HHS Public Access

Author manuscript

Ther Drug Monit. Author manuscript; available in PMC 2017 December 01.

Published in final edited form as:

Ther Drug Monit. 2016 December ; 38(6): 791–795. doi:10.1097/FTD.0000000000000329.

Therapeutic Drug Monitoring of Lopinavir in HIV-infected Children on Second-line Antiretroviral Therapy in Asia

Linda Aurpibul, MD[1], **Sirinya Teerananchai, PhD**[2], **Wasana Prasitsuebsai, MD**[2], **Tavitiya Sudjaritruk, MD. ScM, PhD**[1,3], **Pope Kosalaraksa, MD**[4], **Nia Kurniati, MD**[5], **Khanh Huu Truong, MD**[6], **Viet Chau Do, MD**[7], **Lam Van Nguyen, MD**[8], **Kulkanya Chokephaibulkit, MD**[9], **Thida Singtoroj, PhD**[10], and **Stephen J. Kerr, PhD**[2,11,12] **on behalf of the TASER-Pediatrics Study Group**

[1]Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand [2]The HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT), The Thai Red Cross AIDS Research Centre, Bangkok, Thailand [3]Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand [4]Srinagarind Hospital, Khon Kaen University, Khon Kaen, Thailand [5]Cipto Mangunkusumo General Hospital, Jakarta, Indonesia [6]Children's Hospital 1, Ho Chi Minh City, Vietnam [7]Children's Hospital 2, Ho Chi Minh City, Vietnam [8]National Hospital of Pediatrics, Hanoi, Vietnam [9]Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand [10]TREAT Asia/amfAR–the Foundation for AIDS Research, Bangkok, Thailand [11]The Kirby Institute, UNSW Australia, Sydney, Australia [12]Department of Global Health, Academic Medical Center, University of Amsterdam, Amsterdam Institute for Global Health and Development, Amsterdam, The Netherlands

Abstract

Background—Failure rates of second-line boosted protease inhibitor antiretroviral therapy regimens in children rise over time. Therapeutic drug monitoring (TDM) can contribute to assessments of adherence. The authors assessed the performance characteristics of the US DHHS-recommended lopinavir (LPV) concentration of 1.0 mg/L for predicting virologic failure (VF) and intermediate-to-high level LPV resistance in Asian children.

Materials and Methods—LPV concentration, HIV RNA level, and adherence data from study participants in Indonesia, Thailand, and Vietnam receiving second-line LPV-based ART and followed for ≥24 weeks were analyzed.

Results—A total of 223 children at a median age of 10.4 (interquartile range, IQR 7.9–13.4) years were enrolled, 61% were male. Their mean CD4 was 842±438 cells/mm^3, and the median LPV duration was 2.5 (IQR 1.3–4.2) years. Five out of 84 (6%) and 18 out of 139 (13%) children had LPV trough and random concentrations <1.0 mg/L at study week 24. Using either of these trough or random LPV concentrations, a cutoff at 1.0 mg/L gave an area under the receiver operating characteristics (AROC) curve of 0.69 in predicting VF with sensitivity of 44% (95% CI 23–66) and specificity of 94% (95% CI 89–97). Seven of 21 with VF and resistance results

Corresponding author: Linda Aurpibul, MD, **Contact information:** Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand 50200. lindaa@rihes.org, Telephone: +66-53-894138; Fax: +66-53-894138.

available had ≥1 major protease inhibitor mutation. Multivariate logistic regression found LPV concentrations <1.0 mg/L (OR 6.47; 95% CI 2.15–19.50, P = 0.001) and CD4 ≤20% (OR 2.83; 95% CI 1.01–7.89, P = 0.05) were independently associated with HIV RNA >1000 copies/mL. No factors predicted major LPV resistance mutations.

Conclusions—The authors support that the DHHS target LPV concentration of <1.0 mg/L is predictive of virologic failure (VF), but not of the presence of major LPV mutations.

Keywords

lopinavir; pharmacokinetics; HIV; children; antiretroviral treatment

Introduction

The number of HIV-infected children engaging in HIV care and treatment has been increasing worldwide. An earlier survey performed by the TREAT Asia Pediatric Network in six Asian countries reported that 20% of children who commenced antiretroviral treatment (ART) had failed first-line regimens and were on their second ART regimens, using mostly protease inhibitors (PI) [1]. Data from Thailand have suggested that the effectiveness of second-line PI regimens in children was 74–77% by 24 months of treatment [2,3]. The increasing failure rate of second-line boosted PI regimens over time raises questions about the need for more aggressive treatment monitoring. Known risk factors for ART failure include high baseline viral load, low baseline CD4 cell count, inadequate drug concentrations, and non-adherence [4,5]. There is no single standard method to measure treatment adherence. In this context, therapeutic drug monitoring (TDM) may contribute to adherence assessments and inform decisions on when to conduct resistance testing. According to the US Department of Health and Human Services (DHHS) guidelines (Last updated Mar 1, 2016), the suggested minimum target trough concentration of lopinavir (LPV) in patients with drug-susceptible HIV virus is 1 mcg/mL [6]. A study among adults in South Africa supported that a LPV plasma level of 1 mcg/mL was the appropriate threshold concentration that maximized the sensitivity and specificity for predicting virologic failure [7].

The primary objective of this study was to explore whether this recommendation was applicable to children and adolescents receiving LPV as part of second-line ART in an Asian cohort. The secondary objective was to determine whether TDM of LPV would be useful as a proxy for adherence and/or justification for genotypic resistance testing in this population.

Materials and methods

The TREAT Asia Studies to Evaluate Resistance in Pediatrics study (TASER-P; ClinicalTrials.gov Identifier: NCT01788891) is a longitudinal observational cohort study to monitor Asian children and adolescents for treatment failure while on second-line antiretroviral treatment (ART). The study was approved by the institutional review boards at all sites. All patients and their caregivers provided written informed consent and assent before taking part in this study. Patients were enrolled either at the time of switching to or after they had already switched to a second-line ART regimen due to first-line failure.

Clinical and laboratory data were captured during follow-up visits at enrollment and weeks 24, 48, 72, 96, and 120 after enrollment. Plasma samples were collected at each visit for HIV RNA levels, and a stored sample would be sent for genotyping when a patient had HIV RNA ≥1,000 copies/mL. Plasma TDM of antiretroviral drugs was performed at week 24 in all patients with the aim to determine its correlation with HIV RNA levels, which were analysed at the same time point for all study participants. At other visits, the patient was called back for blood sampling for TDM whenever the HIV RNA was ≥1,000 copies/mL. For all TDM samples, blood collection was advised to be carried out within 30 minutes before the next ART dose in order to obtain a trough concentration. The actual time since the last dose to the time of blood draw was recorded. After collection, blood samples were mixed and sent to the laboratory within 6 hours in lithium heparin tubes. Blood samples were centrifuged at 1800 g for 10 minutes at 20°C. Plasma was divided and transferred to labeled polypropylene tubes and stored at −20°C until shipping to the HIV-NAT Research Laboratory. This lab participates in an international inter-laboratory quality control program for therapeutic drug monitoring in HIV infection (KKGT) for external quality control [8]. Plasma concentrations of LPV were determined by a validated high-performance liquid chromatography (HPLC) assay with ultraviolet detection, and a lower limit of quantification of 0.105 mg/L.

Adherence assessments were done at each study visit using the following methods: the World Health Organization's (WHO) visual analogue scale (VAS) for adherence, questionnaires including 3-day recall, and pill count. Adherence counseling was provided at every visit. Study participants in Indonesia, Thailand, and Vietnam receiving second-line LPV-based ART and followed for ≥24 weeks after study enrollment were included in the analysis. For this study, virologic failure (VF) was defined as having HIV RNA >1,000 copies/mL to get enough cases with VF for conducting the analysis. Mutations were interpreted using the Stanford University HIV Drug Resistance Database; clinically significant major PI-resistance mutations associated with either high- or low-level resistance were assessed. All LPV concentrations from samples collected at week 24 were used to plot receiver operating characteristics (ROC) curves for VF. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), and area under the ROC (AROC) as a measure of correctly predicting outcomes (VF) were calculated. Multiple logistic regression was used to determine associations with occurrence of VF. Covariates with P <0.1 were adjusted for in a multivariate model and statistical significance was identified using a two-sided P value of 0.05. Data were analyzed using Stata 13.1 (StataCorp., College Station, TX, USA) and SAS 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

A total of 223 children and adolescents were included; 219 (98%) were perinatally HIV-infected (Table 1). Forty-four percent had WHO stage 3–4 disease at the time of second-line ART initiation. The median (IQR) duration on LPV at the start of the study was 2.9 years (1.6–4.2). There were three children enrolled at the time of switching to LPV; the other 220 (99%) had been on LPV prior to entry. At enrollment 12 children had HIV RNA >1000 copies/mL, including 10 who were on LPV and two who were just switched to LPV. At the time of this analysis, corresponding to week 24 after TASER-P enrollment, ≥95% treatment

adherence was reported by VAS in 200/223 (90%) and by pill count in 192/223 (86%) study participants. The median (IQR) LPV daily dose used was 19.3 mg/kg (16.2–22.6). All except the 4 samples collected more than 16 hours post dose were included in the analysis; the median (IQR) time after the last dose was 13.2 (12.6–14.2) hours. There were 84 samples collected within 12±0.5 hours post dose; the median (IQR) time after the last dose was 12 (11.8–12.2) hours. The median LPV concentrations were 16.0 (14.8–20.5) and 19.5 (16.4–22.9) mg/L among those who received LPV prior to and those who were switched at TASER-P enrollment, respectively.

During the course of the study, 37 children had HIV RNA >1000 copies/mL, which met the definition of VF in this analysis. Twenty-three children met the criterion for VF at week 24; five (22%) had adherence <95% by both pill count and VAS, four (17%) reported missed doses at that study visit, and four (17%) reported taking medication more than one hour later than the set time in the previous three months. Of these, 21(91%) had at least one documented HIV RNA >1000 copies/mL after switching to LPV-including regimens before TASER-P enrollment. Five out of 84 children with LPV concentrations from specimens collected within 12±0.5 hours post dose LPV concentrations available at week 24 had VF. A threshold of 1.0 mg/L had a sensitivity of 66.7% (95% CI, CI 9.4–99.2), specificity of 96.3% (95% CI 89.6–99.2), and an AROC of 0.82 (95% CI 0.49–1.00) for detecting VF (Table 2a). When including LPV concentrations from specimens collected within 16 hours post dose to predict virologic failure at week 24, a threshold of 1.0 mg/L had a sensitivity of 43.5% (95% CI 23.2–65.5), specificity of 93.5% (95% CI 89.1–96.5), and an AROC of 0.69 (95%CI 0.58–0.79) for detecting VF (Table 2b). There were 14 other children who developed VF after study week 24. Using LPV concentrations from specimens collected within 16 hours post dose to predict virologic failure at week 24 or later on, a cutoff at 1.0 mg/L gave an AROC curve of 0.71 (95% CI 0.62–0.81) in predicting VF with sensitivity of 53.6% (95% CI 33.9–72.5) and specificity of 89.3% (95% CI 84.2–93.2) (Table 2c).

Twenty-one of 23 participants with HIV RNA >1000 copies/mL at week 24 of the study had resistance results available. Of these, seven (33%) had major PI mutations (I54V, M46I, V82A or S, N88S, L76V, L90M); their duration on LPV ranged from 1.7 to 5.3 years. The two with LPV concentrations <1.0 mg/L had low-level resistance, while the other five with LPV concentrations >1.0 mg/L had intermediate to high-level resistance. Two of seven reported missed doses in the last three days; one child reported adherence on VAS of 96% while the others reported 100% adherence, and pill count revealed 100% adherence for all seven.

After study week 24, the 14 additional children (7%) who developed VF had been on LPV between 0.8 and 5.8 years before HIV RNA >1000 copies/mL was detected. Two of 14 (14%) had major PI mutations (M46LM, V82A); both had LPV >1.0 mg/L. Five of 14 (35%) children with VF had LPV plasma concentrations below the 1.0 mg/L cutoff. All five reported no missing doses in the three days prior to the clinic visit, and the genotypic resistance results showed susceptibility to LPV. Pill count revealed 100% adherence in two, ≤90% adherence in two, and was not available in the remaining participants.

Univariate analysis showed that three factors had an association with HIV RNA >1000 copies/mL. These were LPV concentrations <1.0 mg/L, CD4 <20% at second-line switch, and any missed dose within three days before the HIV-RNA measurement. Age,duration on LPV, WHO staging, and adherence assessment by pill count or VAS were not associated with HIV RNA >1000 copies/mL. In a multivariate model, after adjusting for any reported missed doses within the previous three days, LPV concentrations <1.0 mg/L (OR 6.47; 95% CI 2.15–19.50, P = 0.001) and CD4 ≤20% (OR 2.83; 95% CI 1.01–7.89, P = 0.05) were independently associated with HIV RNA >1000 copies/mL. No factors predicted the presence of major LPV resistance mutations.

Discussion

In this study population, an LPV concentration cutoff of 1.0 mg/L had a sensitivity of 44% and specificity of 94% in predicting VF with a cutoff of >1000 copies/mL. Low LPV concentrations were associated with VF, but not with the presence of major PI mutations.

The relationship between LPV concentrations and virologic failure was previously described in an adult study from South Africa [7]. They reported that among 37 of 93 patients on second-line ART containing boosted LPV, all of those who had virologic failure (HIV RNA level >1,000 copies/mL) had low LPV exposure either in plasma or hair samples, while only two of them had major PI mutations. The NPV, or the probability that those with LPV plasma concentration >1.0 mg/L did not have virologic failure in their study was 86%. In this analysis, the sensitivity and specificity of using LPV concentration cutoff at 1.0 mg/L for detecting VF were 66.7 and 96.3%, respectively. This means that by using this cutoff, we would be able to identify 67 out of 100 patients who had VF; and 96 out of 100 patients who had no VF.

Our findings are also consistent with a pediatric study in South Africa where HIV-infected children aged between 4 and 42 months initiated on LPV-based regimens were followed up to 52 weeks. They observed that with LPV concentrations <1.0 mg/L, the hazard ratio for having HIV RNA >400 copies/mL was 2.3 times higher when compared to LPV concentrations ≥1.0 mg/L [9]. In our study, we found that the odds of having HIV RNA >1000 copies/mL among those with plasma LPV concentrations <1.0 mg/L was 6.47 times when compared to those with plasma LPV concentrations ≥1.0 mg/L. Thus, our findings support the use of the DHHS target trough concentration of LPV for Asian children. Together with other adherence measurements, TDM may be added in certain cases with unexpected suboptimal treatment response to determine whether drug exposure is adequate.

Only 22% of children with low LPV concentrations reported decreased adherence to medication either by pill count or self-report. Some reported missing doses in the past three months prior to the study visit when the LPV level was obtained. Self-reported adherence is a simple and convenient measure, but may be subject to how it is assessed. Our finding was similar to the US report from a study in HIV-infected adults, where investigators found that random PI concentrations were independently predictive of virologic response, while 27% of patients on unboosted single PI regimens had undetectable PI concentrations [10]. In that study, the self-reported adherence rates were as high as 91% [10]. TDM may be useful in

such cases where there are discrepancies between self-report and/or pill count adherence assessments and immunologic and/or virologic outcomes.

We found no individual factor associated with the presence of major LPV resistance mutations. This might be explained by the potency and higher resistance barriers of PIs, or the lack of drug pressure due to very poor or non-adherence. This shows that additional interventions, such as intensive adherence counseling, can successfully result in resuppression and ongoing regimen potency. Further study is needed to assess whether these low LPV concentrations persist even with better adherence; in which case other pharmacokinetic and pharmacodynamics factors may be playing a role.

Our study has a number of limitations, including low incidence of VF. In addition, not all TDM samples were collected at the same time point, and because of timing of doses and travelling times to clinic, some samples were collected >12 hours after the previous dose was taken. Nevertheless, by including all these children, the precision of the 95% CI around the AROC was improved, and the estimates were significantly better than would be expected by chance. We also defined VF with a single HIV RNA >1000 copies/mL, as our focus was on identifying those at greatest risk of failure and not specifically to follow criteria for switch. However, our data do support that the DHHS target LPV concentration of <1.0 mg/L is predictive of non-suppression, but not of the presence of major LPV mutations. The study highlights the challenges in reliably assessing adherence in children and adolescents.

Acknowledgments

The study is an initiative of TREAT Asia, a program of amfAR, The Foundation for AIDS Research, with support from ViiV Healthcare, the AIDS LIFE Association, and the US National Institutes of Health's National Institute of Allergy and Infectious Diseases, Eunice Kennedy Shriver National Institute of Child Health and Human Development, and National Cancer Institute, as part of the International Epidemiologic Databases to Evaluate AIDS (IeDEA; U01AI069907).

The authors gratefully acknowledge the participation of the children and adolescents and their families, as well as contributions of all study staff.

TASER-Pediatrics Network: K.H. Truong and T.P.K. Le, Children's Hospital 1, Ho Chi Minh City, Vietnam; V.C. Do, V.T. An, and T.M. Ha, Children's Hospital 2, Ho Chi Minh City, Vietnam; W. Prasitsuebsai, S. Kerr, T. Bunupuradah, A. Avihingsanon, T. Jupimai, N Thammajaruk, and C Ruengpanyathip, HIV-NAT, the Thai Red Cross AIDS Research Centre, Bangkok, Thailand; L.V. Nguyen and K.D.T. Khu, National Hospital of Pediatrics, Hanoi, Vietnam; N. Kurniati and D. Muktiarti, Cipto Mangunkusumo General Hospital, Jakarta, Indonesia; P. Kosalaraksa, P. Lumbiganon, and C. Sopharak, Division of Infectious Diseases, Department of Pediatrics, Khon Kaen University, Khon Kaen, Thailand; T. Sudjaritruk, V. Sirisanthana, and L. Aurpibul, Research Institute for Health Sciences and Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; K. Chokephaibulkit, S. Sricharoenchai, and N. Kongstan, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; A.H. Sohn, N. Durier, and T. Singtoroj, TREAT Asia/amfAR–The Foundation for AIDS Research, Bangkok, Thailand.

References

1. Prasitsuebsai W, Bowen AC, Pang J, et al. TREAT Asia Pediatric Program. Pediatric HIV clinical care resources and management practices in Asia: A regional survey of the TREAT Asia Pediatric Network. AIDS Patient Care and STDs. 2010; 24:127–131. [PubMed: 20059355]
2. Puthanakit T, Jourdain G, Suntarattiwong P, Chokephaibulkit K, Siangphoe U, Suwanlerk T, et al. High virologic response rate after second-line boosted protease inhibitor-based antiretroviral therapy regimens in children from a resource limited setting. AIDS Res Ther. 2012; 9:20. [PubMed: 22709957]

3. Bunupuradah T, van der Lugt J, Kosalaraksa P, et al. HIV-NAT 017 Study Team. Safety and efficacy of a double-boosted protease inhibitor combination, saquinavir and lopinavir/ritonavir, in pretreated children at 96 weeks. Antivir Ther. 2009; 14:241–248. [PubMed: 19430099]

4. Ajose O, Mookerjee S, Mills EJ, et al. Treatment outcomes of patients on second-line antiretroviral therapy in resource-limited settings: a systematic review and meta-analysis. AIDS. 2012; 26:929–38. [PubMed: 22313953]

5. Suaysod R, Ngo-Giang-Huong N, Salvadori N, et al. Treatment failure in HIV-infected children on second-line protease inhibitor-based antiretroviral therapy. Clin Infect Dis. 2015; 61:95–101. [PubMed: 25838288]

6. Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf. Accessed Apr 25, 2016 [Table 17, page 64]

7. van Zyl GU, van Mens TE, McIlleron H, et al. Low lopinavir plasma or hair concentrations explain second-line protease inhibitor failures in a resource-limited setting. J Acquir Immune Defic Syndr. 2011; 56:333–339. [PubMed: 21239995]

8. Burger D, Teulen M, Eerland J, et al. The International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma: a global proficiency testing program. Ther Drug Monit. 2011; 33:239–243. [PubMed: 21383652]

9. Moholisa RR, Schomaker M, Kuhn L, et al. Plasma lopinavir concentrations predict virological failure in a cohort of South African children initiating a protease-inhibitor-based regimen. Antivir Ther. 2014; 19:399–406. [PubMed: 24518130]

10. Yasuda JM, Miller C, Currier JS, et al. California Collaborative Treatment Group. The correlation between plasma concentrations of protease inhibitors, medication adherence and virological outcome in HIV-infected patients. Antivir Ther. 2004; 9:753–761. [PubMed: 15535413]

Table 1

Characteristics of study participants (n = 223)

Characteristics	At initiation of an LPV-based regimen	At the time of TASER-P study enrollment	Children with virologic failure at any time after study entry[a]	Children with LPV concentration <1.0 mg/L (either trough or random)
Number of patients	223	223	37	15
Age in years, median (IQR)	7.4 (5.2–10.14)	10.4 (7.9–13.4)	11.3 (7.9–14.5)	10.3 (4.2–13.9)
Male sex, n (%)	136 (61)		23 (62)	11 (73)
Country, n (%)				
Thailand	90 (40)		14 (38)	9 (60)
Vietnam	112 (50)		20 (54)	5 (33)
Indonesia	21 (9)		3 (8)	1 (7)
WHO clinical staging, n (%)[b]				
1–2	100 (45)		20 (54)	7 (47)
3–4	99 (44)		15 (41)	7 (47)
CD4 lymphocyte count, cells/mm^3 n (%)	190 (85)	194 (87)	34 (92)	14 (93)
Median (IQR)	293 (146–596)	759 (569–1066)	583 (415–763)	482 (410–625)
CD4 lymphocyte percentage, n (%)	175 (79)	172 (77)	31 (84)	14 (93)
Median (IQR)	13 (7– 20)	25 (19–31)	20 (12–24)	16 (12–20)
Log$_{10}$ HIV RNA level, copies/mL, n (%)	169 (76)	223 (100)	37 (100)	15 (100)
Median (IQR)	5.0 (4.3–5.6)	2.4 (1.6–2.5)	4.4 (3.7–4.9)	4.9 (4.7–5.3)
Duration on LPV, years				
Median (IQR)		2.5 (1.3–4.1)	2.9 (1.5–4.4)	1.6 (0.8–2.6)
6–<24 months		48 (22)	6 (16)	6 (40)
≥24 months		175 (78)	31 (84)	9 (60)
LPV dose (mg/kg)				
Median (IQR)		19.4 (16.2–22.6)	21.1 (16.3–24.2)	17.1 (14.9–24.6)
BW <20 kg		22.1 (18.9–24.6)	24.2 (21.3–26.7)	24.6 (21.3–26.7)
BW 20–30 kg		19.5 (17.2–22.9)	22.2 (18.9–23.3)	18.9 (15.3–38.1)
BW >30 kg		17.3 (13.8–20.8)	16.2 (14.2–20.7)	16.1 (14.7–17.1)
LPV concentration (mg/L)				
Median (IQR)		6.9 (4.0–9.7)	6.5 (0.1–12.5)	0.1 (0.1–0.1)
Body surface area (m^2), n (%)		222 (99.6)	37 (100)	15 (100)
Median (IQR)		1 (0.8–1.22)	1 (0.8–1.2)	0.9 (0.6–1.3)
Adherence assessment, n (%)				
Pill count ≥95%		192 (86)	29 (83)	11 (79)
VAS report ≥95%		200 (90)	30 (86)	10 (77)

[a] For this study virologic failure (VF) was defined as having HIV RNA >1,000 copies/mL

[b] Some unknown or missing data.

WHO World Health Organization; IQR Interquartile range; BW body weight; mg milligrams; kg kilograms; VL viral load; lopinavir; VAS Visual analogue scale.

Table 2

Performance characteristics of LPV TDM in predicting virologic failure[a], based on LPV concentrations recommended in the US DHHS guidelines[b]

LPV concentration	≥1 mg/L	<1 mg/L	Total
a. Using LPV concentrations from specimens collected within 12±0.5 hours post dose			
No virologic failure	78	1	79
Virologic failure	3	2	5
Total	81	3	84
Sensitivity (95% CI)		**66.7% (9.4%–99.2%)**	
Specificity (95% CI)		**96.3% (89.6%–99.2%)**	
ROC area		**0.82 (0.49–1.00)**	
b. Using LPV concentrations from specimens collected within 16 hours post dose to predict virologic failure at week 24.			
No virologic failure	183	13	196
Virologic failure	13	10	23
Total	196	23	219
Sensitivity (95% CI)		**43.5% (23.2%–65.5%)**	
Specificity (95% CI)		**93.5% (89.1%–96.5%)**	
ROC area		**0.69 (0.58–0.79)**	
c. Using LPV concentrations from specimens collected within 16 hours post dose to predict virologic failure at week 24 or subsequent visits.			
No virologic failure	183	22	205
Virologic failure	13	15	28
Total	196	37	233
Sensitivity (95% CI)		**53.6% (33.9%–72.5%)**	
Specificity (95% CI)		**89.3% (84.2%–93.2%)**	
ROC area		**0.71 (0.62–0.81)**	

[a]For this study virologic failure (VF) was defined as having HIV RNA >1,000 copies/mL.

[b]Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf. Accessed Apr 25, 2016 [Table 17, page 64].

LPV lopinavir; TDM therapeutic drug monitoring; CI confidence interval; ROC Receiver operating characteristic