and *SLC22A6*) produced statistically significant decreases in objective function value (OFV) and therefore a multivariable analysis was not possible. Changes in OFV resulting from the univariable addition of covariates into the model along with corresponding mathematical descriptions are summarised as Supplementary material (Supplementary Table S1). Of note, despite the lack of statistical significance, raltegravir CL/F was reduced by 21% (Fig. 2A) in patients with low *UGT1A1* activity compared to those with normal/reduced activity (reference population; normal and reduced combined due to  $\leq 10\%$  difference in population CL/F values between the two groups). Fixed and random effects obtained for the final raltegravir model and visual predictive check (VPC) are presented (Table 2 and Fig. 1). Goodness-of-fit plots are also shown (Supplementary Fig. S1).

Predicted mean ( $\pm$ s.d.; CV%) raltegravir  $AUC_{0-12}$ ,  $C_{max}$ ,  $C_{12}$  and half-life were 8.70 mg.h/L (8.20; 94%), 1.44 mg/L (0.68; 47%),

**Table 1.** Clinical characteristics, demographics and genetic polymorphisms of patients included in the population pharmacokinetic model of raltegravir for the NEAT001/ANRS143 pharmacokinetic sub-study [data expressed as median (range) unless stated otherwise]

Parameter	
Included for modelling ( $n$ )	349
Sex [ $n$ (%)]	
Male	306 (87.7)
Female	43 (12.3)
Age (years)	37 (20–71)
Weight (kg)	72 (41–135)
CD4 + T cell count (cells/ $mm^3$ )	340 (5–780)
HIV-RNA ( $\log_{10}$ copies/mL)	4.82 (3.11–6.31)
Ethnicity [ $n$ (%)]	
Caucasian	288 (82.5)
Black	44 (12.6)
Asian	8 (2.3)
Other	9 (2.6)
<i>SLC22A6</i> 453G>A (rs4149170) [ $n$ (%)]	
GG	216 (61.9)
GA	68 (19.5)
AA	9 (2.6)
Missing	56 (16.0)
<i>SLC22A6</i> 728C>T (rs11568626) [ $n$ (%)]	
CC	285 (81.7)
CT	6 (1.7)
TT	2 (0.6)
Missing	56 (16.0)
<i>UGT1A1</i> *28 (rs8175347) [ $n$ (%)]	
*1/*1, *1/*36 (normal enzyme activity)	109 (31.2)
*1/*28, *28/*36, *36/*37 <sup>a</sup> (reduced enzyme activity)	115 (33.0)
*28/*28 <sup>a</sup> (low enzyme activity)	40 (11.5)
*36/*36 <sup>b</sup> (unknown enzyme activity)	1 (0.3)
Missing	84 (24.1)

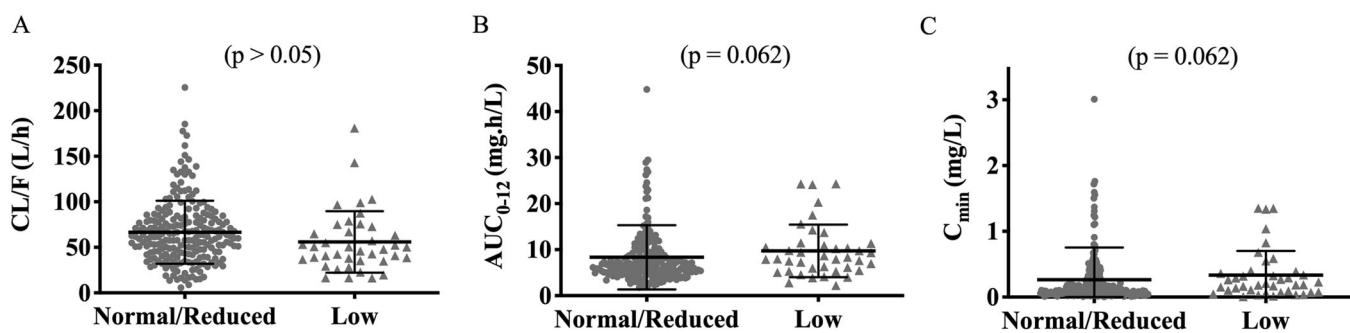
<sup>a</sup>Reduced enzyme activity consists of genotype \*1/\*6, \*1/\*28, \*1/\*37, \*28/\*36, \*36/\*37 and low enzyme activity consists of \*28/\*28, \*28/\*37, \*37/\*37. Note that no patients in this cohort had the \*1/\*6 or \*1/\*37 genotypes (reduced) or \*28/\*37 or \*37/\*37 genotypes (low).

<sup>b</sup>The impact of \*36/\*36 on *UGT1A1* enzyme activity is unknown and therefore was excluded from the population pharmacokinetic covariate analysis.

0.29 mg/L (0.60; 205%) and 9.13 h (3.94; 43%), respectively; median (range)  $T_{max}$  was 1.50 h (1.00–2.00). Substantial interindividual variability was observed in the  $C_{12}$  estimates. A post-hoc analysis was performed to determine the impact of *UGT1A1*\*28 on predicted raltegravir  $AUC_{0-12}$  and  $C_{12}$  [low activity ( $n=40$ ) vs. normal/reduced activity as reference ( $n=224$ )]. Geometric mean ratio (90% CI), back-transformed from log values were 1.34 (0.99–1.84;  $p=0.062$ ) and 1.32 (0.99–1.77;  $p=0.062$ ) respectively, suggesting a modest increase, although not statistically significant, in  $AUC_{0-12}$  and  $C_{12}$  of patients with low activity *UGT1A1* genotype compared to the reference genotype (Fig. 2B, C).

### Pharmacokinetic–pharmacodynamic analysis

The analysis of raltegravir PK parameters included 349 patients of which 58 had virological failure (VF; 16.6%). We found no



**Fig. 2 Scatter plots of raltegravir pharmacokinetic parameters stratified by UGT1A1 activity.** Mean and SD of raltegravir **A** apparent oral clearance (CL/F), **B** exposure (AUC<sub>0-12</sub>) and **C** minimum concentration (C<sub>min</sub>; concentration 12 hours post-dose) in patients with low UGT1A1 activity compared to patients with normal and reduced UGT1A1 activity combined ( $p > 0.05$ ).

**Table 2.** Population pharmacokinetic parameter estimates and relative standard errors (RSE) for the final raltegravir model ( $n = 349$  individuals). Of the covariates tested none fulfilled the statistical criteria to remain in the model.

Parameter	Estimate (RSE%)
Fixed effects	
CL/F (L/h)	55.8 (4.1)
V <sub>c</sub> /F (L)	194 (6.5)
Q/F (L/h)	13.0 (4.0)
V <sub>p</sub> /F (L)	117 (0.6)
k <sub>a</sub> (h <sup>-1</sup> )	1.12 (13.0)
Random effects	
IV CL/F (%)	62.7 (12.1)
Residual error	
Proportional (%)	69.9 (7.0)

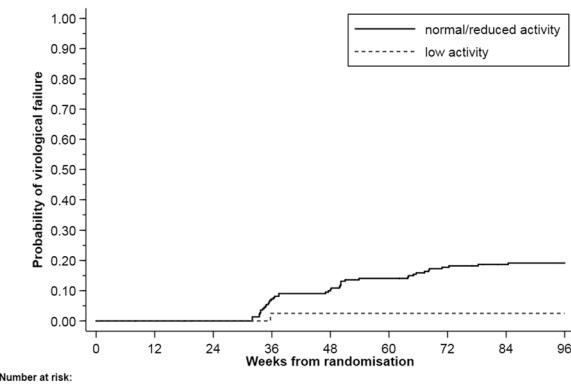
$$\text{RSE} = (\text{SE}_{\text{ESTIMATE}}/\text{ESTIMATE}) \times 100.$$

CL/F apparent oral clearance, V<sub>c</sub>/F apparent volume of distribution of the central compartment, Q/F intercompartmental clearance, V<sub>p</sub>/F volume of the peripheral compartment, k<sub>a</sub> absorption rate constant IV, interindividual variability.

significant association of raltegravir C<sub>12</sub> or AUC<sub>0-12</sub> with time to VF overall (multivariable HR: 0.72 per log<sub>10</sub> mg/L increase (95% CI 0.44–1.17),  $p = 0.181$ ; and 0.48 per log<sub>10</sub> mg.h/L increase (95% CI 0.17–1.38),  $p = 0.173$ , respectively). Results were similar when censoring after switch from randomised regimen, after multiple imputation of missing PK parameters or when analysing time to the trial primary endpoint (results not shown). Similarly, we did not see an association between raltegravir PK parameters and change in CD4 cell count from baseline (C<sub>12</sub>: -1.3 (95% CI -41.0 to 38.4) cells/mm<sup>3</sup> per log<sub>10</sub> mg/L increase,  $p = 0.940$ ; AUC<sub>0-12</sub>: -0.6 (95% CI -77.9 to 76.7) cells/mm<sup>3</sup> per log<sub>10</sub> mg.h/L increase,  $p = 0.99$ ).

#### Adverse events

Thirty-two of 349 participants (9.2%) experienced grade 2 or higher triglycerides by week 96, and we found a higher risk with higher raltegravir AUC<sub>0-12</sub> (HR 6.24 per log<sub>10</sub> mg/L increase; 95% CI 1.88 to 20.72;  $p = 0.003$ ). Fifty participants had creatine kinase grade 2 or higher, however, there was no association with raltegravir AUC<sub>0-12</sub> (HR 1.11 per log<sub>10</sub> mg/L increase; 95% CI 0.42 to 2.98;  $p = 0.83$ ). There also was no association between raltegravir AUC<sub>0-12</sub> and LDL levels post-randomisation (-0.10 mmol/L (95% CI -0.33 to 0.13) per log<sub>10</sub> mg/L increase;  $p = 0.39$ ). Similarly, no significant associations were seen with raltegravir C<sub>max</sub> (results not shown).



**Fig. 3 Kaplan-Meier plot of multivariable Cox regression analysis.** Probability of virological failure in patients with low UGT1A1 activity compared to normal and reduced UGT1A1 activity combined at week 96. ( $p = 0.012$ ).

#### Integrase resistance

Fifteen of 349 participants experienced VF with integrase resistance mutations by week 96. We found no significant association of raltegravir C<sub>12</sub> or AUC<sub>0-12</sub> with time to detection of integrase resistance mutations (HR, adjusted for baseline CD4 and HIV-1 viral load: 0.70 per log<sub>10</sub> mg/L increase (95% CI 0.42–1.16),  $p = 0.163$ ; and 0.17 per log<sub>10</sub> mg.h/L increase (95% CI 0.01–2.07),  $p = 0.164$ , respectively).

#### Genetic association analysis

In the 264 participants assessed, a significantly lower incidence of VF by week 96 was seen in patients with low UGT1A1 activity (1 failure; cumulative risk 2.5%) compared to those with normal/reduced activity (42 failures; 19.2%),  $p = 0.012$  (Fig. 3). This association remained significant after adjusting for baseline CD4 count (HR = 0.14 [95%CI 0.02–0.99]  $p = 0.048$ ), but not when adjusted for baseline HIV-1 viral load (HR = 0.17;  $p = 0.082$ ) or both baseline CD4 and HIV-1 viral load (HR = 0.18;  $p = 0.089$ ).

Integrase resistance mutations were detected in 1/40 (cumulative risk 2.6%) participants with low UGT1A1 activity versus 13/224 (6.3%) in patients with normal/reduced activity (unadjusted HR 0.39;  $p = 0.363$ ; adjusted for baseline CD4 and HIV-1 viral load: HR 0.83;  $p = 0.856$ ). We did not find any association between UGT1A1\*28 genotype and any of the adverse events (results not shown).

#### DISCUSSION

We developed a popPK model of raltegravir, administered as 400 mg twice daily, using data from the NEAT001/ANRS143 study

[20]. Raltegravir was best described by a 2-compartment model with first-order absorption but both 1- and 2-compartment models have been reported [22, 23]. The estimated mean  $AUC_{0-12}$ ,  $C_{max}$  and  $C_{12}$  were comparable to those achieved in the phase III QDMRK study, with raltegravir 400 mg twice daily [24]. Similarly, raltegravir  $T_{max}$  and half-life were in line with literature values [25] and estimated CL/F was within the range of previous popPK studies, although reported estimates are highly variable (e.g. 39.1, 60.2, 80.6 L/h) [22, 23, 26]. Considerable interindividual variability was observed (CL/F:62.7%,  $C_{12}$ :205%), which is expected with raltegravir.

Sex-related differences such as higher gastric pH and lower P-glycoprotein (P-gp) expression in females [27], ethnicity-related differences due to variable plasma protein binding and P-gp expression [28], and age-related changes such as reduced renal and hepatic clearance [29] can potentially influence raltegravir PK. However, the clinical effects of such differences have not been observed in most studies [26, 30]. Similarly, in our study, sex, weight and ethnicity did not influence raltegravir CL/F. In contrast, a popPK analysis showed a 55% higher raltegravir relative bioavailability in females and a 65% lower V/F in Caucasians, however this contributed little to the reduction in parameter variability [22].

Raltegravir has a marked inter and intraindividual variability, especially with  $C_{min}$  concentrations [10], complicating PK/PD analyses. In the QDMRK study, a correlation between  $C_{min}$  and viral suppression was observed in patients receiving raltegravir 800 mg once daily, but not in patients receiving 400 mg twice daily [24]. In our study, where raltegravir was dosed 400 mg twice daily, we did not observe any significant associations between raltegravir secondary PK parameters ( $C_{12}$  or  $AUC_{0-12}$ ) and time to VF or change in CD4 from baseline, possibly due to the substantial interindividual variability observed. PK sampling was performed at single time points, 4 and 24 weeks post-therapy initiation. Due to marked variability and potential changes in adherence over time, the calculated PK parameters may not be appropriate for association with a 96-week PD endpoint. Studies using PK sampling over multiple longitudinal time points could potentially overcome these complications and help establish a clearer PK/PD relationship of raltegravir [31].

$AUC_{0-12}$  was directly proportional to  $\geq$ grade 2 triglycerides seen in 9.2% of the patients by week 96 and to our knowledge, this is the first time this association has been observed. Raltegravir, however, is generally well-tolerated and adverse events rarely lead to treatment discontinuation [32]. Compared to other antiretrovirals, raltegravir has a favourable lipid profile, with minimal increases in total cholesterol and triglycerides [2].

Polymorphisms altering the TA repeat expansion in the TATAA box of the *UGT1A1* gene, such as *UGT1A1\*28* and *UGT1A1\*6*, have been shown to influence *UGT1A1* enzyme activity, resulting in changes in the PK and PD of *UGT1A1* substrates. *UGT1A1\*6* has been reported to be associated with a higher dolulegravir  $C_{min}$  and *UGT1A1\*6* and *UGT1A1\*28* with a higher incidence of neuropsychiatric adverse events in those receiving dolulegravir [33]. *UGT1A1\*28* linked to increased toxicity of the anti-cancer drug irinotecan, is also well documented [34].

Studies investigating the influence of *UGT1A1* polymorphisms on raltegravir have produced mixed results. The first study to investigate this association ( $n=57$ ) demonstrated an elevation in  $C_{min}$  (91%) in patients with the *UGT1A1\*28/\*28* genotype compared to *UGT1A1\*1/\*1* [14]. Similarly, work conducted by Belkhir et al. ( $n=104$ ) observed higher raltegravir exposure and lower glucurononconjugation rate in *UGT1A1\*28* carriers compared to *UGT1A1\*1* [13]. However, several other studies failed to show any influence of *UGT1A1\*28* on raltegravir PK [10, 16, 17]. Our study did not demonstrate a significant relationship between raltegravir PK and the genetic polymorphisms studied. The influence of *SLC22A6* and *UGT1A1* genotypes on CL/F did not

fulfil the criteria for inclusion in the popPK model, although patients homozygous for *UGT1A1\*28* had 21% lower typical value of CL/F, corresponding to slightly higher  $AUC_{0-12}$  (GMR: 1.34) and  $C_{12}$  (GMR: 1.32), compared to combined low/reduced activity genotype. The impact of this polymorphism, however, was more pronounced on VF with a significantly lower incidence in those homozygous for *UGT1A1\*28* ( $p=0.012$ ), even after adjusting for baseline CD4, although clinical consequence may be questionable given that the association was lost when adjusted for both baseline CD4 cell count and HIV-1 viral load. To our knowledge, this was the first study to assess the influence of *UGT1A1\*28* on integrase resistance mutations and we did not see significant associations. Our findings suggest that a high intraindividual variability in raltegravir PK may mask the effects of genetic polymorphisms on single drug concentration profiles, reinforcing the need for PK investigations using multiple longitudinal time points.

A new film-coated tablet containing 600 mg of raltegravir with optimised exposure and bioavailability has been evaluated in the ONCEMRK study that demonstrated the non-inferiority of a once-daily 1200 mg (two 600 mg tablets) raltegravir-containing regimen to the standard regimen of 400 mg twice daily for initial therapy in terms of efficacy and safety [5, 35]. HIV suppression was similar in both groups despite a significant difference in median  $C_{min}$  concentrations: 113 nM (IQR 63–211) for 1200 mg once daily versus. 543 nM (309–1135) for 400 mg twice daily [36]. *UGT1A1\*28* could have a similar influence on the PK and PD of the new formulation, which needs to be investigated.

There are several limitations to our study. Raltegravir PK has been shown to be influenced by the fat content of food through a change in gastric pH, however, the clinical relevance of this interaction is questionable [37]. In our study, we did not have the data to investigate this association. Furthermore, the limited sampling scheme of one sample per patient within a dosing interval necessitating the use of priors may have influenced the parameter estimates. Although popPK is the preferred method for dealing with sparse data the prior subroutine was implemented in order to allow partition of the random effects. Indeed it has been suggested from studies in mice that at least two samples within a dosing interval are needed to adequately estimate random effects [38] (i.e. separate interindividual and residual variability). Furthermore, the available priors from the literature may not be informative for the study population and a degree of model misspecification was evident particularly during the absorption phase where data was sparsest. Despite the limitations, it is important to note that priors were not used for estimation of raltegravir CL/F (the main parameter of interest and from which  $AUC_{0-12}$  was derived), the model described the central tendency of raltegravir concentrations well and parameter estimates were consistent with literature values, providing confidence in the model and predictions.

In conclusion, there were no significant correlations between the PK and PD of raltegravir. The influence of *UGT1A1\*28* was more profound on the incidence of VF than on raltegravir PK, possibly masked by intraindividual variability. These findings emphasise the importance of including multiple longitudinal time points while evaluating PK/PD relationships and investigating genetic associations on raltegravir PK.

## METHODS

### Patients and pharmacokinetic sampling

Between August 2010 and September 2011, 805 HIV-infected, treatment-naïve males and non-pregnant females were enrolled into NEAT 001/ANRS 143 (NCT01066962), a randomised, open-label trial, from 78 clinical sites across 15 European countries. Recruitment criteria have been detailed previously [20]. Briefly, patients without any major IAS-USA resistance mutations with plasma HIV viral load  $>1000$  copies/mL and CD4 count

below 500 cells/mm<sup>3</sup>, unless presenting a symptomatic HIV infection were suitable to participate in the study. Patients with abnormal laboratory results, hepatic or renal insufficiency or suffering from co-infections (e.g. tuberculosis, hepatitis) were excluded. All patients received darunavir/ritonavir and were randomized 1:1 to raltegravir 400 mg twice daily (NRTI-sparing regimen) or tenofovir disoproxil fumarate/emtricitabine (standard regimen). In this sub-study, only patients randomised to the raltegravir arm were included (darunavir, ritonavir, tenofovir disoproxil fumarate and emtricitabine are presented separately) [39]. Single blood samples were taken at week 4 and 24 to obtain plasma for drug measurement. Raltegravir plasma concentrations were determined by a validated LC-MS/MS method [40] with a LLQ of 0.0117 mg/L.

## Ethics

NEAT 001/ANRS 143 was conducted in accordance with the Declaration of Helsinki and ethical approval was obtained locally from study sites. All study participants provided written informed consent [20].

## Genotyping

Genomic DNA was extracted from blood samples using the QI Amp DNA mini kit (Qiagen, West Sussex, UK). DNA was quantified using NanoDrop (Thermo Fisher Scientific, Wilmington, DE, USA). Genotyping was conducted using RT-PCR on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Hercules, CA, USA). The PCR procedure consisted of denaturation (95 °C; 10 min), 50 cycles of amplification (92 °C; 15 s) and annealing (60 °C; 1.5 min) [41]. Taqman genotype master mix and assays, *SLC22A6* 453G>A (rs419170, designed using Custom TaqMan® Assay Design Tool) and *SLC22A6* 728C>T (rs11568626, C\_25598602\_40) were purchased from Life Technologies (Paisley, Renfrewshire, UK). Opticon Monitor software (v. 3.1, Bio-Rad Laboratories) was used to obtain allelic discrimination plots and identify genotypes. *UGT1A1* was genotyped using the Sequenom MassARRAY platform and iPLEX Pro UGT1A1-TA assays (Sequenom Laboratories, San Diego, CA, USA). Similar to methods described by Lee et al. [42], 20 ng of genomic DNA was amplified by PCR and then treated with shrimp alkaline phosphatase to inactivate unincorporated nucleotides. Using iPLEX Gold Reaction Cocktail, single base extension reaction was performed followed by spotting onto SpectroCHIP II. Data were analysed by MassARRAY TYPER software (v. 4.0.20, Sequenom Laboratories).

## Population pharmacokinetic modelling

Raltegravir plasma concentration-time data were analysed using nonlinear mixed effects (NONMEM v. 7.3, ICON Development Solutions, Ellicott City, MD, USA) with FOCE-I estimation [43]. The \$PRIORITY subroutine was implemented due to the sparseness of the sampling with single samples drawn per patient on two separate clinic visits, 4 and 24 weeks after therapy initiation. Parameter estimates and corresponding variances from a previous popPK analysis were used as priors [22].

Covariates including weight, age, sex, ethnicity and *UGT1A1*\*28, *SLC22A6* 453G>A and *SLC22A6* 728C>T genotypes were primarily investigated by univariable analysis for associations with raltegravir CL/F. If covariates were significant they were progressed to multivariable analysis. Genotypes were parameterised and the common allele homozygotes were used as reference to compare heterozygotes and rare allele homozygotes. Studies have demonstrated the influence of *UGT1A1* polymorphisms on *UGT1A1* activity. Studies assessing promoter activity have shown that a TA insertion to give TA<sub>7</sub> (*UGT1A1*\*28) reduces gene transcription compared to the wild type TA<sub>6</sub> (*UGT1A1*\*1) [44, 45]. TA<sub>8</sub> (*UGT1A1*\*37) repeats cause lower transcription compared to TA<sub>7</sub> and TA<sub>5</sub> (*UGT1A1*\*36) cause higher transcription compared to the wild type [46]. Moreover, the *UGT1A1* protein is seen to be twofold lower in *UGT1A1*\*28/\*28 compared to those having the wild type. Based on the *UGT1A1* activity, the patients in this study were grouped as normal (\*1/\*1, \*1/\*36), reduced (\*1/\*6, \*1/\*28, \*1/\*37, \*28/\*36, \*36/\*37) and low (\*28/\*28, \*28/\*37 or \*37/\*37) in accordance with the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines [46]. Missing genetic data were included as a separate fixed effect to maximise data use [47].

To distinguish the difference between nested models a decrease in the minimal OFV ( $-2 \log$  likelihood) of at least 3.84 units was necessary ( $p = 0.05$ ,  $\chi^2$  distribution, 1 d.f.). A forwards inclusion process was used to incorporate significant covariates followed by backwards elimination, retaining the biologically plausible covariates that produced an increase in OFV of at least 10.83 units ( $p = 0.001$ ,  $\chi^2$  distribution, 1 d.f.). This threshold

was chosen in order to vigorously test the relationships observed, given sparseness of the concentration-time data per patient.

A VPC was performed to evaluate the overall model suitability by performing 1000 simulations of the raltegravir dataset using Perl-speaks-NONMEM software (PsN; version 3.4.2) [48] and plotted with Xpose4 [49] in RStudio (version 1.1.383). The final model was used to predict raltegravir  $AUC_{0-12}$ ,  $C_{max}$ ,  $C_{12}$ , and half-life for each patient included in the model. In addition to the popPK assessment of the relationship between raltegravir CL/F and *UGT1A1*\*28, a post-hoc analysis was performed to evaluate the influence of *UGT1A1* polymorphisms on model predicted raltegravir  $AUC_{0-12}$  and  $C_{12}$  using geometric mean ratios (GMR) and 90% confidence intervals. The analysis was performed on log-transformed data and subsequently back-transformed and presented as linear values.

## Pharmacokinetic–pharmacodynamic analysis

The primary PD endpoint was VF interpreted as change of any element of the initial randomised regimen before week 32 due to documented inadequate virological response (defined as reductions of  $<1 \log_{10}$  copies/mL in HIV-1 RNA by week 18 or HIV-1 RNA  $\geq 400$  copies/mL at or week 24); failure to achieve virological response  $\leq 50$  copies/mL by week 32; HIV-1 RNA of 50 copies/mL or higher at any time after 32 weeks, confirmed by a second measurement). Multivariable Cox regression was utilised to assess the association between model-predicted  $\log_{10}(C_{12})$  or  $\log_{10}(AUC_{0-12})$  and time to VF, adjusting for sex, age, mode of HIV infection, ethnicity, country, baseline CD4+ cell count, and baseline HIV-1 RNA. Various sensitivity analyses were also performed: a) censoring analysis time when any component of the initial randomised treatment was stopped; b) multiple imputation of missing PK parameters (using the same factors as described above plus the event indicator and the Nelson–Aalen estimator) [50].

Similar analyses were performed to evaluate the influence of *UGT1A1* polymorphisms on VF to week 96. As an additional PD endpoint, we investigated the relationship between change in CD4 cell count from baseline to week 96 with  $\log_{10}(C_{12})$  or  $\log_{10}(AUC_{0-12})$  using multivariable linear regression models adjusting for baseline CD4 and other factors as described above.

## Adverse events

Multivariable Cox models were used to analyse the association between model-predicted  $\log_{10}(C_{max})$  or  $\log_{10}(AUC_{0-12})$  with predefined adverse event endpoints, grade 2 or higher creatine kinase or triglycerides (time from randomisation to first occurrence). Generalised estimating equations (GEEs) were used to analyse the association of the same PK parameters and LDL levels post-randomisation. Lipids were measured at baseline, and then at weeks 2, 4, 8, 12, 24, 48 and 96 post-randomisation. Creatine kinase was additionally measured at weeks 32, 64 and 80. All analyses were adjusted for the corresponding laboratory value at baseline. Similar analyses were performed to assess the association between *UGT1A1* genotypes and adverse events.

## Integrase resistance

Genotypic testing was requested in case of VF or any single VL  $> 500$  copies/mL at or after week 32 [51]. Integrase mutations were interpreted according to the 2014 IAS-USA list of mutations [52]. Kaplan-Meier analyses and Cox regression were performed to assess the association of raltegravir PK parameters and *UGT1A1* genotypes on integrase resistance, assuming that patients who did not experience VF did not develop resistance.

## DATA AVAILABILITY

The data that support the findings of these analyses are available upon reasonable request from the authors and with the permission of the trial sponsor and coordinating investigator.

## CODE AVAILABILITY

The NONMEM code for the final model describing raltegravir PK can be found in the Supplementary Material.

## REFERENCES

- Steigbigel RT, Cooper DA, Teppler H, Eron JJ, Gatell JM, Kumar PN, et al. Long-term efficacy and safety of raltegravir combined with optimized background

therapy in treatment-experienced patients with drug-resistant HIV infection: week 96 results of the BENCHMRK 1 and 2 phase III trials. *Clin Infect Dis.* 2010;50:605–12.

- Rockstroh JK, Lennox JL, DeJesus E, Saag MS, Lazzarin A, Wan H, et al. Long-term treatment with raltegravir or efavirenz combined with tenofovir/emtricitabine for treatment-naïve human immunodeficiency virus-1-infected patients: 156-week results from STARTMRK. *Clin Infect Dis.* 2011;53:807–16.
- Ryom L, Cotter A, De Miguel R, Béguin C, Podlekareva D, Arribas JR, et al. 2019 update of the European AIDS Clinical Society Guidelines for treatment of people living with HIV version 10.0. *HIV Med.* 2020;21:617–24.
- DHHS. Guidelines for the use of antiretroviral agents in adults and adolescents with HIV. 2021; 40. <https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf>.
- Cahn P, Sax PE, Squires K, Molina J-M, Ratanasuwan W, Rassoul M, et al. Raltegravir 1200 mg once daily vs 400 mg twice daily, with emtricitabine and tenofovir disoproxil fumarate, for previously untreated HIV-1 infection: week 96 results from ONCEMRK, a randomized, double-blind, noninferiority trial. *J Acquir Immune Defic Syndr.* 2018;78:589–98.
- Kassahun K, McIntosh I, Cui D, Hreniuk D, Merschman S, Lasseter K, et al. Metabolism and disposition in humans of raltegravir (MK-0518), an anti-AIDS drug targeting the human immunodeficiency virus 1 integrase enzyme. *Drug Metab Dispos.* 2007;35:1657–63.
- Anker M, Corales RB. Raltegravir (MK-0518): a novel integrase inhibitor for the treatment of HIV infection. *Expert Opin Investig Drugs.* 2008;17:97–103.
- Burger DM. Drug-drug interactions with raltegravir. *Eur J Med Res.* 2009;14:17–21.
- Burger DM. Raltegravir: a review of its pharmacokinetics, pharmacology and clinical studies. *Expert Opin Drug Metab Toxicol.* 2010;6:1151–60.
- Siccardi M, D'Avolio A, Rodriguez-Novo S, Cuenca L, Simiele M, Baitto L, et al. Intrapatient and interpatient pharmacokinetic variability of raltegravir in the clinical setting. *Ther Drug Monit.* 2012;34:232–5.
- Messiaen P, Wensing AMJ, Fun A, Nijhuis M, Brusselaers N, Vandekerckhove L. Clinical use of HIV integrase inhibitors: a systematic review and meta-analysis. *PLoS One.* 2013;8:e52562.
- Elliot E, Chirwa M, Boffito M. How recent findings on the pharmacokinetics and pharmacodynamics of integrase inhibitors can inform clinical use. *Curr Opin Infect Dis.* 2017;30:58–73.
- Belkhir L, Seguin-Devaux C, Elens L, Pauly C, Gengler N, Schneider S, et al. Impact of UGT1A1 polymorphisms on Raltegravir and its glucuronide plasma concentrations in a cohort of HIV-1 infected patients. *Sci Rep.* 2018;8:7359.
- Wenning L, Petry A, Kost J, Jin B, Breidinger S, DeLepeleire I, et al. Pharmacokinetics of raltegravir in individuals with UGT1A1 polymorphisms. *Clin Pharm Ther.* 2009;85:623–7.
- Yagura H, Watanabe D, Ashida M, Kushida H, Hirota K, Ikuma M, et al. Correlation between UGT1A1 polymorphisms and raltegravir plasma trough concentrations in Japanese HIV-1-infected patients. *J Infect Chemother.* 2015;21:713–7.
- Hirano A, Ikemura K, Takahashi M, Shibata M, Amioka K, Nomura T, et al. Lack of correlation between UGT1A1 \*6, \*28 genotypes, and plasma raltegravir concentrations in Japanese HIV type 1-infected patients. *AIDS Res Hum Retroviruses.* 2012;28:776–9.
- Neely M, Decosterd L, Fayet A, Lee JSF, Margol A, Kanani M, et al. Pharmacokinetics and pharmacogenomics of once-daily raltegravir and atazanavir in healthy volunteers. *Antimicrob Agents Chemother.* 2010;54:4619–25.
- Moss DM, Kwan WS, Liptrott NJ, Smith DL, Siccardi M, Khoo SH, et al. Raltegravir is a substrate for SLC22A6: a putative mechanism for the interaction between raltegravir and tenofovir. *Antimicrob Agents Chemother.* 2011;55:879–87.
- Moss DM, Neary M, Owen A. The role of drug transporters in the kidney: lessons from tenofovir. *Front Pharm.* 2014;5:1–14.
- Raff F, Babiker AG, Richert L, Molina J-M, George EC, Antinori A, et al. Ritonavir-boosted darunavir combined with raltegravir or tenofovir-emtricitabine in antiretroviral-naïve adults infected with HIV-1: 96 week results from the NEAT001/ANRS143 randomised non-inferiority trial. *Lancet.* 2014;384:1942–51.
- Chen S, St Jean P, Borland J, Song I, Yeo AJ, Piscitelli S, et al. Evaluation of the effect of UGT1A1 polymorphisms on dolutegravir pharmacokinetics. *Pharmacogenomics.* 2014;15:9–16.
- Arab-Alameddine M, Fayet-Mello A, Lubomirov R, Neely M, di Iulio J, Owen A, et al. Population pharmacokinetic analysis and pharmacogenetics of raltegravir in HIV-positive and healthy individuals. *Antimicrob Agents Chemother.* 2012;56:2959–66.
- Wang L, Soon GH, Seng K-Y, Li J, Lee E, Yong E-L, et al. Pharmacokinetic modeling of plasma and intracellular concentrations of raltegravir in healthy volunteers. *Antimicrob Agents Chemother.* 2011;55:4090–5.
- Rizk ML, Hang Y, Luo W-L, Su J, Zhao J, Campbell H, et al. Pharmacokinetics and pharmacodynamics of once-daily versus twice-daily raltegravir in treatment-naïve HIV-infected patients. *Antimicrob Agents Chemother.* 2012;56:3101–6.
- Iwamoto M, Wenning LA, Petry AS, Laethem M, De Smet M, Kost JT, et al. Minimal effects of ritonavir and efavirenz on the pharmacokinetics of raltegravir. *Antimicrob Agents Chemother.* 2008;52:4338–43.
- Vera JH, Jackson A, Dickinson L, Else L, Barber T, Mora-Peris B, et al. The pharmacokinetic profile of raltegravir-containing antiretroviral therapy in HIV-infected individuals over 60 years of age. *HIV Clin Trials.* 2015;16:39–42.
- Nicolas J-M, Espie P, Molimard M. Gender and interindividual variability in pharmacokinetics. *Drug Metab Rev.* 2009;41:408–21.
- Johnson JA. Predictability of the effects of race or ethnicity on pharmacokinetics of drugs. *Int J Clin Pharm Ther.* 2000;38:53–60.
- Mangoni AA, Jackson SHD. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. *Br J Clin Pharm.* 2003;57:6–14.
- Brainard DM, Wenning LA, Stone JA, Wagner JA, Iwamoto M. Clinical pharmacology profile of raltegravir, an HIV-1 integrase strand transfer inhibitor. *J Clin Pharm.* 2011;51:1376–402.
- Nettles RE, Kieffer TL, Parsons T, Johnson J, Cofrancesco J, Gallant JE, et al. Marked intraindividual variability in antiretroviral concentrations may limit the utility of therapeutic drug monitoring. *Clin Infect Dis.* 2006;42:1189–96.
- Elzi L, Erb S, Furrer H, Cavassini M, Calmy A, Vernazza P, et al. Adverse events of raltegravir and dolutegravir. *AIDS.* 2017;31:1853–8.
- Yagura H, Watanabe D, Kushida H, Tomishima K, Togami H, Hirano A, et al. Impact of UGT1A1 gene polymorphisms on plasma dolutegravir trough concentrations and neuropsychiatric adverse events in Japanese individuals infected with HIV-1. *BMC Infect Dis.* 2017;17:622.
- Takano M, Sugiyama T. UGT1A1 polymorphisms in cancer: impact on irinotecan treatment. *Pharmgenom Pers Med.* 2017;10:61–68.
- Krishna R, Rizk ML, Larson P, Schulz V, Kesisoglou F, Pop R. Single- and multiple-dose pharmacokinetics of once-daily formulations of raltegravir. *Clin Pharm Drug Dev.* 2018;7:196–206.
- Cahn P, Kaplan R, Sax PE, Squires K, Molina J-M, Avihingsanon A, et al. Raltegravir 1200 mg once daily versus raltegravir 400 mg twice daily, with tenofovir disoproxil fumarate and emtricitabine, for previously untreated HIV-1 infection: a randomised, double-blind, parallel-group, phase 3, non-inferiority trial. *Lancet HIV.* 2017;4:e486–e494.
- Brainard DM, Friedman EJ, Jin B, Breidinger SA, Tillan MD, Wenning LA, et al. Effect of low-, moderate-, and high-fat meals on raltegravir pharmacokinetics. *J Clin Pharm.* 2011;51:422–7.
- Ette EL, Kelman AW, Howie CA, Whiting B. Analysis of animal pharmacokinetic data: performance of the one point per animal design. *J Pharmacokinet Biopharm.* 1995;23:551–66.
- Dickinson L, Gurjar R, Stöhr W, Bonora S, Owen A, D'Avolio A, et al. 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. *J Antimicrob Chemother.* 2020;75:628–39.
- D'Avolio A, Baitto L, Siccardi M, Sciandra M, Simiele M, Oddone V, et al. An HPLC-PDA method for the simultaneous quantification of the HIV integrase inhibitor raltegravir, the new nonnucleoside reverse transcriptase inhibitor etravirine, and 11 other antiretroviral agents in the plasma of HIV-infected patients. *Ther Drug Monit.* 2008;30:662–9.
- Olagunju A, Bolaji O, Amara A, Else L, Okafor O, Adejuwogbe E, et al. Pharmacogenetics of pregnancy-induced changes in efavirenz pharmacokinetics. *Clin Pharm Ther.* 2015;97:298–306.
- Lee LS-U, Seng K-Y, Wang L-Z, Yong W-P, Hee K-H, Soh TI, et al. Phenotyping of UGT1A1 activity using raltegravir predicts pharmacokinetics and toxicity of irinotecan in FOLFIRI. *PLoS One.* 2016;11:e0147681.
- Beal SL, Sheiner L. NONMEM users guide. *ICON Dev Solutions, Ellicott City, Maryland, USA.*
- Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med.* 1995;333:1171–5.
- Hsieh T-Y, Shiu T-Y, Huang S-M, Lin H-H, Lee T-C, Chen P-J, et al. Molecular pathogenesis of Gilbert's syndrome: decreased TATA-binding protein binding affinity of UGT1A1 gene promoter. *Pharmacogenet Genom.* 2007;17:229–36.
- Gammal R, Court M, Haidar C, Iwuchukwu O, Gaur A, Alvarellos M, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for UGT1A1 and atazanavir prescribing. *Clin Pharm Ther.* 2016;99:363–9.
- Dickinson L, Amin J, Else L, Boffito M, Egan D, Owen A, et al. Comprehensive pharmacokinetic, pharmacodynamic and pharmacogenetic evaluation of once-daily Efavirenz 400 and 600 mg in treatment-naïve HIV-infected patients at 96 weeks: results of the ENCORE1 study. *Clin Pharmacokinet.* 2016;55:861–73.
- Lindbom L, Pihlgren P, Jonsson N. PsN-Toolkit—a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. *Comput Methods Prog Biomed.* 2005;79:241–57.
- Jonsson EN, Karlsson MO. Xpose—an S-PLUS-based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. *Comput Methods Prog Biomed.* 1998;58:51–64.

50. White IR, Royston P. Imputing missing covariate values for the Cox model. *Stat Med*. 2009;28:1982–98.
51. Lambert-Niclot S, George EC, Pozniak A, White E, Schwimmer C, Jessen H, et al. Antiretroviral resistance at virological failure in the NEAT 001/ANRS 143 trial: Raltegravir plus darunavir/ritonavir or tenofovir/emtricitabine plus darunavir/ritonavir as first-line ART. *J Antimicrob Chemother*. 2016;71:1056–62.
52. Wensing AM, Calvez V, Ceccherini-Silberstein F, Charpentier C, Günthard HF, Paredes R, et al. 2014 Update of the drug resistance mutations in HIV-1. *Top Antivir Med*. 2017;27:111–21.

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## AUTHOR CONTRIBUTIONS

RG: Extracted DNA, performed genotyping, wrote the manuscript, performed statistical analysis. LD: Built popPK models, performed statistical analysis, wrote the manuscript. DC: Performed *UGT1A1* genotyping. WS: Performed statistical analysis, wrote the manuscript. SB: Performed pharmacokinetic bioanalysis, reviewed manuscript. AO: Funded study, supervised pharmacogenetic analysis, reviewed data, reviewed manuscript. AD: Performed pharmacokinetic bioanalysis, reviewed data, reviewed manuscript. AC: Managed study, reviewed statistical analysis, reviewed manuscript. NDC: Enroled patients, reviewed manuscript. GF: Enroled patients, reviewed manuscript. LV: Enroled patients, reviewed manuscript. GD: Designed study, enroled patients, reviewed manuscript. AP: Designed study, enroled patients, reviewed manuscript. CS: Reviewed protocol, managed study, reviewed manuscript. FR: Designed study, enroled patients, reviewed manuscript. MB: Lead pharmacokinetic sub-study, designed study, enroled patients, reviewed manuscript.

## COMPETING INTERESTS

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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. FR has 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. NDC has received one research grant from Gilead. 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. CS 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.

## ADDITIONAL INFORMATION

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