Analysis of Nevirapine (NVP) Resistance in Ugandan Infants Who Were HIV Infected Despite Receiving Single-Dose (SD) NVP versus SD NVP Plus Daily NVP Up to 6 Weeks of Age to Prevent HIV Vertical Transmission

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Background. Single-dose nevirapine (SD NVP) at birth plus NVP prophylaxis for the infant up to 6 weeks of age is superior to SD NVP alone for prevention of vertical transmission of human immunodeficiency virus (HIV) through breastfeeding. We analyzed NVP resistance in HIV-infected Ugandan infants who received either SD NVP or extended NVP prophylaxis.

Methods. We tested plasma HIV by using a genotyping assay (ViroSeq; Celera Diagnostics), a phenotypic resistance assay (PhenoSense; Monogram Biosciences), and sensitive point mutation assay (LigAmp, for K103N, Y181C, and G190A).

Results. When infants were 6 weeks old, ViroSeq detected NVP resistance in a higher proportion of infants in the extended NVP arm than in the SD NVP arm (21 of 25 [84%] vs. 12 of 24 [50%]; $P = .01$). Similar results were obtained with LigAmp and PhenoSense. In both study arms, infants who were HIV infected at birth frequently had NVP resistance detected. In contrast, infants in the extended NVP arm who were HIV infected after birth were more likely to have resistance detected at 6 weeks, compared with infants in the SD NVP arm. The use of extended NVP prophylaxis was also associated with detection of NVP resistance by ViroSeq at 6 months (7 of 7 [100%] infants in the extended NVP arm had resistance detected, compared with 1 of 6 [16.7%] infants in the SD NVP arm; $P = .005$).

Conclusions. The use of extended NVP prophylaxis was associated with increased selection for and persistence of NVP resistance in HIV-infected Ugandan infants.

Single-dose nevirapine (SD NVP) can reduce the risk of mother-to-child transmission (MTCT) of HIV [1, 2] but may lead to the emergence of resistant variants in infants who are HIV infected despite prophylaxis. After exposure to SD NVP, 46% of Ugandan infants [3] and 87% of Malawian infants [4] who were HIV infected had NVP-resistant variants detected in plasma samples obtained at 6–8 weeks of age. NVP resistance has also been observed in infants who received SD NVP in South Africa [5] and India [6]. The most common NVP-resistance mutation detected in samples obtained from infants after receipt of SD NVP is Y181C, although other

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mutations are also seen [3, 5, 6]. NVP resistance can persist in some infants for a year or more after receipt of SD NVP [3, 5]. In many resource-limited settings, more than half of HIV-infected infants die by 2 years of age [7]. Therefore, antiretroviral treatment may be indicated for NVP-exposed infants before NVP-resistant variants can fade. In women, clinical studies show that prior use of SD NVP does not compromise the efficacy of subsequent antiretroviral treatment, provided that sufficient time elapses between SD NVP exposure and initiation of the antiretroviral treatment [8, 9]. In one study, HIV-infected infants with prior exposure to SD NVP responded poorly to antiretroviral therapy initiated before 6 months of age, compared with infants without prior SD NVP exposure [9]. In another study, children who were exposed to SD NVP (median age, 1.7 years) and children who were not exposed to SD NVP (median age, 7.8 years) were able to achieve virologic suppression on NVP-based highly active antiretroviral therapy [10].

MTCT of HIV during breastfeeding accounts for one-third to one-half of infant infections [11, 12] and is associated with high maternal viral load, cell-free and cell-associated HIV in breast milk, low maternal CD4 cell count, mastitis, infant coinfections, mixed feeding, and long duration of breastfeeding [13–18]. SD NVP does not prevent the majority of postnatal HIV infections. NVP-resistant variants have been detected in the breast milk of some women after exposure to SD NVP [19–21]. Transmission of NVP-resistant HIV through the breast milk has been documented [3], but there are few data that describe resistance in late-infected infants.

The Six-Week Extended Nevirapine (SWEN) study, performed in Uganda, Ethiopia, and India, compared the efficacy of SD NVP alone to that of SD NVP plus an infant regimen of up to 6 weeks of NVP prophylaxis (daily NVP from day 8 to day 42) with respect to the regimens' ability to prevent HIV transmission by breastfeeding [22]. In a modified intent-to-treat analysis that excluded infants who were HIV infected at birth, the extended NVP regimen was 46% more effective at preventing MTCT by 6 weeks of age and was associated with a significant (27%) reduction in HIV transmission or infant mortality during the first 6 months of life, compared with SD NVP alone [22]. One concern about the use of extended NVP prophylaxis was that it might enhance selection of NVP-resistant HIV, compared with the use of SD NVP alone. In this report, we analyzed NVP resistance in infants who received either SD NVP alone or SD NVP plus up to 6 weeks of NVP to prevent MTCT of HIV in the Ugandan component of the 3-country SWEN trial. Three different laboratory methods were used for analysis of NVP resistance: (1) a Food and Drug Administration–cleared, population sequencing–based, HIV genotyping assay (ViroSeq HIV Genotyping System; Celera Diagnostics), (2) a sensitive point mutation assay (LigAmp), and (3) a commercial phenotypic resistance assay (PhenoSense HIV assay; Monogram Biosciences).

METHODS

Source of study samples. Samples were obtained from infants in National Clinical Trial 00639938, "Nevirapine Study for the Prevention of Maternal-Infant HIV Transmission in Uganda" [22]. In this trial, pregnant, HIV-infected women and their infants were randomized to 1 of 3 regimens for prevention of MTCT of HIV. The study arms were as follows: (1) the SD NVP arm (the HIVNET 012 regimen, in which SD NVP was administered to the mother in labor and to the infant after birth), (2) the extended NVP arm (in which SD NVP was administered to the mother and the infant and NVP was administered to the infant daily on days 8–42), and (3) the HIVIGLOB arm (in which SD NVP was administered to the mother and infant and antenatal HIV immune globulin was given to the mother at 37–38 weeks gestation and to the infant shortly after birth). Infants in all 3 study arms received daily multivitamins for up to 6 weeks. Infants were tested for HIV infection at birth (by age 7 days), at 2 and 6 weeks of age, and at subsequent study visits. The intervention (daily NVP or daily multivitamins) was discontinued if HIV infection was confirmed in an infant before the 6-week visit. Some infants in the extended NVP arm who were HIV infected at birth received multiple doses of NVP (median, 14 doses) before HIV infection was confirmed and the regimen was discontinued. Analysis of NVP resistance in this report was limited to HIV-infected infants in the SD NVP and extended NVP study arms.

Laboratory methods. HIV genotyping was performed using the ViroSeq HIV Genotyping System (version 2.6), as described elsewhere [3]. Bidirectional sequence data was obtained at the positions of NVP-resistance mutations for 47 of 49 infants tested. HIV subtyping was performed by phylogenetic analysis of *pol* region sequences [3]. Polymerase chain reaction products generated in the ViroSeq system were also analyzed by using the LigAmp assay to detect and quantify HIV variants with NVP-resistance mutations (K103N [AAC], 0.5% assay cutoff; Y181C [TGT], 1% assay cutoff; and G190A [GCA], 0.5% assay cutoff). The LigAmp assay was performed as described elsewhere [23], with the following exception: for Y181C detection, 2 upstream oligonucleotides were used because of sequence diversity at the oligonucleotide binding site. Sequences of oligonucleotides used in the LigAmp assay are shown in table 1. Assays for phenotypic drug susceptibility and HIV replication capacity were performed at a commercial laboratory with the PhenoSense HIV assay, as described elsewhere [24].

Statistical methods. The baseline characteristics of infants included in the primary analysis and the proportion of infants with each mutation (or combination of mutations) were compared by study arm. Baseline characteristics were also compared for infants included in this study and infants whose samples were not available for analysis. The statistical significance for comparisons of proportions was assessed by using Fisher's exact test.

Table 1. Ligation oligonucleotides used in the LigAmp assay.

Mutation and oligonucleotide, by subtype	Oligonucleotide sequence (5'→3')
Subtype A	
K103N	
Upstream	tail-AGGAATACCACATCCAGCAGGTCTAAAAAAGGAC
Downstream	Phos-AAATCAGTAACAGTACTAGATGTGGGGG
Y181C	
Upstream 1	tail-CCTTTAGATCACAAAATCCAGAAATAATTATATG
Upstream 2	tail-CCTTTAGATCACAAAATCCAGAAATGATTATATG
Downstream	Phos-TCAATACATGGATGACTTGTATGTAGGA-tail
G190A	
Upstream	tail-TTATCTATCAATACATGGATGACTTGTATGTGGC
Downstream	Phos-ATCTGATTTAGAAATAGGGCAGCATAGA-tail
Subtype D	
K103N	
Upstream	tail-AGGAATACCACATCCTGCAGGGCTAAAAAAGGAC
Downstream	Phos-AAATCAGTAACAGTACTGGATGTGGGTG
Y181C	
Upstream 1	tail-CTTTTAGAAAACAAAATCCAGAAATGGTTATATG
Upstream 2	tail-CTTTTAGAAAACAAAATCCAGAAATAGTTATATG
Downstream	Phos-TCAATACATGGATGATTTGTATGTAGGA-tail
G190A	
Upstream	tail-TTATCTATCAATACATGGATGATTTGTATGTGGC
Downstream	Phos-ATCTGACTTAGAAATAGGGCAGCATAGA-tail

NOTE. Different LigAmp oligonucleotides were used for HIV subtype A and D samples. For intersubtype-recombinant samples, oligonucleotides were selected on the basis of the HIV subtype in the region of interest. HIV subtype A oligonucleotides were used for analysis of 1 subtype G sample from a late-infected infant. Upstream oligonucleotides contain a 5' tail with the following sequence: 5'-ACTGTAAAACGACGGCCAGTGTTCCCCT-CAAACTGGCAGATGCACG. Downstream oligonucleotides contain a 3' tail with the following sequence: 5'-TGGTCATAGCTGTTTCCTGCA. The upstream oligonucleotide tail includes binding sites for an M13 primer and a real-time polymerase chain reaction (PCR) probe (underlined). The downstream oligonucleotide is phosphorylated at the 5' end (Phos), and contains an M13 binding site. M13 primers and a real-time PCR probe are used to detect the ligated oligonucleotides in the detection step of the LigAmp reaction [23]. For detection of Y181C, 2 different upstream oligonucleotides were used in the ligation reaction (upstream 1 and upstream 2, 1:1 mixture).

The Mann-Whitney rank sum test was used to compare medians. Analysis was performed in Stata (version 8; StataCorp). Associations were considered statistically significant at $P < .05$.

Informed consent. The guidelines of the US Department of Health and Human Services and the authors' institutions were followed in the conduct of this research. Informed consent was obtained from all study subjects for participation in the SWEN trial in Uganda. The study was approved by institutional review boards in Uganda and at the Johns Hopkins University School of Medicine.

RESULTS

Study cohort. Samples were available for 49 (71.0%) of 69 infants who received a diagnosis of HIV infection by 6 weeks of age (24 in the SD NVP arm and 25 in the extended NVP arm). We compared the clinical and laboratory characteristics of the 49 infants included in this study with those of the 20 infants who did not have samples available for analysis (table 2). There was no significant difference between these 2 groups in terms of study arm, maternal viral load prior or maternal CD4 cell count prior to receipt of NVP, median number of NVP doses received, or infant viral load. The proportion of infants who were HIV infected at birth was higher among those included in this study (27 of 49 [55.1%]) than among those who did not have samples available for resistance testing (6 of 20 [30%]). However, this difference was not statistically significant ($P = .07$).

In the extended NVP arm, daily NVP dosing was discontinued as soon as a diagnosis of HIV infection was confirmed. The median number of NVP doses administered was 14 for infants who received a diagnosis of HIV infection at birth (17 infants; range, 3–33 doses), 14 doses for infants who received a diagnosis of HIV infection at 2 weeks of age (5 infants; range, 7–26 doses),

Table 2. Clinical characteristics of infants who were infected with HIV by 6 weeks of age.

Characteristic	Overall ($N = 69$)	All HIV-infected infants, by sample availability: No sample ($n = 20$)	All HIV-infected infants, by sample availability: Sample tested ($n = 49$)	P[a]	Infants with samples tested for resistance, by study arm: SD NVP ($n = 24$)	Infants with samples tested for resistance, by study arm: Extended NVP ($n = 25$)	P[b]
Enrolled in SD NVP study arm	36 (52.2)	12 (60.0)	24 (49.0)	.44	NA	NA	NA
Maternal $\log_{10}$ viral load at enrollment, median, copies of HIV RNA/mL	5.1	5.0	5.1	.62[c]	5.0	5.1	.53[c]
Maternal CD4 cell count at enrollment, median, cells/μL	353	367.5	349	.90[c]	299	392	.53[c]
Infant age at time of HIV infection diagnosis							
Birth, i.e., ⩽7 days	33 (47.8)	6 (30)	27 (55.1)	.07[d]	10 (41.7)	17 (68)	.09[d]
2 weeks	11 (15.9)	4 (20)	7 (14.3)	.72[d]	2 (8.3)	5 (20)	.42[d]
6 weeks	25 (36.2)	10 (50)	15 (30.6)	.17[d]	12 (50)	3 (12)	.005[d]
No. of NVP doses received, median (range)[e]	14 (0–36)	18 (0–36)	14 (3–35)	.48[c]	NA	14 (3–35)	
First infant $\log_{10}$ viral load after diagnosis of HIV infection, median, copies of HIV RNA/mL	5.5	5.6	5.5	.89	5.6	5.3	.19
HIV subtype							
A		NA	25 (51.0)		12 (50)	13 (52)	.99[d]
C		NA	1 (2.0)		1 (4.2)	0 (0)	.49[d]
D		NA	13 (26.5)		8 (33.3)	5 (20)	.35[d]
Recombinant		NA	10 (20.4)		3 (12.5)	7 (28.0)	.29[d]

NOTE. Data are no. (%) of subjects, unless otherwise indicated. Extended NVP, extended nevirapine; NA, not applicable; SD NVP, single-dose nevirapine.

[a] Comparison of infants with no sample and infants who had a sample tested for resistance.
[b] Comparison of infants in the SD NVP arm vs. the extended NVP arm.
[c] By Mann-Whitney rank sum test.
[d] By Fisher's exact test.
[e] Median number of NVP doses for infants in the extended arm only.

and 34 doses for infants who received a diagnosis of HIV infection at 6 weeks of age (3 infants; range, 21–35 doses).

We next compared the same clinical and laboratory characteristics described above, as well as HIV subtype, for the 24 infants in the SD NVP arm and the 25 infants in the extended NVP arm who were included in this study (table 2). There was no significant difference with respect to maternal viral load or maternal CD4 cell count prior to receipt of NVP, infant viral load, or infant HIV subtype distribution between these 2 groups. The proportion of infants who received a diagnosis of HIV infection at birth was lower in the SD NVP arm than in the extended NVP arm (10 of 24 [41.7%] vs. 17 of 25 [68%]), but this difference was not statistically significant ($P = .09$). The proportion of infants who received a diagnosis of HIV infection at 6 weeks of age was higher in the SD NVP arm (12 of 24 [50%]) than in the extended NVP arm (3 of 25 [12%]) ($P = .005$), reflecting the reduced risk of postnatal HIV transmission in the extended NVP arm [22].

Analysis of NVP resistance with the ViroSeq HIV Genotyping System. HIV genotyping results were obtained for all 49 infants who had 6-week samples available for testing. A higher proportion of infants in the extended NVP arm had at least 1 NVP-resistance mutation detected, compared with infants in the SD NVP arm (21 of 25 [84%] vs. 12 of 24 [50%]; $P = .01$) (table 3 and figure 1*A*). This difference was also observed in the subset of infants who received a diagnosis of HIV infection at 2 or 6 weeks of age (7 of 8 [88.9%] infants in the extended NVP arm had NVP-resistance mutations detected, compared with 5 of 14 [35.7%] infants in the SD NVP arm; $P = .03$) (figure 1*B*). In contrast, in the subset of infants who were HIV-infected at birth, the proportion of infants who had NVP-resistance mutations detected was similar in the 2 study arms (14 of 17 [82.3%] in the extended NVP arm vs. 7 of 10 [70%] in the SD NVP arm; $P = .39$) (figure 1*B*). For all 49 infants analyzed, the detection of NVP-resistance mutations was not associated with subtype (A vs. D) ($P = .99$). In the extended NVP arm, there was no asso-

Table 3. Results of genotypic testing for nevirapine resistance for infant plasma samples obtained at 6 weeks, for the ViroSeq and LigAmp assays.

Test type, variable	Total	Study arm		*P*[a]
		SD NVP	Extended NVP	
ViroSeq				
No. of infants tested	49	24	25	
⩾ 1 NVP-resistance mutation detected	33	12 (50)	21 (84)	.01
K101E only	1	0	1	
K103N only	4	1	3	
V106A only	1	1	0	
V106M only	1	1	0	
Y181C only	12	4	8	
Y188C only	1	1	0	
G190A only	1	0	1	
⩾ 2 NVP-resistance mutations detected	12	4 (16.7)	8 (32)	.18
K103N and Y181C	4	3	1	
K103N, V106A, and Y181C	2	1	1	
K103N and Y188C	2	0	2	
Y181C and G190A	2	0	2	
K103N and V106A	1	0	1	
V106A and Y181C	1	0	1	
LigAmp[b]				
No. of infants tested	44	20	24	
⩾ 1 NVP-resistance mutation detected	26	7 (35)	19 (79)	.005
Infants with ⩾ 0.5% K103N	13	3 (15)	10 (41.7)	
Median % of viral population with K103N	4.7	4.2	5.2	
Infants with ⩾ 1% Y181C	22	7 (35)	15 (62.5)	
Median % of viral population with Y181C	15.6	10.8	20.5	
Infants with ⩾ 0.5% G190A	9	3 (15)	6 (25)	
Median % of viral population with G190A	3.2	1.6	5.6	

NOTE. Data are no. of subjects or no. (%) of subjects, unless otherwise indicated. Extended NVP, extended nevirapine; SD NVP, single-dose nevirapine.

[a] By Fisher's exact test, for comparison of the 2 study arms.

[b] LigAmp results over 100% were adjusted to 100%. Median values were calculated only for samples in which the mutation was detected.

ciation between the detection of NVP-resistance mutations and the number of NVP doses received (median, 14 doses for infants who had resistance mutations detected vs. 17.5 doses for infants who did not have resistance mutations detected; $P = .85$).

Analysis of K103N, Y181C, and G190A using the LigAmp assay. We used a point mutation assay, LigAmp, to detect and quantify HIV variants with the K103N, Y181C, and G190A NVP-resistance mutations. Forty-four (89.7%) of 49 6-week samples for which ViroSeq results were obtained (see above) were tested with the LigAmp assay. Two subtype C samples were not tested, and 3 infants did not have sufficient samples remaining for analysis with LigAmp. For the 44 samples tested with both ViroSeq and LigAmp, 31 (91.2%) of the 34 K103N, Y181C, and G190A mutations detected by ViroSeq were also detected by LigAmp; 2 K103N mutations and 1 Y181C mutation were encoded by unusual nucleotide codons and were not detected by LigAmp. LigAmp detected 13 additional NVP-resistance mutations that were below the level of detection of the ViroSeq system.

We compared the proportion of infants who had Y181C, K103N, or G190A mutations detected by LigAmp in the 2 study arms. A higher proportion of infants in the extended NVP arm had ⩾1 mutation detected, compared with infants in the SD NVP arm (19 of 24 [79%] vs. 7 of 20 [35%]; $P = .005$) (table 3). In the extended NVP arm, the detection of any NVP-resistance mutation (K103N, Y181C, or G190A) was not associated with the number of NVP doses received (median of 14 doses among those with ⩾1 mutation detected vs. 21 doses among those with no mutations detected; $P = .14$). In the 2 study arms, there was no significant difference in the median level (i.e., the percentage of the viral population with the mutation) for any of the 3 mutations (table 3).

When data from infants in the 2 study arms were combined, a higher proportion of infants infected with HIV subtype A had

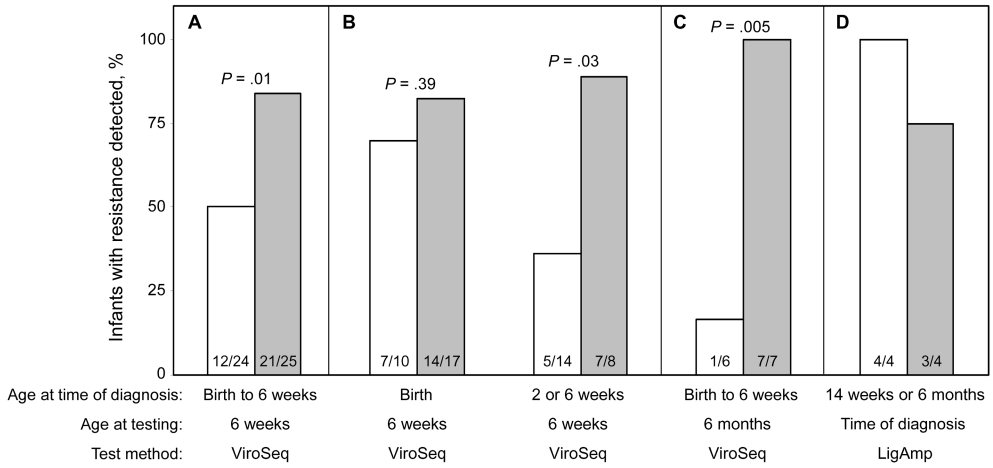

Figure 1. Nevirapine (NVP) resistance results for samples from infants in the single dose NVP (SD NVP) study arm *(white bars)* and the extended NVP prophylaxis study arm *(shaded bars)*. The proportions shown are the no. of infants who had NVP-resistance mutations identified/the no. of infants tested. The *P* values for comparison of the proportion in each study arm (by Fisher's exact test) are shown above the bars. The first 2 panels show the proportion of infants who had NVP-resistance mutations detected by ViroSeq at 6 weeks of age. *A* shows the results for all infants tested, and *B* shows the results for infants who received a diagnosis of HIV infection at birth (left) and for infants who were HIV uninfected at birth and received a diagnosis of HIV infection at age 2 weeks or 6 weeks (right). *C,* Proportion of infants who had NVP-resistance mutations detected by ViroSeq at 6 months of age. *D,* Proportion of late-infected infants who had NVP-resistance mutations (K103N, Y181C, or G190A) detected by the LigAmp assay at the time HIV infection was diagnosed.

NVP-resistance mutations detected by LigAmp, compared with infants infected with subtype D (17 of 23 [73.9%] vs. 5 of 12 [41.7%]), but this difference was not statistically significant ($P = .08$). Among infants who had ⩾1 mutation detected, the median level of each of the 3 NVP-resistance mutations was as follows: for infants who were HIV-infected at birth, K103N was detected in 4.4% of the viral population, Y181C in 25%, and G190A in 3%; for infants who received a diagnosis of HIV infection at 2 or 6 weeks of age, K103N was detected in 5.4% of the viral population, Y181C in 2.6%, and G190A in 18.7%.

Analysis of phenotypic drug resistance. Phenotypic resistance results were obtained with the PhenoSense assay for 42 (85.7%) of 49 available samples. Phenotypic NVP resistance was more frequently detected among infants in the extended NVP arm than those in the SD NVP arm (19 of 22 [86.3%] vs. 9 of 20 [45%]; $P = .005$). Results from the ViroSeq and PhenoSense assays (resistance detected vs. not detected) were the same for all but 1 of the 42 infants who had results available for both assays; this infant had the Y181C mutation detected by ViroSeq as a mixture, but did not have phenotypic resistance detected. There was only 1 infant who had an NVP-resistance mutation detected by LigAmp alone; in samples from this infant, the LigAmp result showed mutation Y181C in 1.4% of the viral population.

The PhenoSense assay also provides quantitative information about drug resistance. In this study, among infants with phenotypic NVP resistance, the median fold change in the IC_{50} for NVP was higher in the extended NVP arm than in the SD NVP arm (143 vs. 66), but the result was not statistically significant ($P = .30$). Many of the infants who had phenotypic NVP resistance were also identified as having cross-resistance to other nonnucleoside reverse transcriptase inhibitors (NNRTIs) (10 [35.7%] of 28 had HIV that was cross-resistant to delavirdine [DLV], 2 [7.1%] of 28 had HIV that was cross-resistant to efavirenz [EFV], and 12 [42.8%] of 28 had HIV that was cross-resistant to EFV and DLV). Among the 42 infants for whom PhenoSense results were available, we found no difference in the median HIV replication capacity according to study arm (both were 33%), and we found no significant difference in the median replication capacity measured in infants who had NVP-resistant mutations detected by ViroSeq versus infants who did not have such mutations detected (35% vs. 33%; $P = .74$). This result suggests that presence of resistance mutations in some HIV variants does not significantly impact the HIV replication capacity of the viral population.

Interestingly, 3 infants in the extended NVP arm did not have NVP-resistance mutations detected by ViroSeq, PhenoSense, or LigAmp. Two of these infants were HIV infected at birth; these infants received 5 and 14 doses of NVP, respectively. One of these infants received a diagnosis of HIV infection at 6 weeks and received 21 doses of NVP.

Analysis of NVP-resistance mutations in infants at 6 months. We used the ViroSeq system and LigAmp assay to analyze the persistence of NVP-resistance mutations at 6 months. Of the 33 infants who had 6-week ViroSeq results available, 17 were excluded because they had initiated antiretroviral therapy before 6 months of age, 1 infant died before 6 months of age, 1 infant was lost to follow-up, and 1 infant had no sample available. Therefore, this analysis included 13 infants. In the SD

NVP arm, 1 (16.7%) of 6 infants had a NVP-resistance mutation detected by ViroSeq (Y181C), and 2 additional infants had a NVP-resistance mutation detected by LigAmp only (mutation Y181C for both infants, detected at 1.4% and 3.5%, respectively) (figure 1*C*). In contrast, all of the 7 infants in the extended NVP arm had NVP-resistance mutations detected by ViroSeq at 6 months (1 of 6 vs. 7 of 7; $P = .005$).

Analysis of NVP resistance in late-infected infants. There were 16 infants who were HIV uninfected at 6 weeks but found to be HIV infected by either 14 weeks ($n = 15$) or 6 months ($n = 1$). Samples collected at the time HIV infection was diagnosed were available for 8 (50%) of these 16 infants (4 in the SD NVP arm and 4 in the extended NVP arm). Only 1 (12.5%) of the 8 infants analyzed had NVP-resistance mutations detected by ViroSeq (1 infant in the extended NVP arm who received a diagnosis at 14 weeks had the K103N mutation detected). In contrast, 7 (87.5%) of the 8 late-infected infants had ⩾1 mutation detected by LigAmp (4 had K103N detected, 1 had G190A detected, 1 had K103N and Y181C detected, and 1 had K103N and G190A detected, figure 1*D*). The infant who did not have resistance mutations detected by either assay was in the extended NVP arm; 34 doses of NVP were administered to this infant, and the infant received a diagnosis of HIV infection at 14 weeks.

DISCUSSION

In this study, infants in the extended NVP arm were more likely to have genotypic and phenotypic NVP resistance identified at 6 weeks of age than infants in the SD NVP arm. Many of the infants who had phenotypic resistance to NVP identified also had HIV that was cross-resistant to DLV and/or EFV. The majority of HIV-infected infants who are exposed to SD NVP prophylaxis and require antiretroviral treatment by 6 months of age respond poorly to NNRTI-based antiretroviral regimens [9]. Infants with prior SD NVP exposure may respond better to NVP-based therapy if it is initiated later [10]. In this study, infants in the extended NVP arm were more likely to have resistance detected at 6 months, compared with infants in the SD NVP arm. Although this study was limited by the small number of infants tested at 6 months ($n = 13$), this finding suggests that NVP-based antiretroviral therapy may be more likely to fail in infants who are HIV infected despite extended NVP prophylaxis than in infants exposed to SD NVP alone.

The association between study arm and the detection of resistance mutations at 6 weeks of age was only seen among infants who were HIV uninfected at birth and received a diagnosis of HIV infection at 2 weeks or 6 weeks. Among that subset of infants, those who received extended NVP prophylaxis were more likely to develop NVP resistance. In contrast, infants who were HIV infected prior to NVP exposure (i.e., infants who were HIV positive at birth) often had NVP-resistance mutations identified at 6 weeks, regardless of whether they received the SD NVP regimen (SD NVP arm) only or the SD NVP regimen plus daily NVP until their HIV infection was confirmed (extended NVP arm; median, 14 doses of NVP; range, 3–33 doses). These results suggest that infants who are HIV infected in utero can receive several doses of NVP without significantly increasing their risk of selecting for NVP resistance.

We also analyzed NVP resistance in late-infected infants (i.e., infants who received a diagnosis of HIV infection at 14 weeks or 6 months of age; 4 infants in each study arm). All but 1 of the late-infected infants had NVP-resistance mutations detected by the sensitive LigAmp assay at the time HIV infection was diagnosed. The 4 late-infected infants in the extended NVP arm received 34–36 doses of NVP. For infants in the SD NVP arm, and for the 1 infant in the extended NVP arm who received a diagnosis of HIV infection at 6 months, NVP would have been cleared from the infant's body by the time of HIV infection. Therefore, in these cases, one can assume that the resistant strain was transmitted to the infant through breastfeeding. For the other 3 infants (those in the extended NVP arm who received a diagnosis of HIV infection at 14 weeks), it is possible that an NVP-susceptible strain was transmitted to the infant shortly after the 6-week visit (before NVP had cleared), with subsequent selection of NVP-resistant HIV in the infant.

The SWEN trial demonstrated that extended NVP prophylaxis is more effective than SD NVP for preventing MTCT of HIV. This study shows that use of extended NVP prophylaxis is associated with increased emergence and persistence of NVP-resistant HIV variants in Ugandan infants. The SWEN trial also included trials performed in India and Ethiopia that used the same NVP-based regimens for preventing MTCT of HIV. Even though the trial design (e.g., timing of infant HIV testing and sample collection) and resistance-testing methods were different in the Ugandan and Indian components of the SWEN study, HIV-infected infants in the Indian trial who were HIV uninfected at birth but received a diagnosis of HIV infection by 6 weeks were also more likely to have NVP resistance if they had received extended NVP prophylaxis [25]. Clinical studies are needed to determine the impact of extended NVP prophylaxis on subsequent antiretroviral treatment in HIV-infected infants.

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