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Dolutegravir and elvitegravir plasma concentrations following cessation of drug intake

Emilie Elliot[1,2], Alieu Amara[2], Akil Jackson[1,2], Graeme Moyle[1], Laura Else[2], Saye Khoo[2], David Back[2], Andrew Owen[2] and Marta Boffito[1,3]*

[1]*St Stephen's Centre, Chelsea and Westminster Hospital, London, UK;* [2]*University of Liverpool, Liverpool, UK;* [3]*Imperial College, London, UK*

*Corresponding author. St Stephen's Centre—Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH, UK. Tel: +44(0)20 33156506; Fax:+44(0)20 33155628; E-mail: marta.boffito@chelwest.nhs.uk

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Objectives: To evaluate dolutegravir and elvitegravir/cobicistat pharmacokinetics in HIV-negative volunteers up to 10 days after drug cessation.

Methods: Healthy volunteers received 50 mg of dolutegravir once-daily for 10 days, then underwent a 9 day wash-out period, and then received elvitegravir/cobicistat as part of Stribild® (245 mg of tenofovir, 200 mg of emtricitabine, 150 mg of elvitegravir and 150 mg of cobicistat) for 10 days. Serial pharmacokinetic (PK) sampling occurred prior to the final dose of each course and at regular intervals for up to 216 h (10 days) after drug cessation. Concentrations were determined by LC-MS/MS, and PK parameters were illustrated as geometric mean and 90% CI.

Results: Seventeen volunteers completed the study. For dolutegravir, plasma terminal elimination $t_{1/2}$ to the last measurable concentration (within 216 h) was longer than its $t_{1/2}$ within the dosing interval (0–24 h): 14.3 h (12.9–15.7 h) versus 23.1 h (19.7–26.6 h); conversely, the terminal elimination $t_{1/2}$ for elvitegravir was lower than its $t_{1/2}$ within the dosing interval (0–24 h): 10.8 h (9.7–13.0 h) versus 5.2 h (4.7–6.1 h). Dolutegravir concentrations were above the protein-adjusted (PA) IC_{90} (64 ng/mL) in 100% of subjects after 36 and 48 h and in 94% after 60 and 72 h. All subjects had detectable dolutegravir concentrations at 96 h, a mean of 23.5% above the IC_{90}. Elvitegravir concentrations were above the PA IC_{95} (45 ng/mL) in 100% of subjects at 24 h, 65% at 36 h but 0% after 48 h.

Conclusions: Our data show marked differences in the elimination rates of dolutegravir and elvitegravir following treatment interruption, which is likely to impact the extent to which drug doses can be delayed or missed. They suggest that clinical differences may emerge in patients who have suboptimal adherence.

Introduction

HIV-1 integrase strand transfer inhibitors (InSTIs) are the newest class of approved ART. As HIV-1 integrase has no equivalent in host cells, InSTIs exhibit minimal related interference with normal cellular processes and, as such, their safety profile differs from that of other ART classes.[1] Consequently, InSTIs are increasingly favoured over older drug classes as they demonstrate high efficacy and tolerability with low toxicity when prescribed in combination with two nucleoside or nucleotide reverse transcriptase inhibitors.

Whilst raltegravir was the first licensed InSTI, elvitegravir and, more recently, dolutegravir have now been approved for use in the USA and Europe.[2] Elvitegravir is prescribed in combination with the cytochrome P450 3A4 (CYP3A4) inhibitor cobicistat, which enhances elvitegravir exposure enabling once-daily dosing. Both elvitegravir and dolutegravir are available in fixed-dose combinations taken once-daily, facilitating the potential for adherence.[3]

Despite advances in ART facilitating better adherence, delayed or omitted doses still occur, potentially compromising virological control and risking the emergence of drug resistance. Therefore, plasma pharmacokinetic (PK) data after cessation of antiretroviral drugs are important to understand the management of late and missed doses. Drug persistence in plasma is dependent on its $t_{1/2}$ (which itself depends on CL and *V*).[4] Antiretroviral agents with longer $t_{1/2}$ may be more forgiving and allow for forgotten doses, especially if drug concentrations remain above sub-therapeutic concentrations until the patient reinitiates drug intake.

In addition to information on the 'forgiveness' of dosing regimens, PK data after cessation of drug intake may inform the appropriateness of specific compounds for HIV pre-exposure prophylaxis and for alternative treatment strategies tailored to facilitate adherence, such as were seen in the FOTO study.[5] That study showed that short-cycle structured treatment interruptions (dosing for 5 days consecutively followed by a 2 day

break) with ART containing tenofovir/emtricitabine and efavirenz, all long $t_{1/2}$ agents, was non-inferior to daily therapy whilst being preferred by patients, with no virological failures reported. More recent data from the BREATHER study of developing and developed countries demonstrated similar efficacy in adolescents on efavirenz.[6]

In vivo data for dolutegravir and elvitegravir/cobicistat concentration decay after intake cessation have not been previously described. In this study, we independently evaluated the plasma PK parameters of once-daily dolutegravir and once-daily elvitegravir/cobicistat in HIV-negative healthy volunteers up to 10 days following cessation of drug intake.

Materials and methods

Participants

Written informed consent was obtained from male and non-pregnant, non-lactating female healthy volunteers aged between 18 and 65 years and with a BMI between 18 and 35 kg/m^2. Participants were excluded if they had any significant acute or chronic medical illness; abnormal physical examination, ECG or clinical laboratory determinations; positive screens for HIV, hepatitis B or C; current or recent (within 3 months) gastrointestinal disease; clinically relevant alcohol or drug use that the investigator felt would adversely affect compliance with trial procedures; exposure to any investigational drug or placebo within 3 months of the first dose of the study drug; use of any other drugs, including over the counter medications and herbal preparations, within 2 weeks before the first dose of the study drug; and previous allergy to any of the constituents of the pharmaceuticals administered during the trial.

Study design

This was a 38 day, open-label, two-phase PK trial conducted at the St Stephen's Centre, Chelsea and Westminster Hospital, London, UK. The study protocol was approved by the London Westminster Research Ethics Committee as well as the Medicines and Healthcare Products Regulatory Agency in the UK and was conducted according to Good Clinical Practice and the Declaration of Helsinki (EudraCT 2014-001421-33, NCT02219217).

At screening, participants had a clinical assessment and routine laboratory investigations performed. The safety and tolerability of study medications were evaluated throughout the trial using the NIAID Division of AIDS table for grading the severity of adult and paediatric adverse events to characterize abnormal findings (published 2004), vital signs, physical examinations and clinical laboratory investigations.

After successful screening, participants were administered 50 mg of dolutegravir once daily for 10 days for the first phase of the study. They were admitted to the unit on day 10. Blood samples for dolutegravir PK assessment were taken before the final dose in the morning of day 10 and at 2, 4, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168, 192 and 216 h post dose.

After the wash-out period of 9 days, on day 20, all subjects were administered Stribild® (245 mg of tenofovir disoproxil fumarate, 200 mg of emtricitabine, 150 mg of elvitegravir and 150 mg of cobicistat) once daily for 10 days for the second phase of the trial, and blood samples were taken at the same intervals as above, prior to and over 216 h after the last dose.

On the PK days, the study medication was taken with a standardized breakfast (626 kcal) and 240 mL of water and subjects were admitted for 12 h, after which they could leave the unit and return at regular intervals to complete sampling over 9 days.

Compliance with study drug administration was assessed through pill counting by the study staff.

Analytical and PK methods

Plasma collection of dolutegravir, elvitegravir and cobicistat

Blood samples were collected into lithium heparin-containing blood tubes (6 mL) at each timepoint, immediately inverted several times and then kept on ice or refrigerated until centrifugation. Within 30 min of blood collection, each blood sample was centrifuged for 10 min at 1200 **g** at 4°C. Plasma was then aliquotted equally into three 2.0 mL tubes (Sarstedt, Germany) and stored at −20°C. Samples were shipped on dry ice to the Liverpool Bioanalytical Facility (Good Clinical Laboratory Practice accredited) for analysis.

Quantification of plasma dolutegravir, elvitegravir, cobicistat

Concentrations of dolutegravir, elvitegravir and cobicistat in plasma were determined using liquid–liquid extraction (with methyl tertiary-butyl ether) of analyte and internal standard (d5-dolutegravir, d6-elvitegravir, or quinoxaline) using validated HPLC tandem MS and analytical conditions substantially as previously described.[7] The lower limit of quantification (LLOQ) was 0.75 ng/mL for all components. For concentrations below the assay LLOQ, a value of one-half of the quantification limit (0.325 ng/mL) was used.

The assay was validated over a calibration range of either 10–4000 or 0.75–20 ng/mL (for concentrations below the LLOQ of the initial assay). Accuracy (percentage bias) was between 98.0% and 104.6% (dolutegravir), 101.8% and 106.7% (elvitegravir) and 99.8% and 106.2% (cobicistat), and precision was between 4.6% and 6.2% (dolutegravir), 4.3% and 5.6% (elvitegravir) and 5.0% and 6.0% (cobicistat).

Pharmacokinetic and statistical analysis

The calculated PK parameters for dolutegravir, elvitegravir and cobicistat were the plasma concentration measured 24 h after the observed dose (C_{24}), the maximum observed plasma concentration (C_{max}) and the area under the plasma concentration curve from 0 to 24 h (AUC_{0-24}). The $t_{1/2}$ was determined from the elimination phase within the normal dosing interval of 0–24 h and as a terminal elimination $t_{1/2}$ to the last measurable concentration within 216 h. All PK parameters were calculated using actual blood sampling time and non-compartmental modelling techniques (WinNonlin Phoenix, version 6.1; Pharsight, Mountain View, CA).

Descriptive statistics, including geometric mean (GM) and 90% CI were calculated for dolutegravir, elvitegravir and cobicistat PK parameters. GMs were compared with the suggested therapeutic targets that were established *in vitro* and are available in the current literature for each drug. These targets are the protein binding-adjusted (PA) IC_{90} for dolutegravir (64 ng/mL) and the PA IC_{95} for elvitegravir (45 ng/mL).[8,9]

Interindividual variability in drug PK parameters was expressed as a percentage coefficient of variation [CV, (standard deviation/mean)×100].

Results

Study population

Seventeen participants completed all phases of the study. The median age was 39 years (range 26–52 years), and the median BMI was 26 kg/m^2 (range 19–34 kg/m^2). Twelve participants were female; nine described themselves as white and eight as black. No serious breach to the protocol was recorded during the study. The study drugs were well tolerated, and no grade 3 or 4 adverse event was reported.

Plasma dolutegravir, elvitegravir and cobicistat pharmacokinetics

GM plasma concentration versus time curves for dolutegravir, elvitegravir and cobicistat are shown in Figure 1, and PK parameters summarized in Table 1.

Dolutegravir plasma pharmacokinetics

The GM terminal elimination $t_{1/2}$ for dolutegravir was 23.1 h (90% CI 19.7–26.6 h). This value was higher than the $t_{1/2}$ measured over the dosing interval of 24 h (GM 14.3 h; 90% CI 12.9–15.7 h).

The PA IC_{90} for dolutegravir is 64 ng/mL.[8] GM plasma dolutegravir concentrations were measured above this value at 24, 36, and 48 h post drug intake cessation (Table 2). At 60 and 72 h post drug intake cessation, 16 out of 17 subjects had dolutegravir concentrations above the IC_{90} (Table 2). At 96 h post dose, dolutegravir GM concentration fell below the IC_{90} (52.2 ng/mL, range 6.9–153.0 ng/mL), with four subjects remaining above the IC_{90} (Table 2).

Elvitegravir plasma pharmacokinetics

Elvitegravir GM terminal elimination $t_{1/2}$ to the last measurable concentration was 5.2 h (90% CI 4.7–6.1 h), which was lower than the $t_{1/2}$ measured over the dosing interval of 24 h (GM 10.8 h, 90% CI 9.7–13.0 h).

The suggested PA IC_{95} for elvitegravir is 45 ng/mL.[9] All subjects had elvitegravir concentrations above the IC_{95} at 24 h post dose. Elvitegravir GM plasma concentration was above the IC_{95} 36 h post drug cessation (GM 57 ng/mL, range 11–296 ng/mL); however, only 11/17 subjects had elvitegravir concentrations above the IC_{95}. The elvitegravir GM concentration fell below the IC_{95} at 48, 60 and 72 h post drug intake cessation, and elvitegravir concentrations were below the LLOQ in all study participants at 96 h post the final dose (Table 2).

Cobicistat plasma pharmacokinetics

The GM terminal elimination $t_{1/2}$ to the last measurable concentration for cobicistat was 18.2 h (90% CI 16.2–26.0 h). This value

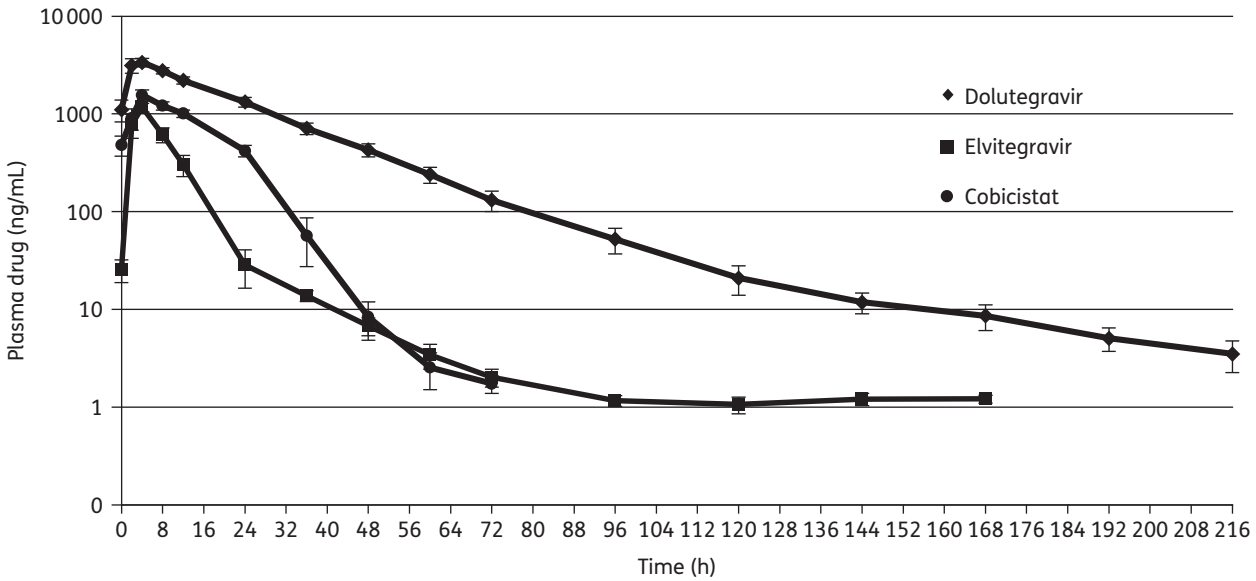

Figure 1. GM drug concentration-time curves of dolutegravir, elvitegravir and cobicistat. Vertical bars represent 90% CIs.

Table 1. Plasma dolutegravir and elvitegravir/cobicistat PK parameters

	Dolutegravir		Elvitegravir		Cobicistat	
PK parameters	GM (90% CI)	CV%	GM (90% CI)	CV%	GM (90% CI)	CV%
T_{max} (h)	3.1 (2.4–3.9)	55	4.5 (4.1–4.6)	39	3.1 (2.9–3.7)	30
C_{max} (ng/mL)	3908 (3571–4245)	21	1675 (1557–1884)	24	127 (1184–1437)	24
AUC_{0-24} (ng·h/mL)	55505 (51368–59642)	18	22965 (21483–25592)	22	10686 (9692–12522)	32
C_{24} (ng/mL)	1324 (1178–1470)	27	419 (387–501)	32	28 (24–48)	85
C_{48} (ng/mL)	427 (362–499)	35	8 (8–15)	78	7 (6–9)	47
$t_{1/2}$ (0–24) (h)	14.3 (12.9–15.7)	23	10.8 (9.7–13.0)	31	3.54 (3.3–3.9)	20
$t_{1/2}$ (last) (h)	23.1 (19.7–26.6)	16	5.2 (4.7–6.1)	18	18.2 (16.2–26.0)	57

C_{48}, 48 h post-dose concentration.

Table 2. Summary of dolutegravir and elvitegravir concentrations (expressed as GM) and detectability at significant timepoints

Time after last dose (h)	Variable	Dolutegravir (IC_{90} 64 ng/mL)	Elvitegravir (IC_{95} 45 ng/mL)
24	GM concentration (ng/mL)	1324	419
	Proportion detectable in plasma	100% (17/17)	100% (17/17)
	Proportion above IC_{90} or IC_{95}	100% (17/17)	100% (17/17)
36	GM concentration (ng/mL)	711	57
	Proportion detectable in plasma	100% (17/17)	100% (17/17)
	Proportion above IC_{90} or IC_{95}	100% (17/17)	65% (11/17)
48	GM concentration (ng/mL)	427	8.3
	Proportion detectable in plasma	100% (17/17)	94% (16/17)
	Proportion above IC_{90} or IC_{95}	100% (17/17)	0%
60	GM concentration (ng/mL)	240	2.5
	Proportion detectable in plasma	100% (17/17)	76% (13/17)
	Proportion above IC_{90} or IC_{95}	94% (16/17)	0%
72	GM concentration (ng/mL)	131	1.7
	Proportion detectable in plasma	100% (17/17)	53% (9/17)
	Proportion above IC_{90} or IC_{95}	94% (16/17)	0%
96	GM concentration (ng/mL)	52.2	—
	Proportion detectable in plasma	100% (17/17)	0%
	Proportion above IC_{90} or IC_{95}	23.5% (4/17)	—

was higher than the $t_{1/2}$ measured over the dosing interval of 24 h (GM 3.5 h, 90% CI 3.3–3.9).

Discussion

We report here the steady-state plasma pharmacokinetics of dolutegravir at 50 mg once daily and elvitegravir at 150 mg once daily boosted by cobicistat (150 mg) over 216 h following drug intake cessation in 17 male and female healthy volunteers. Our data fully characterize for the first time the PK forgiveness of the two newest InSTIs.

Following achievement of steady-state, GM dolutegravir concentrations remained above the suggested plasma PA IC_{90} of 64 ng/mL for up to 72 h post drug intake cessation, with most subjects (94%) showing concentrations above the PA IC_{90} at this time. The GM concentration for elvitegravir was above the suggested PA IC_{95} of 45 ng/mL at 24 and 36 h post drug cessation, with 65% of participants above this cut-off at the latter timepoint, but it had fallen below the PA IC_{95} by 48 h post dose (with no participant above this cut-off then). It is important to note that these agents have no established minimum effective therapeutic concentrations. Being above the (partially PA) *in vitro* PA IC_{95} does not imply that the exposures are sufficient for a fully effective *in vivo* drug exposure, especially when optimal drug exposures are needed during induction of virological control.

Plasma interindividual variability (CV) in dolutegravir and elvitegravir C_{24} was 27% and 32%, respectively. These values are lower than those measured for the third available InSTI, raltegravir (53%–220%).[10]

Interestingly, whilst dolutegravir is not dependent on a booster and concentrations are persistent in the systemic circulation for a prolonged period, concentrations of elvitegravir were observed to drop when the concentrations of its booster cobicistat fell below a certain level. The terminal $t_{1/2}$ (0 to 216 h) was longer than the $t_{1/2}$ within the dosing interval (0–24 h) for dolutegravir, whilst the opposite was true for elvitegravir (23.1 versus 14.3 h and 5.2 versus 10.8 h, respectively). One explanation could be that this phenomenon is due to saturation of metabolic processes at higher concentrations, meaning a change in rate of CL as cobicistat concentrations fall to non-saturating levels.

Of note, the single-tablet formulations of dolutegravir and elvitegravir contain partner NRTIs of varying $t_{1/2}$ values. We have previously shown the plasma $t_{1/2}$ of abacavir and lamivudine to be 3–4 h and 5.7 h, respectively, with intracellular half-lives of the active triphosphorylated metabolite of abacavir (carbovir) and the active triphosphorylated metabolite of lamivudine to be 14.1 h and 19 h, respectively.[11] Exposures differ between male and female subjects. The longer $t_{1/2}$ of tenofovir and emtricitabine, both in plasma and PBMC (31 h and 37 h and 164 h and 39 h, respectively),[2–5] may also be important to the clinical forgiveness of these regimens and the specific resistance mutations that are observed at failure. Dolutegravir is currently available in co-formulation with abacavir and lamivudine; it is interesting to note that the $t_{1/2}$ of carbovir and the active triphosphorylated metabolite of lamivudine match dolutegravir's $t_{1/2}$ both within the dosing interval (14 h) and to the last measurable concentration (23 h).

In patients with chronic diseases, poor adherence to medications has been shown to be common,[12] and in the context of HIV infection, the potential repercussions can be serious since if drug concentrations drop to sub-therapeutic levels after missed doses, there is a risk of emergence of drug-resistant HIV strains, which limit future therapeutic options. Great efforts are made to support and encourage patients with respect to the importance of adherence, but it is often unclear how delayed a dose was or how many doses can be omitted before efficacy is lost. Therefore, identifying persistence of therapeutic concentrations is essential for robust patient care. Additionally, understanding the PK attributes of a drug and, more specifically, PK forgiveness can also allow identification of potential candidates for pre-exposure prophylaxis and alternative treatment strategies where optimal dosing frequency needs to be characterized.

Nevertheless, one of the limitations of this study is that it was carried out in healthy volunteers to avoid dose delays in patients infected with HIV. As such, PK/pharmacodynamic conclusions or speculation on what *in vivo* concentrations are needed to maintain efficacy cannot be robustly drawn. Ideally, pharmacodynamic data are required to draw definite conclusions on how late a drug dose can be or on whether drug doses can be missed without the risk of failure.

Indeed, discrepancies in antiretroviral drug pharmacokinetics between healthy volunteers and people living with HIV have been previously described, particularly for the PIs.[13] Such differences are thought to be related to physiological variability in several parameters between the two populations, including CYP450 activity and α-1-acid glycoprotein expression.[13] Since dolutegravir and elvitegravir are new drugs, data on differences in concentrations between healthy volunteers and HIV-infected subjects have not been presented to date. However, today there are no data indicating any significant difference in pharmacokinetics of dolutegravir or elvitegravir between healthy and HIV-infected subjects.

A further limitation of the study is the use of cut-off values calculated from *in vitro* data to define therapeutic concentrations *in vivo*. However, minimum effective concentrations of dolutegravir and elvitegravir have not been established, and the PA IC_{90} and PA IC_{95} are currently the only surrogates available to define efficacy in the context of drug exposure.

Additionally, the same *in vitro* threshold is not available for both drugs, the PA IC_{90} and PA IC_{95}, for dolutegravir and elvitegravir respectively, are today the only reference values available to define drug efficacy in the context of drug exposure.

Finally, dolutegravir was administered alone in this study, whilst elvitegravir/cobicistat were co-administered as part of a single tablet combination therapy with tenofovir/emtricitabine. Dolutegravir administered within an abacavir/lamivudine fixed combination was considered not justified, based on the risk of abacavir hypersensitivity in HIV-negative subjects[14] and the known lack of effect of backbone NRTIs on the pharmacokinetics of either elvitegravir or dolutegravir.

Of note, it is not impossible that a minority of patients had residual exposure to low-dose dolutegravir at the start of the elvitegravir phase of our study, but this exposure is likely to be minimal, as PK sampling for elvitegravir was carried out 19 days after the last dose of dolutegravir and, importantly, dolutegravir has no known impact on elvitegravir/cobicistat metabolic pathways.

In conclusion, our data show that there are marked differences in the elimination rates of dolutegravir and elvitegravir following treatment interruption. These differences are likely to affect the extent to which drug doses can be delayed or missed, and they suggest that clinical differences may emerge in patients who have suboptimal adherence. However, the net risk or benefit of these elimination characteristics depends very much upon all the components of the regimen.

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References

1 Blanco JL, Whitlock G, Milinkovic A *et al*. HIV integrase inhibitors: a new era in the treatment of HIV. *Expert Opin Pharmacother* 2015; **16**: 1313–24.

2 Perry CM. Elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate single-tablet regimen (Stribild®): a review of its use in the management of HIV-1 infection in adults. *Drugs* 2014; **74**: 75–97.

3 Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the Use of Antiretroviral Agents in HIV-1-infected Adults and Adolescents*. Department of Health and Human Services. http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf.

4 Brunton LL, Lazo JS, Parker KL. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. New York: McGraw-Hill, 2006.

5 Cohen CJ, Colson AE, Sheble-Hall AG *et al*. Pilot study of a novel short-cycle antiretroviral treatment interruption strategy: 48-week results of the five-days-on, two-days-off (FOTO) study. *HIV Clin Trials* 2007; **8**: 19–23.

6 Butler KM. ART with weekends off is noninferior to continuous ART in young people on EFV+2NRTI. In: *Abstracts of the 2015 Conference on Retroviruses and Opportunistic Infections (CROI 2015), Seattle, WA*, 2015. Oral abstract 38LB. http://www.croiconference.org. International Antiviral Society–USA, San Francisco, CA, USA.

7 Else L, Watson V, Tjia J *et al*. Validation of a rapid and sensitive high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010; **878**: 1455–65.

8 Cottrell ML, Hadzic T, Kashuba AD. Clinical pharmacokinetic, pharmacodynamic and drug-interaction profile of the integrase inhibitor dolutegravir. *Clin Pharmacokinet* 2013; **52**: 981–94.

9 Ramanathan S, Mathias AA, German P *et al*. Clinical pharmacokinetic and pharmacodynamic profile of the HIV integrase inhibitor elvitegravir. *Clin Pharmacokinet* 2011; **50**: 229–44.

10 Brainard DM, Wenning LA, Stone JA *et al*. Clinical pharmacology profile of raltegravir, an HIV-1 integrase strand transfer inhibitor. *J Clin Pharmacol* 2011; **51**: 1376–402.

11 Moyle G, Boffito M, Fletcher C *et al*. Steady-state pharmacokinetics of abacavir in plasma and intracellular carbovir triphosphate following administration of abacavir at 600 milligrams once daily and 300 milligrams twice daily in human immunodeficiency virus-infected subjects. *Antimicrob Agents Chemother* 2009; **53**: 1532–8.

12 Coleman CI, Roberts MS, Sobieraj DM *et al*. Effect of dosing frequency on chronic cardiovascular disease medication adherence. *Curr Med Res Opin* 2012; **28**: 669–80.

13 Dickinson L, Khoo S, Back D. Differences in the pharmacokinetics of protease inhibitors between healthy volunteers and HIV-infected persons. *Curr Opin HIV AIDS* 2008; **3**: 296–305.

14 Ziagen (abacavir). *Summary of Product Characteristics*. London: ViiV Healthcare UK Ltd. 2 July 2015.