Plasma Tenofovir, Emtricitabine and Rilpivirine and Intracellular Tenofovir Diphosphate and Emtricitabine Triphosphate Pharmacokinetics Following Drug Intake Cessation

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Abstract

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

Antimicrobial Agents and Chemotherapy

Introduction

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

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

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

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

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

Materials and Methods

Study population

Male or non-lactating, non-pregnant females aged 18 to 65 years with a body mass index (BMI) of 18-35 kg/m^2 who provided written informed consent were eligible for enrolment. Exclusion criteria included the presence of any significant acute or chronic medical illness; a positive screen for hepatitis B, C or HIV; evidence of organ dysfunction or abnormal physical examination; abnormalities in vital signs, ECG or clinical laboratory parameters; current or recent (within three months) gastrointestinal disease; clinically relevant alcohol or drug use (including positive urine drug screen) or those considered by the Investigator to affect compliance with trial procedures; exposure to any investigational drug or placebo within three months of first dose of study drug; use of any other drugs including over-the-counter medications and herbal preparations within two weeks of the first dose of study drug; known allergy to any constituents of study drug; or females of childbearing potential not using effective non-hormonal birth control methods.

Study design

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

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

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

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

Analytical methods

Plasma collection for tenofovir, emtricitabine and rilpivirine quantification

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

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

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

Quantification of tenofovir and emtricitabine and rilpivirine in plasma

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

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

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

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

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

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

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

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

Final model parameters were used to predict IC TFV-DP and FTC-TP concentration-time profiles for the present study between 0-168 hours. Plasma PK parameters were fixed to individual Bayesian estimates for the present study and population parameters obtained for the relationship between drug in plasma and IC anabolites were used as prior information. Predictions were made utilising the $SIMULATION option of NONMEM.

Statistical Analysis

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

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

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

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

Results

Study population

Eighteen participants (11 female; 61%) completed the study. Median (range) age, weight, BMI, serum creatinine and CrCL were 31 years (19-47), 75 kg (60-105), 24 kg/m^2 (21-31),

73 µmol/L (57-104) and 103 ml/min/1.73 m^2 (78-146), respectively. Participants described themselves as Caucasian (n=10), Black-Caribbean (n=2), Black-African (n=2), Asian (n=1), Hispanic (n=1) and mixed ethnicity (n=2). Study drug was well tolerated and no grade 3 or 4 adverse events were reported.

Plasma tenofovir, emtricitabine and rilpivirine pharmacokinetics

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

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

Prediction of intracellular tenofovir diphosphate and emtricitabine triphosphate concentrations

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

A diagrammatic summary of the model structure used for both drugs is shown (Fig. S1). Plasma tenofovir and emtricitabine were best described by a two-compartment oral model, parameterised by apparent oral clearance (CL/F), apparent volume of the central (V_c/F) and peripheral compartments (V_p/F), intercompartmental clearance (Q/F) and absorption rate constant (k_a). Due to lack of information in absorption phase the k_a of both drugs were fixed to literature values; 1.05 h^{-1} and 0.53 h^{-1} for tenofovir and emtricitabine, respectively (9, 13). Residual variability was described by a proportional error model and inclusion of interindividual variability (IIV) was supported on CL/F and V_p/F for tenofovir and CL/F, V_c/F and V_p/F for emtricitabine. TFV-DP and FTC-TP from the EFV study were described by first-order rate constants, k_{24} (uptake and conversion to phosphorylated form) and k_{40} (loss of anabolite). IIV was included on k_{24} and a proportional error model described residual variability.

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

state (or with efavirenz) and increased by 33% with food (or with rilpivirine; Table S2). Inclusion of weight on clearance and volume parameters using allometric scaling significantly improved the tenofovir model as did addition of CrCL (linear function) on tenofovir CL/F. CrCL was significantly associated with emtricitabine CL/F (linear function).

Population parameters from the final models are shown (Table S2). Diagnostic plots suggested that both models adequately described the data and was confirmed by the visual predictive checks with 90% and 92% of observed plasma tenofovir and TFV-DP concentrations, respectively within the 90% prediction interval and 92% and 94% of plasma emtricitabine and FTC-TP concentrations, respectively within the prediction interval (Figure S3).

Intracellular tenofovir diphosphate and emtricitabine triphosphate pharmacokinetics

Geometric mean predicted TFV-DP and FTC-TP concentrations over 168 hours are illustrated (Fig. 2) and PK parameters shown (Table 1). Model-derived terminal elimination half-lives (0-168 hours) for TFV-DP and FTC-TP were 116 (4.8 days) and 37 hours (1.5 days), respectively.

IC TFV-DP and FTC-TP target concentrations for HIV suppression are not known, however HIV prevention targets have been determined using PK data from the iPrEx trial. Ninety percent risk reduction was associated with 16 fmol/10^6 and 3.7 pmol/10^6 viable cells for TFV-DP and FTC-TP, respectively (15). At 24, 36, 48 and 72 hours after stopping drug predicted TFV-DP were <16 fmol/10^6 cells in 6%, 0%, 1% and 22% of individuals, respectively whilst 56%, 78%, 83% and 83%, respectively were below 3.7 pmol/10^6 cells for predicted FTC-TP.

Discussion

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

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

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

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

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

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

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

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

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

Interpretation of these data is limited by the lack of fully validated target concentrations at which virological suppression (or prevention) occurs for rilpivirine and IC TFV-DP and FTC-TP. Therefore, the time at which virological control could be lost (or transmission occurs) or how long a dose could be delayed was not attainable. Using PK data from iPrEx, an IC TFV-DP concentration of 16 fmol/10^6 viable cells was associated with 90% HIV risk reduction (15). This target was also applied to data obtained from the Cell-PrEP study which investigated the achievement and maintenance of protective concentrations of tenofovir/emtricitabine in uninfected men who have sex with men. After stopping drug at day 30, 80% and 48% of individuals were above this concentration at 2 and 7 days post drug cessation, respectively (28). In comparison, predicted TFV-DP from the present study were ≥16 fmol/10^6 cells in 94% and 72% of volunteers, 2 and 7 days after stopping drug.

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

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

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Conflicts of Interest

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

HMY has received travel bursaries from Gilead

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

GM has been on the Speaker Bureau for Janssen, Bristol Myers Squibb, Gilead and Merck and an advisor for Tobira, Merck and Teva

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AA has none to declare

ZK has none to declare

CH has none to declare

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Figure Legends

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)

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

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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)

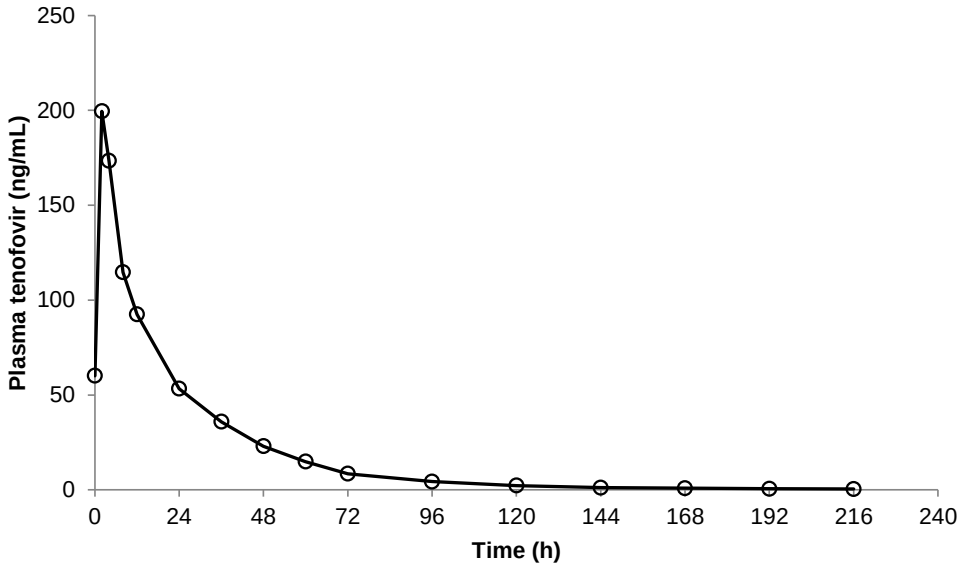
		Plasma		
Parameter	***Units***	***Tenofovir***	***Emtricitabine***	***Rilpivirine***
AUC_{0-24}	ng.h/mL	2573 (2342-3208)	8537 (7860-11955)	2116 (1929-2527)
CV%		*40*	*53*	*34*
AUC_{0-last}	ng.h/mL	4249 (3860-5325)	11126 (10169-15075)	7271 (6635-8761)
CV%		*41*	*50*	*36*
C_{max}	ng/mL	227 (208-280)	1260 (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		

Parameter[a]	***Units***	***Tenofovir diphosphate***	***Units***	***Emtricitabine triphosphate***
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*

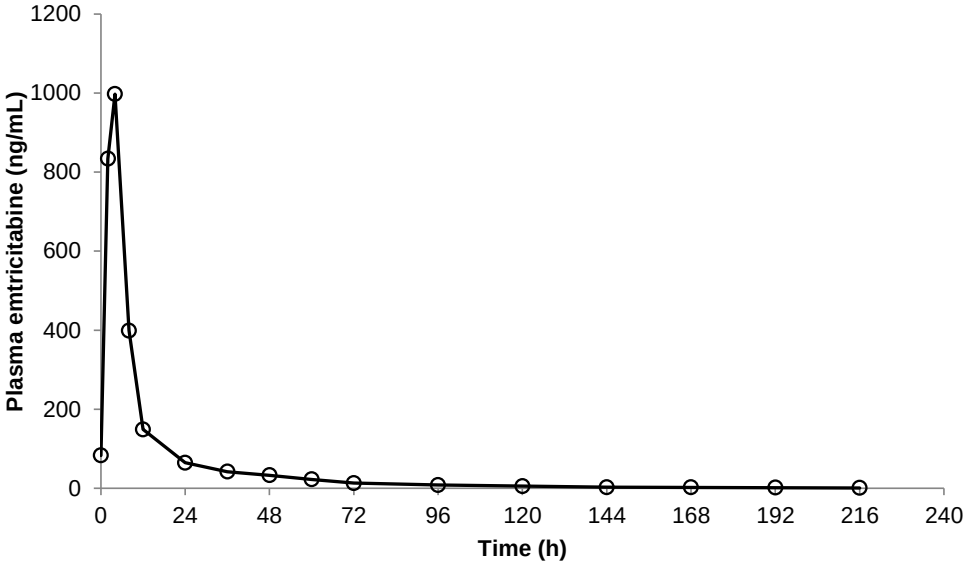
AUC_{0-24}, AUC_{0-168}: area under the curve over 24 hours or 168 hours post-dose; AUC_{0-last}: area under the curve to the last measureable concentration within 216 hours (0-216 for plasma rilpivirine); C_{max}: maximum concentration; C_{24}: concentration 24 hours post-dose; TE half-life: terminal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

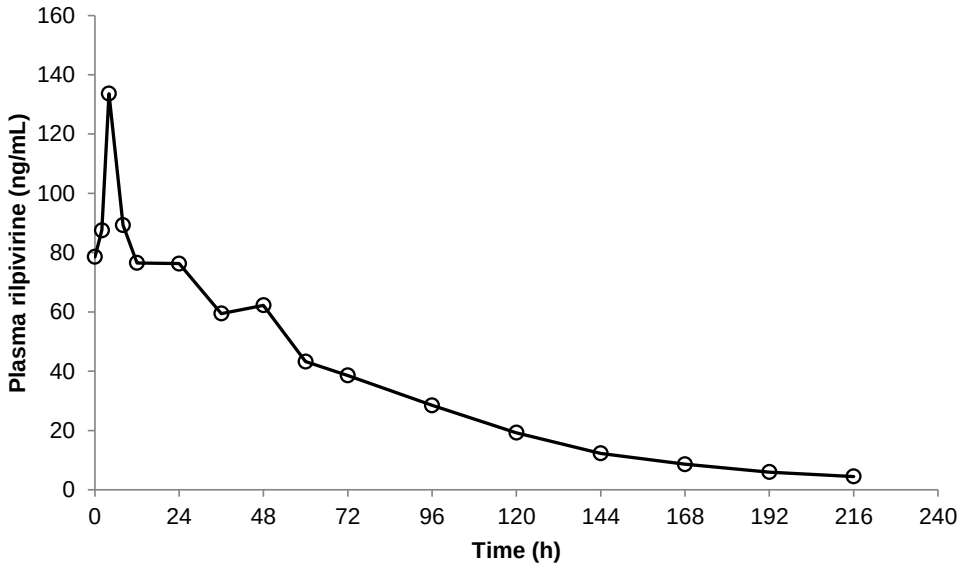
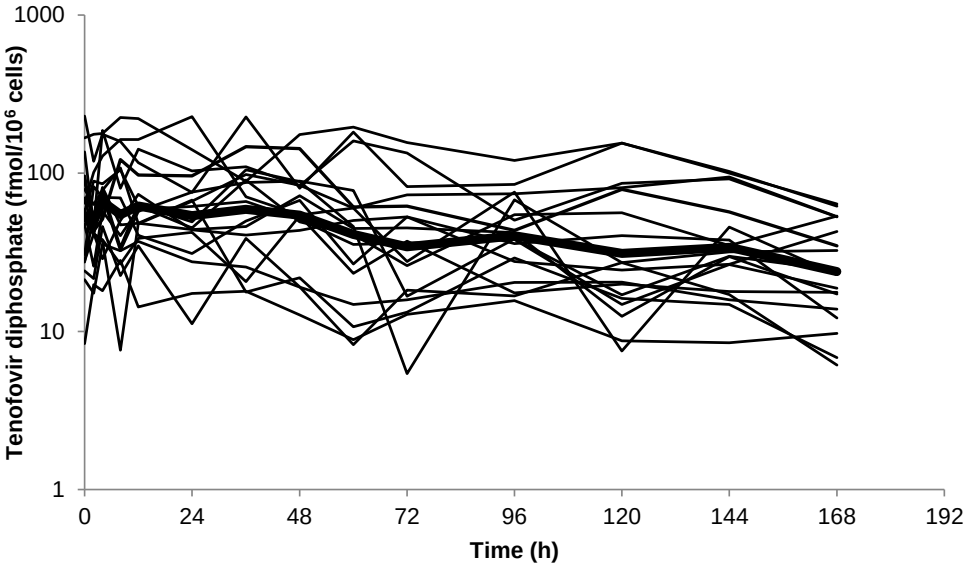


FIG 1 Geometric mean plasma (**A**) tenofovir, (**B**) emtricitabine and (**C**) rilpivirine concentrations over 216 hours following drug intake cessation in healthy volunteers (n=18)

(A)



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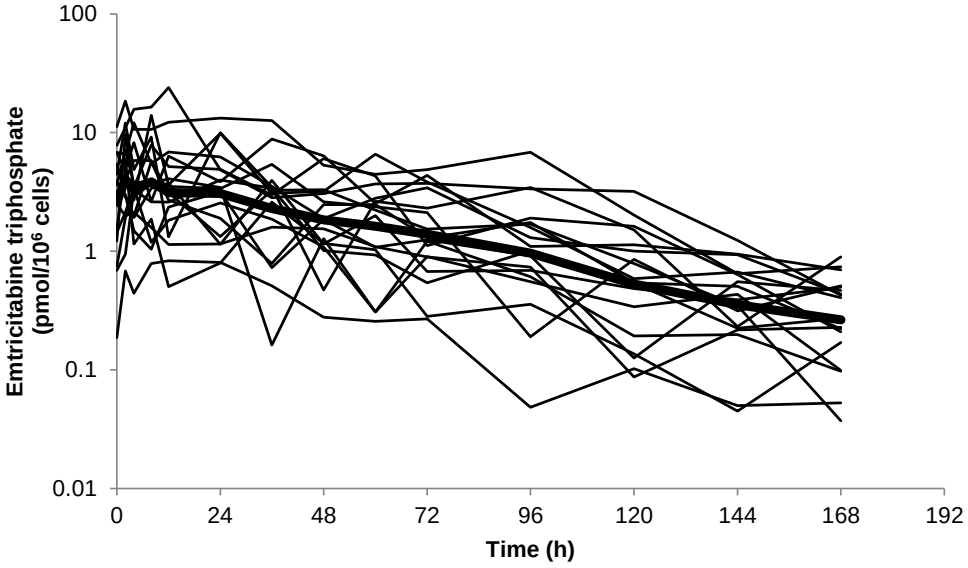

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FIG 2 Individual predicted intracellular (**A**) tenofovir diphosphate and (**B**) emtricitabine triphosphate concentrations over 168 hours following drug intake cessation in healthy volunteers on a log-linear scale (n=18; individual concentration-time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