Association of Virologic Failure and Nonnucleoside Reverse Transcriptase Inhibitor Resistance Found in Antiretroviral-Naive Children Infected With Human Immunodeficiency Virus and Given Efavirenz-Based Treatment

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Among 66 antiretroviral-naive children aged <3 years with human immunodeficiency virus (HIV) or coinfecting with HIV and tuberculosis and initiating efavirenz-based antiretroviral therapy (ART), non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance was detected before ART in 5 (7.6%). Virologic failure occurred in 2 of these children; they were last tested at 16 and 24 weeks of ART. Pre-ART NNRTI resistance was not associated with virologic failure.

Keywords. efavirenz; HIV drug resistance; HIV-infected children; HIV/TB coinfection; NNRTI resistance.

Tuberculosis (TB) is the most common cause of death in hospitalized adults and children living with human immunodeficiency virus (HIV), accounting for approximately one-third of all deaths in this population [1]. Early initiation of HIV antiretroviral therapy (ART) for patients with

HIV-associated TB is critical for reducing the incidence of morbidity and death [1]. For children living with HIV/TB coinfection, especially those in a low- or middle-income country (LMIC), where the disease burden from HIV and TB is high, options for ART are limited because of interactions of most antiretroviral drugs (ARVs) with the anti-TB drugs rifampicin and isoniazid [1, 2]. Alternative ARVs are often more expensive, have not been well studied, or are unavailable for young children [2].

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) included in most ART regimens for individuals aged >3 years who are receiving anti-TB cotreatment [1]. Unlike lopinavir-ritonavir, a protease inhibitor (PI) used in the recommended first-line ART for children aged <3 years, and nevirapine (NVP), an NNRTI used in alternative regimens, EFV has fewer interactions with anti-TB therapy [1, 2]. Although drug resistance mutations (DRMs) commonly selected through NVP-based prevention of perinatal transmission (PPT) strategies are predicted to diminish rates of HIV replication suppression by EFV-based ART, a recent clinical trial found that switching NVP-exposed children aged 3 to 6 years from PI-based to EFV-based ART was effective for maintaining viral suppression [3]. In the context of LMICs, EFV costs less, is easier to administer, and is better tolerated than PI-based regimens [1].

To explore the potential use of EFV in younger populations, protocol P1070 was conducted by the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network to study the effect of genetic polymorphisms on EFV metabolism and establish EFV dosing guidelines for children aged <3 years [4]. As part of protocol P1070, the present study investigated the frequency of pre-ART drug resistance (PDR) to NNRTIs in children with HIV or HIV/TB coinfection who, according to their history, were not exposed to NNRTIs in utero via maternal use or after delivery. Also, because these children initiated EFV-based ART, we aimed to determine if children with NNRTI-associated DRMs had a greater rate of virologic failure (VF) or EFV-selected NNRTI DRMs.

METHODS

Study Design

Briefly, children qualified for enrollment if they had a diagnosis of HIV or HIV/TB coinfection, were 3 to 36 months of age and initiating ART, and had no exposure to NNRTIs in utero via maternal use or postnatal prophylaxis for PPT, unless they were ≥24 months of age. Participants were treated with an ART regimen containing EFV plus 2 nucleoside reverse transcriptase inhibitors (NRTIs). TB treatment included

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rifampicin, isoniazid, and pyrazinamide with or without ethambutol. *CYP2B6* genotyping and therapeutic pharmacokinetic monitoring were performed to achieve an EFV area under the curve in a 24-hour period (AUC_{0-24h}) between 35 and 180 $\mu\text{g}\cdot\text{h}/\text{mL}$. The study followed participants for 24 weeks between 2010 and 2015 [4] or until the end of anti-TB therapy if the only access to EFV was through the study. VF was defined as having an HIV RNA level of ≥ 400 copies/mL and was assessed after 8, 16, and 24 weeks of ART. HIV drug resistance testing was performed on specimens obtained at 0, 4, and 24 weeks of ART.

HIV Resistance Genotyping

Dried blood spots (DBSs) prepared on Whatman FTA cards (GE Healthcare Life Sciences, Chicago, Illinois) were processed as described [5], and ≥ 100 viral templates were amplified by nested HIV-1 *pol* DNA polymerase chain reaction (PCR) with first-round (forward, CCTACACCTGTCAACATAATTGG, and reverse, CAYTTGTCAGGATGGAGTTCATA) and second-round (forward, AATTAAAGCCAGGAATGGATGG, and reverse, CAAAGGAATGGAGGTTCTTTCTGATG) primers. If a DBS specimen was not available, RNA was extracted from 0.4 to 1 mL of plasma using the QIAamp viral RNA minikit (Qiagen, Valencia, California). Reverse transcription and PCR were carried out using the PrimeScript One Step RT-PCR kit (TaKaRa Bio USA, Inc, Mountain View, California). PCR products from each specimen were tested for HIV-1 NNRTI DRMs (K103N, V106M, Y181C, and G190A) using an oligonucleotide ligation assay (OLA) optimized for HIV-1 subtype C [5]. For specimens with an “indeterminate” result according to OLA (negative reaction for both mutant and wild-type genotypes), the genotype at the codon(s) in question was determined by consensus sequencing.

Statistical Methods

Demographic information and virologic outcome were compared between participants with the wild-type or PDR genotype at enrollment using the Fisher exact or a Mann–Whitney U test as appropriate.

RESULTS

Sixty-six participants enrolled in IMPAACT P1070 were tested for NNRTI DRMs. Participants were recruited from India, South Africa, and Zimbabwe. Sixty-five of them had a documented history of no previous NNRTI exposure, and 1 enrolled at 35 months of age after single-dose NVP at birth. The median age and HIV RNA level at enrollment were 19 months (range, 3–36 months [interquartile range (IQR), 11.8–27 months]) and 5.8 $\log_{10}$ copies/mL (range, 3.7–6.7 $\log_{10}$ copies/mL [IQR, 5.3–6.0 $\log_{10}$ copies/mL]), respectively. We found no differences in age, country, or clinical status between participants with wild-type genotype or those with PDR at enrollment (Table 1).

At enrollment, NNRTI DRMs were detected by OLA in 5 participants (7.6% [95% confidence interval, 2.9%–16.9%]), 4 infected with HIV and 1 coinfecting with HIV and TB. In all cases, PDR mutations were detected at a single codon, K103N ($n = 3$) or G190A ($n = 2$). The same mutation was detected in subsequent specimens from these children at a similar or decreased proportion of their HIV quasiespecies. The median mutant frequency at baseline was 98% (range, 6.5%–100%) and decreased to 15% (range, 3%–48%) at the last study visit for 3 children for whom specimens obtained at 16 to 24 weeks of ART were available (Table 2).

The plasma HIV RNA level was >400 copies/mL in 4 (7.7%) of 52 and 1 (2.4%) of 42 children who remained in the study

Table 1. Baseline Demographic Characteristics and Virologic Outcome of Efavirenz-Based ART According to Genotype at Enrollment

Variable	Total Cohort (N = 66)	PDR Genotype ^a (N = 5)	Wild-Type Genotype ^b (N = 61)	P
Age (median [IQR]) (mo) ^b	19 (11.8–27)	19 (15–20)	19 (11.5–27)	.88
Plasma HIV RNA (median [IQR]) ($\log_{10}$ copies/mL)	5.84 (5.29–6.00)	6.00 (5.84–6.19)	5.80 (5.25–6.00)	.22
Efavirenz AUC (median [IQR]) ($\mu\text{g}\cdot\text{h}/\text{mL}$) ^b	119.1 (67.7–160.8)	119.1 (75.5–201.7)	117.4 (62.0–160.0)	.79
Country (n [%])				.50
India	19 (28.8)	2 (40.0)	17 (27.9)	
South Africa	31 (47.0)	1 (20.0)	30 (49.2)	
Zimbabwe	16 (24.2)	2 (40.0)	14 (22.9)	
Clinical status (n [%])				1.00
HIV infection only	50 (75.8)	4 (80.0)	46 (75.4)	
HIV/TB coinfection	16 (24.2)	1 (20.0)	15 (24.6)	
Plasma HIV RNA level, ≥ 400 copies/mL (n/N [%]) ^b				
8 wk	26/56 (46.4)	3/5 (60.0)	23/51 (45.1)	.66
16 wk	4/52 (7.7)	0/5 (0.0)	4/47 (8.5)	1.00
24 wk	1/42 (2.4)	0/3 (0.0)	1/39 (2.6)	1.00

Abbreviations: ART, antiretroviral therapy; AUC, area under the curve; HIV, human immunodeficiency virus; IQR, interquartile range; PDR, pre-ART drug resistance; TB, tuberculosis.

^aOne participant with PDR (100% K103N at 0 and 4 weeks) and documented history of nevirapine exposure experienced ART suppression of HIV replication at study week 16.

^bAge and efavirenz AUC were not available from 6 participants excluded from the parent P1070 study because they received efavirenz in a formulation not allowed by the protocol; all 6 of these patients had a wild-type genotype at enrollment and were excluded from the virologic outcome analysis.

Table 2. Enrollment Characteristics, Mutant Frequency Detected by OLA, and Virologic Outcome Through 24 Weeks of EFV-Based ART in Children With Pre-ART NNRTI Drug Resistance

Infant No.	Age at Enrollment (mo)	EFV AUC (μg·h/mL)	Mutation Detected by OLA	Mutant Detected in HIV Quasispecies of Individual According to Time on EFV ART (%)				VL According to Time on EFV ART (copies/mL)			
				0 wk	4 wk	16 wk	24 wk	0 wk	8 wk	16 wk	24 wk
29 ^a	35	75.45	K103N	100	100	NS	NS	685 321	561	<400	<400
35	11	63.71	G190A	6.5	3	3	LTFU	>750 000	1333	<400	LTFU
40	19	339.48	G190A	98	88	NS	15	1 553 660	<400	<400	<400
48	15	201.73	K103N/S	100	NS	NS	NS	1 888 422	977	<400	<400
59	20	119.06	K103N	86	25	48	LTFU	251 000	<400	<400	LTFU

Abbreviations: AUC, area under the curve; ART, antiretroviral therapy; EFV, efavirenz; HIV, human immunodeficiency virus; LTFU, lost to follow-up or exited the study before week 24 of ART; NNRTI, non-nucleoside reverse transcriptase inhibitor; NS, no dried blood spot specimen was collected or available for drug resistance testing; OLA, oligonucleotide ligation assay; VL, plasma HIV RNA viral load.

^aThis infant was exposed to single-dose nevirapine at birth and was coinfectd with HIV and tuberculosis.

at 16 and 24 weeks of EFV ART, respectively. Three of the 4 patients with VF at 16 weeks continued in the study and had a viral load (VL) of <400 copies/mL at 24 weeks; the other patient was lost to follow-up. The child with VF at 24 weeks had a VL of 404 copies/mL. The proportions of participants with a plasma HIV RNA level of ≥400 copies/mL at weeks 8, 16, and 24 among those with PDR versus those with wild-type virus at enrollment were not significantly different (Table 1). All 5 participants with PDR achieved viral suppression by week 16 of EFV ART, and 3 with follow-up remained virologically suppressed at week 24 (Table 2). Children with VF at weeks 16 or 24 did not have DRMs detected by OLA.

DISCUSSION

Of the 66 children in this study aged <3 years with HIV or HIV/TB coinfection and initiating EFV-based ART, the frequency of NNRTI PDR was lower than previously reported rates in young children, although many of those reported received NVP as prophylaxis for PPT. Important to note is that in this small study, a single NNRTI mutation was not associated with increased VF in those receiving EFV-based ART.

Studies of pediatric PDR among PPT-unexposed children in Africa near the time of this study (2010–2013) reported rates that ranged from 7.7% in Uganda to 26.8% in Togo [6], and a systematic review and meta-analysis that included children from more than 13 countries in sub-Saharan Africa estimated a prevalence of NNRTI PDR of 9.7% [7]. The lower rate of PDR that we observed could be attributed to our use of OLA, designed to detect only 4 DRMs that confer high-level resistance to NNRTIs selected by NVP or EFV [5], whereas other studies used consensus sequencing and counted DRMs that conferred any level of reduced susceptibility to NNRTIs [6, 8, 9].

In this study, the rate of VF with EFV ART was lower than that in previous studies with children on NNRTI-ART [8, 9], and all children with PDR achieved viral suppression by 16 weeks of ART. A Nigerian study of PPT-unexposed children initiating NNRTI ART reported that 33% of them had an HIV

RNA level of >1000 copies/mL within a 2-year period, and the rate was 58.3% among those with PDR [8]. A 6-month study of Malian children reported a similar rate of VF and a significant association between NNRTI PDR and VF (odds ratio, 22.9), although unreported exposure to ARV drugs was suspected [9]. Most children in these studies received NVP-based ART. The mutation Y181C is frequently selected in infants exposed to NVP for PPT and has been associated with VF of subsequent NVP-based ART [10]. In our cohort, only K103N and G190A were detected, which is likely because these resistance mutations were transmitted from their mothers and not selected by PPT. Worth noting is that phenotypic assays have found that Y181C confers intermediate resistance to EFV [11]. Also relevant is that studies of adults in which NVP- and EFV-containing regimens were compared found that virologic and clinical outcomes with EFV-based ART were superior to those with NVP-based ART [12], and adults with a single NNRTI PDR did not seem to experience increased VF compared to those with wild-type variants [13, 14]. In addition, although interpatient pharmacokinetic variability and inadequate drug levels could contribute to VF in children, EFV doses in the P1070 protocol were adjusted to optimize drug exposure, and all participants with PDR achieved a concentration higher than the minimum target [4]. Our results suggest that despite increasing rates of transmitted NNRTI resistance in LMICs, EFV should not be ruled out as a treatment option, especially in HIV/TB-coinfectd children. Integrase inhibitors, namely raltegravir, are not always available for infants, and lopinavir-ritonavir ART poses adherence challenges in young children, interacts with anti-TB drugs, and costs more than EFV. In the setting of NVP exposure for PPT, however, selection of drug resistance in infants could include multiple DRMs and thus increase the risk of VF with subsequent EFV ART. Switching NVP-exposed children aged 3 to 6 years from PI-based ART to EFV-based ART was effective at maintaining viral suppression [3], but additional studies are needed to assess the efficacy of EFV in younger children who had PPT or have transmitted resistance.

Limitations of this study include its small sample size, high rate of early study discontinuation, and relatively short study duration, which limited the power to detect associations between PDR and VF. Also, although the OLA is more sensitive than consensus sequencing for detecting DRMs, variable numbers of viral templates amplified from DBSs could have decreased the sensitivity of detection of mutant variants at low frequency. Last, the OLA tested only 4 major NNRTI DRMs, which might have resulted in an underestimation of PDR in this population.

Despite these limitations, our results suggest that PDR at a single NNRTI codon that confers high-level resistance might not confer VF in young children prescribed EFV-based ART. With the limited number of ARVs available for pediatric treatment in LMICs, an optimized EFV regimen could provide an effective option that is more accessible than the currently recommended PI-based ART for children aged <3 years, especially in settings with high rates of TB coinfection.

Notes

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