ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2009, p. 1937–1943 Vol. 53, No. 5
0066-4804/09/$08.00+0 doi:10.1128/AAC.01064-08


# Pilot Pharmacokinetic Study of Human Immunodeficiency Virus-Infected Patients Receiving Tenofovir Disoproxil Fumarate (TDF): Investigation of Systemic and Intracellular Interactions between TDF and Abacavir, Lamivudine, or Lopinavir-Ritonavir▽

Alain Pruvost,[1]* Eugènia Negredo,[2] Frédéric Théodoro,[1] Jordi Puig,[2] Mikaël Levi,[1,3] Rafaela Ayen,[4] Jacques Grassi,[1] and Bonaventura Clotet[2,4]

*CEA, iBiTecS, SPI, Laboratoire d'Etude du Métabolisme des Médicaments, Gif sur Yvette, F-91191,[1] and SPI-BIO, Parc d'Activité du Pas du Lac, 10 bis avenue Ampère, F-78180 Montigny le Bretonneux,[3] France, and Lluita contra la SIDA Foundation[2] and Irsicaixa Foundation,[4] Germans Trias i Pujol University Hospital, Badalona, Barcelona, Spain*

Received 7 August 2008/Returned for modification 20 October 2008/Accepted 23 February 2009

**Previous work has demonstrated the existence of systemic interaction between tenofovir (TFV) disoproxil fumarate (TDF) and didanosine as well as between TDF and lopinavir-ritonavir (LPV/r). Here we investigated TDF interactions with the nucleoside reverse transcriptase inhibitors (NRTIs) lamivudine (3TC) and abacavir (ABC), comparing both the concentrations of nucleoside/nucleotide reverse transcriptase inhibitors in plasma and the intracellular concentrations of their triphosphate metabolites (NRTI-TP) for human immunodeficiency virus-infected patients receiving these NRTIs with TDF and after 4 weeks of TDF interruption. We also looked at interactions between TDF–ABC and LPV/r, comparing patients receiving or not receiving LPV/r. Blood samples were taken at baseline and at 1, 2, and 4 h after dosing. Liquid chromatography-tandem mass spectrometry was used to measure NRTIs and NRTI-TPs. Statistical analyses were performed on pharmacokinetic parameters: the area under the concentration-time curve from 0 to 4 h ($AUC_{0-4}$), the maximum concentration of the drug ($C_{max}$), and the residual concentration of the drug at the end of the dosing interval ($C_{trough}$) for plasma and the $AUC_{0-4}$ and $C_{trough}$ for intracellular data. Among the groups of patient discontinuing TDF, the very long intracellular half-life of elimination (150 h) of TFV-DP (the diphosphorylated metabolite of TFV, corresponding to a triphosphorylated species) was confirmed. Comparison between groups as well as the longitudinal study showed no significant systemic or intracellular interaction between TDF and ABC or 3TC. Significant differences were observed between patients receiving LVP/r and those receiving nevirapine. For ABC, plasma exposure was decreased (40%) under LVP/r, while, in contrast, plasma exposure to TFV was increased by 50% and the intracellular TFV-DP $AUC_{0-4}$ was increased by 59%. A trend for a gender effect was observed for TFV-DP at the intracellular level, with higher and $C_{trough}$ values for women.**

Highly active antiretroviral therapy regimens include at least three antiretroviral agents combining a nucleoside/nucleotide reverse transcriptase inhibitor [N(t)RTI] backbone with a protease inhibitor (PI) or a nonnucleoside reverse transcriptase inhibitor (NNRTI) (51). The issue of drug-drug interaction between NRTIs, including N(t)RTIs, and also between N(t)RTIs and PIs or NNRTIs needs to be addressed (37).

All these drugs or prodrugs could interact at the systemic level (absorption, metabolism, distribution, or elimination) or at the cellular level through membrane transport, or through intracellular metabolism for the N(t)RTIs.

Several in vitro and in vivo intracellular interactions have been reported between NRTIs sharing the same metabolic pathways for thymidine analogues (14, 17) and for cytidine analogues (24).

More recently, a significant interaction between didanosine (ddI) and tenofovir (TFV) disoproxil fumarate (TDF) has been clinically demonstrated (6, 23, 29, 32), showing a higher plasma exposure to ddI when it is combined with TDF than when it is given alone. As a result, the ddI dosage was reduced to 250 mg in combination with TDF, allowing plasma to reach the same exposure to ddI as that obtained when 400 mg ddI was given alone (i.e., without TDF). Intracellular investigation of this new combination (35) has shown no significant difference between the levels of ddATP (the triphosphate metabolite of ddI) reached with 250 mg of ddI in combination with TDF and those reached with 400 mg of ddI without TDF, further proving that the dose adjustment of ddI was correct.

To explain the ddI-TDF interaction, an interesting hypothesis based on the inhibition of the purine nucleoside phosphorylase (PNP) by TFV phosphate was proposed (39). This enzyme, which is involved in the catabolism of the purine bases inosine and guanine, when inhibited by phosphorylated forms of TFV, would lead to increased levels of ddI (a purine analogue) in addition to increased concentrations of GTP and consequently dGTP (2). The corresponding increased levels of dGTP might be responsible for a decrease in ribonucleotide reductase activity and for mitochondrial DNA damage, which would be the main cause of the $CD4^+$ cell depletion reported when TDF and ddI are combined (3, 30). Nevertheless, recent

* Corresponding author. Mailing address: CEA, IBiTec-S, Service de Pharmacologie et d'Immunoanalyse, PC no. 18, F-91191 Gif sur Yvette, France. Phone: 33 1 69081312. Fax: 33 1 69085907. E-mail: alain.pruvost@cea.fr.

▽ Published ahead of print on 9 March 2009.

in vitro data showed no alteration in endogenous deoxynucleoside triphosphate (dNTP) pools by TDF (41, 46).

The aim of this pilot study was first to explore possible interactions between TDF and nucleoside reverse transcriptase inhibitors (NRTIs) other than ddI (i.e., lamivudine [3TC] and abacavir [ABC]) and also to confirm a previous observation regarding a potential interaction between TDF and lopinavir-ritonavir (LPV/r) (34). Just after the initiation of this pilot study, a publication reporting the intracellular pharmacokinetics of a triple-nucleoside regimen in human immunodeficiency virus (HIV)-infected patients (15), trying to explain the early lack of virologic response to this combination (12), showed no intracellular drug interaction between TDF, ABC, and 3TC. Finally, it was also reported in other work (22) that coadministration of TDF and LPV/r resulted in increased TFV exposure at the plasma level.

In this study, by using validated liquid chromatography-tandem mass spectrometry (LC–MS-MS) techniques, we confirmed the previously reported lack of interaction between 3TC, ABC, and TDF at both the plasma and the intracellular level. We also evaluated, in HIV-infected patients, the interaction between TDF and LPV/r at both the plasma and the intracellular level; previously, this interaction was described for healthy volunteers at the plasma level only. As secondary objectives, we investigated possible interactions between 3TC, ABC, and LPV/r or nevirapine (NVP) at the plasma and intracellular levels. Finally, on the basis of the hypothesis of PNP inhibition by TFV phosphate, we measured and compared the intracellular dGTP concentrations between groups of patients.

(The data reported in this study were presented in part as poster and abstract 56 at the 8th International Workshop on Clinical Pharmacology of HIV Therapy, Budapest, Hungary, 16 to 18 April 2007.)

## MATERIALS AND METHODS

**Patients.** The study enrolled subjects who had been receiving a triple highly active antiretroviral therapy regimen for more than 42.2 (±8.2) months, including 300 mg of TDF once daily, taken with food; 300 mg of 3TC once daily or 300 mg of ABC twice daily; and an NNRTI (NVP; 400 mg once daily) or a PI (LPV/r; 400 and 100 mg, respectively, twice daily). Patients were seen consecutively between January 2005 and August 2005 in the HIV Unit of Germans Trias i Pujol University Hospital (Badalona, Spain). Patients who consented were included in the study and were classified depending on the NRTI combination. Exclusion criteria were suspected improper drug adherence, treatment discontinuation, or treatment changes during the previous 3 months.

Patients were asked to come fasting to the clinical unit and then took antiretroviral therapy after the first blood draw (baseline). After that, they ate a breakfast consisting of a mini-sandwich and coffee or juice.

**Study design.** This was a single-center, pilot cross-sectional study followed by a longitudinal prospective study (after 4 weeks of TDF interruption).

**(i) Cross-sectional study.** The four groups were assigned treatment regimens as follows: group 1 (seven patients, five male and two female) received TDF, 3TC, and LPV/r; group 2 (eight patients, five male and three female) received TDF, 3TC, and NVP; group 3 (seven patients, five male and two female) received TDF, ABC, and LPV/r; and group 4 (five patients, all male) received TDF, ABC, and NVP.

**(ii) Longitudinal study of patients discontinuing TDF.** TDF was withdrawn for 4 weeks in order to assess the pharmacokinetic (PK) interactions between TDF and ABC or between TDF and 3TC, only for patients receiving LPV/r (groups 1 and 3), in order to avoid viral rebound during the dual therapy. For groups 1 and 3, measurements of plasma ABC or 3TC levels and intracellular carbovir triphosphate (CBV-TP) or 3TC-TP levels were repeated after this 4-week interruption.

Blood samples were taken from all subjects at baseline (just before dosing) and at 1, 2, and 4 h postdosing.

**Chemicals.** 3TC, 3TC-TP, ABC, and CBV-TP were from GlaxoSmithKline (Research Triangle Park, NC). TFV and TFV-DP (the diphosphorylated metabolite of TFV, corresponding to a triphosphorylated species) were from Gilead Sciences (Foster City, CA). 2-Chloroadenosine and 2-chloro-ATP, both used as internal standards (IS), were from Sigma-Aldrich (St Quentin-Fallavier, France).

**Analytical methodology and performance. (i) NRTIs in plasma.** Calibration standards were made in blank human plasma (Biopredic, Rennes, France) at concentrations ranging from the lower limit of quantification (LLOQ), 5.0 ng/ml, to the upper limit of quantification, 1,500 ng/ml, with quality controls at 15.0 (low), 800 (intermediate), and 1,400 (high) ng/ml. On the day of analysis, samples were thawed; 50 μl of IS solution (3,750 ng/ml in water) was added to a 200-μl aliquot of plasma diluted with 200 μl of 0.1 N HCl in a polypropylene tube; and the mixture was vortex mixed. Solid-phase extraction was performed on Oasis MCX 60-mg, 3-ml cartridges (Waters, Saint-Quentin-en-Yvelines, France) using a positive-pressure processor (Speedisk 48; Mallinckrodt Baker, Paris, France). After the cartridge was washed with 2 ml of acetonitrile and conditioned with 2 ml of water, the sample (450 μl) was loaded under low pressure. Cartridges were washed first with 2 ml of 0.1 N HCl and then with 2 ml of acetonitrile and were dried under a stream of nitrogen for 30 s. Analytes were eluted with a threefold 500-μl methanol–2% ammonia solution. The extract sample was evaporated to dryness at 50°C in a Turbovap LV evaporator (Zymark, Roissy, France). Extracts reconstituted with 200 μl of mobile phase A (see below) were held at +4°C in the autosampler, and 10 μl was injected into the LC–MS-MS system (Alliance 2795–Quattro Micro; Waters, France). The LC assay method coupled with spectrometric detection (MS-MS) for the simultaneous determination of 3TC, ABC, and TFV in human plasma has been validated and described previously (27). To sum up, LC separation was achieved on a Synergi Polar-RP 4-μm, 50- by 2-mm column (Phenomenex, France) maintained at around 40°C. Gradient analysis was performed from 98% water–formic acid (99.5:0.5, vol/vol) (mobile phase A) up to 80% methanol–formic acid (99.5:0.5, vol/vol) in 4 min at a flow rate of 300 μl/min. MS-MS detection was performed after positive electrospray ionization, with monitoring of ion transitions 230.2→111.7, 287.2→191.1, 288.1→176.03, and 302.05→169.88 for 3TC, ABC, TFV, and 2-chloroadenosine, respectively. The calibration curves were fitted to a $1/x^2$-weighted quadratic regression model for 3TC and ABC. For TFV, a $1/x^2$ weighted linear regression model was used. The LLOQ was 5 ng/ml when 200 μl of the biological sample was used (50 pg on the column). Mean extraction recoveries over the entire calibrated range were 88.7% ± 4.9%, 85.3% ± 3.8%, and 80.3% ± 3.8% for ABC, 3TC, and TFV, respectively. Mean ionization recoveries were 92.7% ± 2.5%, 91.5% ± 13.4%, and 86.3% ± 5.9% for ABC, 3TC, and TFV, respectively. Extraction and ionization recoveries were 92 and 102%, respectively, for the IS at working concentrations. The accuracy and precision values for intra-and interanalytical runs met the international requirements (48). Stability experiments performed with plasma (for 4 h at room temperature and after three freeze-thaw cycles) and processed samples (72 h at +4°C in the autosampler) gave precision and accuracy results within the regulatory limits (48).

**(ii) NRTI-TPs in peripheral blood mononuclear cells (PBMCs).** The purity of the master stock solutions was checked using the same chromatographic system previously described (4) coupled with UV detection (λ, 254 nm). For the simultaneous determination of 3TC-TP, CBV-TP, and TFV-DP levels, the validated LC–MS-MS assay method previously reported (36) was used. For dGTP measurement, a previously described method (16) was used, slightly modified according to the work of Pruvost et al. (36) for LC and MS detection.

**Clinical subject samples.** After thawing at room temperature, patients' plasma samples were treated in the same manner as the calibration standards and quality control samples, as described above. PBMCs collected from 7 to 8 ml of blood from treated patients were kept frozen at −80°C. PBMCs were transferred on dry ice to the analytical laboratory and were subjected to the same treatment as previously described (36).

**Cell counting.** The PBMCs of each clinical sample were counted in our laboratory using a validated biochemical test as previously described (5).

**PK parameters and statistical analysis.** The area under the concentration-time curve from 0 h to the last sampling time, 4 h ($AUC_{0-4}$), was calculated for both N(t)RTIs and NRTI-TPs by using WinNonlin Professional software (version 3.1; Pharsight Corporation, CA). A noncompartmental analysis program and a linear trapezoidal rule were used to compute the AUC. For plasma, the highest of the concentrations of the drug found at the four sampling times was considered the maximum concentration of the drug ($C_{max}$) in plasma and was used for comparison. Since for ABC, 3TC, and TFV, the time to maximum concentration of the drug is around 1 h, we were in the terminal phase of elimination at 4 h. Under these conditions, the $AUC_{0-4}$ was a representative part of the total AUC, which can be used for comparison. Owing to the particularly flat intracellular PK profiles of these NRTI-TPs, $C_{max}$ was not used for compar-

isons of NRTI-TPs. The residual concentration of the drug at the end of the dosing interval ($C_{trough}$) was also used and was the first concentration read at time zero just before the new dosing. Since dGTP is an endogenous compound with low intra-variability (here 16%), the mean concentration for the four sampling times was calculated and used for comparisons between groups. GraphPad Prism (version 5.01; GraphPad Software Inc., CA) was used to report descriptive statistical values such as means, coefficients of variation, geometric least squares means, and 95% confidence intervals. Statistical comparisons by treatment group using the PK parameters discussed above were performed using SigmaStat software (version 3.0.1; SPSS Inc., IL). According to the situation, one-way analysis of variance (Kruskal-Wallis one-way analysis of variance on ranks when the normality test failed), a paired *t* test, or two way analysis of variance was used. Statistical comparisons were accepted as significant at a $P$ value of $<0.05$.

## RESULTS

Twenty-seven patients were included in the cross-sectional part of the study: seven in group 1, eight in group 2, seven in group 3, and five in group 4. Fourteen of these patients (groups 1 and 3) also participated in the longitudinal study. Most patients were men (26% were women), and the mean age was 43.8 ± 9.5 years, with no differences between treatment groups (the mean age of each group differs less than 7.5% from the global mean). All participants were Caucasian. The mean CD4 cell count was 650 ± 367/mm$^3$ (the mean CD4 cell count of each group differs less than 14.2% from the global mean), and all participants maintained viral suppression (<50 copies/ml) throughout the study. On entering into the study, none of the participants presented with an acute infection or another opportunistic disease. The mean (standard deviation) baseline plasma creatinine level was 99.5 (24.5) μmol/liter. For patients participating in the prospective study, no changes were observed at the end of the study, with a mean (standard deviation) plasma creatinine level of 91.9 (24.4) μmol/liter.

**Plasma and intracellular interactions.** To study the possible plasma and intracellular interactions between NRTIs and TDF as well as between NRTIs and the third antiretroviral agent (LPV/r or NVP), we measured the concentrations of both NRTIs in plasma (272 results in 164 plasma samples) and those of NRTI-TPs within the cells (in PBMC samples). Assay performance measurements gave results similar to those obtained during the validation (27, 36). For NRTIs, mean precision and accuracy ranged from 3.3 to 12.8% and from 92.3 to 114%, respectively. For NRTI-TPs, mean precision and accuracy ranged from 9.5 to 19% and from 91.6 to 102%, respectively.

**Effects of TDF on NRTIs and NRTI-TPs in the presence of LPV/r.** To evaluate the impact of TDF on the pharmacokinetics of NRTIs, the PK parameters of group 1 (TDF–3TC–LPV/r) for 3TC and 3TC-TP were compared (by a paired *t* test) with those of the same patients discontinuing TDF. Geometric means (GM) for the 3TC $AUC_{0-4}$, $C_{max}$, and $C_{trough}$ in plasma for group 1 with and without TDF were 5,636 and 6,896 h · ng/ml, 2,360 and 2,448 ng/ml, and 107.3 and 101.6 ng/ml, respectively, showing no significant difference. Comparison of the GM of the intracellular 3TC-TP $AUC_{0-4}$ and $C_{trough}$ (48.2 and 58.0 h · pmol/10$^6$ cells and 10.4 and 12.4 pmol/10$^6$ cells, respectively) led to the same conclusion. Similar observations were made for plasma ABC and intracellular CBV-TP PK parameters by comparing group 3 (TDF–ABC–LPV/r) with group 3 discontinuing TDF. ABC $AUC_{0-4}$, $C_{max}$, and $C_{trough}$ GM were 3,202 and 2,842 h · ng/ml, 1,510 and 1,621 ng/ml, and 57.7 and 36.3 ng/ml, respectively, and CBV-TP $AUC_{0-4}$ and $C_{trough}$ GM were 1,388 and 1,022 h · fmol/10$^6$ cells and 296.1 and 212.3 fmol/10$^6$ cells, respectively, showing no statistically significant difference. It is worth noting that for the two groups discontinuing TDF, after 4 weeks, no quantifiable level of TFV was found in the plasma samples. In contrast, 48% of the PBMC samples (27/56) had quantifiable concentrations of TFV-DP (median, 17 fmol/10$^6$ cells; range, 7 to 39 fmol/10$^6$ cells), allowing us to calculate an intracellular half-life of elimination. The other half of the patients presented intracellular TFV-DP levels that were detectable but below the LLOQ. To include these patients, very likely associated with a shorter TFV-DP half-life, in the analysis, the LOQ/2 value according to their cell counts was also used to evaluate the half-life of TFV-DP; the median half-life was estimated as 150 h, with a range of 120 to 266 h.

**Effects of NRTIs (3TC and ABC) on TFV and TFV-DP.** The effects of 3TC and ABC on the pharmacokinetics of both TFV and TFV-DP were evaluated by comparing group 1 (TDF–3TC–LPV/r) with group 3 (TDF–ABC–LPV/r) and group 2 (TDF–3TC–NVP) with group 4 (TDF–ABC–NVP). The GM of the $AUC_{0-4}$, $C_{max}$, and $C_{trough}$ of TFV for groups 1 and 3 were 962.0 and 845.9 h · ng/ml, 356.0 and 290.2 ng/ml, and 90.6 and 86.3 ng/ml, respectively, and the GM of the $AUC_{0-4}$ and $C_{trough}$ of TFV-DP were 1,150 and 839.8 h · fmol/10$^6$ cells and 247.4 and 213.9 fmol/10$^6$ cells, respectively, showing no significant difference between groups 1 and 3. The same observation was made in the comparison of group 2 versus group 4; the GM of the $AUC_{0-4}$, $C_{max}$, and $C_{trough}$ of TFV for groups 2 and 4 were reported as 581.8 and 637.7 h · ng/ml, 248.6 and 232.4 ng/ml, and 55.4 and 45.8 ng/ml, respectively, while the GM of the $AUC_{0-4}$ and $C_{trough}$ of TFV-DP were 651.9 and 564.2 h · fmol/10$^6$ cells and 176.2 and 108.5 fmol/10$^6$ cells, respectively.

**Effect of LPV/r or NVP on NRTIs and NRTI-TPs.** As shown from the data presented above, we can observe apparent differences in the PK parameters of both TFV and TFV-DP between regimens including LVP/r or NVP. As a result, the effects of LPV/r and NVP on ABC, 3TC, and TFV and their corresponding triphosphate metabolites were investigated. For this purpose, comparisons were performed between groups 1 and 2, groups 3 and 4, and groups 1 plus 3 and 2 plus 4 for plasma and intracellular PK parameters of 3TC and 3TC-TP, ABC and CBV-TP, and TFV and TFV-DP, respectively. PK parameters are reported in Tables 1 and 2 for NRTIs and NRTI-TPs, respectively. For 3TC and 3TC-TP, no significant differences were evidenced. For ABC (group 3 versus group 4), the $C_{max}$ and $AUC_{0-4}$ were decreased by 46% ($P = 0.012$) and 40% ($P = 0.048$), respectively, when ABC was dosed with LVP/r compared to NVP. At the PBMC level, intracellular PK parameters for CBV-TP showed an opposite trend, but it did not reach statistical significance. In contrast, for TFV (groups 1 and 3 versus groups 2 and 4), the $C_{trough}$, $C_{max}$, and $AUC_{0-4}$ were increased by 72% ($P = 0.013$), 33% ($P = 0.033$), and 50% ($P = 0.026$), respectively, when TDF was dosed with LVP/r compared to NVP. This difference was also seen for TFV-DP at the PBMC level, where the $C_{trough}$ and $AUC_{0-4}$ were 57% and 59% higher, respectively, with TDF–LVP/r than with TDF–NVP.

**dGTP concentrations.** Comparison of mean intracellular dGTP concentrations in the same patients with and without

TABLE 1. Plasma PK parameters of lamivudine, abacavir, and tenofovir in the presence of lopinavir-ritonavir or nevirapine

| Drug | No. of patients | PK parameter[a] | | |
|---|---|---|---|---|
| | | $AUC_{0-4}$ (ng · h/ml) | $C_{max}$ (ng/ml) | $C_{trough}$ (ng/ml) |
| 3TC | | | | |
| With LPV/r | 7 | 5,748 (3,932–7,365) | 2,241 (2,014–2,299) | 161 (62.0–178) |
| With NVP | 8 | 4,974 (3,707–7,414) | 1,850 (1,666–2,599) | 84.3 (55.6–99.1) |
| GMR[b] (*P* value) | | 1.11 (0.491) | 1.21 (0.121) | 1.45 (0.232) |
| ABC | | | | |
| With LPV/r | 7 | 3,630 (2,248–3,762) | 1,783 (954.9–1,992) | 42.4 (32.7–181) |
| With NVP | 5 | 5,257 (3,868–7,802) | 2,665 (2,173–3,718) | 76.3 (56.5–135) |
| GMR (*P* value) | | 0.60 (0.048) | 0.54 (0.012) | 0.71 (0.343) |
| TFV | | | | |
| With LPV/r | 14 | 1,006 (612–1,344) | 349.5 (228.2–453.4) | 96.7 (54.6–119) |
| With NVP | 13 | 582.7 (456.7–811.7) | 252.1 (195.6–306.3) | 54.4 (33.5–62.1) |
| GMR (*P* value) | | 1.50 (0.026) | 1.33 (0.033) | 1.72 (0.013) |

[a] Given as median (interquartile range).
[b] GMR, ratio of the GM of the PK parameter of the LPV/r combination to that of the NVP combination.

TDF showed no effect of TDF on the intracellular concentration of dGTP: the GM for group 1 with and without TDF were 117 and 127 fmol/$10^6$ cells, respectively, and the GM for group 3 with and without TDF were 88 and 99 fmol/$10^6$ cells, respectively. Moreover, no gender effect was observed.

**Gender effect.** In order to detect a possible gender effect in the results described above, a two-way analysis of variance taking into account two factors, gender and associated treatment (LPV/r or NVP), was performed. PK parameters are reported in Table 3. For TFV-DP, a significant gender effect ($P = 0.045$) was evidenced at the intracellular level: $AUC_{0-4}$ values for women were 72% and 41% higher than those for men in the LPV/r and NVP groups, respectively. The relative rank order of mean $AUC_{0-4}$ values for TFV-DP was as follows: highest for women with LPV/r (1,745.2 fmol · h/$10^6$ cells), intermediate and similar for men with LPV/r (921.2 fmol · h/$10^6$

TABLE 2. Intracellular PK parameters of 3TC-TP, CBV-TP, and TFV-DP in the presence of lopinavir-ritonavir or nevirapine

| Drug | No. of patients | PK parameter[a] | |
|---|---|---|---|
| | | $AUC_{0-4}$ (fmol · h/$10^6$ cells) | $C_{trough}$ (fmol/$10^6$ cells) |
| 3TC-TP | | | |
| With LPV/r | 7 | 37,390 (33,730–80,810) | 9,951 (8,001–11,460) |
| With NVP | 8 | 35,850 (26,910–59,250) | 7,628 (6,000–11,320) |
| GMR[b] (*P* value) | | 1.18 (0.397) | 1.16 (0.189) |
| CBV-TP | | | |
| With LPV/r | 7 | 1,346 (927.9–2,234) | 298.8 (219.1–472.8) |
| With NVP | 5 | 654.3 (656.0–1,497) | 129.2 (98.30–298.3) |
| GMR (*P* value) | | 1.45 (0.241) | 1.84 (0.119) |
| TFV-DP | | | |
| With LPV/r | 14 | 915.0 (697.7–1,386) | 222.8 (160.4–376.5) |
| With NVP | 13 | 652.2 (476.4–783.6) | 164.6 (116.5–186.9) |
| GMR (*P* value) | | 1.59 (0.013) | 1.57 (0.042) |

[a] Given as median (interquartile range).
[b] GMR, ratio of the GM of the PK parameter of the LPV/r combination to that of the NVP combination.

TABLE 3. Intracellular PK parameters of 3TC-TP and TFV-DP in men and women in the presence of lopinavir-ritonavir or nevirapine

| Drug | No. of patients | $AUC_{0-4}$ | | $C_{trough}$ | |
|---|---|---|---|---|---|
| | | Mean (fmol · h/$10^6$ cells) | CV[a] (%) | Mean (fmol/$10^6$ cells) | CV (%) |
| 3TC-TP | | | | | |
| With LPV/r | | | | | |
| In women | 2 | 86,710 | 67 | 15,310 | 36 |
| In men | 5 | 43,160 | 49 | 9,112 | 12 |
| GMR[b] | | 1.91 | | 1.64 | |
| With NVP | | | | | |
| In women | 3 | 61,520 | 79 | 14,010 | 86 |
| In men | 5 | 38,330 | 43 | 8,185 | 32 |
| GMR | | 1.40 | | 1.42 | |
| *P* value[c] | | 0.084 | | 0.082 | |
| TFV-DP | | | | | |
| With LPV/r | | | | | |
| In women | 4 | 1,745 | 78 | 315 | 35 |
| In men | 10 | 921.2 | 45 | 233.1 | 54 |
| GMR | 1.72 | | | 1.45 | |
| With NVP | | | | | |
| In women | 3 | 829.3 | 30 | 241.5 | 28 |
| In men | 10 | 590.8 | 27 | 137.7 | 36 |
| GMR | | 1.41 | | 1.84 | |
| *P* value[c] | | 0.045 | | 0.039 | |

[a] CV, coefficient of variation.
[b] GMR, ratio of the GM for women to the GM for men.
[c] By two-way analysis of variance.

cells) and women with NVP (829.3 fmol · h/$10^6$ cells), and lowest for men with NVP (590.8 fmol · h/$10^6$ cells). The same observation was made for $C_{trough}$ values ($P = 0.039$), which were 45% and 84% higher for women than for men in the LPV/r and NVP groups, respectively. No such significantly higher exposure was observed for TFV at the plasma level, despite a trend in $AUC_{0-4}$ and $C_{max}$ in the LPV/r group. Also, no statistically significant difference was observed for 3TC in plasma. A trend toward higher 3TC-TP exposure was observed at the intracellular level. Such a comparison was not performed for CBV-TP, because there were too few patients in this group.

## DISCUSSION

In this study, we have been able to describe detailed systemic and intracellular drug-drug interactions between TDF, ABC, and LPV/r.

These data confirm previous reports showing the lack of interaction between TDF, 3TC, and ABC at the level of plasma (11, 21, 49), as well as the lack of interaction at the intracellular level in vitro (38) and in vivo (15).

Interestingly, the median intracellular half-life of elimination of TFV-DP found in this study, 150 h, provides confirmation of our previous estimate of 180 h (35) and is very close to the value reported by Hawkins et al. (15), around 150 h.

By comparing the LPV/r and NVP groups, we have observed a decrease in ABC exposure (40%) when ABC was combined with LPV/r similar to that recently published by Waters et al. (50), who reported a 32% decrease in plasma ABC exposure following the addition of LPV/r for HIV-infected patients on ABC (600 mg once daily). The mechanism of induction of ABC glucuronidation by ritonavir and/or LPV proposed by Waters et al. on the basis of their own work and previous work (10, 31, 44, 45) seems to be well founded. To our knowledge, PK data for intracellular CBV-TP in the presence and absence of LPV/r were not reported. While a lower plasma ABC concentration was observed in this group receiving ABC–LPV/r, our observation of a reverse trend for CBV-TP at the PBMC level was very surprising and certainly deserves confirmation. Globally, as expected, TFV-DP, 3TC-TP, and CBV-TP presented flat PK profiles from 0 to 4 h. However, for a few patients of the LPV/r group, the CBV-TP profile fluctuated more than usual. It seems likely that, for these patients, the intracellular CBV-TP concentration at the 4-h time point was near the $C_{max}$ but was not included in the 0- to 4-h period. Consequently, for CBV-TP, $AUC_{0-4}$ was not a representative part of the total AUC and was possibly underestimated. This does not challenge our findings but rather explains why only a trend was observed for increased intracellular CBV-TP exposure in the presence of LPV/r. Nevertheless, this observation is consistent with the efficacy of the combination containing ABC plus LPV/r in comparison with other nucleoside combinations, as seen in the present study and in the KLEAN study (9).

In contrast to ABC, we may observe an increase of around 50% in plasma TFV exposure when TDF is administered with LPV/r, confirming the systemic interaction between TDF and LPV/r already observed in a previous study (22) conducted with healthy volunteers. In the present study, conducted with chronically treated HIV-infected patients, the effect was a bit more pronounced. But these results in this study cannot be directly compared with those of the crossover drug interaction study with healthy volunteers, and this weak gap could be explained either by differences in the two populations studied (healthy volunteers versus chronically treated patients, age, gender structure, sample size, and diet) or by the way PK parameters were estimated. Nevertheless, regarding our evaluation of PK parameters (over only 4 h instead of 24 h), we may note that the mean $C_{trough}$ with or without LPV/r was 91.8 or 60.8 ng/ml, respectively, which compares well with that reported by Kearney et al., 99.8 or 63.8 ng/ml, respectively. This shows, furthermore, that $C_{trough}$ is not the parameter involved in the difference in the AUC increase. Since $C_{trough}$ is the same in both studies, and considering that the $C_{trough}$ could also be used as the 24-h concentration, because after the last sampling time (4 h) we are in the terminal phase of TFV elimination, as shown by Kearney et al., we may evaluate the $AUC_{0-24}$ for TFV. By doing so, we obtain a mean TFV $AUC_{0-24}$ (without LPV/r) of 2,904 ng · h/ml, a value very close to that for the group of healthy volunteers without LPV/r, reported at 2,870 ng · h/ml. Thus, the use of $AUC_{0-4}$ for group comparison was suitable here. It is worth noting that our results and those of Kearney et al. contradict recently published data (25), where no statistically significant difference was found between PK parameters for HIV-infected patients receiving TDF with and without LPV/r. Since the patient demographic structure (age and gender) was very close to that of our study, the reason for this discrepancy remains unclear to date.

Interestingly, the original contribution of this study is to show that the TFV increase observed at the plasma level is accompanied by a TFV-DP increase of a similar magnitude at the intracellular level. TFV-DP exposure was increased by 59% when LPV/r was coadministered. This confirms the existence of an interaction and demonstrates a direct correlation between plasma TFV levels and intracellular TFV-DP concentrations.

As far as the mechanism of the TDF–LPV/r interaction is concerned, several hypotheses that involve intestinal or renal function have been proposed. In fact, PIs are known to interact with efflux transporters such as P-glycoprotein (Pgp) and multidrug resistance-associated proteins (7, 18, 19, 26, 33, 47). LPV and ritonavir could thus affect TDF absorption by inhibiting Pgp activity, as previously observed in in vitro experiments (43). Other investigators recently reported on a possible renal implication (40), but some of these results are contradictory, and so far there is no clear demonstration of involvement of kidney function in this interference. Nevertheless, in parallel with this increase in exposure, patients had not developed overt adverse effects such as nephrotoxicity (20).

Another original contribution of this paper is the observation that intracellular TFV-DP exposure is significantly higher for women than for men, even if we must remain cautious owing to the low number of women in the patient groups and the $P$ value of 0.045. A similar trend was observed for 3TC-TP but was not statistically significant, very likely due to the lower number of patients receiving 3TC than TDF. Sex-related differences in intracellular concentration for NRTI-TP metabolites have already been reported for zidovudine (1, 42), 3TC (1), and ABC (13, 28); such differences are shown here for TFV-DP and have to be confirmed by other studies. This significant difference was seen only for the triphosphate me-

tabolite at the intracellular level, not for TFV at the plasma level, suggesting a difference in the capacities of men and women to phosphorylate NRTIs and N(t)RTIs, whether directly related to sex hormones or not.

Finally, concerning the possible inhibition of PNP by TDF phosphate metabolites and its possible consequence on an increase in the dGTP pool, no difference was seen in dGTP levels between patients receiving or not receiving TDF. These in vivo results are in agreement with previous in vitro observations showing similar dGTP levels in uninfected human $CD4^+$ and $CD8^+$ T cells treated with ABC, TDF, TDF–ABC, and TDF–ABC–3TC (41) and revealing no effect of TDF alone or with other NRTIs on dNTP pools (46). Of note, TFV-DP was not entirely washed out after 4 weeks in patients of groups 1 and 3. Still, considering the half-life and the washout period, the remaining TFV-DP level was around only 4.5% of the initial TFV-DP level. In addition, TFV monophosphate (TFV-MP) is known to be the main inhibitor of PNP (39). Because there is no enzymatic limiting step in the TFV phosphorylation cascade, TFV-MP levels cannot be higher than those of TVF-DP; the former are approximately fivefold lower than the latter (8). Thus, taking into account the TFV-DP concentration after the washout period, the remaining TFV-MP level would be markedly lower than its $K_i$ for PNP inhibition (39). Therefore, under these conditions, we can reasonably consider that the comparison of dGTP levels as a marker of PNP inhibition (before and after TDF washout) is relevant.

Some limitations of this study have to be pointed out. First of all, the sample sizes of the groups included in this study were small and do not allow high-powered statistical tests. The study design also leads to limitations. For example, the potential impact of TDF on 3TC or ABC was directly investigated with the same patients receiving TDF and after TDF discontinuation, whereas the influence of 3TC or ABC on TDF was indirectly observed by comparison of TDF PK parameters in the two combinations.

Nevertheless, this work clearly shows the interest of addressing both systemic and intracellular metabolism for the understanding of N(t)RTI- and NRTI-based anti-HIV therapies.

## ACKNOWLEDGMENTS

This study was supported by unrestricted grants from GlaxoSmith-Kline and Gilead Sciences, Spain.

We acknowledge the study participants.

## REFERENCES

1. **Anderson, P. L., T. N. Kakuda, S. Kawle, and C. V. Fletcher.** 2003. Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in HIV-infected individuals. AIDS **17:**2159–2168.
2. **Barreiro, P., T. Garcia-Benayas, A. Rendon, S. Rodriguez-Novoa, and V. Soriano.** 2004. Combinations of nucleoside/nucleotide analogues for HIV therapy. AIDS Rev. **6:**234–243.
3. **Barrios, A., A. Rendon, E. Negredo, P. Barreiro, T. Garcia-Benayas, P. Labarga, J. Santos, P. Domingo, M. Sanchez-Conde, I. Maida, L. Martin-Carbonero, M. Nunez, F. Blanco, B. Clotet, M. A. Sambeat, P. Gil, J. Gonzalez-Lahoz, D. Cooper, and V. Soriano.** 2005. Paradoxical $CD4^+$ T-cell decline in HIV-infected patients with complete virus suppression taking tenofovir and didanosine. AIDS **19:**569–575.
4. **Becher, F., A. Pruvost, C. Goujard, C. Guerreiro, J. F. Delfraissy, J. Grassi, and H. Benech.** 2002. Improved method for the simultaneous determination of d4T, 3TC and ddI intracellular phosphorylated anabolites in human peripheral-blood mononuclear cells using high-performance liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. **16:**555–565.
5. **Benech, H., F. Theodoro, A. Herbet, N. Page, D. Schlemmer, A. Pruvost, J. Grassi, and J. R. Deverre.** 2004. Peripheral blood mononuclear cell counting using a DNA-detection-based method. Anal. Biochem. **330:**172–174.
6. **Blanchard, J. N., M. Wohlfeiler, A. Canas, K. King, and J. T. Lonergan.** 2003. Pancreatitis with didanosine and tenofovir disoproxil fumarate. Clin. Infect. Dis. **37:**e57–e62. (Corrected.)
7. **Chandler, B., L. Almond, J. Ford, A. Owen, P. Hoggard, S. Khoo, and D. Back.** 2003. The effects of protease inhibitors and nonnucleoside reverse transcriptase inhibitors on p-glycoprotein expression in peripheral blood mononuclear cells in vitro. J. Acquir. Immune Defic. Syndr. **33:**551–556.
8. **Delaney, W. E., IV, A. S. Ray, H. Yang, X. Qi, S. Xiong, Y. Zhu, and M. D. Miller.** 2006. Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. Antimicrob. Agents Chemother. **50:**2471–2477.
9. **Eron, J., Jr., P. Yeni, J. Gathe, Jr., V. Estrada, E. DeJesus, S. Staszewski, P. Lackey, C. Katlama, B. Young, L. Yau, D. Sutherland-Phillips, P. Wannamaker, C. Vavro, L. Patel, J. Yeo, and M. Shaefer.** 2006. The KLEAN study of fosamprenavir-ritonavir versus lopinavir-ritonavir, each in combination with abacavir-lamivudine, for initial treatment of HIV infection over 48 weeks: a randomised non-inferiority trial. Lancet **368:**476–482.
10. **Fichtenbaum, C. J., J. G. Gerber, S. L. Rosenkranz, Y. Segal, J. A. Aberg, T. Blaschke, B. Alston, F. Fang, B. Kosel, and F. Aweeka.** 2002. Pharmacokinetic interactions between protease inhibitors and statins in HIV seronegative volunteers: ACTG study A5047. AIDS **16:**569–577.
11. **Flaherty, J., B. Kearney, J. Wolf, J. Sayre, and D. Coakley.** 2001. A multiple-dose, randomized, crossover, drug interaction study between tenofovir DF and efavirenz, indinavir, or lopinavir/ritonavir, abstr. 336. Abstr. 1st Int. AIDS Soc. Conf. HIV Pathogenesis Treatment.
12. **Gallant, J. E., A. E. Rodriguez, W. G. Weinberg, B. Young, D. S. Berger, M. L. Lim, Q. Liao, L. Ross, J. Johnson, and M. S. Shaefer.** 2005. Early virologic nonresponse to tenofovir, abacavir, and lamivudine in HIV-infected antiretroviral-naive subjects. J. Infect. Dis. **192:**1921–1930.
13. **Harris, M., D. Back, S. Kewn, S. Jutha, R. Marina, and J. S. Montaner.** 2002. Intracellular carbovir triphosphate levels in patients taking abacavir once a day. AIDS **16:**1196–1197.
14. **Havlir, D. V., R. D. Schrier, F. J. Torriani, K. Chervenak, J. Y. Hwang, and W. H. Boom.** 2000. Effect of potent antiretroviral therapy on immune responses to *Mycobacterium avium* in human immunodeficiency virus-infected subjects. J. Infect. Dis. **182:**1658–1663.
15. **Hawkins, T., W. Veikley, R. L. St. Claire III, B. Guyer, N. Clark, and B. P. Kearney.** 2005. Intracellular pharmacokinetics of tenofovir diphosphate, carbovir triphosphate, and lamivudine triphosphate in patients receiving triple-nucleoside regimens. J. Acquir. Immune Defic. Syndr. **39:**406–411.
16. **Henneré, G., F. Becher, A. Pruvost, C. Goujard, J. Grassi, and H. Benech.** 2003. Liquid chromatography-tandem mass spectrometry assays for intracellular deoxyribonucleotide triphosphate competitors of nucleoside antiretrovirals. J. Chromatogr. B **789:**273–281.
17. **Hoggard, P. G., S. Kewn, M. G. Barry, S. H. Khoo, and D. J. Back.** 1997. Effects of drugs on 2′,3′-dideoxy-2′,3′-didehydrothymidine phosphorylation in vitro. Antimicrob. Agents Chemother. **41:**1231–1236.
18. **Huisman, M. T., J. W. Smit, K. M. Crommentuyn, N. Zelcer, H. R. Wiltshire, J. H. Beijnen, and A. H. Schinkel.** 2002. Multidrug resistance protein 2 (MRP2) transports HIV protease inhibitors, and transport can be enhanced by other drugs. AIDS **16:**2295–2301.
19. **Huisman, M. T., J. W. Smit, H. R. Wiltshire, R. M. Hoetelmans, J. H. Beijnen, and A. H. Schinkel.** 2001. P-glycoprotein limits oral availability, brain, and fetal penetration of saquinavir even with high doses of ritonavir. Mol. Pharmacol. **59:**806–813.
20. **Johnson, M. A., J. C. Gathe, Jr., D. Podzamczer, J. M. Molina, C. T. Naylor, Y. L. Chiu, M. S. King, T. J. Podsadecki, G. J. Hanna, and S. C. Brun.** 2006. A once-daily lopinavir/ritonavir-based regimen provides noninferior antiviral activity compared with a twice-daily regimen. J. Acquir. Immune Defic. Syndr. **43:**153–160.
21. **Kearney, B. P., E. Isaacson, J. Sayre, R. Ebrahami, and A. K. Cheng.** 2003. The pharmacokinetics of abacavir, a purine nucleoside analogue, are not affected by tenofovir DF, abstr. A-1615. Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother.
22. **Kearney, B. P., A. Mathias, A. Mittan, J. Sayre, R. Ebrahimi, and A. K. Cheng.** 2006. Pharmacokinetics and safety of tenofovir disoproxil fumarate on coadministration with lopinavir/ritonavir. J. Acquir. Immune Defic. Syndr. **43:**278–283.
23. **Kearney, B. P., J. R. Sayre, J. F. Flaherty, S. S. Chen, S. Kaul, and A. K. Cheng.** 2005. Drug-drug and drug-food interactions between tenofovir disoproxil fumarate and didanosine. J. Clin. Pharmacol. **45:**1360–1367.
24. **Kewn, S., G. J. Veal, P. G. Hoggard, M. G. Barry, and D. J. Back.** 1997. Lamivudine (3TC) phosphorylation and drug interactions in vitro. Biochem. Pharmacol. **54:**589–595.
25. **Kiser, J., M. Carten, C. Aquilante, P. Anderson, P. Wolfe, T. King, T. Delahunty, L. Bushman, and C. Fletcher.** 2008. The effect of lopinavir/ritonavir on the renal clearance of tenofovir in HIV-infected patients. Clin. Pharmacol. Ther. **83:**265–272.
26. **Lee, C. G., M. M. Gottesman, C. O. Cardarelli, M. Ramachandra, K. T. Jeang, S. V. Ambudkar, I. Pastan, and S. Dey.** 1998. HIV-1 protease inhib-

itors are substrates for the MDR1 multidrug transporter. Biochemistry **37:** 3594–3601.
27. **Levi, M., and A. Pruvost.** 2006. Sensitive HPLC-ESI-MS/MS method for the simultaneous quantitative determination of abacavir, lamivudine and tenofovir in human plasma, abstr. 69. Abstr. 7th Int. Workshop Clin. Pharmacol. HIV Ther.
28. **Moyle, G., C. Fletcher, C. Higgs, I. Song, Y. Lou, J. Lin, G. Yuen, E. Guerini, P. Hay, and M. Boffito.** 2007. An open-label, two-period, crossover, pharmacokinetic (PK) study of abacavir (ABC) and its intracellular anabolite carbovir triphosphate (CBV-TP) following 600 mg once-daily and 300 mg twice-daily administration of ABC in HIV-infected subjects, abstr. 13. Abstr. 8th Int. Workshop Clin. Pharmacol. HIV Ther.
29. **Murphy, M. D., M. O'Hearn, and S. Chou.** 2003. Fatal lactic acidosis and acute renal failure after addition of tenofovir to an antiretroviral regimen containing didanosine. Clin. Infect. Dis. **36:**1082–1085.
30. **Negredo, E., J. Molto, D. Burger, P. Viciana, E. Ribera, R. Paredes, M. Juan, L. Ruiz, J. Puig, A. Pruvost, J. Grassi, E. Masmitja, and B. Clotet.** 2004. Unexpected CD4 cell count decline in patients receiving didanosine and tenofovir-based regimens despite undetectable viral load. AIDS **18:**459–463.
31. **Ouellet, D., A. Hsu, J. Qian, C. S. Locke, C. J. Eason, J. H. Cavanaugh, J. M. Leonard, and G. R. Granneman.** 1998. Effect of ritonavir on the pharmacokinetics of ethinyl oestradiol in healthy female volunteers. Br. J. Clin. Pharmacol. **46:**111–116.
32. **Pecora Fulco, P., and M. A. Kirian.** 2003. Effect of tenofovir on didanosine absorption in patients with HIV. Ann. Pharmacother. **37:**1325–1328.
33. **Perloff, M. D., L. L. Von Moltke, J. E. Marchand, and D. J. Greenblatt.** 2001. Ritonavir induces P-glycoprotein expression, multidrug resistance-associated protein (MRP1) expression, and drug transporter-mediated activity in a human intestinal cell line. J. Pharm. Sci. **90:**1829–1837.
34. **Pruvost, A., E. Negredo, H. Benech, B. Clotet, and J. Grassi.** 2005. Possible interaction between tenofovir and boosted lopinavir; analysis at the intracellular level in HIV infected patients, abstr. 34. Abstr. 6th Int. Workshop Clin. Pharmacol. HIV Ther.
35. **Pruvost, A., E. Negredo, H. Benech, F. Theodoro, J. Puig, E. Grau, E. Garcia, J. Molto, J. Grassi, and B. Clotet.** 2005. Measurement of intracellular didanosine and tenofovir phosphorylated metabolites and possible interaction of the two drugs in human immunodeficiency virus-infected patients. Antimicrob. Agents Chemother. **49:**1907–1914.
36. **Pruvost, A., F. Theodoro, L. Agrofoglio, E. Negredo, and H. Benech.** 2008. Specificity enhancement with LC-positive ESI-MS/MS for the measurement of nucleotides: application to the quantitative determination of carbovir triphosphate, lamivudine triphosphate and tenofovir diphosphate in human peripheral blood mononuclear cells. J. Mass Spectrom. **43:**224–233.
37. **Ray, A. S.** 2005. Intracellular interactions between nucleos(t)ide inhibitors of HIV reverse transcriptase. AIDS Rev. **7:**113–125.
38. **Ray, A. S., F. Myrick, J. E. Vela, L. Y. Olson, E. J. Eisenberg, K. Borroto-Esodo, M. D. Miller, and A. Fridland.** 2005. Lack of a metabolic and antiviral drug interaction between tenofovir, abacavir and lamivudine. Antivir. Ther. **10:**451–457.
39. **Ray, A. S., L. Olson, and A. Fridland.** 2004. Role of purine nucleoside phosphorylase in interactions between 2′,3′-dideoxyinosine and allopurinol, ganciclovir, or tenofovir. Antimicrob. Agents Chemother. **48:**1089–1095.
40. **Rollot, F., E. M. Nazal, L. Chauvelot-Moachon, C. Kelaidi, N. Daniel, M. Saba, S. Abad, and P. Blanche.** 2003. Tenofovir-related Fanconi syndrome with nephrogenic diabetes insipidus in a patient with acquired immunodeficiency syndrome: the role of lopinavir-ritonavir-didanosine. Clin. Infect. Dis. **37:**e174–e176.
41. **Singer, S., D. Irlbeck, A. Pruvost, G. Freeman, G. Yuen, S. Sparks, S. Novick, R. Harvey, and R. Lanier.** 2006. Changes in levels of dNTPs, carbovir-TP and tenofovir-DP in purified human $CD4^+$ and $CD8^+$ peripheral blood T cells following treatment with tenofovir +/− abacavir +/− lamivudine. Abstr. XV Int. HIV Drug Resist. Workshop, abstr. 146.
42. **Stretcher, B. N., A. J. Pesce, P. T. Frame, and D. S. Stein.** 1994. Pharmacokinetics of zidovudine phosphorylation in peripheral blood mononuclear cells from patients infected with human immunodeficiency virus. Antimicrob. Agents Chemother. **38:**1541–1547.
43. **Tong, L., T. K. Phan, K. L. Robinson, D. Babusis, R. Strab, S. Bhoopathy, I. J. Hidalgo, G. R. Rhodes, and A. S. Ray.** 2007. Effects of human immunodeficiency virus protease inhibitors on the intestinal absorption of tenofovir disoproxil fumarate in vitro. Antimicrob. Agents Chemother. **51:** 3498–3504.
44. **Tseng, A., and D. Fletcher.** 1998. Interaction between ritonavir and levothyroxine. AIDS **12:**2235–2236.
45. **van der Lee, M. J., L. Dawood, H. J. ter Hofstede, M. J. de Graaff-Teulen, E. W. van Ewijk-Beneken Kolmer, N. Caliskan-Yassen, P. P. Koopmans, and D. M. Burger.** 2006. Lopinavir/ritonavir reduces lamotrigine plasma concentrations in healthy subjects. Clin. Pharmacol. Ther. **80:**159–168.
46. **Vela, J. E., M. D. Miller, G. R. Rhodes, and A. S. Ray.** 2005. Effect of tenofovir in combination with other anti-HIV NRTIs on intracellular nucleotide pools, poster H-1901. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother.
47. **Vishnuvardhan, D., L. L. Moltke, C. Richert, and D. J. Greenblatt.** 2003. Lopinavir: acute exposure inhibits P-glycoprotein, extended exposure induces P-glycoprotein. AIDS **17:**1092–1094.
48. **Viswanathan, C. T., S. Bansal, B. Booth, A. J. DeStefano, M. J. Rose, J. Sailstad, V. P. Shah, J. P. Skelly, P. G. Swann, and R. Weiner.** 2007. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm. Res. **24:** 1962–1973.
49. **Wang, L. H., G. E. Chittick, and J. A. McDowell.** 1999. Single-dose pharmacokinetics and safety of abacavir (1592U89), zidovudine, and lamivudine administered alone and in combination in adults with human immunodeficiency virus infection. Antimicrob. Agents Chemother. **43:**1708–1715.
50. **Waters, L. J., G. Moyle, S. Bonora, A. D'Avolio, L. Else, S. Mandalia, A. Pozniak, M. Nelson, B. Gazzard, D. Back, and M. Boffito.** 2007. Abacavir plasma pharmacokinetics in the absence and presence of atazanavir/ritonavir or lopinavir/ritonavir and vice versa in HIV-infected patients. Antivir. Ther. **12:**825–830.
51. **Yeni, P. G., S. M. Hammer, M. S. Hirsch, M. S. Saag, M. Schechter, C. C. Carpenter, M. A. Fischl, J. M. Gatell, B. G. Gazzard, D. M. Jacobsen, D. A. Katzenstein, J. S. Montaner, D. D. Richman, R. T. Schooley, M. A. Thompson, S. Vella, and P. A. Volberding.** 2004. Treatment for adult HIV infection: 2004 recommendations of the International AIDS Society—USA Panel. JAMA **292:**251–265.