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Persistent Minority K103N Mutations among Women Exposed to Single-Dose Nevirapine and Virologic Response to Nonnucleoside Reverse-Transcriptase Inhibitor–Based Therapy

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Abstract

Objective—We investigated whether there are long-lasting effects of exposure to single-dose nevirapine (sdNVP) treatment on virologic response to nonnucleoside reverse-transcriptase inhibitor (NNRTI)–based therapy among human immunodeficiency virus (HIV)–infected women.

Methods—An observational epidemiologic study was conducted in Johannesburg, South Africa. Initial and sustained virologic response to NNRTI-based therapy was compared between 94 HIV-infected women who had received sdNVP 18–36 months earlier and 60 unexposed, HIV-infected women who had been pregnant 12–36 months earlier. Viral load was measured every 4 weeks up to week 24 and then every 12 weeks up to week 78. Time to viral suppression (viral load, <50 copies/mL) and confirmed rebound in the viral load (viral load, >400 copies/mL) were compared. Drug resistance was assessed using K103N allele–specific real-time polymerase chain reaction assay and population sequencing.

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Results—Almost all women (97.5% of sdNVP-exposed women and 91.3% of sdNVP-unexposed women; $P = .21$) achieved viral suppression by week 24, and similar percentages of sdNVP-exposed and -unexposed women (19.4% and 15.1%, respectively) experienced viral rebound within 78 weeks after treatment ($P = .57$). K103N was detected with the K103N allele-specific real-time polymerase chain reaction assay among sdNVP-exposed and -unexposed women before treatment; detection was strongly predictive of inadequate viral response: 60.9% of women for whom K103N was detected in either viral RNA or DNA did not experience viral suppression or experienced viral rebound, compared with 15.1% of women for whom K103N was not detected ($P < .001$). After treatment, the M184V mutation occurred less frequently among sdNVP-exposed women than among sdNVP-unexposed women, but the frequency of NNRTI-associated mutations was similar between these groups of women with inadequate virologic response.

Conclusions—Exposure to sdNVP in the prior 18–36 months was not associated with a reduced likelihood of achieving and sustaining viral suppression while receiving NNRTI-based therapy. However, women with minority K103N mutations before treatment had a reduced durability of virologic suppression.

Nevirapine (NVP) is as a key agent in the arsenal of antiretroviral drugs available for the prevention of mother-to-child transmission (PMTCT) of HIV. In its simplest form, single-dose NVP (sdNVP), which consists of administration of 1 dose to the mother and 1 to the child, approximately halves the risk of transmission [1]. Although it is not as effective as longer and more-complex treatment regimens [2], sdNVP has allowed PMTCT access to be expanded in low-resource settings and has been widely used worldwide [3,4]. When added to other PMTCT regimens, NVP increases their efficacy [5], making NVP an attractive adjunct for programs with the resources to offer combination regimens.

Prophylactic use of NVP readily selects viral mutations that promote resistance to nonnucleoside reverse-transcriptase inhibitors (NNRTIs) [6–9] that are recommended as part of first-line adult treatment regimens [10]. NVP added to short-course zidovudine treatment resulted in reduced viral suppression 6 months after the commencement of therapy in a randomized trial from Thailand [11]. A subsequent study from Botswana found that the effects of NVP were confined to pregnant women who had initiated therapy within 6 months after delivery [12]. A study from Zambia reported that sdNVP exposure occurring >6 months before therapy was initiated had no impact on clinical outcomes or CD4 cell count [13]. The proportion of women who have detectable viral drug-resistance mutations decreases after exposure to NVP, providing a rationale for these findings [14–16].

Although decreases in resistance mutations detectable with standard methods (i.e., population sequencing) occur after exposure to NVP, mutations that are detectable by more-sensitive methods persist among a small percentage of women for long periods after exposure [14,16–19]. The consequences of persistent minority resistance mutants on virologic response to therapy have not, to our knowledge, been reported in sdNVP-exposed women.

We present the results of a study conducted in Johannesburg, South Africa, that investigated the effects on initial and sustained virologic responses to NNRTI-based therapy of exposure to sdNVP 18–36 months earlier, a point at which resistance mutations that are detectable by standard methods are expected to have receded but when minority resistance mutations may still be present.

METHODS

Study Design

We conducted a nonrandomized epidemiologic study that compared virologic response to NNRTI-based treatment in women who received sdNVP during a prior pregnancy 18–36

months earlier with that in sdNVP-unexposed women who had been pregnant 12–36 months earlier but who received no antiretroviral drugs for PMTCT. These intervals were selected to investigate distant exposure to NVP, to exclude women who had recent exposures and who were likely to have a poor response (for safety reasons), to select women likely to have resistance mutations at a level less than the detection limits of standard assays, and to produce control subjects who were equally likely to be caregivers of young children.

Study Population

HIV-infected women were screened for study eligibility at 1 site in Johannesburg during the period July 2004 through May 2006. Antiretroviral-naïve women with a CD4 cell count <200 cells/mm³, with a CD4 cell count <350 cells/mm³ plus a World Health Organization (WHO) stage III condition, or with a WHO stage IV condition were eligible to receive antiretroviral therapy. Exclusion criteria were acute hepatitis, elevated liver function test values of grade II or greater, and a history of NVP toxicity. Potential participants were referred from the research site and other neighboring health facilities' HIV treatment, PMTCT follow-up, and pediatric HIV services. Some exposed women were recruited from a prior cohort study of women receiving sdNVP [14,20]. "Exposed" status was confirmed by consulting medical records, if possible; status was confirmed for 23 of 94 women. "Unexposed" status was only accepted if an internally consistent and plausible history was provided by the study participant. All women provided written informed consent, and the study was approved by the institutional review boards of Columbia University (New York, NY) and the University of the Witwatersrand (Johannesburg).

Treatment Regimens

Women were treated with NVP, lamivudine, and stavudine. NVP was introduced at one-half the peak dosage for 14 days and escalated to full dosage if no major toxicities occurred. If concomitant treatment for tuberculosis (rifampicin is part of standard treatment) was required or if liver or skin toxicities occurred, efavirenz was substituted for NVP with counseling about the importance of contraception. Zidovudine was substituted for stavudine in the event of implicated toxicities. Regimens including either NVP or efavirenz are referred to as "NNRTI-based therapy." Trimethoprim-sulfamethoxazole was prescribed if pretreatment CD4 cell counts were <200 cells/mm³.

Special attention was given to adherence counseling. A patient "contract" was drawn up whereby the participant agreed to adhere with medication guidelines. A booklet of HIV treatment information was developed, and participants were evaluated with regard to their understanding of this information before they started therapy. Specially designed pill boxes and diary cards were given to assist with adherence. Each visit included review with the pharmacist about dosing and adherence and a pill count of returned drugs, and all members of the clinical team enquired and offered support about adherence. A second-line regimen of lopinavir-ritonavir, zidovudine, and didanosine was initiated for women who met clinical or virologic end points for treatment failure.

Procedures

Blood specimens were obtained before treatment initiation for measurement of CD4 cell count and viral load. Blood samples were drawn every 4 weeks through week 24 and every 12 weeks thereafter until week 78 for determination of the viral load. If viral loads were unexpectedly high, the participant was recalled and the test repeated. CD4 cell counts were measured every 12 weeks through study week 78. For viral load measurements of HIV RNA, we used the standard assay (lower limit of detection, 400 copies/mL) before commencement of treatment and an ultrasensitive assay (Roche Amplicor, version 1.5; Roche Diagnostics; lower limit of detection, 50 copies/mL) after commencement of treatment. The CD4 cell count was measured

by flow cytometry. Social, clinical, and demographic data were collected at the time of enrollment.

Virologic End Points

Viral suppression was defined as a viral load <50 copies/mL during receipt of NNRTI-based therapy. Women who switched to second-line regimens before they achieved viral suppression were censored. Viral rebound was defined as a confirmed viral load >400 copies/mL (measured with 2 separate, usually sequential samples) after achievement of viral suppression, with the first elevation occurring before week 78. Inadequate virologic responses were defined as either failure to achieve viral suppression or viral rebound.

Drug Resistance Testing

All pretreatment plasma (viral RNA) and cell pellet (proviral DNA) samples were tested for the presence of the K103N mutation by allele-specific real-time PCR (AS-PCR). The genotype was determined for samples with the K103N mutation by population sequencing. Pretreatment and follow-up plasma samples obtained from all women with inadequate virologic responses were tested using both methods.

AS-PCR—Viral RNA was isolated from plasma samples using the MagNa Pure LC Total Nucleic Acid Isolation kit on the MagNa Pure Automated System (Roche Diagnostics). Cellular DNA was isolated from buffy coat using the QIAamp DNA Blood Mini Kit (Qiagen). A nested PCR was performed, as described elsewhere [14], using outer primer combinations pFOR-OUT and pREV-OUT and inner primer combinations pFOR-IN and pREV-IN to generate a 985-bp product. For the reverse-transcription reaction, 5 U of AMV Reverse Transcriptase and 8 U of Protector RNase Inhibitor (Roche) were included in the first-round master mix, and the reaction was performed at 42°C for 1 h before cycling. PCR products were tested for the K103N mutation as described elsewhere [14]. Analysis of synthetic plasmid mixtures and of 221 samples obtained from treatment-naïve subjects yielded a cutoff of detection of 0.2% for the minor variant (mean + 3 SDs). All positive samples were retested, and only those samples that yielded positive results on all 3 repeated tests were considered to be true-positive results.

Population sequencing—The same PCR products mentioned above were sequenced using BigDye Terminator, version 3.1, Cycle Sequencing Kit (Applied Biosystems) on an ABI3100 Genetic Analyzer (Applied Biosystems). Genotypic resistance was defined with use of the Stanford Genotypic Resistance Interpretation Algorithm (<http://hivdb.stanford.edu/pages/algs/HIVdb.html>). Consensus sequences were aligned and manually edited using Sequencher software, version 4.5 (GeneCodes). Multiple alignments were performed using ClustalW XXL (<http://www.ch.embnet.org/software/ClustalW-XXL.html>). Phylogenetic analysis of nucleic acid sequences was performed using PHY-LIP [21] and reference sequences from Los Alamos National Laboratory (<http://www.hiv.lanl.gov>). Sequences from this study are available in GenBank (accession numbers FJ348474-FJ348572).

Statistical Methods

The initial sample size calculations estimated that we required 120 women (equally distributed between sdNVP-exposed and sdNVP-unexposed groups) to detect an association of the same order of magnitude as that in the Thai study [11]. Sample size calculations were revised (requiring 96 sdNVP-exposed and 65 sdNVP-unexposed women) once two-thirds of the sample had been enrolled, in light of the considerably better response to treatment observed and because of difficulties in locating control subjects. The revised sample size was designed

to detect a 2-fold difference (with $\alpha = 0.05$ and $\beta = 0.2$ in a 2-tailed test) between sdNVP-exposed and sdNVP-unexposed groups in virologic failure at 78 weeks (assumed to be 25% among sdNVP-unexposed persons, with an 85% rate of follow-up).

Time to viral suppression and rebound was assessed using Kaplan-Meier methods and log-rank tests. For time to viral suppression, we used the time (in days) from the date of initiation of therapy to the date of the first viral load determination of <50 copies/mL (event) or the last available test (if censored) while the patient was still receiving NNRTI-based treatment as the event-time. Time to rebound in the viral load was calculated as above, except that calculations were restricted to patients who achieved viral suppression; the rebound event was defined as >2 tests noting a viral load >400 copies/mL, with the date of the first elevated test result used to define the event-time. For the global outcome of inadequate virologic response, time from treatment initiation to the first test noting a viral load >400 copies/mL was considered to be the event-time for persons who did not experience viral suppression. Cox Proportional hazards models were used for multivariable analysis. For all other comparisons, χ^2 tests were used for categorical variables, t tests were used for normally distributed continuous variables, and Wilcoxon tests were used for nonnormally distributed continuous variables. Analyses were performed using SAS software (SAS Institute).

RESULTS

Study population

Of 420 women screened, 177 were eligible for treatment and met inclusion criteria, and 166 (94%) were enrolled; 99 were sdNVP exposed, and 67 were sdNVP unexposed (figure 1). After we excluded women who did not initiate treatment or who had no follow-up data, 154 women were retained; 94 were sdNVP exposed, and 60 were sdNVP unexposed. Characteristics of the cohort are shown in table 1. sdNVP-exposed and sdNVP-unexposed women had similar pretreatment CD4 cell counts and viral loads but differed in some characteristics (e.g., more sdNVP-exposed women were employed or had a refrigerator and television, and more sdNVP-unexposed women had household members living with HIV infection) (table 1).

Initial and sustained virologic response

Almost all women achieved a viral load <50 copies/mL by week 24. The cumulative probability of viral suppression by week 24 was 0.975 among sdNVP-exposed women and 0.913 among sdNVP-unexposed women ($P = .21$); among women who experienced viral suppression, the time to suppression was similar (table 2). Among sdNVP-exposed women, there was a borderline trend toward greater suppression if exposure had occurred 24–36 months previously (all of these women experienced viral suppression by month 6), compared with exposure 18–23 months previously (probability of suppression, 0.941) ($P = .048$).

Among women who experienced viral suppression, there was no difference between sdNVP-exposed and sdNVP-unexposed women with regard to the risk of viral rebound by week 78 ($P = .57$). Of 88 sdNVP-exposed women who experienced viral suppression by week 24, 16 did not maintain suppression through week 78 (cumulative probability of rebound, 0.194); we censored data for 7 women who were lost to follow-up. Of 54 sdNVP-unexposed women who achieved viral suppression by week 36, 8 did not maintain suppression (cumulative probability of rebound, 0.151); we censored data for 1 woman who died during week 6 of severe toxicity complications (table 2). Among sdNVP-exposed women, there was no trend between viral rebound and time after exposure. Interestingly, 5 of 16 women in the sdNVP-exposed group who experienced viral rebound subsequently had resuppression of the viral load without having changed their drug regimens after enhanced adherence support was provided. There were no differences between the groups with regard to immunologic response (table 2). The risk of

switching to a second-line treatment regimen was similar among NVP-exposed women (0.098; $n = 8$) and unexposed women (0.138; $n = 8$).

Pretreatment resistance mutations

K103N mutations were detected by AS-PCR in 10 (10.6%) of 94 sdNVP-exposed women before they commenced treatment; the mutations were detected in viral RNA for 10 women (10.6%) and in viral DNA for 3 women (3.2%). K103N mutations were also detected by AS-PCR in 9 (15.0%) of 60 sdNVP-unexposed women before treatment; they were detected in viral RNA for 8 women (13.3%) and in viral DNA for 3 women (5.0%). Samples with the highest percentages of K103N mutations detected by AS-PCR also had K103N mutations detected by population sequencing (table 3).

Detection of K103N mutations by AS-PCR before treatment was a strong predictor of inadequate virologic response (table 4). Eleven (57.9%) of 19 women who were either exposed or unexposed to sdNVP and who had K103N mutations detected by AS-PCR in either viral DNA or RNA had inadequate virologic response; 3 did not experience viral suppression (viral load, <50 copies/mL), and 8 experienced initial suppression followed by rebound (viral load, >400 copies/mL). Despite having K103N mutations detected before treatment, 7 women (36.8%) attained and sustained viral suppression; 1 was lost to follow-up. The cumulative probability of inadequate virologic response by week 78 was 0.609 among women with pretreatment K103N mutations and 0.151 among those without ($P < .001$).

Of all 30 women who experienced an inadequate virologic response, 11 (36.7%) had K103N mutations detected before the commencement of treatment, and only 1 woman (3.3%), who had been exposed to sdNVP, had another major NNRTI-related mutation (Y181C) detected before treatment (table 5).

Posttreatment resistance mutations

The earliest rebound samples (i.e., samples obtained at the time of or soonest after viral rebound) from all women with inadequate virologic response were sequenced (table 5). Phylogenetic analysis revealed that all sequences were HIV-1 subtype C, and all samples from the same individual clustered together. The M184V mutation was more common among sdNVP-unexposed women than sdNVP-exposed women (9 of 12 vs. 6 of 18; $P = .03$), but the majority of all women had at least 1 NNRTI-associated mutation (13 of 18 sdNVP-exposed women vs. 10 of 12 sdNVP-unexposed women; $P = .48$).

Other predictors of virologic response

Viral suppression occurred more rapidly if the pretreatment viral load was $<100,000$ copies/mL ($P < .001$), but the proportion of women who achieved and sustained viral suppression by week 24 was the same. There was a nonsignificant association between the presence of the K103N mutation and lower CD4 cell counts and higher viral loads before treatment. The association between pretreatment presence of the K103N mutation and inadequate virologic response remained after adjustment for viral load and CD4 cell count. There continued to be no association between sdNVP exposure and inadequate virologic response after adjustment for pretreatment viral load, CD4 cell count, and K103N mutation presence. Adjustment for any of the variables that were found to differ between the sdNVP-exposed and sdNVP-unexposed groups (table 1) also did not affect this association. Failure to complete high school, presence of a child at home with HIV infection, and a lack of alcohol use were associated with inadequate viral response in univariate analyses. Inclusion of these factors in multivariable analysis did not change the association between sdNVP exposure status and treatment response.

Poor adherence to treatment, which was defined as returning >20% of pills for any of the 3 drugs or not returning pills, was assessed during the first 4 weeks after commencement of therapy, and treatment adherence rates did not differ between those with or without a later inadequate viral response (6.7% and 4.3% of these women, respectively, had poor adherence to treatment; $P = .58$). Poor adherence determined at the time of viral rebound or at the last visit if viral suppression occurred was significantly associated with inadequate viral response (42.1% of such women had poor adherence), compared with sustained viral suppression (20.9% of such women had poor adherence), among women who did not have K103N mutations detected before the commencement of treatment ($P = .046$). Adjustment for treatment adherence did not affect associations between exposure and virologic response.

DISCUSSION

Exposure to sdNVP 18–36 months before initiation of therapy did not attenuate the likelihood of viral suppression by week 24 or reduce the sustainability of viral suppression. Response to NNRTI-based therapy was excellent among sdNVP-exposed women, >95% of whom attained viral suppression and 80% of whom maintained suppression up to week 78. Our results are consistent with those of other studies that have demonstrated no compromise in treatment response if NVP exposure has occurred >6 months previously [12,13]. Ours is, to our knowledge, the most detailed study of this subject, because it included frequent viral load measurements, close follow-up to 78 weeks, and characterization of drug resistance before treatment—design features that facilitated detection of subtle effects of exposure, should they have been present. Some socioeconomic differences between sdNVP-exposed and sdNVP-unexposed women may be explained by access to health care or by health care-seeking behaviors, but none of these factors masked the association between sdNVP exposure and outcome.

Approximately 10% of sdNVP-exposed women had K103N mutations before treatment that we detected in viral RNA using AS-PCR, a method capable of detecting this point mutation if it is present at levels less than the threshold of population sequencing. A smaller proportion of women (3%) had K103N mutations detected in viral DNA, possibly because we extracted total DNA from buffy coats. Many studies have described “minority variants” that can only be detected with more-sensitive methods [14,17–19,22], but the clinical relevance of these variants among sdNVP-exposed women has not been established. We demonstrate that K103N mutations detectable by AS-PCR were strongly associated with inadequate virologic responses to NNRTI-based therapy. Most women—even those who harbored these mutations—initially experienced viral suppression. However, this suppression was often not sustained among those whose K103N mutations were detected before the commencement of treatment. The association between K103N mutations and viral response was not absolute: ~40% of women with K103N mutations still had durable responses, and one-third of women with inadequate viral responses experienced resuppression of the viral load without a change in regimen, after adherence support was intensified. We tested for the K103N mutation with the AS-PCR assay, because this is the mutation most frequently selected by sdNVP exposure. Minority populations of other resistance mutations may have been present; thus, this study represents a minimum estimate. The incomplete association between mutations and viral suppression, combined with the small proportion of sdNVP-exposed women who harbored mutations before treatment, together explained why treatment responses were not noticeably influenced by a history of sdNVP exposure 18–36 months before the study.

We detected drug resistance mutations among 15% of drug-naïve women who reported no exposure to sdNVP. Ours is not the first study to observe K103N mutations among apparently unexposed women [23–25]. Our study offered no incentive for women to make false claims about exposure, and “social desirability” tendencies would not have had this effect either. We

cannot rule out that women forgot or misreported their treatment histories, but some may have had transmitted (primary) resistance and/or environmental exposures. Many sdNVP-unexposed women had other household members with HIV infection (often children), and inadvertent consumption or sharing of medications may explain the results. False-positive assay results are unlikely to have occurred, because all positive samples were confirmed, and there was a strong association with treatment response even after we excluded samples that also yielded positive results by sequencing. One case involving a high concentration of K103N mutations noted by AS-PCR but a genotype negative for K103N may have resulted from polymorphisms in the primer-binding regions [26]. We noted that K103R mutations interfered with detection of K103N mutations. All K103N genotype-positive samples yielded highly positive results by AS-PCR. As treatment programs expand, community-acquired resistance will become more common, with PMTCT programs serving as only one of the potential sources of resistance mutations.

Single-dose tenofovir plus emtricitabine or zidovudine-lamivudine, when administered after NVP exposure, can reduce selection of resistance mutations [27–29]. Theoretically, reduction in initial selection of mutations should minimize effects on later treatment outcomes. Investigation of the proportion of women who harbor low-level—but persisting—mutations after these “tail” protocols may provide useful confirmation that such interventions minimize effects on treatment outcomes.

In conclusion, 10%–15% of women with previous pregnancies who enter treatment programs have resistance mutations that triple the chances that they will not maintain viral suppression while receiving NNRTI-based therapy. This proportion is sufficiently small such that there are no detectable consequences of sdNVP exposure that has occurred 18–36 months previously. To ensure that only few pregnant women will require therapy in the months that follow delivery, we recommend that eligible pregnant women be screened and commence effective antiretroviral therapy.

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Figure 1.
Flow chart of study population. NVP, nevirapine.

Table 1

Pretreatment characteristics of 154 HIV-infected women who initiated therapy in Johannesburg, South Africa, by single-dose nevirapine (sdNVP) exposure status.

Characteristic	sdNVP- exposed women (n = 94)	sdNVP- unexposed women (n = 60)	P
Age, mean years $\pm$ SD	30.9 $\pm$ 5.15	29.3 $\pm$ 4.93	.05
Interval since most recent pregnancy, median months (IQR)	24.8 (20.4–29.9)	19.1 (14.5–26.3)	<.001
Median parity (IQR)	2 (1–3)	2 (1–3)	.72
WHO stage of HIV infection			
1	22 (23.4)	11 (18.3)	
2	15 (16.0)	12 (20.0)	
3	56 (59.6)	35 (58.3)	
4	1 (1.1)	1 (1.7)	.75
CD4 cell count, median cells/mm ³ (IQR)	159 (85–210)	177 (130–241)	.11
HIV RNA level, median copies/mL (IQR)	96,150 (39,100–299,500)	115,500 (45,600–546,000)	.30
Completion of high school	36 (38.3)	18 (30.0)	.29
Employment	38 (40.4)	10 (16.7)	.002
Married status	19 (20.2)	7 (11.7)	.17
HIV status was disclosed to husband or partner	60 (63.8)	34 (56.7)	.37
Presence reported of household members who do not know subject's HIV status	36 (38.3)	17 (28.3)	.20
Other household members are known to have HIV infection	55 (58.5)	46 (76.7)	.01
Others in household taking antiretroviral therapy	31 (33.0)	23 (38.3)	.50
Child in household known to have HIV infection	26 (27.7)	19 (31.7)	.59
Characteristics of home			
Inside tap present	47 (50.0)	28 (46.7)	.69
Electricity present	82 (87.2)	47 (78.3)	.14
Refrigerator present	70 (74.5)	31 (51.7)	.004
Radio present	77 (81.9)	47 (78.3)	.58
Television present	82 (87.2)	42 (70.0)	.009
Reported alcohol use	22 (23.4)	12 (20.0)	.62
Reported traditional medicine use	11 (11.7)	7 (11.7)	.98
Initial treatment regimen			
NVP ^a	74 (78.7)	44 (73.3)	
Efavirenz	20 (21.3)	16 (26.7)	.44
Good adherence to treatment, ^b proportion of patients (%)			

Characteristic	sdNVP- exposed women (n = 94)	sdNVP- unexposed women (n = 60)	P
First assessment after treatment	87/90 (96.7)	53/57 (93.0)	.31
Week 24	76/82 (92.7)	47/51 (92.2)	.91
Week 52	58/66 (87.9)	39/45 (86.7)	.85

NOTE. Data are no. (%) of women, unless otherwise indicated. Ninety-four women had been exposed to single-dose NVP (sdNVP) 18–36 months earlier, and 60 had not been exposed but had been pregnant 12–36 months earlier. IQR, interquartile range; WHO, World Health Organization.

^aFive sdNVP-exposed women and 8 sdNVP-unexposed women initially started receiving nevirapine and had their regimens switched to efavirenz during the course of the study.

^bDefined as >80% pill count reconciliation.

Table 2

Virologic and immunologic response to nonnucleoside reverse-transcriptase inhibitor (NNRTI)-based therapy among 94 single-dose nevirapine (sdNVP)-exposed women and 60 sdNVP-unexposed women in Johannesburg, South Africa.

Characteristic	sdNVP-exposed women	sdNVP-unexposed women	P
Initial virologic response < 24 weeks ^a			
All women	94	60	
Week 4	28 (0.301)	17 (0.286)	
Week 8	51 (0.552)	27 (0.460)	
Week 12	68 (0.738)	42 (0.721)	
Week 16	84 (0.924)	48 (0.826)	
Week 20	86 (0.949)	51 (0.878)	
Week 24	88 (0.975)	53 (0.913)	.21
No. of women who did not achieve a viral load <50 copies/mL by week 24 ^b	2	4	
No. of women whose data were censored before week 24 ^c	4	3	
Initial virologic response and a nonsustained response at or before week 78 ^d			
All women	88	54 ^b	
Week 24	4 (0.046)	3 (0.057)	
Week 36	9 (0.107)	5 (0.094)	
Week 52	12 (0.144)	7 (0.132)	
Week 64	14 (0.169)	...	
Week 78	16 (0.194)	8 (0.151)	.57
CD4 cell count response ^e			
Median pretreatment CD4 cell count, cells/mm ³	159	177	.11
Week 12			
No. of women	90	57	
Median CD4 cell count, cells/mm ³	295	325	.13
Median increase from pretreatment CD4 cell count, cells/mm ³	123	151	.38
Week 24			
No. of women	87	57	
Median CD4 cell count, cells/mm ³	318	345	.33
Median increase from pretreatment CD4 cell count, cells/mm ³	157	155	.84
Week 36			
No. of women	86	52	
Median CD4 cell count, cells/mm ³	323	366	.10
Median increase from pretreatment CD4 cell count, cells/mm ³	172	177	.79
Week 52			
No. of women	77	51	

Characteristic	sdNVP-exposed women	sdNVP-unexposed women	P
Median CD4 cell count, cells/mm ³	374	371	.16
Median increase from pretreatment CD4 cell count, cells/mm ³	224	224	.91
Week 78			
No. of women	71	47	
Median CD4 cell count, cells/mm ³	414	434	.45
Median increase from pretreatment CD4 cell count, cells/mm ³	273	266	.60

NOTE. Data are no. of women (cumulative probability), unless otherwise indicated.

^aDefined as a viral load <50 copies/mL.

^bOf the 2 sdNVP-exposed women who did not achieve a viral load <50 copies/mL while receiving NNRTI-based therapy, the viral load in 1 had decreased to 104 copies/mL at week 20, she missed the week-24 visit, and when seen again at week 36, she had a viral load of 17,800 copies/mL; the second woman had a viral load of 5370 copies/mL at the week 24 visit, and viral loads had been at similar levels at prior visits during treatment. Of the 4 women who did not experience viral suppression in the sdNVP-unexposed group, the viral loads at 24 weeks were 2050, 25,500, 64,900, and 5880 copies/mL. One subsequently experienced viral suppression at week 36 and was included among the total of 54 women who experienced viral suppression and risked viral rebound.

^cThe censored women in the sdNVP-exposed group included 2 who experienced viral suppression by week 24 but who had been switched to a lopinavir-ritonavir-based regimen earlier because of toxicity and 2 who were lost to follow-up before week 24. The censored women in the sdNVP-unexposed group included 1 who experienced viral suppression by week 24 but who had switched earlier to a lopinavir-ritonavir-based regimen, 1 who was lost to follow-up, and 1 who experienced viral suppression by week 36 while receiving the NNRTI-based regimen; the latter woman was included in the viral suppression population in the analysis of sustained virologic response.

^dNonsustained response was defined as a viral load >400 copies/mL.

^eExcludes all who commenced lopinavir-ritonavir treatment before the CD4 count measurement was determined, who missed the visit, or who did not have CD4 cell count data for the visit.

Table 3

Baseline resistance mutations in RNA and DNA, as detected by K103N allele-specific real-time PCR (AS-PCR) and population sequencing, among single-dose nevirapine (sdNVP)-exposed women and sdNVP-unexposed women.

Subject group	RNA specimens		DNA specimens		Later outcome
	K103N AS-PCR result, ^a %	Genotype study finding ^b	K103N AS-PCR result, ^a %	Genotype study finding ^b	
sdNVP-exposed women					
1	21.0	No mutations	37.6	K103N	Viral suppression
2	10.0	K103R	14.7	K103N, K103R	Viral load rebound
3	8.5	No mutations	<0.2	Not done	Viral suppression
4	7.0	No mutations	8.2	No mutations	Viral load rebound
5	1.7	No mutations	<0.2	No mutations	Viral load rebound
6	1.5	No mutations	<0.2	No mutations	Viral suppression
7	0.9	No mutations	<0.2	Not done	Viral suppression
8	0.4	Y181C	<0.2	Y181C	Viral load rebound and decrease; no change in medication
9	0.3	No mutations	<0.2	Not done	Viral load rebound
10	0.3	No mutations	<0.2	Not done	Viral suppression
sdNVP-unexposed women					
1	82.3	K103N, V106M	<0.2	V106M	No viral suppression
2	41.4	K103N	<0.2	No mutations	Viral suppression
3	18.8	K103N, Y181C, G190A	22.1	K103N, Y181C, G190A	No viral suppression
4	5.8	No mutations	<0.2	Not done	Viral suppression
5	1.8	No mutations	2.8	No mutations	Viral load rebound
6	0.7	No mutations	<0.2	No mutations	Viral load rebound
7	0.4	No mutations	<0.2	No mutations	No viral suppression
8	0.2	Not done	<0.2	Not done	Lost to follow-up
9	<0.2	No mutations	27.4	K103N	Viral load rebound

NOTE. Only women with a positive (defined as >0.2%) K103N AS-PCR result in either RNA or DNA are shown.

^aData are the estimated percentage of the viral population that carried K103N, and positive results are shown in boldface font.

^bMutations shown in boldface font are major NNRTI-associated mutations. K103R is a polymorphism that may interfere with the AS-PCR detection of the K103N mutant.

Table 4

Risk of inadequate viral response to nonnucleoside reverse-transcriptase inhibitor (NNRTI)-based therapy, by pretreatment detection of K103N mutations by allele-specific PCR in DNA and RNA specimens obtained from single-dose nevirapine (sdNVP)-exposed and -unexposed women.

Variable	Inadequate virologic response			Modified inadequate virologic response		
	No. of women	Kaplan-Meier probability	P	No. of women	Kaplan-Meier probability	P
K103N in DNA						
Positive (n = 6)	5	0.833		5	0.833	
Negative (n = 148)	25	0.182	<.001	20	0.145	<.001
K103N in RNA ^a						
Positive (n = 18)	10	0.585		9	0.526	
Negative (n = 135)	20	0.159	<.001	16	0.127	<.001
K103N detected only by AS-PCR, excluding women with positive results of sequencing ^b						
Positive (n = 12)	6	0.542		6	0.542	
Negative (n = 135)	19	0.151	<.001	15	0.119	<.001
sdNVP exposure history						
Exposed (n = 94)	18	0.211		13	0.152	
Unexposed (n = 60)	12	0.209	.89	12	0.209	.31
sdNVP exposure history among women with the K103N mutation detected in either DNA or RNA specimens						
Exposed (n = 10)	5	0.500		4	0.400	
Unexposed (n = 9)	6	0.741	.12	6	0.741	.06
sdNVP exposure history among women for whom the K103N mutation was not detected in either DNA or RNA specimens						
Exposed (n = 84)	13	0.170		9	0.117	
Unexposed (n = 51)	6	0.122	.50	6	0.122	.91

NOTE. Inadequate virologic response was defined as nonachievement of a viral load of <50 copies/mL while receiving NNRTI-based therapy or viral rebound (i.e., a confirmed viral load >400 copies/mL) <78 weeks after achievement of viral suppression. Modified inadequate virologic response is defined as above except that women who experienced resuppression of the viral load without a change in regimen are reclassified as having an adequate response.

^aOne sample could not be tested and was assumed to have yielded negative results in the following analyses, stratified by results in either RNA or DNA.

^bOnly includes those for whom the K103N mutation was detected by AS-PCR in the absence of any evidence of any NNRTI-associated mutation detected by standard population sequencing of RNA or DNA.

Table 5

Detection of resistance mutations in pretreatment and posttreatment plasma viral RNA specimens obtained from women with inadequate virologic responses.

Subject group	Pretreatment specimens		Posttreatment specimens	
	K103N AS-PCR result, ^a %	Genotype study finding ^b	K103N AS-PCR result, ^a %	Genotype study finding ^b
sdNVP-exposed women				
2	10.0	No mutations	99.6	K103N ...
4	7.0	No mutations	100.0	K103N M184V
5	1.7	No mutations	100.0	K103N M184V
9	0.3	No mutations	<0.2	Y181C M184V
8 ^c	0.4	Y181C	96.1	K103N ...
11	<0.2	No mutations	100.0	K103N ...
12	<0.2	No mutations	99.8	K103N ...
13	<0.2	No mutations	98.8	K103N, Y181C M184V, D67N
14	<0.2	No mutations	91.7	K103N, V106M ...
15	<0.2	No mutations	34.2	K103N ...
16	<0.2	No mutations	1.5	No mutations ...
17	<0.2	Y181C	<0.2	Y181C K219EN
18 ^c	<0.2	No mutations	<0.2	G190A M184V
19 ^c	<0.2	No mutations	<0.2	Y181H ...
20 ^c	<0.2	No mutations	<0.2	No mutations M184IV
21 ^c	<0.2	No mutations	<0.2	No mutations ...
22	<0.2	No mutations	<0.2	No mutations ...
23	<0.2	No mutations	<0.2	No mutations ...
sdNVP-unexposed women				
1	82.3	K103N, V106M	99.9	K103N, V106M, Y181C, K238T M184V
3	18.8	K103N, Y181C, G190A	100.0	K103N, Y181C M184V
5	1.8	No mutations	<0.2	Y181C M184I, D67N
6	0.7	No mutations	<0.2	V106M, F227L ...

Pretreatment specimens			Posttreatment specimens		
Subject group	K103N AS-PCR		Genotype study finding ^b		
	result, ^a %	Genotype study finding ^b	K103N AS-PCR result, ^a %		NRTI resistance mutation(s)
7	0.4	No mutations	100.0	K103N	M184V, M41L
10	<0.2 ^d	No mutations	<0.2	V106M	M184V
11	<0.2	No mutations	19.5	Y181C	...
12	<0.2	No mutations	0.3	No mutations	M184V
13	<0.2	No mutations	<0.2	Y181C	M184V
14	<0.2	No mutations	<0.2	V106M	M184V
15	<0.2	No mutations	<0.2	G190A	M184V
16	<0.2	No mutations	<0.2	No mutations	...

^aData are the estimated percentage of the viral population that was K103N, and positive results are shown in boldface font.

^bFor genotyping results, only mutations usually defined as major NNRTI-associated mutations are shown.

^cParticipants who achieved viral suppression (viral load, <50 copies/mL), a rebound in the viral load (confirmed viral load, >400 copies/mL), and subsequent resuppression without a change in therapy.

^dThis participant had the K103N mutation detected by AS-PCR and genotype studies of DNA specimens (see table 3).