Nevirapine Resistance in Previously Nevirapine-Unexposed HIV-1-Infected Kenyan Infants Initiating Early Antiretroviral Therapy

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Abstract

Nevirapine (NVP) resistance occurs frequently in infants following NVP use in prevention of mother-to-child transmission (PMTCT) regimens. However, among previously NVP-unexposed infants treated with NVP-antiretroviral therapy (ART), the development and impact of NVP resistance have not been well characterized. In a prospective clinical trial providing early ART to HIV-infected infants < 5 months of age in Kenya (OPH03 study), we followed NVP-unexposed infants who initiated NVP-ART for 12 months. Viral loads were assessed and resistance determined using a population-based genotypic resistance assay. Of 99 infants screened, 33 had no prior NVP exposure, 22 of whom were initiated on NVP-ART. Among 19 infants with follow-up, seven (37%) infants developed resistance: one at 3 months and six at 6 months after ART initiation. The cumulative probability of NVP resistance was 5.9% at 3 months and 43.5% at 6 months. Baseline HIV RNA levels ($p=0.7$) and other characteristics were not associated with developing resistance. Post-ART, higher virus levels at visits preceding the detection of resistance were significantly associated with increased detection of resistance ($p=0.004$). Virus levels after 6 and 12 months of ART were significantly higher in infants with resistance than those without ($p=0.007$, $p=0.030$, respectively). Among infants without previous NVP exposure, development of NVP resistance was frequent and was associated with virologic failure during the first year of ART. Earlier development of NVP resistance in infants than in adults initiating NVP-ART may be due to longer viremia following ART or inadequate NVP levels resulting from NVP lead-in dosing. The development of NVP resistance may, in part, explain the superiority of protease inhibitor-based ART in infants.

Introduction

EARLY ANTIRETROVIRAL THERAPY (ART) initiation in infants results in long-term viral suppression, maintenance of high CD4 T cells, and a slower progression to AIDS and mortality.¹⁻⁶ Thus, the World Health Organization (WHO) guidelines recommend ART for all infants younger than 5 years of age regardless of clinical, immunologic, or virologic criteria.^{7,8} Current WHO guidelines recommend protease inhibitor (PI)-based ART for children less than 3 years of age, regardless of prior non-nucleoside reverse transcriptase inhib-

itor (NNRTI) exposure.^{7,8} However, in many settings nevirapine (NVP) continues to be used in NVP-unexposed infants because of its lower cost and drug availability at clinic sites.^{2,9}

In adults without prior NVP exposure, NVP and PI regimens in initial ART result in comparable virologic suppression. A multisite randomized clinical trial (RCT) among adult women without prior NVP exposure in seven African countries (OCTANE) demonstrated equivalent virologic efficacy in those treated with NVP-based ART versus PI-based ART.⁹ However, women in the NVP-ART arm had higher rates of treatment discontinuation and drug resistance.⁹ There are

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conflicting data regarding NVP- vs. PI-ART among NVP-unexposed children. In a multisite pediatric RCT (P1060) comparing NVP- versus PI-based ART in NVP-unexposed children, there were more primary events (virologic failure or treatment discontinuation by 24 weeks) in the NVP-ART than PI-ART arm (40.8% versus 19.3%).¹⁰ In this study, among a subset of 32 children who received NVP-ART, 66% had NVP resistance at the time of virologic failure and none of the children who failed PI-based ART developed protease resistance.¹⁰ In contrast, the PENPACT-1 trial noted no difference in virologic outcomes in NVP-unexposed children treated with NNRTI- or PI- based ART regimens.¹¹

Infants have higher pre-ART HIV RNA viral levels than adults¹² and may experience suboptimal drug levels,^{13,14} which could account for the higher rate of virologic failure, mortality, and drug resistance seen in infants¹⁰ than adults.⁹ Furthermore, younger infants may have slower viral decay rates than older children,¹⁵ and this could explain differences between the P1060 RCT and the PENPACT-1 trial, the latter of which had a broader age range and higher median age. These previous studies have examined NVP resistance at the time of virologic failure. To date, detailed longitudinal assessments of the development of NVP resistance in infants without previous exposure to NVP have not been available. We examined the development of NVP resistance in a cohort of HIV-infected infants with no previous NVP exposure who were initiated on NVP-ART and followed for 12 months, and evaluated the associations between the development of NVP resistance and subsequent viral suppression.

Materials and Methods

Study population and sample collection

HIV-1-infected infants <5 months of age were enrolled in an RCT initiated in September 2007 in Nairobi, Kenya [Optimizing Pediatric HIV-1 Therapy 03 (OPH03): NCT00428116], as has been described previously.¹⁶ Briefly, mother-infant pairs were approached at antenatal and postnatal Nairobi City Council clinics for recruitment in the study following screening for HIV status. In addition, HIV-infected infants identified following admission to Kenyatta National Hospital were recruited. Following informed consent, a filter paper blood sample was obtained for confirmation of infant HIV-1 status. Additional eligibility criteria were (1) not yet received ART, except for prevention of mother-to-child transmission (PMTCT), (2) age <5 months, and (3) caregiver planning to reside in Nairobi for >36 months. Ethical approval was obtained from the University of Washington and the Kenyatta National Hospital/University of Nairobi Institutional Review Boards.

At enrollment, caregivers of infants provided demographic information. Exposure of mother or infant to NVP for PMTCT was based on caregiver self-report and was confirmed by review of medical records when possible. Blood samples were collected from infants and mothers for CD4, viral load, and resistance testing. Three adherence and counseling sessions were provided to all caregivers prior to ART initiation. For hospitalized infants, ART initiation was deferred until stabilization and hospital discharge.

Infants were initiated on ART consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and either an NNRTI or a PI. NRTIs included lamivudine and either zidovudine or

abacavir. Children exposed to NVP-PMTCT were prescribed a PI regimen including ritonavir-boosted lopinavir (LPV/r), while children without previous exposure to NVP-PMTCT and not on TB medications were prescribed an ART regimen including nevirapine. Nevirapine was administered in a classic dose-escalation strategy, in which infants were given a dose of 160 to 200 mg/m² of body surface area once daily for 14 days and then increased to twice daily, according to WHO guidelines.¹⁷

Following ART initiation, blood samples were collected from infants at 1 and 3 months after initiation of ART and every 3 months thereafter for up to 2 years. CD4 levels were determined in real time every 6 months, and HIV-1 viral load testing was done retrospectively on the 1 month and quarterly samples. Adherence to therapy was monitored by caregiver report (any missed dose in the past 3 days or 2 weeks) and when available by weights of medicine bottles returned to the study pharmacists. Blood samples were collected from mothers of the infants at 12 months after infant enrollment in the study.

For the present substudy, we included subjects without previous exposure to NVP-PMTCT and initiated on NVP-ART. We analyzed data during the first 12 months of ART.

Laboratory assays

HIV-1 DNA filter paper assays were performed in Nairobi using a highly specific and sensitive combined “in-house” PCR specific to *pol* and *gag*.^{18,19}

Plasma was separated from whole blood, aliquoted, and stored at –80°C until use. One aliquot was shipped to Seattle in liquid nitrogen for viral load monitoring. Total CD4% and CD4 cell counts were obtained using FACS Calibur (Becton Dickinson). Plasma HIV-1 RNA levels were determined using the Gen-Probe HIV-1 Viral Load Assay (San Diego, CA), which has been validated for detection of HIV-1 subtypes prevalent in Kenya.²⁰

Genotypic testing. All baseline samples and any follow-up samples testing with HIV RNA viral load >1,000 copies/ml were evaluated for genotypic resistance in Nairobi or Seattle using an “in-house” population-based sequencing method as described previously.²¹ Briefly, viral RNA was extracted from 140 μ l of plasma using a QiAmp viral RNA kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. A nested reverse transcriptase-polymerase chain reaction (RT-PCR), followed by a second PCR, was performed to amplify 645 bp of HIV-1 *pol*.²¹ The PCR products of correct size were confirmed by gel electrophoresis and purified and sequenced by dideoxynucleoside-based analysis using a Big Dye terminator kit (Applied Biosystems) and ABI Prism 3100 equipment. Genotypic resistance was defined as the presence of resistance mutations associated with impaired drug susceptibility using the Stanford Genotypic Resistance Interpretation Algorithm (<http://hivdb.stanford.edu/>). Codons included major NNRTI mutations at K103N, Y188L, Y181C, and G190A, and major NRTI mutations, M184V, K65R, and thymidine analog mutations (TAMs). For subjects with detected resistance, samples from earlier time points with viral loads <1,000 copies/ml were evaluated to further refine the timing of the first detection of resistance.

HIV-1 subtypes were determined using the NCBI subtyping tool (www.hiv.lanl.gov), and phylogenetic trees were

constructed from *pol* sequences with PAUP* version 4.0 Beta10,²² by creating a neighbor-joining phylogenetic tree with reference sequences from the Los Alamos National Laboratory HIV Database (www.hiv.lanl.gov/).

For infants with detected resistance, viral sequences from the mothers' samples were isolated and compared to rule out that resistance was transmitted.

After 6 months on ART, virologic failure was defined by sustained viral loads of >1,000 copies/ml or having last available sample >1,000 copies/ml.

Statistical analysis

Cumulative probabilities of timing of first detected resistance were estimated using Kaplan–Meier analysis, and Cox regression was used to evaluate characteristics for association with the development of resistance.

Pre-ART baseline characteristics included infant demographics (age and gender), anthropometric measures [weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ) z-scores], clinical (WHO stage) and immunological measures (CD4 percentage and count), HIV subtype and viral load, and maternal CD4 count and ART regimen. Follow-up characteristics included infant's ART regimen and adherence to ARVs, breastfeeding status, and HIV-1 RNA viral loads at 1 month post-ART initiation and quarterly thereafter. HIV-1 RNA viral load and adherence were modeled as time-varying covariates, as the values from the interval prior to the evaluation of resistance.

Subsequent virus levels post-ART initiation were compared between children who did and did not develop resistance using Wilcoxon rank sum tests. The proportions of children experiencing viral failure were compared using a Fisher's exact test.

Results

Study population and baseline characteristics

Ninety-nine HIV-infected infants were enrolled in the parent randomized clinical trial (OPH03 study) at <5 months of age, of whom 33 infants were determined to have neither maternal nor infant NVP-PMTCT exposure, and 26 initiated ART. Of these, four infants initiated PI-ART because they were also on medications for TB and 22 infants initiated NVP-ART. Nineteen had follow-up samples available and were included in the present substudy (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/aid). Seventeen infants were initiated on NVP/3TC/AZT regimens and two infants on NVP/3TC/ABC.

Infants had a median WAZ of -3.21 , HAZ of -2.12 , and WHZ of -0.88 (Table 1). Of infants 53% had HIV WHO clinical stage III/IV, and the median CD4% was 19% (interquartile range, IQR; 15, 25). The median HIV RNA viral load was $6.7 \log_{10}$ copies/ml (IQR, 6.1, 7.0). The majority of infants were infected with clade A (63%), and others had clades C (16%), D (11%), and intersubtype recombinants (11%) based on polymerase gene sequences (Table 1).

Development of NVP resistance in NVP unexposed infants

Enrollment samples from 17 of the 19 infants were available, all of which indicated no detectable resistance. One infant had a

TABLE 1. BASELINE CHARACTERISTICS OF NEVIRAPINE-UNEXPOSED INFANTS

Characteristic (N = 19)	Median (IQR) or n (%)
Infant characteristics	
Female	8 (42)
Age at enrollment, months	3.8 (2.4–4.2)
Weight-for-age z-score (WAZ)	-3.21 (-3.73 – -1.34)
Height-for-age z-score (HAZ)	-2.12 (-3.25 – -0.93)
Weight-for-height z-score (WHZ)	-0.88 (-3.07 – -0.12)
HIV WHO Clinical Stage	10 (53)
III/IV	
CD4 ⁺ T cells, %	19 (15–25)
CD4 ⁺ T cell count, cells/ μ l	1,311 (760–1,939)
Plasma HIV-1 RNA, copies/ml ^a	5,300,000 (1,280,450–9,189,600)
Plasma HIV-1 RNA, $\log_{10}$ copies/ml ^a	6.72 (6.11–6.96)
HIV-1 subtype	
A	12 (63)
C	3 (16)
D	2 (11)
Recombinant	2 (11)
ART regimen	
NVP/3TC/AZT	17 (89)
NVP/3TC/ABC	2 (11)
Maternal characteristics	
Age, years	25 (23–31)
Secondary or college education	11 (58)
Housewife or unemployed	18 (95)
CD4 ⁺ T cell count, cells/ μ l ^b	417 (201–486)
On ARV regimen	1 (5)
Breastfeeding	
Infant ever breastfed	18 (95)
Duration of breastfeeding, months ^c	10 (6–15)

^aInformation was unavailable for two mother–infant pairs.

^bInformation was unavailable for one mother–infant pair.

^cThree mothers reported ever breastfeeding their infants, but did not report the duration of breastfeeding.

small volume of enrollment plasma available for viral load (VL) testing only, and one infant did not have an enrollment sample available; however, both of these infants had samples at 1-month post-ART initiation that indicated no resistance, and were thus considered to have no resistance at baseline.

Following initiation of ART, the infants contributed 78 samples during the first year on ART; 25 samples had VL <1,000 copies/ml, yielding 53 samples for resistance testing (including one sample with VL unavailable). Of the 53 samples, 51 samples were amplified by PCR, of which 19 samples had resistance detected. For the seven infants with resistance detected, we examined three additional samples at earlier time points with viral loads <1,000 copies/ml to better estimate the timing of the first detection of resistance. One sample did not amplify, one amplified with no resistance detected, and one amplified with resistance detected. Overall, 20 samples had resistance detected. These samples were from seven infants, of whom two infants had resistance detected in two samples, four in three samples, and one in four samples (Table 2). One infant had

TABLE 2. INFANT VIRAL LOAD (COPIES/ML) AND TYPE OF RESISTANCE MUTATIONS AMONG INFANTS WHO DEVELOPED RESISTANCE

PTID	Parameter	Enrollment	Month 1	Month 3	Month 6	Month 9	Month 12
03-0011	VL c/ml R/Mut	No sample No sample	94,400 None	208,800 K103N M184V	559,400 K103N M184V	569,200 K103N M184V	166,600 K103N M184V
03-0015	VL c/ml R/Mut	3,421,150 None	52,285 None	4,470 None	358,140 Y181C A98AG M184V	1,228,500 Y181C A98G M184V	2,056,800 Y181C A98G M184V <i>K70KR</i> <i>D67DN</i> <i>T215NSTY</i>
	<i>TAMs</i>						
03-0025	VL c/ml R/Mut	4,996,750 None	10,825 None	10,780,500 None	99,315 Y188L M184V	219,725 Y188L M184V	101,995 Y188L M184V
03-0043	VL c/ml R/Mut	7,883,300 None	152,045 None	36,200 None	1,262,000 Y188L D67DG <i>K70R</i>	608,500 None	994,500 Y188*FLY M184MV T215I <i>D67DN</i> <i>K70KR</i> <i>K219EK</i>
	<i>TAMs</i>						
03-0045 ^a	VL c/ml R/Mut	17,734,500 None	88,290 None	16,175 None	412,230 Y181C D67G M184V	170,190 Y181C D67G M184V	1,045,600 Y181C D67DG M184V
03-0054	VL c/ml R/Mut	1,2466,650 None	1,580 None	885 —	1,704,500 K103N	695,600 K103N M184IMV	168,115 K103N M184IMV
03-0075 ^b	VL c/ml R/Mut	164,200 None	4,260 None	690 —	320 G190A K65R	19,385 G190A K65KR M184MV <i>D67DN</i>	No sample No sample
	<i>TAMs</i>				<i>M41LM</i>		

^aThe mother of this infant was on ARVs at enrollment, but the regimen could not be ascertained.

^bOne infant had initial first detected sample at month 9; however, this subject had a sample from an earlier time point with viral load < 1,000 copies/ml and resistance detected, and so the timing of first detection was considered as month 6.

All seven infants had additional M184V (NRTI) mutations; this confers high-level resistance to lamivudine (3TC) and emtricitabine (FTC), but increases susceptibility to zidovudine (AZT) and tenofovir (TDF).

Note: refer to Supplementary Table S1 for infant viral load (copies/ml) and PCR amplification among infants who did not develop resistance.

Bold: sample was unavailable.

Italics: HIV-1 plasma viral load < 1,000 copies/ml and resistance testing not performed.

the first detection of NVP resistance at 3 months and six infants had the first detection at 6 months on ART.

Overall follow-up was 12.2 person-years, and the incidence of first detection of NVP resistance was 57.5 cases per 100 person-years. The cumulative probability was 5.9% at 3 months and 43.5% at 6 months (Fig. 1). Four infants were censored before month 12: one subject was lost to follow-up, two died, and one had the regimen switched due to TB medications (Supplementary Table S1).

Mutations present in the seven infants who developed resistance

All seven infants developed major NNRTI-resistant mutations that conferred high-level resistance to NVP: two infants each developed mutations at codons K103N, Y181C, and Y188L, and one infant developed a G190A mutation. In each infant, only one major NVP mutation was noted. These

major NVP resistance mutations were also detectable in samples at visits after the development of resistance.

Additionally among all seven infants who developed NNRTI resistance, M184V NRTI resistance mutations were also detected during follow-up. Four of the seven infants continued to accumulate additional NRTI mutations after the first detection of resistance. Three of these four infants with additional NRTI mutations had TAMs—one infant developed one TAM while two developed at least three TAMs. Overall, seven of the 17 infants who were prescribed AZT-containing regimens developed resistance (including three with TAMs), while neither of the two prescribed ABC-containing regimens did so.

Samples from mothers of infants developing NVP resistance

Among the group of infants who developed NVP resistance, all mothers reported ever breastfeeding their infants.

FIG. 1. Time to first detection of nevirapine (NVP) resistance mutations. The at-risk table is below the Kaplan–Meier figure. The number of subjects at risk at each time point is shown, and the number of failures at each time point is in parentheses. The numbers censored (lost/died) are shown in the figure as numbers, at their last follow-up (censor) times. One infant developed NVP resistance at 3 months and six infants at 6 months while on antiretroviral therapy (ART). The cumulative probability of developing NVP resistance among this group of 19 infants was 5.9% at 3 months and 43.5% at 6 months.

One mother reported the cessation of breastfeeding prior to enrollment, and six mothers reported breastfeeding until infants were a range of 6–39 months old. At enrollment only one of the mothers reported receiving postnatal ART, but the type of regimen could not be ascertained.

Six of the seven mothers had plasma samples available at baseline, and the relatedness of the viral sequences from each of the mother–infant pairs was assessed using phylogenetic analysis. All mother–infant pair sequences clustered together on a single node of the phylogenetic tree (data not shown), and no resistance was detected in any of the mother’s samples.

Correlates of NVP resistance

The timing of first detection of NVP resistance was not significantly associated with infant gender, age, growth measures, WHO stage, or CD4 percentage, nor with caregiver age or sociodemographics (Table 3). Pre-ART HIV RNA levels were not significantly correlated with the detection of resistance ($p=0.7$). Infants with subtype C infection were significantly more likely to have NVP resistance than infants with subtype A ($p=0.002$).

Following ART initiation virus levels declined, both among the group of seven who developed resistance and among those who did not (Fig. 2a and b). Higher virus levels at the visit preceding the assessment of resistance were significantly associated with increased detection of resistance ($p=0.004$). For every log increase in viral load the risk of resistance at the next visit increased by 56%. Compared to infants without mutations, children who developed mutations had smaller declines in viral loads at 1 month (2.73 vs. 2.06 log₁₀ copies/ml; $p=0.088$) and 3 months (3.27 vs. 2.63 log₁₀ copies/ml; $p=0.045$) post-ART initiation.

Caregiver-reported adherence in the interval since the last assessment of resistance was not correlated with resistance ($p=0.9$). Mothers of infants who developed resistance reported no missed doses of ART at 45 (94%) of 48 monthly

visits, and pharmacy-based adherence at 20 visits ranged from 80% to 110%.

NVP resistance and subsequent viral suppression

After 6 and 12 months on ART, viral levels were significantly higher in infants with NVP resistance than those without ($p=0.007$, $p=0.030$, respectively) (Fig. 2c). All seven infants with resistance experienced virologic failure compared with two of the nine infants without NVP resistance ($p=0.003$). Among the infants without NVP resistance one had an HIV-1 RNA level of 1,815 copies/ml at month 12 but was undetectable at month 15, and one infant experienced a viral blip at month 12 that subsequently declined to undetectable levels.

Discussion

In this study of HIV-infected infants who were not previously exposed to NVP-PMTCT and initiated on NVP-ART, seven of the 19 infants with follow-up (37%) developed NVP resistance during 1 year of ART. Higher virus levels at the preceding visit were significantly associated with increased detection of resistance, suggesting that the slow decay of virus in ART-treated infants and potentially inadequate NVP levels at initiation may have contributed to resistance in these ART-treated infants. The presence of NVP resistance had a significant impact on subsequent virus levels, with all infants experiencing viral failure by 12 months compared with 22% of infants who had no NVP resistance detected.

The proportion of infants with resistance detected in our study was similar to those who developed resistance in Cohort 2 of the P1060 study.¹⁰ In that study, 31% (45) of 147 NVP-unexposed children initiated on NVP-ART had virologic failure at 24 weeks.¹⁰ Of the 45 children, 32 had samples available for resistance testing and of those 21 (65%) children had resistant mutations detected (19 had NNRTI

TABLE 3. INFANT AND MATERNAL CHARACTERISTICS AND INCIDENCE OF NEVIRAPINE RESISTANCE

Characteristic	N	Hazard ratio (95% CI) ^a	p-value ^a
Baseline infant characteristics			
Female	11	Ref.	—
Male	8	2.14 (0.43–10.77)	0.4
Age			
< 3 months	12	Ref.	—
≥ 3 months	7	1.38 (0.32–5.87)	0.7
Weight-for-age z-score (WAZ)			
≥ -2	7	Ref.	—
< -2	12	1.84 (0.41–8.25)	0.4
Height-for-age z-score (HAZ)			
≥ -2	9	Ref.	—
< -2	10	1.57 (0.36–6.82)	0.5
HIV WHO Clinical Stage			
Grade I/II	9	Ref.	—
Grade III/IV	10	1.16 (0.25–5.28)	0.9
CD4 ⁺ T cells%			
≥ 25%	5	Ref.	—
< 25%	14	2.01 (0.19–21.68)	0.6
HIV-1 subtype			
A	12	Ref.	—
C	3	14.02 (2.65–74.22)	0.002
D	2	2.36 (0.17–31.97)	0.5
Recombinant	2	1.62 (0.23–11.21)	0.6
Baseline maternal characteristics			
Age			
> 25 years	8	Ref.	—
≤ 25 years	11	1.47 (0.27–8.12)	0.7
Secondary or college education	9	Ref.	—
Primary education	10	2.29 (0.44–11.98)	0.3
CD4 ⁺ T cell count			
≥ 350 cells/ μ l	10	Ref.	—
< 350 cells/ μ l	8	3.91 (0.77–19.94)	0.101
Infant viral load			
Baseline HIV-1 plasma viral load, per log ₁₀ copies/ml increase	17	0.76 (0.22–2.60)	0.7
HIV-1 plasma viral load, per log ₁₀ copies/ml increase ^b	—	1.56 (1.15–2.12)	0.004
Adherence			
Caregiver-reported missed dose ^b	—	1.13 (0.26–4.86)	0.9

^aHazard ratios and *p*-values are from Cox regression models comparing each category to the referent category.

^bLog₁₀ HIV-1 viral load and adherence are modeled as time-varying covariates, as the values from the interval prior to the evaluation of resistance.

mutations and two children had only NRTI mutations) at the time of virologic failure.¹⁰

HIV-infected infants have elevated viral levels and slower viral suppression during ART compared to HIV-infected adults.^{9,10,12,15} One reason for the rapid development of resistance in our study may be that the children were in their infancy (<5 months of age) and had elevated levels of HIV RNA viral levels (median of 5,300,000 copies/ml) prior to ART. The combination of high HIV viral RNA levels with antiretroviral drug pressure during early therapy, and the slower time to viral suppression, likely led to the development of resistance early in infection in the infants. In addition, infants may have poorer adherence leading to slower viral decline; although infant adherence was not associated with resistance in this study, it is challenging to measure. We observed a higher incidence of resistance among infants with subtype C infection; however, only three infants were infected HIV-1 subtype C. This is consistent with a prior study

in which subtype C was associated with a higher risk of NVP resistance among mothers exposed sd-NVP for PMTCT.²³

Infants in our study were administered NVP in a classic-dose escalation strategy, possibly receiving suboptimal levels of NVP during the first 2 weeks of therapy and contributing to the development of NVP resistance. Dose escalation may spare toxicity to children, as demonstrated in the CHAPAS-1 study in which children (median age 5 years) who were randomized to receive full-dose NVP more frequently developed skin rash than those receiving dose escalation.²⁴ Balancing the risks of toxicity versus resistance will need to be considered in future dosing regimens.

The development of broad NRTI resistance mutations in infants who developed NNRTI-resistant mutations has the potential to impact the efficacy of second-line ART regimens. Specifically, four of seven infants maintained on failing NVP-based ART accumulated additional NRTI mutations between the first detection of resistance and 12 months

FIG. 2. HIV-1 RNA plasma viral load. HIV-1 RNA plasma viral load levels are presented for subjects with mutations detected during follow-up (a) and without mutations detected (b). A smoothed (lowess) curve (c) illustrates higher virus levels in the group with mutations. After 6 and 12 months on ART, viral levels were significantly higher in infants with NVP resistance than those without ($p=0.007$, $p=0.030$, respectively). All seven infants with resistance experienced virologic failure by 12 months on ART compared with two of the nine infants without NVP resistance ($p=0.003$).

follow-up. This has implications regarding how quickly failing regimens should be identified and switched and underscores the critical need for viral assessment among infants on ART, particularly those on NNRTI regimens.

A limitation of our study was the use of population-based sequencing for the detection of resistance, which does not detect low-level resistance. Therefore, the incidence of resistance in infants was likely underestimated. Infants were monitored using clinical and immunologic criteria required by national guidelines at the time, with HIV RNA viral levels analyzed retrospectively. Viral load or resistance monitoring in real time is useful for the evaluation of ART adherence and ART effectiveness.

A Ugandan study in children with viral RNA monitoring in real time identified 34% of children as having virologic failure while on first-line NNRTI-based ART.²⁵ Furthermore, when the children with virologic failure were targeted for resistance testing, resistance was detected either before initiation of ART or at the time of failure.²⁵ The strengths of the present study include the unique cohort of young HIV-infected infants (<5 months of age) initiated on early NVP-ART, with detailed sampling making it possible to examine for resistance, and to measure HIV RNA viral levels and cofactors. Additionally, the availability of maternal samples from the infants in our cohort permitted us to conduct detailed phylogenetic analysis of maternal and infant viral sequences to identify linkage. These findings also suggested that NVP resistance was not transmitted from mother to child.

Overall, our study demonstrated a high incidence of NVP resistance during 12 months of NVP-ART in this cohort of young NVP-unexposed infants who were initiated on NVP-based ART, and demonstrated that resistance compromised subsequent viral suppression. The poor rate of virologic suppression underscores the importance of regular monitoring of viral levels in early treated HIV-infected infants and may, in part, explain the superiority of protease inhibitor-based ART.^{26,27} Our study suggests that 6-month viral load and resistance monitoring in children would detect NVP resistance and enable earlier changes in therapy among children on NVP-ART.

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Author Disclosure Statement

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