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1 **Plasma Tenofovir, Emtricitabine and Rilpivirine and Intracellular Tenofovir**
2 **Diphosphate and Emtricitabine Triphosphate Pharmacokinetics Following Drug Intake**
3 **Cessation**

4

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24

25 **Abstract**

26 Pharmacokinetic (PK) data describing a prolonged time-course of antiretrovirals in plasma
27 and peripheral blood mononuclear cells (PBMCs) are important for understanding and
28 management of late or missed doses and to assess appropriateness of compounds for pre-
29 exposure prophylaxis (PrEP). This study aimed to evaluate the PK of coformulated tenofovir
30 DF, emtricitabine and rilpivirine in plasma and intracellular (IC) anabolites, tenofovir
31 diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP) in healthy volunteers up to 9
32 days after drug cessation. Individuals received daily tenofovir DF/emtricitabine/rilpivirine
33 (245/200/25 mg) for 14 days. Drug intake was stopped and serial sampling occurred prior to
34 the final dose and up to 216 hours (9 days) after stopping drug. Concentrations were
35 quantified and PK parameters calculated. Eighteen volunteers completed the study.
36 Geometric mean (90% CI) tenofovir and emtricitabine terminal elimination plasma half-life
37 within 216 hours was longer than 0-24 hours [tenofovir: 31 (27-40) vs. 13.3 (12.5-15.1)
38 hours; emtricitabine: 41 (36-54) vs. 6.4 (5.9-7.6) hours]. Model-predicted IC half-lives (0-
39 168) were 116 (TFV-DP) and 37 hours (FTC-TP). Plasma rilpivirine at 216 hours was 4.5
40 ng/mL (4.2-6.2) and half-lives 0-216 and 0-24 were 47 (41-59) and 35 (28-46) hours,
41 respectively. These data contribute to our understanding of drug behaviour following
42 treatment interruption however adherence to therapy should be promoted. Validated plasma
43 and IC target concentrations are necessary to allow interpretation with respect to sustained
44 virus suppression or HIV prevention.

45 **Introduction**

46 The challenge of maintaining a high level of adherence to antiretroviral therapy has been
47 aided, in part, by the development of fixed dose combination tablets suitable for once daily
48 dosing, such as Atripla[®] [tenofovir disoproxil fumarate (DF)/emtricitabine/efavirenz; Gilead
49 Sciences Ltd, London, UK] and Eviplera[®] (or Complera[®]; tenofovir
50 DF/emtricitabine/rilpivirine; Gilead Sciences Ltd, London, UK and Gilead Sciences Inc.,
51 Foster City, CA, USA). Eviplera[®], containing rilpivirine, a non-nucleoside reverse
52 transcriptase inhibitor (NNRTI), and tenofovir DF and emtricitabine (a nucleotide and
53 nucleoside reverse transcriptase inhibitor, respectively) is approved in Europe for treatment
54 of HIV-infected adults without NNRTI-associated resistance mutations, or mutations
55 associated with tenofovir or emtricitabine resistance, and viral loads $\leq 100,000$ copies/mL (1).
56 In the US it has been approved for therapy-naïve adults with viral load $\leq 100,000$ copies/mL
57 and for switching virologically suppressed patients (viral load < 50 copies/mL) under certain
58 conditions (2). Moreover, encouraging therapeutic outcomes and improved lipid profiles have
59 been observed switching suppressed patients to tenofovir DF/emtricitabine/rilpivirine from
60 tenofovir DF/emtricitabine/efavirenz or raltegravir-based regimens (3).

61

62 Despite the inroads made into improving patient adherence to therapy, delays in drug intake
63 or missed doses can occur as a result of individual circumstances e.g. busy lifestyle or
64 personal problems, risking viral rebound and resistance emergence. Key pharmacokinetic
65 (PK) characteristics such as a prolonged elimination half-life, are likely to be more forgiving
66 of late or missed doses, however PK data under these conditions are lacking, particularly for
67 coformulated regimens. Although patients are instructed to maintain a high level of
68 adherence, information regarding drug persistence in plasma and cells following treatment
69 interruption could potentially improve management of late or missed doses.

70

71 Data describing persistence of drugs within plasma, cells and other physiological
72 compartments are also essential for HIV prevention strategies such as pre-exposure
73 prophylaxis (PrEP), determining which drugs may have suitable PK properties. Coformulated
74 tenofovir DF/emtricitabine (Truvada[®]; Gilead Sciences Ltd, London, UK) was approved by
75 the US Food and Drug Administration in 2012 for use as PrEP in high risk individuals and
76 those engaging in sexual activity with HIV-infected partners (4). An intramuscularly
77 administered, long acting formulation of rilpivirine is also under investigation as a PrEP
78 agent (5).

79

80 Tenofovir (administered as tenofovir disoproxil fumarate and rapidly converted by esterases
81 following absorption to tenofovir) and emtricitabine are prodrugs that require intracellular
82 (IC) phosphorylation to their active anabolites. While tenofovir is a monophosphate analogue
83 requiring two phosphorylation steps to tenofovir diphosphate (TFV-DP), emtricitabine
84 triphosphate (FTC-TP) is formed by three endogenous enzymatic steps (6). Concentrations of
85 parent compounds in plasma and of TFV-DP and FTC-TP within peripheral blood
86 mononuclear cells (PMBC) have been reported in combination with efavirenz (Atripla[®]) over
87 9.5 days after stopping therapy in healthy volunteers (7) however, their PK profiles
88 coformulated with rilpivirine after stopping medication have not been evaluated. Moreover,
89 rilpivirine plasma PK and terminal half-life after drug cessation has not been previously
90 investigated.

91

92 The primary aim of this study was to evaluate plasma PK of tenofovir, emtricitabine and
93 rilpivirine and IC TFV-DP and FTC-TP PK in healthy, HIV negative volunteers over nine
94 days following drug intake cessation.

95

96 **Materials and Methods**

97 *Study population*

98 Male or non-lactating, non-pregnant females aged 18 to 65 years with a body mass index
99 (BMI) of 18-35 kg/m² who provided written informed consent were eligible for enrolment.
100 Exclusion criteria included the presence of any significant acute or chronic medical illness; a
101 positive screen for hepatitis B, C or HIV; evidence of organ dysfunction or abnormal physical
102 examination; abnormalities in vital signs, ECG or clinical laboratory parameters; current or
103 recent (within three months) gastrointestinal disease; clinically relevant alcohol or drug use
104 (including positive urine drug screen) or those considered by the Investigator to affect
105 compliance with trial procedures; exposure to any investigational drug or placebo within
106 three months of first dose of study drug; use of any other drugs including over-the-counter
107 medications and herbal preparations within two weeks of the first dose of study drug; known
108 allergy to any constituents of study drug; or females of childbearing potential not using
109 effective non-hormonal birth control methods.

110

111 *Study design*

112 This was a 23 day (excluding screening and follow up), open-label, single-treatment arm, PK
113 study, carried out at the PK Unit of St Stephen's Centre, Chelsea & Westminster Foundation
114 Trust (London, UK). The study was reviewed and approved by the National Research Ethics
115 Service (NRES Chelsea, London) and trial conduct was in accordance with the Declaration of
116 Helsinki (EudraCT 2012-002781-13).

117

118 Routine laboratory tests were performed at screening and drug safety and tolerability were
119 assessed throughout the study period according to the NIAID Division of AIDS (ACTG)

120 grading scale for adverse events (grade 1, mild - grade 4, life-threatening) in addition to
121 monitoring of vital signs, physical examinations and clinical laboratory investigations.

122

123 Following a 10 hour overnight fast on study day 1 (baseline visit), participants were
124 administered tenofovir DF/emtricitabine/rilpivirine (245/200/25 mg) with a 533 kcal
125 breakfast. All participants continued tenofovir DF/emtricitabine/rilpivirine once daily at
126 home and adherence was monitored by questionnaire and pill-count. On day 14, individuals
127 were admitted to the research unit and blood collection for drug quantification commenced
128 immediately before (within 10 minutes) the final tenofovir DF/emtricitabine/rilpivirine dose
129 (pre-dose, 0 hours). Samples were drawn at 2, 4, 8 and 12 hours after stopping the drug.
130 Subjects were discharged thereafter, returning to provide 24, 36, 48, 60, 72, 96, 120, 144,
131 168, 192 and 216 hours samples. All visits to the unit included documentation of concomitant
132 medications and adverse events. A final follow-up visit between days 30 and 36 reviewed
133 adverse events, vital signs and clinical laboratory assessments.

134

135 *Analytical methods*

136 *Plasma collection for tenofovir, emtricitabine and rilpivirine quantification*

137 Blood was collected into lithium heparin Vacutainer blood collection tubes which were
138 immediately inverted several times, placed in a light-protective container, and kept on ice or
139 refrigerated until centrifugation. Samples were centrifuged (10 minutes, 1200 g, 4°C) within
140 30 minutes of collection and plasma stored in light-protective amber-coloured tubes (-20°C)
141 prior to shipping on dry ice to the Good Clinical Laboratory Practice (GCLP)-accredited
142 Liverpool Bioanalytical Facility (Liverpool, UK) for analysis.

143

144 *Peripheral blood mononuclear cell isolation for TFV-DP and FTC-TP quantification*

145 PBMCs were obtained as previously described (7). There was a technical issue generating the
146 cell counts which meant that IC TFV-DP and FTC-TP could not be determined by
147 bioanalytical methods.

148

149 *Quantification of tenofovir and emtricitabine and rilpivirine in plasma*

150 Plasma tenofovir, emtricitabine and rilpivirine were determined using fully validated liquid
151 chromatography-tandem mass spectrometry (LC-MS/MS) methods (7, 8). Lower limit of
152 quantification (LLQ) was 0.5 ng/mL and assay precision was <15% for all three drugs.

153

154 *Modelling and prediction of TFV-DP and FTC-TP concentrations in peripheral blood*
155 *mononuclear cells*

156 Modelling of plasma tenofovir and emtricitabine linked to their IC anabolites (TFV-DP,
157 FTC-TP) has been previously described using various approaches (9-11). This methodology
158 was explored to allow prediction of TFV-DP and FTC-TP, up to 168 hours (7 days)
159 following drug cessation, from plasma data.

160

161 Separate models were developed for tenofovir and emtricitabine using nonlinear mixed
162 effects modelling (NONMEM v. 7.2, ICON Development Solutions, Ellicott City, MD, USA)
163 (12), and initial parameter estimates for plasma data were taken from the literature (9, 13).

164

165 Plasma tenofovir and emtricitabine and time-matched TFV-DP and FTC-TP concentrations
166 from a previous study investigating tenofovir, emtricitabine and efavirenz PK (Atripla®)
167 following drug cessation in healthy volunteers (EFV study) (7) were used as prior
168 information to describe the relationship between plasma and IC anabolite concentrations. All
169 data from both studies were modelled simultaneously. Plasma and IC concentrations between

170 0-156 hours (6.5 days) for the EFV study and plasma concentrations between 0-168 hours (7
171 days) for the present study were included as this provided the majority of samples above
172 assay LLQ. Samples <LLQ between 0-156 and 0-168 hours were excluded from the
173 modelling process.

174

175 The influence of covariates: age, weight, BMI, serum creatinine, creatinine clearance [CrCL;
176 calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI
177 formula (14)], sex, ethnicity and food intake (drug administration under fasted vs. fed
178 conditions) on plasma tenofovir and emtricitabine PK were investigated. To accept a model
179 with one extra parameter a decrease in the minimal objective function value (OFV) of at least
180 3.84 units was required ($p=0.05$, χ^2 distribution, 1 d.f.). A backwards elimination step was
181 performed once significant covariates were included; biologically plausible covariates
182 producing an increase in OFV (>6.64 units; $p=0.01$, χ^2 distribution, 1 d.f.) upon removal
183 were retained.

184

185 To evaluate the models, 90% prediction intervals (P5-P95) using final parameter estimates
186 were generated from 1000 simulated individuals with the same distribution of covariates as
187 the original dataset and observed data were superimposed. At least 90% of observed data
188 within the prediction interval was representative of an adequate model.

189

190 Final model parameters were used to predict IC TFV-DP and FTC-TP concentration-time
191 profiles for the present study between 0-168 hours. Plasma PK parameters were fixed to
192 individual Bayesian estimates for the present study and population parameters obtained for
193 the relationship between drug in plasma and IC anabolites were used as prior information.

194 Predictions were made utilising the \$SIMULATION option of NONMEM.

195

196 ***Statistical Analysis***

197 This was an exploratory study and no formal sample size calculation was performed. It was
198 estimated that sixteen subjects completing the study would allow for relevant conclusions.

199

200 Area under the concentration-time curve 0-24 hours post-dose (AUC_{0-24}) and to the last
201 measureable time point within 216 hours (AUC_{0-last}), maximum concentration (C_{max}) and
202 concentration 24 hours post-dose (C_{24}) were calculated for plasma tenofovir, emtricitabine
203 and rilpivirine using non-compartmental methods (WinNonlin Phoenix v. 6.3, Pharsight
204 Corporation, Mountain View, CA, USA). Terminal elimination half-life was determined to
205 the last measureable time point within 216 hours.

206

207 AUC_{0-24} and 0-168 (AUC_{0-168}), C_{max} and C_{24} were calculated as outlined above for TFV-DP
208 and FTC-TP using model predicted concentrations. Terminal elimination half-life was
209 calculated using the formula: $\ln(2)/k_{40}$ (k_{40} : rate constant for loss or elimination of TFV-DP
210 or FTC-TP; Fig. S1).

211

212 Pharmacokinetic parameters were summarised as geometric mean (90% CI) and
213 interindividual variability expressed as co-efficient of variation [CV%; (standard
214 deviation/mean)*100].

215

216 **Results**

217 *Study population*

218 Eighteen participants (11 female; 61%) completed the study. Median (range) age, weight,
219 BMI, serum creatinine and CrCL were 31 years (19-47), 75 kg (60-105), 24 kg/m² (21-31),

220 73 $\mu\text{mol/L}$ (57-104) and 103 ml/min/1.73 m² (78-146), respectively. Participants described
221 themselves as Caucasian (n=10), Black-Caribbean (n=2), Black-African (n=2), Asian (n=1),
222 Hispanic (n=1) and mixed ethnicity (n=2). Study drug was well tolerated and no grade 3 or 4
223 adverse events were reported.

224

225 *Plasma tenofovir, emtricitabine and rilpivirine pharmacokinetics*

226 Of 288 samples, 20 (7%; 120-216 hours), 5 (2%; 168-216 hours) and 1 (0.3%; 192 hours)
227 were below the LLQ for tenofovir, emtricitabine and rilpivirine, respectively. Nine, 15 and
228 17 individuals had quantifiable tenofovir, emtricitabine and rilpivirine at all sampling time
229 points between 0-216 hours after stopping drug.

230

231 Geometric mean plasma concentrations over time for tenofovir, emtricitabine and rilpivirine
232 are shown (Fig. 1) and PK parameters summarised (Table 1). Geometric mean (90% CI)
233 terminal elimination half-life to the last measureable time-point within 216 hours was
234 markedly longer than that of 0-24 hours for tenofovir and emtricitabine [30.7 h (27.2-39.7)
235 vs. 13.3 h (12.5-15.1) and 40.5 h (35.8-53.5) vs. 6.44 h (5.88-7.55)]. Elimination half-life 0-
236 216 was slightly longer than 0-24 for rilpivirine [47.2 h (41.3-59.3) vs. 34.6 h (28.4-45.9)].
237 Rilpivirine geometric mean (90% CI) C₂₁₆ was 4.53 ng/mL (4.22-6.15). A therapeutic cut-off
238 has not been defined for rilpivirine but 50 ng/mL has been suggested based on unpublished
239 data (5). At 24 and 36 hours (representing a 12 hour delayed dose), 2/18 (11%) and 6/18
240 (33%) of participants had concentrations below 50 ng/mL; 7/18 (39%) and 11/18 (61%) were
241 below this value 72 hours after stopping the drug.

242

243 *Prediction of intracellular tenofovir diphosphate and emtricitabine triphosphate*
244 *concentrations*

245 Sixteen healthy volunteers (5 female; n=11 Caucasian, n=2 Black-African, n=2 mixed
246 ethnicity, n=1 Asian) from the EFV study were included. Median (range) age, weight, BMI,
247 serum creatinine and CrCL were 32 years (21-57), 81 kg (54-109), 27 kg/m² (21-35), 81
248 μmol/L (60-112) and 102 ml/min/1.73 m² (72-126), respectively. Of 206 and 207 evaluable
249 plasma and intracellular samples, the EFV study (7) contributed 203 and 206 plasma
250 tenofovir and emtricitabine concentrations (<LLQ: n=3, n=0; respectively) and 183 and 207
251 TFV-DP and FTC-TP concentrations (<LLQ: n=24, n=0, respectively). The present study
252 contributed 245 (n=6 <LLQ) and 250 (n=1 <LLQ) plasma tenofovir and emtricitabine
253 concentrations to the models.

254

255 A diagrammatic summary of the model structure used for both drugs is shown (Fig. S1).
256 Plasma tenofovir and emtricitabine were best described by a two-compartment oral model,
257 parameterised by apparent oral clearance (CL/F), apparent volume of the central (V_c/F) and
258 peripheral compartments (V_p/F), intercompartmental clearance (Q/F) and absorption rate
259 constant (k_a). Due to lack of information in absorption phase the k_a of both drugs were fixed
260 to literature values; 1.05 h⁻¹ and 0.53 h⁻¹ for tenofovir and emtricitabine, respectively (9, 13).
261 Residual variability was described by a proportional error model and inclusion of
262 interindividual variability (IIV) was supported on CL/F and V_p/F for tenofovir and CL/F,
263 V_c/F and V_p/F for emtricitabine. TFV-DP and FTC-TP from the EFV study were described
264 by first-order rate constants, k₂₄ (uptake and conversion to phosphorylated form) and k₄₀ (loss
265 of anabolite). IIV was included on k₂₄ and a proportional error model described residual
266 variability.

267

268 Inclusion of a food (or partner drug) effect on relative bioavailability (F1) for tenofovir but
269 not emtricitabine significantly improved the fit. F1 was fixed to 1 (*i.e.* 100%) for the fasted

270 state (or with efavirenz) and increased by 33% with food (or with rilpivirine; Table S2).

271 Inclusion of weight on clearance and volume parameters using allometric scaling

272 significantly improved the tenofovir model as did addition of CrCL (linear function) on

273 tenofovir CL/F. CrCL was significantly associated with emtricitabine CL/F (linear function).

274

275 Population parameters from the final models are shown (Table S2). Diagnostic plots

276 suggested that both models adequately described the data and was confirmed by the visual

277 predictive checks with 90% and 92% of observed plasma tenofovir and TFV-DP

278 concentrations, respectively within the 90% prediction interval and 92% and 94% of plasma

279 emtricitabine and FTC-TP concentrations, respectively within the prediction interval (Figure

280 S3).

281

282 *Intracellular tenofovir diphosphate and emtricitabine triphosphate pharmacokinetics*

283 Geometric mean predicted TFV-DP and FTC-TP concentrations over 168 hours are

284 illustrated (Fig. 2) and PK parameters shown (Table 1). Model-derived terminal elimination

285 half-lives (0-168 hours) for TFV-DP and FTC-TP were 116 (4.8 days) and 37 hours (1.5

286 days), respectively.

287

288 IC TFV-DP and FTC-TP target concentrations for HIV suppression are not known, however

289 HIV prevention targets have been determined using PK data from the iPrEx trial. Ninety

290 percent risk reduction was associated with 16 fmol/10⁶ and 3.7 pmol/10⁶ viable cells for

291 TFV-DP and FTC-TP, respectively (15). At 24, 36, 48 and 72 hours after stopping drug

292 predicted TFV-DP were <16 fmol/10⁶ cells in 6%, 0%, 1% and 22% of individuals,

293 respectively whilst 56%, 78%, 83% and 83%, respectively were below 3.7 pmol/10⁶ cells for

294 predicted FTC-TP.

295 **Discussion**

296 Concentrations in plasma of tenofovir, emtricitabine, and for the first time rilpivirine, have
297 been demonstrated over 9 days (216 hours) after stopping tenofovir
298 DF/emtricitabine/rilpivirine intake in healthy, HIV-negative adults. Prediction of IC TFV-DP
299 and FTC-TP concentrations from plasma data was also achieved utilising modelling and
300 simulation and prior information from a previous, similar study (7).

301

302 A therapeutic cut-off for sustained viral suppression has not been defined for rilpivirine, but
303 50 ng/mL has been suggested based on an unpublished analysis of phase III trials in which 50
304 ng/mL was the upper limit of the lowest quartile of trough concentrations in which
305 virological response was lowest (5). Eleven percent, 33% and 39% of individuals had
306 concentrations below this threshold value 24, 36 and 48 hours after stopping drug,
307 respectively. However, these data should be interpreted with caution given that 50 ng/mL is
308 not a validated target concentration. The long elimination half-lives of 35 hours (0-24) and 47
309 hours (0-216) determined as part of this study are consistent with that previously reported for
310 rilpivirine [45 hours (16, 17)]. The data presented indicate that rilpivirine exhibits PK
311 properties that may allow forgiveness for delayed dosing in some patients however,
312 individuals should be instructed to adhere to licensed dosing guidelines.

313

314 Tenofovir plasma exposure was higher in the present study compared to that obtained by
315 Jackson *et al.*, in healthy volunteers stopping therapy [AUC_{0-last}: 4249 vs. 2895 ng.h/mL (7)]
316 and was highlighted during the modelling process. The two studies were conducted at the
317 same research unit and bioanalysis occurred at the same laboratory. However, the NNRTI in
318 the fixed dose combination was different between studies (efavirenz vs. rilpivirine) as were
319 the food intake conditions. The EFV study was conducted under fasting conditions (2 hours

320 prior and after drug intake); however rilpivirine must be administered with food in order to
321 achieve optimal absorption. Tenofovir exposure, as a component of tenofovir
322 DF/emtricitabine/rilpivirine has been shown to increase by 38% following a standard meal
323 (540 kcal) (1). A non-clinically relevant increase in plasma tenofovir of 24% has also been
324 reported upon co-administration with rilpivirine, potentially through mild inhibition of the
325 renal transmembrane transporters responsible for tenofovir renal elimination (18). However,
326 an interaction has not been observed during co-administration with efavirenz (19). Inclusion
327 of a food effect on F1 (relative bioavailability) improved the tenofovir model, resulting in a
328 33% higher F1 for the present study compared to the EFV study. This could also be attributed
329 to the interaction with rilpivirine or a combination of both a food and partner drug effect.
330 Emtricitabine PK parameters were within the ranges previously reported and is known to be
331 unaffected by food intake or co-administration with rilpivirine (1, 7). Terminal elimination
332 half-lives to the last measureable time point within 216 hours for both nucleosides were
333 considerably longer than over 0-24 hours (tenofovir: 31 vs. 13 hours; emtricitabine: 41 vs. 6
334 hours) and were also in agreement with values from the earlier study (7).

335

336 Due to issues with PBMC cell counts, TFV-DP and FTC-TP could not be directly quantified
337 however a modelling approach was explored using the observed plasma tenofovir and
338 emtricitabine concentrations and data from another study as prior information. The model
339 was relatively simplistic using an effect compartment for TFV-DP or FTC-TP linked to the
340 plasma compartment by a rate constant (k_{24}) describing a number of processes including the
341 uptake and metabolism of tenofovir and emtricitabine. Given that tenofovir monophosphate
342 and emtricitabine diphosphate were not measured this helped limit problems with
343 identifiability of model parameters. A similar model structure has recently been used to
344 describe tenofovir and TFV-DP in healthy female volunteers (20). An indirect response

345 model has previously been used to describe plasma tenofovir and IC TFV-DP in HIV patients
346 (9); however this model was not supported by our data. A simulation study reported by
347 Madrasi *et al.*, investigated a mechanistic model for tenofovir focusing more on describing
348 saturable uptake and metabolism in PBMCs using literature values (11). Despite the
349 simplistic nature of the model used for the current analysis, it performed well for both drugs
350 and parameters generally agreed with the literature, but could be updated is further data
351 became available (9, 13, 21).

352

353 The parameters describing the IC anabolites (k_{24} , k_{40} , variability in k_{24}) were estimated using
354 data generated from a previous study (7), but also incorporated the individual predicted
355 plasma PK parameters determined for the present study. The plasma PK parameters drive the
356 prediction of TFV-DP and FTC-TP in the model. Therefore, the predicted TFV-DP PK
357 parameters were slightly higher than those reported by Jackson *et al.*, (7) because plasma
358 tenofovir was also higher. Unsurprisingly, FTC-TP parameters were similar between studies
359 given the agreement in plasma PK parameters. A limitation of the modelling is that external
360 datasets are required to further evaluate the models; however it is noteworthy that the TFV-
361 DP and FTC-TP predictions are within ranges previously reported, including the PrEP
362 population for TFV-DP (15, 20, 22)

363

364 Evaluation of antiretroviral PK forgiveness and persistence within physiological
365 compartments is also important for methods of HIV prevention, such as PrEP. Favourable PK
366 characteristics, including prolonged elimination half-lives are beneficial for PrEP agents
367 allowing for once daily or less frequent dosing in order to aid adherence. Based on outcomes
368 reported from the iPrEx and Partners PrEP trials, Truvada[®] (tenofovir/emtricitabine) was
369 approved as a PrEP regimen in the US (23, 24). A long acting, parenteral formulation of

370 rilpivirine is under development and investigations have begun to determine its suitability as
371 a PrEP compound. Single dose rilpivirine PK in plasma and male (600 mg) and female (300,
372 600, 1200 mg) genital tracts were assessed and shown to persist up to 84 days. The effect of
373 rilpivirine concentrations in female genital tract fluid on HIV replication was also explored
374 *ex-vivo* (5). Studies to further evaluate long acting rilpivirine as PrEP are planned
375 [ClinicalTrials.gov Identifier: NCT02165202 (25)] or ongoing [ClinicalTrials.gov Identifier:
376 NCT01656018 (26, 27)]. Furthermore, rilpivirine oral formulation (with or without tenofovir
377 and emtricitabine) may be used in the context of PrEP for short periods of time (e.g. as an
378 oral lead in dose for safety reasons or as an alternative to long acting PrEP), therefore
379 knowledge of drug exposures after stopping drug and PK forgiveness may help to plan for
380 this eventuality.

381

382 Interpretation of these data is limited by the lack of fully validated target concentrations at
383 which virological suppression (or prevention) occurs for rilpivirine and IC TFV-DP and
384 FTC-TP. Therefore, the time at which virological control could be lost (or transmission
385 occurs) or how long a dose could be delayed was not attainable. Using PK data from iPrEx,
386 an IC TFV-DP concentration of 16 fmol/10⁶ viable cells was associated with 90% HIV risk
387 reduction (15). This target was also applied to data obtained from the Cell-PrEP study which
388 investigated the achievement and maintenance of protective concentrations of
389 tenofovir/emtricitabine in uninfected men who have sex with men. After stopping drug at day
390 30, 80% and 48% of individuals were above this concentration at 2 and 7 days post drug
391 cessation, respectively (28). In comparison, predicted TFV-DP from the present study were
392 ≥ 16 fmol/10⁶ cells in 94% and 72% of volunteers, 2 and 7 days after stopping drug.

393

394 As this study evaluated drug PK after stopping treatment it could not be conducted in HIV-
395 infected patients and assessment of viral load after treatment interruption could not be
396 performed. Translation from the present findings requires further study in patient populations
397 where pharmacodynamic endpoints can be investigated given that PK between HIV-infected
398 and healthy individuals may differ (17). Although, this study contributes significantly to our
399 understanding of drug behaviour when therapy is stopped and the long elimination half-lives
400 determined for all three drugs is encouraging.

401

402 Adherence to antiretroviral therapy should be promoted in order to maintain optimal
403 virological control, however, persisting plasma PK of tenofovir, emtricitabine and rilpivirine
404 and IC TFV-DP and FTC-TP demonstrated by this study may provide a potentially forgiving,
405 coformulated regimen for individuals that may miss or delay an occasional dose, as well as a
406 prospective PrEP candidate.

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409

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413

414 **Conflicts of Interest**

415 LD is supported by PreDiCT-TB and has received a travel bursary from Gilead Sciences Ltd

416 HMY has received travel bursaries from Gilead

417 AJ since completion of the study has become an employee of Gilead Sciences

418 GM has been on the Speaker Bureau for Janssen, Bristol Myers Squibb, Gilead and Merck

419 and an advisor for Tobira, Merck and Teva

420 LE, SK, and DB have received research grants and/or travel bursaries from Merck, Bristol

421 Myers and Squibb, GlaxoSmithKline, Pfizer, Abbott, ViiV, Boehringer Ingelheim and

422 Janssen Pharmaceuticals

423 AA has none to declare

424 ZK has none to declare

425 CH has none to declare

426 MB has received travel and research grants from and has been an adviser for Janssen, Roche,

427 Pfizer, ViiV, Bristol-Myers Squibb, Merck Sharp & Dohme and Gilead

428 **References**

- 429 1. **Gilead Sciences Ltd.** 2014. EVIPLERA[®] (200 mg/25 mg/245 mg film coated tablets)
430 Summary of Product Characteristics. Available at:
431 <http://www.medicines.org.uk/EMC/medicine/25518/SPC/Eviplera+200+mg+25+mg+245+mg+film+coated+tablets/> (last accessed 23/01/2015).
432
- 433 2. **Gilead Sciences.** 2014. COMPLERA[®] (emtricitabine, rilpivirine, tenofovir disoproxil
434 fumarate) tablets, for oral use - Highlights of Prescribing Information. Available at:
435 https://www.gilead.com/~media/Files/pdfs/medicines/hiv/complera/complera_pipdf (last
436 accessed 23/01/2015).
- 437 3. **Pinnetti C, Di Giambenedetto S, Maggiolo F, Lorenzini P, Fabbiani M, Tommasi C,**
438 **Latini A, Ammassari A, Loiacono L, Sterrantino G, Bellagamba R, Boumis E,**
439 **Antinori A, Zaccarelli M.** 2014. Simplification to co-formulated
440 rilpivirine/emtricitabine/tenofovir in virologically suppressed patients: Data from a
441 multicenter cohort. *J Int AIDS Soc* **17**:19812.
- 442 4. **US Food and Drug Administration.** 2012. FDA approves first drug for reducing the
443 risk of sexually acquired HIV infection - FDA News Release. Available at:
444 <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm312210.htm> (last
445 accessed 23/01/2015).
- 446 5. **Jackson AG, Else LJ, Mesquita PM, Egan D, Back DJ, Karolia Z, Ringner-Nackter**
447 **L, Higgs CJ, Herold BC, Gazzard BG, Boffito M.** 2014. A compartmental
448 pharmacokinetic evaluation of long-acting rilpivirine in HIV-negative volunteers for pre-
449 exposure prophylaxis. *Clin Pharmacol Ther* **96**:314-323.
- 450 6. **Piliero PJ.** 2004. Pharmacokinetic properties of nucleoside/nucleotide reverse
451 transcriptase inhibitors. *J Acquir Immune Defic Syndr* **37 Suppl 1**:S2-S12.

- 452 7. **Jackson A, Moyle G, Watson V, Tjia J, Ammara A, Back D, Mohabeer M, Gazzard**
453 **B, Boffito M.** 2013. Tenofovir, emtricitabine intracellular and plasma, and efavirenz
454 plasma concentration decay following drug intake cessation: implications for HIV
455 treatment and prevention. *J Acquir Immune Defic Syndr* **62**:275-281.
- 456 8. **Else LJ, Tjia J, Jackson A, Penchala SD, Egan D, Boffito M, Khoo SH, Back DJ.**
457 2014. Quantification of rilpivirine in human plasma, cervicovaginal fluid, rectal fluid and
458 genital/rectal mucosal tissues using liquid chromatography-tandem mass spectrometry.
459 *Bioanalysis* **6**:1907-1921.
- 460 9. **Baheti G, Kiser JJ, Havens PL, Fletcher CV.** 2011. Plasma and intracellular
461 population pharmacokinetic analysis of tenofovir in HIV-1-infected patients. *Antimicrob*
462 *Agents Chemother* **55**:5294-5299.
- 463 10. **Hirt D, Pruvost A, Ekouevi DK, Urien S, Arrive E, Kone M, Nerrienet E, Nyati M,**
464 **Gray G, Kruy LS, Blanche S, Dabis F, Treluyer JM.** 2011. Very high concentrations
465 of active intracellular phosphorylated emtricitabine in neonates (ANRS 12109 trial, step
466 2). *Antimicrob Agents Chemother* **55**:2953-2960.
- 467 11. **Madrasi K, Burns RN, Hendrix CW, Fossler MJ, Chaturvedula A.** 2014. Linking the
468 population pharmacokinetics of tenofovir and its metabolites with its cellular uptake and
469 metabolism. *CPT Pharmacometrics Syst Pharmacol* **3**:e147.
- 470 12. **Beal S, Sheiner LB.** 1989-1998. NONMEM[®] Users Guide. ICON Development
471 Solutions, Ellicott City, Maryland, USA.
- 472 13. **Valade E, Treluyer JM, Bouazza N, Ghosn J, Foissac F, Benaboud S, Fauchet F,**
473 **Viard JP, Urien S, Hirt D.** 2014. Population pharmacokinetics of emtricitabine in HIV-
474 1-infected adult patients. *Antimicrob Agents Chemother* **58**:2256-2261.

- 475 14. **Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, Kusek**
476 **JW, Eggers P, Van Lente F, Greene T, Coresh J, Ckd EPI.** 2009. A new equation to
477 estimate glomerular filtration rate. *Ann Intern Med* **150**:604-612.
- 478 15. **Anderson PL, Glidden DV, Liu A, Buchbinder S, Lama JR, Guanira JV, McMahan**
479 **V, Bushman LR, Casapia M, Montoya-Herrera O, Veloso VG, Mayer KH,**
480 **Chariyalertsak S, Schechter M, Bekker LG, Kallas EG, Grant RM, iPrEx Study T.**
481 2012. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in
482 men who have sex with men. *Sci Transl Med* **4**:151ra125.
- 483 16. **Garvey L, Winston A.** 2009. Rilpivirine: a novel non-nucleoside reverse transcriptase
484 inhibitor. *Expert Opin Investig Drugs* **18**:1035-1041.
- 485 17. **Janssen-Cilag Ltd.** 2014. EDURANT[®] (25 mg film-coated tablets) Summary of Product
486 Characteristics. Available at:
487 <http://www.medicines.org.uk/EMC/medicine/25490/SPC/Edurant+25+mg/> (last accessed
488 23/01/2015).
- 489 18. **Hoetelmans R, Van Heeswijk R, Kestens D, Stevens M, Peeters M, Williams P,**
490 **Bastiaanse L, Buffels R, Woodfall B.** 2005. Pharmacokinetic interaction between the
491 novel non-nucleoside reverse transcriptase inhibitor TMC278 and tenofovir disoproxil
492 fumarate in healthy volunteers. In: 3rd IAS Conference on HIV Pathogenesis and
493 Treatment July 24-27 2005, Rio de Janeiro, Brazil. Abstract WePe3.3C15.
- 494 19. **Droste JA, Kearney BP, Hekster YA, Burger DM.** 2006. Assessment of drug-drug
495 interactions between tenofovir disoproxil fumarate and the nonnucleoside reverse
496 transcriptase inhibitors nevirapine and efavirenz in HIV-infected patients. *J Acquir*
497 *Immune Defic Syndr* **41**:37-43.
- 498 20. **Burns RN, Hendrix CW, Chaturvedula A.** 2015. Population pharmacokinetics of
499 tenofovir and tenofovir-diphosphate in healthy women. *J Clin Pharmacol* **55**:629-638.

- 500 21. **Jullien V, Treluyer JM, Rey E, Jaffray P, Krivine A, Moachon L, Lillo-Le Louet A,**
501 **Lescoat A, Dupin N, Salmon D, Pons G, Urien S.** 2005. Population pharmacokinetics
502 of tenofovir in human immunodeficiency virus-infected patients taking highly active
503 antiretroviral therapy. *Antimicrob Agents Chemother* **49**:3361-3366.
- 504 22. **Wang LH, Begley J, St Claire RL, 3rd, Harris J, Wakeford C, Rousseau FS.** 2004.
505 Pharmacokinetic and pharmacodynamic characteristics of emtricitabine support its once
506 daily dosing for the treatment of HIV infection. *AIDS Res Hum Retroviruses* **20**:1173-
507 1182.
- 508 23. **Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, Tappero JW,**
509 **Bukusi EA, Cohen CR, Katabira E, Ronald A, Tumwesigye E, Were E, Fife KH,**
510 **Kiarie J, Farquhar C, John-Stewart G, Kakia A, Oduyo J, Mucunguzi A, Nakku-**
511 **Joloba E, Twesigye R, Ngure K, Apaka C, Tamooch H, Gabona F, Mujugira A,**
512 **Panteleeff D, Thomas KK, Kidoguchi L, Krows M, Revall J, Morrison S, Haugen**
513 **H, Emmanuel-Ogier M, Ondrejcek L, Coombs RW, Frenkel L, Hendrix C, Bumpus**
514 **NN, Bangsberg D, Haberer JE, Stevens WS, Lingappa JR, Celum C, Partners Pr**
515 **EPST.** 2012. Antiretroviral prophylaxis for HIV prevention in heterosexual men and
516 women. *N Engl J Med* **367**:399-410.
- 517 24. **Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, Goicochea P,**
518 **Casapia M, Guanira-Carranza JV, Ramirez-Cardich ME, Montoya-Herrera O,**
519 **Fernandez T, Veloso VG, Buchbinder SP, Chariyalertsak S, Schechter M, Bekker**
520 **LG, Mayer KH, Kallas EG, Amico KR, Mulligan K, Bushman LR, Hance RJ,**
521 **Ganoza C, Defechereux P, Postle B, Wang F, McConnell JJ, Zheng JH, Lee J,**
522 **Rooney JF, Jaffe HS, Martinez AI, Burns DN, Glidden DV, iPrEx Study T.** 2010.
523 Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N*
524 *Engl J Med* **363**:2587-2599.

- 525 25. **PATH**. 2014. Phase II safety and acceptability of an investigational injectable product,
526 TMC278LA, for pre-exposure prophylaxis. Available at:
527 <https://clinicaltrials.gov/ct2/show/NCT02165202?term=rilpivirine&rank=6> (last accessed
528 26/01/2015).
- 529 26. **Janssen Research & Development**. 2015. A study to evaluate safety, acceptability,
530 pharmacokinetics, and ex vivo pharmacodynamics of TMC278 long acting formulation
531 in HIV-1 seronegative participants. Available at:
532 <https://clinicaltrials.gov/ct2/show/NCT01656018?term=rilpivirine&rank=3> (last accessed
533 26/01/2015).
- 534 27. **McGowan I, Siegel S, Duffill K, Shetler C, Dezzutti C, Richardson-Harman N,**
535 **Abebe K, Back D, Else L, Herrick A, Williams P, Rehman KK, Cranston RD**. 2014.
536 A phase 1 open label safety, acceptability, pharmacokinetic, and pharmacodynamic study
537 of intramuscular TMC278 LA (the MWRI-01 Study). In: HIV Research for Prevention
538 (HIV R4P) October 28-31 2014, Cape Town, South Africa. Abstract OA27.06 LB.
- 539 28. **Seifert SM, Glidden DV, Meditz AL, Castillo-Mancilla JR, Gardner EM,**
540 **Predhomme JA, Rower C, Klein B, Kerr BJ, Guida LA, Zheng JH, Bushman LR,**
541 **Anderson PL**. 2015. Dose Response for Starting and Stopping HIV Preexposure
542 Prophylaxis for Men Who Have Sex With Men. *Clin Infect Dis* **60**:804-810.

543 **Figure Legends**

544

545 **FIG 1** Geometric mean plasma (A) tenofovir, (B) emtricitabine and (C) rilpivirine
546 concentrations over 216 hours following drug intake cessation in healthy volunteers (n=18)

547

548 **FIG 2** Individual predicted intracellular (A) tenofovir diphosphate and (B) emtricitabine
549 triphosphate concentrations over 168 hours following drug intake cessation in healthy
550 volunteers on a log-linear scale (n=18; individual concentration-time profiles generated by
551 modelling and simulation). The bold line represents the geometric mean concentration-time
552 profile

Table 1 Summary of plasma tenofovir, emtricitabine, rilpivirine and intracellular tenofovir diphosphate and emtricitabine triphosphate pharmacokinetic parameters obtained following drug intake cessation (n=18). Data presented as geometric mean (90% CI)

Plasma				
<i>Parameter</i>	<i>Units</i>	<i>Tenofovir</i>	<i>Emtricitabine</i>	<i>Rilpivirine</i>
AUC ₀₋₂₄	ng.h/mL	2573 (2342-3208)	8537 (7860-11955)	2116 (1929-2527)
CV%		40	53	34
AUC _{0-last}	ng.h/mL	4249 (3860-5325)	11126 (10169-15075)	7271 (6635-8761)
CV%		41	50	36
C _{max}	ng/mL	227 (208-280)	1260 (1148-1925)	139 (128-168)
CV%		38	65	35
C ₂₄	ng/mL	53.3 (48.8-71.1)	64.7 (58.2-97.3)	76.3 (68.7-94.8)
CV%		48	65	41
TE half-life	h	30.7 (27.2-39.7)	40.5 (35.8-53.5)	47.2 (41.3-59.3)
CV%		48	51	46

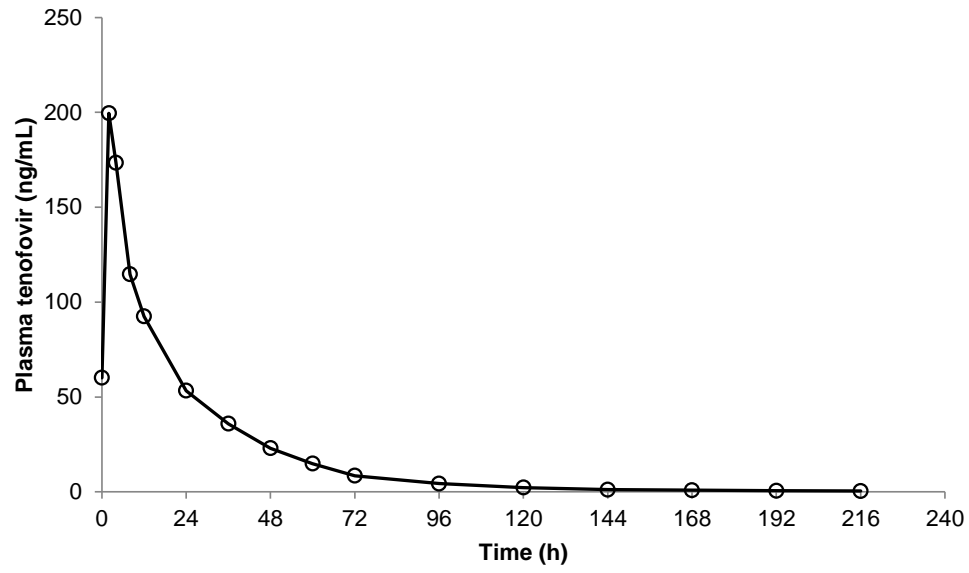
Intracellular

<i>Parameter^a</i>	<i>Units</i>	<i>Tenofovir diphosphate</i>	<i>Units</i>	<i>Emtricitabine triphosphate</i>
AUC ₀₋₂₄	fmol.h/10 ⁶ cells	1456 (1302-2193)	pmol.h/10 ⁶ cells	87.8 (79.2-150)
CV%		66		80
AUC ₀₋₁₆₈	fmol.h/10 ⁶ cells	7495 (6792-11486)	pmol.h/10 ⁶ cells	273 (252-440)
CV%		66		70
C _{max}	fmol/10 ⁶ cells	92.2 (83.8-135)	pmol/10 ⁶ cells	6.15 (5.73-10.5)
CV%		60		75
C ₂₄	fmol/10 ⁶ cells	54.0 (48.2-87.9)	pmol/10 ⁶ cells	3.07 (2.88-5.63)
CV%		75		83

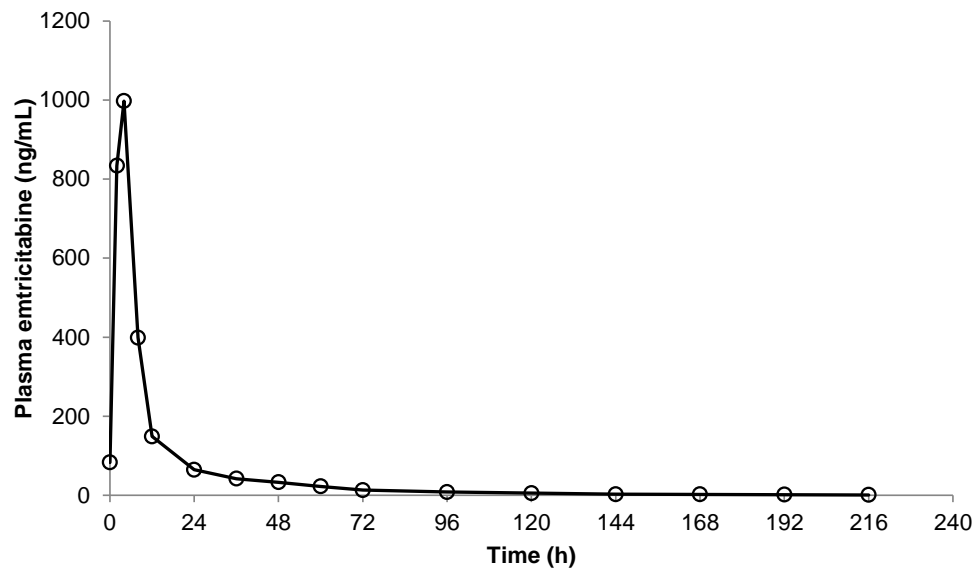
AUC₀₋₂₄, AUC₀₋₁₆₈: area under the curve over 24 hours or 168 hours post-dose; AUC_{0-last}: area under the curve to the last measureable concentration within 216 hours (0-216 for plasma rilpivirine); C_{max}: maximum concentration; C₂₄: concentration 24 hours post-dose; TE half-life: terminal elimination half-life to the last measureable concentration within 216 hours (0-216 for plasma rilpivirine)

^a Parameters determined by non-compartmental analysis using concentration-time profiles generated by means of modelling and simulation

(A)



(B)



(C)

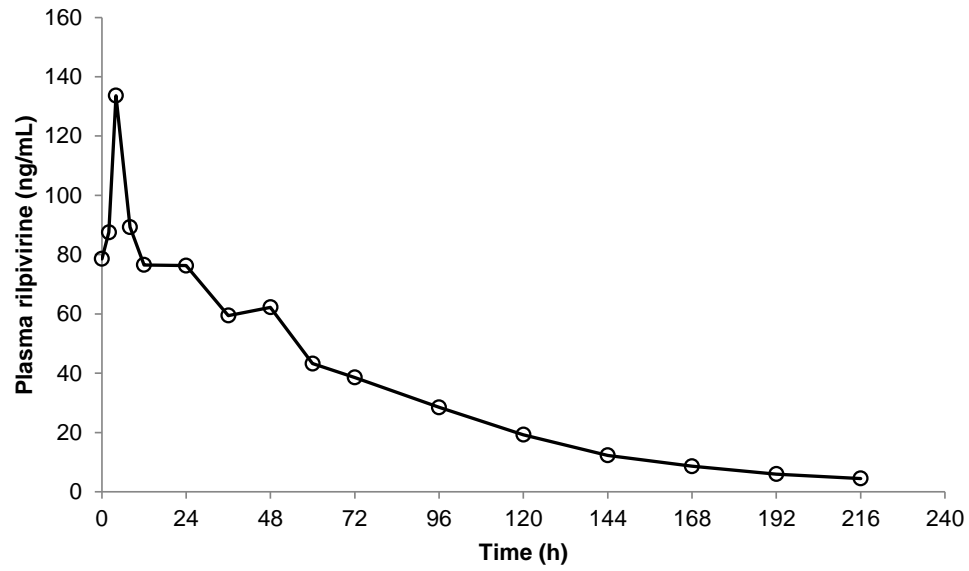
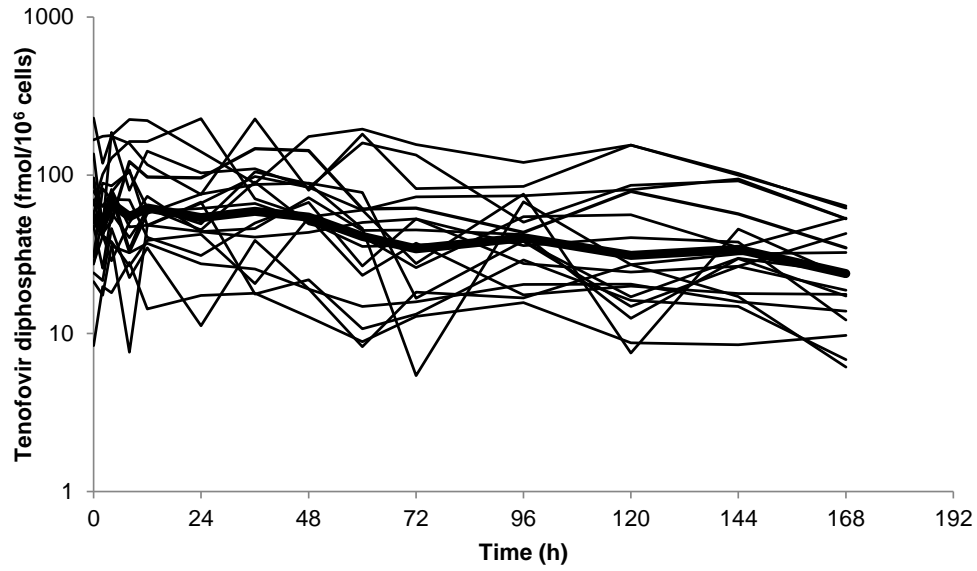


FIG 1 Geometric mean plasma (A) tenofovir, (B) emtricitabine and (C) rilpivirine concentrations over 216 hours following drug intake cessation in healthy volunteers (n=18)

(A)



(B)

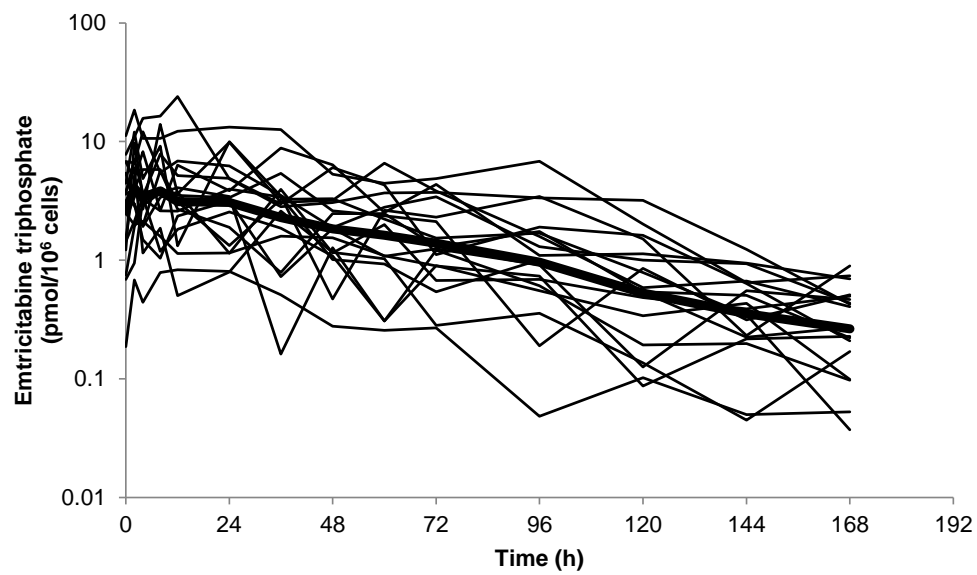


FIG 2 Individual predicted intracellular (**A**) tenofovir diphosphate and (**B**) emtricitabine triphosphate concentrations over 168 hours following drug intake cessation in healthy volunteers on a log-linear scale (n=18; individual concentration-time profiles generated by modelling and simulation). The bold line represents the geometric mean concentration-time profile