Detection of K103N in Ugandan women after repeated exposure to single dose nevirapine

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Objectives: Use of single dose nevirapine (SD NVP) for prevention of HIV-1 mother-to-child transmission (pMTCT) is associated with selection of K103N-containing HIV variants. Repeat use of SD NVP for pMTCT may influence emergence and persistence of NVP-resistant variants.

Design: K103N-containing variants were studied in 48 Ugandan women who received SD NVP in the HIVNET 012 trial, and were re-exposed to SD NVP in one ($n = 44$) or two ($n = 4$) subsequent pregnancies during a 5-year follow-up study.

Methods: Samples were analyzed using the LigAmp assay (assay cutoff: 0.5% K103N).

Results: Among 44 women who were re-exposed to SD NVP in one subsequent pregnancy, 37.8% had K103N detected within 1 year of SD-NVP re-exposure. Detection of K103N was independently associated with detection of K103N 6–8 weeks after the first SD NVP exposure and with pre-NVP viral load. The portion of women with undetectable K103N by 2 years after SD NVP administration was similar after first versus second use of SD NVP for pMTCT. K103N was undetectable in 93.2% of evaluable women by 3 years of re-exposure. Only two of four women who received SD NVP in two pregnancies during the follow-up study had K103N detected after the last SD NVP exposure.

Conclusions: K103N was detected in some women within 1 year of SD NVP re-exposure, but faded from detection in most women by 3 years after re-exposure. Detection of K103N by 1 year after SD NVP re-exposure was associated with prior selection of K103N-containing variants and with pre-NVP viral load.

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Introduction

Single dose (SD) nevirapine (NVP) is widely used in resource-limited settings for prevention of HIV-1 mother-to-child transmission (pMTCT) [1–3]. In some women, exposure to SD NVP is associated with selection of NVP-resistant HIV-1 strains [4]. K103N is the most common NVP resistance mutation detected in women after SD NVP [5,6], and K103N-containing variants can persist at low levels in some women for a year or more after SD NVP exposure [7,8]. A slower rate of fading of K103N-containing variants has been shown to be associated with a higher baseline (pre-NVP) viral load and HIV-1 subtype (D>A) [8]. Persistence of K103N-containing variants after SD NVP could potentially reduce the efficacy of non-nucleoside reverse transcriptase (NNRTI)-based regimens for future treatment of HIV-1 infection. It is not known whether K103N-containing

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variants persist for longer periods in women who have received SD NVP for pMTCT and are then re-exposed to SD NVP for pMTCT in a subsequent pregnancy.

In this sub-study, we use a sensitive and quantitative point mutation assay (LigAmp) to examine selection and persistence of K103N-containing variants in women after a second or third exposure to SD NVP for pMTCT.

Methods

Study participants

In HIVNET 012, 306 antiretroviral drug-naive Ugandan women received SD NVP for pMTCT; 232 (75.8%) of those women were subsequently enrolled in a long-term follow-up study with study visits up to 5 years after their original SD NVP exposure (Extended Mother and Child Follow-up; Amendment II for HIVNET 012, follow-up study). Fifty women became pregnant during the 5-year follow-up study and received SD NVP for pMTCT. Two of the 50 women were excluded from the analysis in this sub-study: one woman had no samples available for testing and one woman had samples that failed to amplify in the LigAmp assay. The remaining 48 women included 44 women who were re-exposed to SD NVP in one pregnancy during the 5-year follow-up study and four women who were re-exposed to SD NVP in two pregnancies during the follow-up study. Two of the 48 women received two doses of NVP (rather than SD NVP) in a subsequent pregnancy, due to false labor. In this sub-study, these were treated as a single re-exposure. The 48 women included 27 with subtype A, one with subtype C, 16 with subtype D, and four with inter-subtype recombinant HIV-1. Among these 48 women, the median time between the initial SD NVP exposure in the HIVNET 012 trial and the first SD NVP re-exposure in the follow-up study was 3.6 years (range, 2.0–5.2 years). All four women who were re-exposed to SD NVP in two pregnancies during the follow-up study had subtype A infection.

Laboratory methods

HIV-1 subtyping was performed in previous studies by phylogenetic analysis of HIV-1 *pol* region sequences [5]. K103N-containing variants in plasma were detected and quantified using the LigAmp assay as described previously [9], using an assay cutoff of 0.5% K103N. Oligonucleotides used in the LigAmp assay for analysis of inter-subtype recombinant strains were selected based on the HIV-1 subtype in the region near codon 103 of HIV-1 reverse transcriptase.

Statistical methods

Two sample chi-squared tests were used to compare characteristics of women who had NVP resistance detected after their initial SD NVP exposure versus those who did not. Logistic regression modeling was used to compute odds ratios for correlates of detection of K103N after re-exposure to SD NVP. All statistical analyses were conducted using SAS (version 9.1; SAS Institute Inc., Cary, North Carolina, USA).

Informed consent

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 subjects for participation in HIVNET 012 and in the follow-up study, and both studies were approved by Institutional Review Boards in Uganda and at Johns Hopkins University School of Medicine.

Results

LigAmp testing scheme

We analyzed K103N-containing variants in women who received SD NVP in the HIVNET 012 trial, and were re-exposed to SD NVP for pMTCT during a 5-year follow-up study (see Methods). We used the LigAmp assay to detect and quantify K103N-containing HIV-1 variants in plasma samples from these women. Samples collected 6–8 weeks after the initial SD NVP exposure in the HIVNET 012 trial were tested in a previous study [10]. In the follow-up study, samples were collected at annual visits 2 to 5 years after the initial SD NVP exposure in the HIVNET 012 trial, and therefore did not occur at fixed times following SD NVP re-exposure. In this sub-study, we analyzed samples collected at the last study visit prior to SD NVP re-exposure and at the first study visit after SD NVP re-exposure (all available samples). If K103N was detected after SD NVP re-exposure, a sample from the next study visit was analyzed. For each woman, if K103N was not detected, samples from subsequent study visits were not tested. For the purpose of data analysis, we grouped results from samples collected less than 1 year, 1–2 years, or 2–3 years after SD NVP re-exposure (Fig. 1).

Detection of K103N in women prior to single dose nevirapine re-exposure

Among the 48 women in this sub-study, 24 had a sample collected less than 1 year prior to SD NVP re-exposure (baseline sample). Among these 24 women, the mean time between the initial SD NVP exposure and collection of the baseline sample was 3.2 years (range, 2.0–4.0 years). Only two of the 24 women had K103N detected prior to SD NVP re-exposure. In both cases, the level of K103N was near the assay cut-off for K103N detection (0.8 and 0.5% K103N).

Detection of K103N in women within 1 year after single dose nevirapine re-exposure

We next used the LigAmp assay to detect and quantify K103N-containing HIV variants in women after SD NVP re-exposure. Among the 44 women who were re-exposed to SD NVP in a single pregnancy in the follow-up study, 37 women had a sample collected within

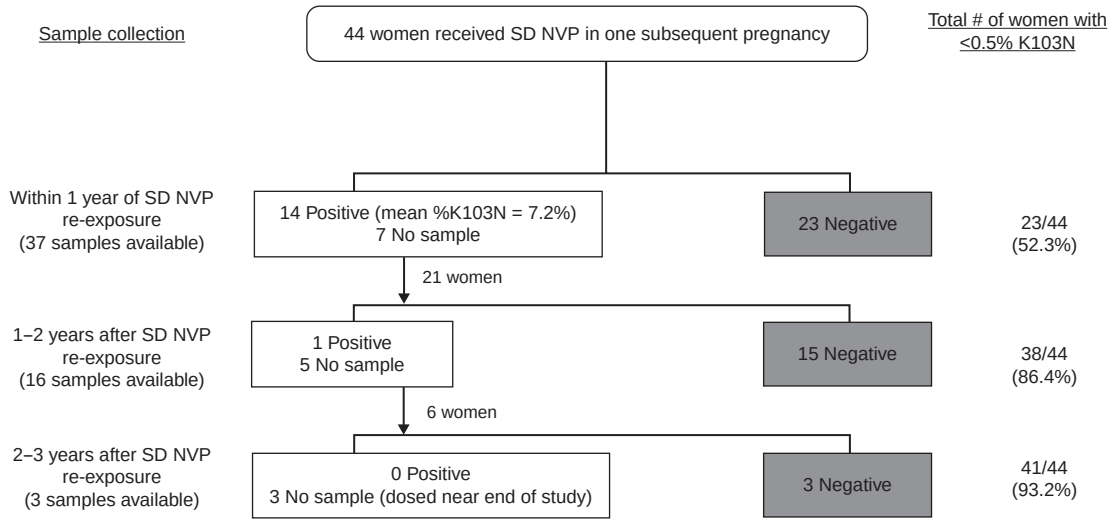

Fig. 1. Overview of analysis of K103N-containing variants in women after re-exposure to single dose nevirapine (SD NVP). Forty-four women enrolled in a follow-up study of the HIVNET 012 trial received SD NVP in one pregnancy during a 5-year follow-up period (see Methods). K103N-containing variants were detected and quantified in those women using the LigAmp assay. For each woman, if K103N was not detected after SD NVP re-exposure (< 0.5% K103N), samples from subsequent study visits were not analyzed. The figure indicates the number of women tested at each study visit who had positive results (Positive: K103N was detected at ≥ 0.5% with the LigAmp assay), negative results (Negative: K103N was not detected with the LigAmp assay), or no sample available for testing (No sample). Numbers in the column to the right of the figure indicate the cumulative number and percentage of women who had less than 0.5% K103N detected within 1 year, 1–2 years, or 2–3 years after SD NVP re-exposure.

1 year of SD NVP re-exposure. The mean time between SD NVP re-exposure and sample collection was 5.8 months for the 37 women. K103N was detected at ≥ 0.5% in 14 (37.8%) of 37 women tested, including nine (45%) of 20 women with subtype A, four (33.3%) of 12 women with subtype D, one (25%) of four women with inter-subtype recombinant HIV-1, and no woman (one in group) with subtype C. The mean % K103N detected within 1 year of SD NVP re-exposure was 7.2% (median = 3.2%). K103N was below the level of detection in the remaining 23 women. The mean time between SD NVP re-exposure and sample collection was similar among women who did versus did not have K103N detected (4.6 months vs. 6.5 months, $P = 0.13$).

As the sample collection was timed to the initial SD NVP exposure, and not to receipt of SD NVP in the subsequent pregnancy, we did not have samples available to examine the selection of K103N-containing variants shortly after SD NVP re-exposure (e.g. at 6–8 weeks postpartum). For this reason, if K103N was not detected in the first available sample after SD NVP re-exposure, we were not able to determine whether K103N-containing variants had been selected at an earlier time point and then faded below the level of detection, or had not been selected after SD NVP re-exposure.

Among the 37 women who had a sample collected within 1 year of SD NVP re-exposure, we next analyzed the association of detection of K103N after SD NVP re-exposure with a number of variables, including: detection of SD NVP in the same woman after her initial SD NVP exposure in the HIVNET 012 trial, pre-NVP HIV viral load, HIV subtype (D versus A), the time between the initial SD NVP exposure and SD NVP re-exposure in the follow-up study, and the time between SD NVP re-exposure and collection of the sample that was used to analyze K103N after SD NVP re-exposure. Note that LigAmp results from 6–8 weeks after the initial SD NVP exposure were not available for two of the 37 women.

All of the 14 women who had K103N detected within 1 year of SD NVP re-exposure had K103N measured 6–8 weeks after their initial SD NVP exposure. K103N was detected in 11 (78.6%) of those 14 women. In contrast, among the 23 women who had undetectable K103N within 1 year of SD NVP re-expsoure, only five (23.8%) of 21 evaluable women had K103N detected 6–8 weeks after their initial SD NVP exposure ($P = 0.0014$).

In univariate models, detection of K103N after SD NVP re-exposure was significantly associated with detection of K103N 6–8 weeks after the initial SD NVP exposure [odds ratio (OR), 11.7; 95% confidence interval (CI), 2.3–59.5] and $\log_{10}$ viral load (OR, 6.6; 95% CI, 1.6–27.1), but not with any of the other variables (Table 1). A multivariate logistic regression model revealed that each of these variables was independently associated with detection of K103N after SD NVP re-exposure: detection of K103N 6–8 weeks after the initial SD NVP exposure (OR, 6.1; 95% CI, 1.02–36.4) and $\log_{10}$ viral load (OR, 4.6; 95% CI, 1.04–19.9; Table 1).

Table 1. Logistic models for detection of K103N within 1 year of single dose nevirapine (SD NVP) re-exposure[a].

Predictor(s)	Univariate models				Multivariate model			
	N	OR	95% CI	P-value	*N*	OR	95% CI	P-value
Detection of K103N 6–8 weeks after initial SD NVP exposure	35	11.7	2.3–59.5	0.003	34	6.1	1.02–36.4	0.047
Log_{10} viral load	34	6.6	1.6–27.1	0.008	34	4.6	1.04–19.9	0.044
Subtype D versus A	30	0.6	0.1–2.9	0.56				
Months between initial SD NVP exposure and SD NVP re-exposure	35	1.0	0.9–1.0	0.43				
Months between SD NVP re-exposure and sample collection	35	0.9	0.7–1.1	0.20				

CI, confidence interval; OR, odds ratio.
[a]*N*, number of observations in the model.

Fading of K103N after single dose nevirapine re-exposure

Among the 21 women who had either detectable K103N ($n=14$) or no sample available for analysis within 1 year of SD NVP re-exposure ($n=7$), 16 women had a sample collected 1–2 years after SD NVP re-exposure. K103N was detected in only one woman; that woman also had K103N detected in the previous sample. The remaining 15 women did not have detectable K103N. Among the six women who had either detectable K103N ($n=1$) or no sample available for analysis 1–2 years after SD NVP re-exposure ($n=5$), three had a sample collected 2–3 years after SD NVP re-exposure. None of the three women tested 2–3 years after SD NVP re-exposure had detectable K103N. The remaining three women were re-exposed to SD NVP near the end of the follow-up study, precluding collection of additional samples.

Overall, K103N was below the level of detection in 23 (52.3%) of the 44 women within 1 year of SD NVP re-exposure, in 38 (86.4%) of the 44 women 1–2 years after SD NVP re-exposure, and in 41 (93.2%) of the 44 women 2–3 years after SD NVP re-exposure (Fig. 1). The results from the 14 women who had K103N detected in at least one sample after SD NVP re-exposure are shown in Fig. 2a.

This sub-study also included four women who received SD NVP in two pregnancies during the follow-up study (Fig. 2b). In two of these women, K103N was still detected 1 year after re-exposure to NVP. One of those women had 0.7% K103N after the first re-exposure (3-year visit), but had undetectable K103N after her second re-exposure (4-year visit; panel a). The other woman, who received a double dose of NVP in her first subsequent pregnancy due to false labor, had 51.7 and 4.3% K103N at the 3- and 4-year visits, respectively. At the 5-year visit, K103N was detected at 15.4% after her second re-exposure (panel b). K103N was not detected in the other two women who were multiply re-exposed to SD NVP (panels c and d).

Fading of K103N after first versus second use of single dose nevirapine

In a previous study, we analyzed K103N-containing variants in 144 women who were enrolled in the same HIVNET 012 follow-up study, but who were not re-exposed to SD NVP for pMTCT (i.e. women who did not become pregnant during the follow-up study). K103N was undetectable in 80% of women tested 2 years after SD NVP. Using a statistical model that considered data from all study visits (Weibull model), we estimated that the probability of fading (lack of detection) of K103N within 2 years of an initial SD NVP exposure was 87.6% (95% CI, 78.9–93.1%) [8]. This was similar to data collected within 2 years after a second SD NVP exposure in this sub-study (86.4%, Fig. 1). Among the 144 women who were not re-exposed to SD NVP, a higher pre-NVP viral load and HIV-1 subtype D (compared to subtype A) were each associated with slower fading of K103N [8]. There was no significant difference in either the baseline (pre-NVP) viral load (4.3 logs versus 4.3 logs, $P=0.89$) or the portion of women with subtype D ($56/144=38.9\%$ versus $16/44=36.4\%$; $P=0.76$, chi-squared) among the 144 women with only one SD NVP exposure versus the 44 women who were re-exposed to SD NVP. Based on these data, we found no evidence for a slower rate of fading after second versus first use of SD NVP for pMTCT.

Discussion

In HIV subtype B, variants with the K103N mutation are relatively fit [11] and can persist for long periods following drug exposure [12]. Even so, in the absence of continued drug selection, those variants may be replaced with wild type virus over time.

We used a sensitive point mutation assay to analyze K103N-containing variants after re-exposure to SD NVP for pMTCT in a second pregnancy. K103N was detected in 37.8% of women within 1 year after SD NVP re-exposure. In a multivariate logistic regression model, pre-NVP viral load and detection of K103N 6–8 weeks after the initial SD NVP exposure independently predicted detection of K103N after SD NVP re-exposure. It is not clear why emergence of K103N after an initial SD NVP exposure predicts detection of K103N after a subsequent SD NVP exposure. This association could reflect maternal (host) factors that influence NVP exposure (e.g. absorption, concentration, or clearance of

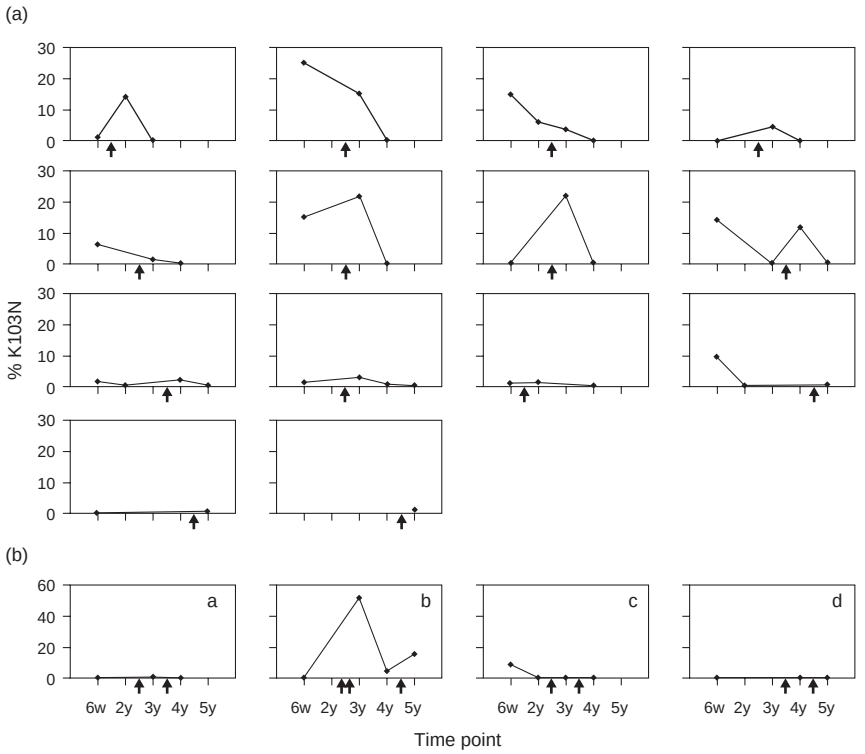

Fig. 2. Level of K103N-containing variants detected in women who were re-exposed to single dose nevirapine (SD NVP) in a subsequent pregnancy. (A) Fourteen women who were re-exposed to SD NVP in a single pregnancy during the follow-up study had K103N detected after SD NVP re-exposure. Each graph shows the % K103N detected at each study visit in one of those women. Arrows indicate when re-exposure to SD NVP occurred. (B) Results from four women who were re-exposed to SD NVP in two pregnancies during the follow-up study are shown (panels a–d). One woman received a double dose of NVP in a subsequent pregnancy (rather than SD NVP) due to false labor (double arrow, panel b). In panels a and b, study visits at 6–8 weeks (6w) and at 2, 3, 4, and 5 years (2y, 3y, 4y, 5y) after the initial SD NVP exposure are indicated on the x-axis.

the drug), viral factors (e.g. genetic properties of individual viral strains that influence the fitness of K103N-containing variants in the presence or absence of the drug), or establishment of a reservoir of K103N-containing variants after the initial SD NVP exposure.

In previous studies of the HIVNET 012 cohort, HIV subtype (D>A) was associated with more frequent detection of K103N after the initial SD NVP exposure (among 144 women with subtype A and 94 women with subtype D [10]) and with slower fading of K103N after the initial SD NVP exposure (among 88 women with subtype A and 66 women with subtype D [8]). In this study, we did not find an association between HIV subtype and detection of K103N after SD NVP re-exposure. This may reflect the smaller number of women with subtype A and D in the follow-up study (19 women with subtype A and 11 women with subtype D).

K103N was undetectable in most women by 2–3 years after re-exposure to SD NVP for pMTCT in a second pregnancy. When these data were compared with data from a previous study of women in the same HIVNET 012 cohort who were not re-exposed to SD NVP, the portion of women with undetectable K103N appeared to be similar following first versus second SD NVP use. As the sample collection was not timed to SD NVP re-exposure in the HIVNET 012 follow-up study, it was not possible to compare the levels of K103N-containing variants shortly after first versus second SD NVP use. PBMC samples were not stored in the HIVNET 012 trial. Therefore, we were not able to evaluate whether K103N-containing variants were archived in cellular DNA in these women.

Recent studies suggest that SD NVP remains effective for pMTCT with repeat use [13,14], and that the response of women to NVP-based treatment regimens is not compromised by a single prior SD NVP exposure, provided that there is sufficient time between SD NVP exposure and treatment initiation [15–18]. Some studies suggest that it may be sufficient to wait 6 months after SD NVP exposure before starting antiretroviral therapy with an NNRTI-containing regimen [19]. Our previous finding, that HIV subtype influences persistence of K103N-containing variants after SD NVP [8], suggests that the optimal time to treatment initiation after SD NVP may vary in different geographic regions, depending on which subtypes are prevalent.

This sub-study suggests that persistence of K103N-containing variants is similar after first versus second use of SD NVP for pMTCT. Further studies are needed to determine the optimal time for treatment initiation in women who received SD NVP in one or more pregnancies, and to determine whether repeated use of SD NVP for pMTCT compromises future treatment of the mother with an NNRTI-containing regimen.

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