Determinants of Viral Resuppression or Persistent Virologic Failure After Initial Failure With Second-Line Antiretroviral Treatment Among Asian Children and Adolescents With HIV

Sirinya Teeraananchai,^{1,2} Stephen J. Kerr,^{1,2} Monica Gandhi,³ Viet Chau Do,⁴ Lam Van Nguyen,⁵ Dan Ngoc Hanh Tran,⁶ Pope Kosalaraksa,⁷ Thida Singtoroj,⁸ Narukjaporn Thammajarak,¹ Thidarat Jupimai,¹ and Annette H. Sohn⁹; on behalf of the TASER Pediatrics Study

¹HIV Netherlands Australia Thailand Research Collaboration, Thai Red Cross AIDS Research Centre, Bangkok, Thailand; ²Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; ³Division of HIV, Infectious Diseases, and Global Medicine, San Francisco General Hospital, University of California San Francisco; ⁴Children's Hospital 2, Ho Chi Minh City, Vietnam; ⁵National Hospital of Pediatrics, Hanoi, Vietnam; ⁶Children's Hospital 1, Ho Chi Minh City, Vietnam; ⁷Division of Infectious Diseases, Department of Pediatrics, Faculty of Medicine, Khon Kaen University, Thailand; and ⁸TREAT Asia/amfAR-Foundation for AIDS Research, Bangkok, Thailand

Of 56 children with perinatally acquired human immunodeficiency virus (HIV) who had been prescribed second-line protease inhibitor–based antiretroviral therapy and had ≥ 1 previous episode of viral failure (HIV RNA, ≥ 1000 copies/mL), 46% had ≥ 1 , 34% had ≥ 2 , and 23% had ≥ 3 consecutive episodes of viral failure during the 2 years of follow-up. Two of these children experienced a major protease inhibitor mutation.

Keywords. adherence; hair concentration; HIV; second-line ART; virologic failure; virologic resuppression.

In 2017, approximately 110 000 children younger than 15 years in the Asia-Pacific region were living with human immunodeficiency virus (HIV) [1]. Those with perinatally acquired HIV

(PHIV) are a unique population in their need for lifelong antiretroviral therapy (ART), which is associated with treatment fatigue and poorer adherence [2–5]. Adolescents with PHIV on second-line ART are of particular concern, because failing these regimens could result in losing key antiretroviral drug options at a relatively younger age. Third-line or salvage regimens also can be more complex to acquire or administer (eg, more pills, multiple times per day), which further endangers adherence. We aimed to determine the outcomes of persistent virologic failure (VF), viral resuppression, and factors associated with VF, including metrics of objective adherence, in a cohort of Asian children and adolescents with PHIV on second-line ART.

METHODS

Study Population and Procedures

The Prospective Monitoring of Second-Line Antiretroviral Therapy Failure and Resistance in Children (TASER-P) study was a longitudinal cohort study performed to monitor 277 children and adolescents receiving second-line ART between 2011 and 2014 in southeast Asia [6]. We conducted an extension study to continue monitoring those children and adolescents with a history of ≥ 1 episode of postsuppression VF (HIV RNA, ≥ 1000 copies/mL) during the main cohort study in Thailand and Vietnam. The general procedures during the extension study were unchanged from those of the parent study [6, 7]; patients had a study follow-up visits every 6 months, when they underwent HIV RNA testing, and adherence measurements were obtained by self-/guardian report (visual analog scale [VAS]), pill counts, and hair antiretroviral concentrations. Therapeutic drug monitoring (TDM) via plasma levels and genotypic resistance testing was done locally at each site if the patient's HIV RNA results met VF criteria. The Stanford algorithm was used to interpret drug-resistance mutations. Patients were censored if they died or were switched to a third-line regimen as a result treatment failure (Supplemental Figure 1).

Persistent VF was defined as ≥ 2 VF episodes during the study period. Viral resuppression was defined as a post-VF HIV RNA concentration of <1000 copies/mL. Undetectable HIV RNA was defined using site-specific thresholds based on locally available laboratory tests (Thailand, <20 and <40 HIV RNA copies/mL; Vietnam, <250 and <300 HIV RNA copies/mL). Lopinavir (LPV) hair concentrations were measured in the University of California San Francisco Hair Analytical Laboratory using validated assays approved by the Division of AIDS Clinical Pharmacology and Quality Assurance program [8].

Statistical Analysis

We documented the characteristics in participants with persistent VF (ie, ≥ 2 consecutive VF episodes) versus those who had

Received 6 December 2018; editorial decision 30 April 2019; accepted 3 May 2019; Published online May 24, 2019.

Previous presentation of results. This work was presented in part at the 9th IAS Conference on HIV Science between July 24, 2017, in Paris, France.

Correspondence: S. Teeraananchai, PhD, HIV-NAT Research Collaboration, 104 Ratchadumri Rd, Pathumwan, Bangkok, Thailand, 10330 (sirinya.t@hivnat.org).

Journal of the Pediatric Infectious Diseases Society 2020;9(2):253–6

© The Author(s) 2019. Published by Oxford University Press on behalf of The Journal of the Pediatric Infectious Diseases Society. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

DOI: 10.1093/jpids/piz034

viral resuppression during follow-up. We then used generalized estimating equations with a logit link and exchangeable correlation matrix to assess factors associated with VF over follow-up. This model was restricted to those receiving LPV and who had at least 1 visit during the extension phase. Covariates assessed in the model to examine VF during follow-up were sex, age, CD4 count, adherence according to a VAS and/or pill count, hair LPV concentration, and body surface area (in m²). Those with a *P* value of <.10 in univariate analysis were adjusted for in a multivariate model.

RESULTS

A total of 56 children and adolescents with PHIV had at least 1 episode of VF while on protease inhibitor (PI)-based second-line ART in the main cohort study and were included in the extension phase. Among these children (Table 1), 63% were male; 12 (21%) were receiving care in Thailand and 44 (79%) in Vietnam. The median age at enrollment into the extension study was 7.3 years (interquartile range [IQR], 5.1–10.0 years). At second-line ART initiation, CD4 test results were available for 53 (95%) children (median CD4 count, 282 cells/μL [IQR, 146–504 cells/μL]), and HIV RNA test results were available for

43 (77%) children (median HIV RNA concentration, 5.1 log 10 copies/mL [IQR, 4.4–5.7 log 10 copies/mL]) (Table 1). Of the 56 extension study enrollees, 31 (76%) underwent viral genotyping at the time of VF in the main cohort study; 2 of the viruses genotyped harbored a major V82A PI mutation, whereas the other viruses were wild type.

At enrollment into the extension study, the median age was 11.6 years (IQR, 9.3–14.2 years), and the median lifetime duration on second-line ART was 3.9 years (IQR, 2.7–6.1 years). Fifty-one (91%) of the 56 children were on an LPV-based regimen, and 5 (9%) children were on an atazanavir-based regimen. Of the 51 (91%) children for whom CD4 test results were available, the median CD4 count was 689 cells/μL (IQR, 386–1096 cells/μL).

During the extension study period, 2 children switched to third-line ART, and 1 other child died before the second visit. Overall, 54% (30 of 56) of the children remained virologically suppressed (28 of them had an undetectable HIV RNA by site-specific thresholds); the median reported adherence was 100% (IQR, 100–100%) according to both the VAS and pill counts. Among the 46% (26 of 56) who experienced a new VF episode during study follow-up, 73% (19 of 26) had at least 2 consecutive

Table 1. Characteristics of Patients at Second-Line Switch, Enrollment Into the Extension Study, and Subsequent Episodes of VF During Follow-up^a

Characteristic	At Switch to Second-Line ART	At Enrollment Into Extension Phase	During Follow-up in Extension Phase			
			No New Episode of VF ^b	New VF Episode	2 VF Episodes	≥3 VF Episodes
n (%)	56	56	30 (54) ^c	26 (46)	19 (34)	13 (25) ^d
Male (n [%])	35 (63)	35 (63)	8 (27)	13 (50)	8 (42)	6 (46)
Age (median [IQR]) (years)	7.3 (5.1–10.0)	11.6 (9.3–14.2)	12.1 (10.1–13.5)	13.9 (10.3–16.8)	14.6 (10.8–17.5)	15.7 (14.7–18.4)
CD4% (median [IQR]) (n)	11 (6–16) (45)	22 (17–27) (49)	24 (19–28)	18 (6–24)	6 (4–21)	14 (2–20)
CD4 count (median [IQR]) (cells/μL) (n)	282 (146–504) (53)	689 (386–1096) (51)	819 (599–1111)	463 (105–714)	244 (61–562)	239 (38–332)
HIV RNA concentration (median [IQR]) (log 10 copies/mL) (n)	5.1 (4.4–5.7) (43)	2.6 (2.4–3.6)	1.3 (1.3–2.0)	4.2 (3.5–5.2)	4.5 (3.7–5.2)	4.7 (4.2–4.9)
HIV RNA ≥ 1000 log 10 copies/mL (% [n])	95 (41)	39 (22)	0	100	100	100
Duration on second-line ART (median [IQR]) (years)	NA	3.9 (2.7–6.1)	3.5 (2.7–6.8)	4.1 (2.7–5.2)	4.4 (3.3–6.0)	5.7 (3.8–6.1)
Adherence according to pill count (median [IQR]) (%)	NA	100 (100–100)	100 (100–100)	100 (90–100)	94 (83–100)	90 (80–100)
Adherence according to pill count ≥ 95%	NA	48 (87)	30 (100)	17 (68)	8 (47)	5 (42)
Adherence according to VAS (median [IQR]) (%)	NA	100 (99–100)	100 (100–100)	99 (90–100)	99 (80–100)	90 (80–100)
Adherence according to VAS ≥ 95%	NA	46 (82)	30 (100)	15 (58)	12 (63)	6 (46)
TDM available (n [%])	NA	21 (88)	NA	23 (88)	18 (95)	13 (100)
All LLOQ (n [%])	NA	9 (43)	NA	10 (43)	11 (61)	8 (62)
LPV based for TDM	NA	20	NA	22	17	11
LPV concentration (median [IQR])	NA	8.9 (3.7–12.3)	NA	7.8 (5.3–9.9)	11.9 (8.2–13.6)	8.2 (6.0–9.9)
LLOQ (n [%])	NA	8	NA	9	11	6
Genotyping results available (n [%])	31 (76)	21 (95)	NA	24 (92)	18 (95)	13 (100)
Hair concentration testing	NA	52 (96)	24 (80)	24 (92)	16 (84)	11 (85)
LPV based for hair testing	NA	49	24	23	15	9
Hair LPV concentration (median [IQR])	NA	9.8 (4.5–13.4)	13.1 (10.8–14.9)	3.6 (2.2–7.8)	5.0 (0.4–7.5)	1.3 (0.3–10.4)
Hair LPV normalized concentration (median [IQR])	NA	10.2 (4.9–15.0)	14.2 (12.2–17.3)	3.7 (2.2–8.0)	5.2 (0.5–7.4)	1.4 (0.3–10.9)
LLOQ (n [%])	NA	3	0	3	2	2

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; LLOQ, lower limit of quantification (<0.05 ng/mg); LPV, lopinavir; NA, not applicable; TDM, therapeutic drug monitoring; VAS, visual analog scale; VF, viral failure (defined as consecutive episodes of VF).

^aTwo children were on atazanavir; their results are not shown here.

^bResult at the last visit.

^cOf these 30 children, 28 had an undetectable HIV RNA concentration by site-specific thresholds.

^dThe denominator for this group (N = 51) changed over time, because 2 children were switched to third-line ART, 1 child died, and 2 children were lost to follow-up.

VF episodes, and 68% (13 of 19) of these children had ≥ 3 consecutive VF episodes.

Among those with ≥ 3 consecutive VF episodes, adherence decreased from the first to the third episode (68% to 42% according to pill count; 58% to 46% according to the VAS). At the VF episodes, 43% to 62% of the children had random LPV plasma concentrations below the lower limit of quantification (LLOQ) (Table 1). The median LPV hair concentration in children who remained suppressed throughout the study period was higher (13.1 ng/mg [IQR, 10.8–14.9 ng/mg]) than in those who developed VF at the first new VF episode (3.6 ng/mg [IQR, 2.2–7.8 ng/mg]), the second episode (5.0 ng/mg [IQR, 0.4–7.5 ng/mg]), and the third or later VF episode (1.3 ng/mg [IQR, 0.3–10.4 ng/mg]).

A total of 26 children in the extension phase experienced 58 VF episodes (Supplemental Figure 2). Resistance genotype test results were available for 24 (92%) children during 55 (95%) of the VF episodes. Nucleoside reverse transcriptase inhibitor (NRTI) mutations included M184V (46%), K70R (29%), and T215Y/F (21%); 50% had ≥ 1 thymidine analog, and 58% were considered still susceptible to tenofovir. Two (8.7%) of 23 children had a virus that had acquired ≥ 1 new major PI mutation (ie, M46I, V82A, or N88NS). It should be noted that 88% of the patients with persistent VF in our study had a virus that remained susceptible to LPV-based treatment regimens.

We assessed the factors associated with VF among the subset of children on LPV-based ART for whom hair concentration results were available (51 [91%] of 56). In an adjusted multivariate model, the odds of any new episode of VF were increased 5-fold (odds ratio, 5.22 [95% confidence interval, 1.18–23.05]) in those with a CD4 count of <350 cells/ μ L compared to those with a CD4 count of ≥ 350 cells/ μ L. Self-reported adherence of $<95\%$ according to the VAS and a lower LPV concentration in hair (<6.85 ng/mg) were both associated with VF (Table 2).

DISCUSSION

Although half of our patients on second-line ART had a repeat VF episode, 25% had persistent VF over ≥ 3 consecutive episodes over ≥ 18 months; the virus was resuppressed in the remainder of them. Only a few of the children developed a major PI resistance mutation, and half of them had an NRTI resistance mutation. Those who maintained a high level of adherence, as verified by their LPV hair concentration, remained virologically suppressed.

Our findings of the generally poor reliability of self-reported adherence relative to plasma TDM and hair drug levels are similar to findings in other studies and highlight the lack of a practical and reliable adherence-measurement tool for children and adolescents. Although time and effort go into documenting levels of self-reported adherence, there is an emerging preference for simplifying pediatric adherence measurements

Table 2. Characteristics Associated With VF Among Those on a Lopinavir-Based Regimen*

Covariate	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P	aOR (95% CI)	P
Female sex	2.26 (0.81–6.30)	.12		
Age		.005		.24
<11 years	Reference		Reference	
11–14 years	1.03 (0.41–2.59)		0.99 (0.22–4.39)	
≥ 14 years	4.39 (1.54–12.55)		1.78 (0.35–9.06)	
Lopinavir hair concentration		<.001		<.001
<6.85 ng/mg	Reference		Reference	
6.85–11.60 ng/mg	0.05 (0.01–0.21)		0.07 (0.02–0.25)	
11.60–14.60 ng/mg	0.03 (0.01–0.13)		0.07 (0.02–0.27)	
≥ 14.60 ng/mg	0.02 (0.01–0.10)		0.03 (0.01–0.15)	
Body surface area		.38		
<0.90 m ²	Reference			
0.90–1.11 m ²	1.04 (0.26–4.13)			
1.11–1.26 m ²	1.47 (0.34–6.32)			
≥ 1.26 m ²	2.40 (0.57–10.14)			
CD4 count		.001		.029
<350 cells/ μ L	5.88 (2.27–15.25)		5.22 (1.18–23.05)	
≥ 350 cells/ μ L	Reference		Reference	
Unknown	1.00 (0.17–6.06)		2.09 (0.43–10.12)	
VAS		.001		.026
$\geq 95\%$	0.16 (0.06–0.46)		0.04 (0.004–0.47)	
<95%	Reference		Reference	

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio; VAS, visual analog scale; VF, virologic failure.

*Factors associated with VF for the subset of children on an LPV-based ART regimen for whom hair concentration results were available during follow-up (N = 51).

to “perfect versus not perfect” as an indicator that tailored interventions are needed [9, 10]. It is important also that these interventions be focused on the individual(s) primarily responsible for daily medicines, whether that person is an adult caregiver or the child or adolescent. Applying principles of differentiated care to youth with a previous history of viremia can further help to focus limited adherence-support resources on those at the greater risk of treatment failure. Although TDM and hair testing are not routinely available in resource-limited settings outside of research contexts, the broader availability of viral load testing makes more frequent monitoring among adolescents with a history of previous VF and “imperfect” adherence a priority.

The strengths of our study are in its longitudinal design in prolonged follow-up of children and adolescents on second-line ART after well-characterized episodes of viremia along with the incorporation of both subjective and objective adherence metrics for evaluating the VF and resistance-testing results. The limitations of our study include the small sample size, restriction of our multivariate modelling to those on an LPV-based regimen, and lack of data on social and behavioral factors that could affect adherence, including information on who was primarily responsible for ensuring adherence (eg, caregiver versus child/adolescent).

CONCLUSION

The viral loads were resuppressed in half of the children and adolescents living with HIV in our cohort with a previous history of VF while on PI-based second-line ART, but the other half of the children experienced repeat VF episodes that were clearly verified to be related to poor adherence. However, viral resistance to PIs emerged infrequently, likely because of a lack of selective drug pressure, and NRTI resistance did not seem to be as substantial a factor in the context of LPV-based second-line ART. Nevertheless, prolonged virologic and treatment failure will lead to progressive clinical and immunologic decline, which reduces the chances that adolescents with PHIV will maintain the benefits of effective ART in terms of decreased risk of morbidity and death. Without more effective and better-targeted interventions to improve adherence, we risk losing many more young people with PHIV.

Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

Notes

Acknowledgments. We gratefully acknowledge the participation of the children, adolescents, and their families and the contributions of all study staff.

TASER-Px Steering Committee Members. K. H. Truong (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), S. Kerr, A. Avihingsanon, N. Thammajaruk, and C. Ruengpanyathip (HIV Netherlands Australia Thailand Research Collaboration, Thai Red Cross AIDS Research Centre, Bangkok, Thailand), L. V. Nguyen and K. D. T. Khu (National Hospital of Pediatrics, Hanoi, Vietnam), P. Kosalaraksa, P. Lumbiganon, and C. Sopharak (Division of Infectious Diseases, Department of Pediatrics, Khon Kaen University, Khon Kaen, Thailand), and A. H. Sohn, J. Ross, and T. Singtoroj, TREAT Asia/amfAR-Foundation for AIDS Research, Bangkok, Thailand.

Financial support. This study is an initiative of TREAT Asia, a program of amfAR, The Foundation for AIDS Research, with support from ViiV Healthcare, and additional support from LIFE+ and the US National

Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases (NIAID), Eunice Kennedy Shriver National Institute of Child Health and Human Development, as part of the International Epidemiology Databases to Evaluate AIDS (IeDEA) (grant U01AI069907). Hair-level assays and testing were also supported by NIAID/NIH grant 2R01AI098472 (principal investigator, M. G.).

Disclaimer. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above.

Potential conflicts of interest. A. H. S. has received support to her institution for travel and grants from ViiV Healthcare. All other authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. UNAIDS. Global Report: UNAIDS report on the global AIDS epidemic 2017. 2017. Available at: <http://aidsinfo.unaids.org/>
2. Jobanputra K, Parker LA, Azih C, et al. Factors associated with virological failure and suppression after enhanced adherence counselling, in children, adolescents and adults on antiretroviral therapy for HIV in Swaziland. *PLoS One* **2015**; 10:e0116144.
3. Xu L, Munir K, Kanabkaew C, Le Coeur S. Factors influencing antiretroviral treatment suboptimal adherence among perinatally HIV-infected adolescents in Thailand. *PLoS One* **2017**; 12:e0172392.
4. MacDonell K, Naar-King S, Huszti H, Belzer M. Barriers to medication adherence in behaviorally and perinatally infected youth living with HIV. *AIDS Behav* **2013**; 17:86–93.
5. Prasitsuebsai W, Sethaputra C, Lumbiganon P, et al; TAPHOD ACASI Study Group of IeDEA Asia-Pacific. Adherence to antiretroviral therapy, stigma and behavioral risk factors in HIV-infected adolescents in Asia. *AIDS Care* **2018**; 30:727–33.
6. Prasitsuebsai W, Teeraananchai S, Singtoroj T, et al. Treatment outcomes and resistance patterns of children and adolescents on second-line antiretroviral therapy in Asia. *J Acquir Immune Defic Syndr* **2016**; 72:380–6.
7. Prasitsuebsai W, Kerr SJ, Truong KH, et al. Using lopinavir concentrations in hair samples to assess treatment outcomes on second-line regimens among Asian children. *AIDS Res Hum Retroviruses* **2015**; 31:1009–14.
8. DiFrancesco R, Rosenkranz SL, Taylor CR, et al. Clinical pharmacology quality assurance program: models for longitudinal analysis of antiretroviral proficiency testing for international laboratories. *Ther Drug Monit* **2013**; 35:631–42.
9. Vreeman RC, Nyandiko WM, Liu H, et al. Measuring adherence to antiretroviral therapy in children and adolescents in western Kenya. *J Int AIDS Soc* **2014**; 17:19227.
10. Vreeman RC, Wiehe SE, Pearce EC, Nyandiko WM. A systematic review of pediatric adherence to antiretroviral therapy in low- and middle-income countries. *Pediatr Infect Dis J* **2008**; 27:686–91.