Sensitive Drug-Resistance Assays Reveal Long-Term Persistence of HIV-1 Variants with the K103N Nevirapine (NVP) Resistance Mutation in Some Women and Infants after the Administration of Single-Dose NVP: HIVNET 012

Tamara Flys,[1] Dwight V. Nissley,[2,3] Cassidy W. Claasen,[3] Dana Jones,[1] Chanjuan Shi,[1] Laura A. Guay,[1] Philippa Musoke,[4] Francis Mmiro,[5] Jeffrey N. Strathern,[3] J. Brooks Jackson,[1] James R. Eshleman,[1] and Susan H. Eshleman[1]

[1]Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, and [2]Gene Regulation and Chromosome Biology Laboratory, Reverse Transcription and Molecular Biology Section, Basic Research Program, Science Applications International Corporation–Frederick, [3]National Cancer Institute–Frederick, Frederick, Maryland; Departments of [4]Paediatrics and [5]Obstetrics and Gynaecology, Makerere University, Kampala, Uganda

(See the editorial commentary by Hammer and the articles by Johnson et al. and Eshleman et al., on pages 1–3, 16–23, and 30–6, respectively.)

Background. The HIV Network for Prevention Trials (HIVNET) 012 trial showed that NVP resistance (NVPR) emerged in some women and children after the administration of single-dose nevirapine (SD-NVP). We tested whether K103N-containing human immunodeficiency virus (HIV)–1 variants persisted in women and infants 1 year or more after the administration of SD-NVP.

Methods. We analyzed samples from 9 women and 5 infants in HIVNET 012 who had NVPR 6–8 weeks after the administration of SD-NVP. Samples were analyzed with the ViroSeq system and with 2 sensitive resistance assays, LigAmp and TyHRT.

Results. ViroSeq detected the K103N mutation in 8 of 9 women and in 2 of 5 infants. LigAmp detected the K103N mutation at low levels in 8 of 9 women and in 4 of 5 infants. K103N was not detected by ViroSeq 12–24 months after the administration of SD-NVP but was detected by LigAmp in 3 of 9 women and in 1 of 5 infants. K103N was also detected in those samples by use of the TyHRT assay.

Conclusions. K103N-containing variants persist in some women and infants for 1 year or more after the administration of SD-NVP. Sensitive resistance assays may provide new insight into the impact of antiretroviral drug exposure on HIV-1 evolution.

Short antiretroviral drug regimens can reduce the risk of mother-to-child transmission (MTCT) of HIV-1 in resource-limited settings. In the HIV Network for Prevention Trials (HIVNET) 012 regimen, women receive single-dose nevirapine (SD-NVP) during labor and infants receive SD-NVP shortly after birth. This regimen is simple, safe, inexpensive, and effective for the prevention of MTCT (pMTCT) of HIV-1 [1]. A disadvantage of this regimen is the emergence of NVP resistance (NVPR) after the administration of SD-NVP.

Received 14 December 2004; accepted 30 March 2005; electronically published 1 June 2005.

Potential conflicts of interest: C.S., J.R.E., and S.H.E. are coinventors of the LigAmp assay, and Johns Hopkins University has filed a patent application with the US Patent and Trademark Office. The inventors may receive royalty payments if the patent is awarded and licensed.

Reprints or correspondence: Dr. Susan H. Eshleman, Dept. of Pathology, Johns Hopkins Medical Institutions, Ross Bldg. 646, 720 Rutland Ave., Baltimore, MD 21205 (seshlem@jhmi.edu).

The Journal of Infectious Diseases 2005;192:24–9
© 2005 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/2005/19201-0006$15.00

Financial support: HIV Network for Prevention Trials, sponsored by the US National Institute of Allergy and Infectious Diseases (NIAID), the National Institutes of Health (NIH), and the Department of Health and Human Services (DHHS) through contracts with Family Health International (N01-AI-35173), Fred Hutchinson Cancer Research Center (N01-AI-45200), and Makerere University (NOI-AI-35173-417); HIV Prevention Trials Network, sponsored by the NIAID, the National Institutes of Child Health and Human Development, the National Institute on Drug Abuse, the National Institute of Mental Health, and the Office of AIDS Research of the NIH, DHHS (grants U01-AI-46745 and U01-AI-48054); Adult AIDS Clinical Trials Groups, sponsored by the NIH, Division of AIDS, NIAID (grant U01-AI-38858); NIH (grant R01-HD042965-01 to S.H.E.); National Cancer Institute, NIH (grant R01 CA81439 to J.R.E. and contract NO1-CO-12400); Maryland Cigarette Restitution Fund (grant to J.R.E.); Canadian Institute of Health Research (fellowship to C.S.).

Downloaded from https://academic.oup.com/jid/article/192/1/24/872933 by guest on 20 August 2022

Table 1. Ligation oligonucleotides and primers used in the LigAmp assay for detection of the K103N mutation (AAA→AAC) in HIV-1 subtype A and D.

The table is available in its entirety in the online edition of the *Journal of Infectious Diseases.*

In HIVNET 012, NVPR was detected in 25% of women [2] and in 46% of infants 6–8 weeks after delivery [3]. Emergence of NVPR after the administration of SD-NVP was subsequently observed in other studies. Results of a recent study suggest that women with prior exposure to SD-NVP may have a reduced virologic response to treatment regimens that contain non-nucleoside reverse-transcriptase (RT) inhibitors (NNRTIs) [4]. It is not known whether prior exposure to SD-NVP reduces the efficacy of treatment regimens that contain NVP in HIV-1–infected children or the efficacy of SD-NVP for pMTCT in subsequent pregnancies. NVP-resistant variants selected in women by SD-NVP could also potentially be transmitted to infants by breast-feeding or to others in the community.

Few studies have evaluated the persistence of NVPR after the administration of SD-NVP. In HIVNET 012, samples obtained 12–24 months after the administration of SD-NVP were available for 11 women and 6 infants who had NVPR at 6–8 weeks after the administration of SD-NVP. No NVPR mutations were detected in those samples [3]. In the HIVNET 023 trial, variants with NVPR mutations could not be detected in almost all women by 6 months postpartum [5]. However, in a South African cohort, 55 (35%) of 155 women who had NVPR at 7 weeks postpartum still had detectable NVPR at 6 months postpartum [6]. Those studies were performed with assays based on population sequencing that are relatively insensitive for the detection of drug-resistant HIV-1 variants present in conjunction with wild-type *(wt)* HIV-1. In the present exploratory study, we used sensitive resistance assays to analyze the emergence and persistence of HIV-1 variants with the K103N mutation in a subset of women and infants in the HIVNET 012 cohort.

SUBJECTS, MATERIALS, AND METHODS

Study subjects. The human experimentation guidelines of the US Department of Health and Human Services and the authors' institutions were followed in the conduct of the research. Women were antiretroviral drug naive and did not receive any other antiretroviral therapy, which is consistent with the standard of care in Uganda at the time the trial was performed. Samples from 9 women and 5 infants who had available samples from long-term follow-up were analyzed. These study subjects had been selected for follow-up in a previous study, after they were found to have NVPR in samples obtained 6–8 weeks after delivery [3]. Samples obtained before the administration of SD-NVP from 40 additional women in HIVNET 012 were also analyzed.

HIV-1 genotyping and subtyping. HIV-1 genotyping was performed with the ViroSeq HIV-1 Genotyping System (Celera Diagnostics), and HIV-1 subtyping was performed by phylogenetic analysis of *pol* region sequences [2]. Some samples were genotyped in studies published elsewhere [3, 7].

Detection of the K103N mutation using the LigAmp assay. The LigAmp assay involves mutation-specific ligation of 2 oligonucleotides to a DNA template, followed by detection of the ligated product by real-time polymerase chain reaction (PCR) [8] (table 1). The LigAmp assay was optimized for the detection of the K103N mutation in HIV-1 subtypes A and D (figures 1 and 2). The conditions used for analysis are described in the Appendix, which is not provided in the print edition. The limit of detection for the K103N mutation in both subtypes was 0.08% (mean + 3 SDs of the result obtained for *wt* DNA). The assay cutoff was set at 0.1% mutant. For analysis of plasma samples, PCR products generated with ViroSeq were used as template DNA and were analyzed in triplicate. To permit analysis of the same samples with the TyHRT assay, PCR products were generated without dUTP. A standard curve was included in each experiment and was used to quantify the percentage of K103N mutations in each sample (figure 2*C* and 2*D*).

Detection of NVPR mutations using the yeast TyHRT assay. The TyHRT assay was used to screen libraries of HIV-1 RT-containing variants for RT activity and NVP susceptibility [9]. Plasmids from NVP-resistant variants were sequenced to identify NVPR mutations. A general description of the assay and the conditions used for analysis are provided in the Appendix, which is not provided in the print edition.

RESULTS

Detection of NVPR mutations using ViroSeq. Plasma samples were analyzed using an assay based on population sequencing (ViroSeq) (figures 3*A* and 4*A* and table 2). The women and infants selected for the present study all had NVPR mutations detected 6–8 weeks after the administration of SD-NVP [3].

Samples obtained from women before the administration of SD-NVP and at delivery contained *wt* HIV-1 (no NVPR mutations were detected) (figure 3*A*). Genotyping results for 5 women were obtained 1 week after the administration of SD-NVP. None of the samples had HIV-1 variants with the K103N mutation. Samples from 2 women had HIV-1 variants with other NVPR mutations (1 had Y181C and 1 had Y181C and G190A). Samples obtained at 6–8 weeks after the administra-

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases.*

Figure 1. HIV-1 sequences from Ugandan women (M) and infants (I). The figure and legend are available in their entirety in the online edition of the *Journal of Infectious Diseases.*

Downloaded from https://academic.oup.com/jid/article/192/1/24/872933 by guest on 20 August 2022

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases.*

Figure 2. Analysis of control plasmids and a representative plasma sample by use of the LigAmp assay. The figure and legend are available in their entirety in the online edition of the *Journal of Infectious Diseases.*

tion of SD-NVP from 8 of 9 women had HIV-1 variants with the K103N mutation and from 4 of 9 women had HIV-1 variants with other NVPR mutations (3 had K103N and Y181C and 1 had V108I). Samples obtained 12–24 months after the administration of SD-NVP from all 9 women had *wt* HIV-1.

Samples obtained at birth from all 5 infants had *wt* HIV-1 (figure 4*A*). Samples obtained at 6–8 weeks after the administration of SD-NVP from 2 of 5 infants had HIV-1 variants with the K103N mutation, and those from 4 of 5 infants had HIV-1 variants with other NVPR mutations (2 had Y181C, 1 had K103N and Y181C, and 1 had Y181C and Y188C). Samples obtained 14–16 weeks after the administration of SD-NVP were available for 4 of 5 infants. One infant had HIV-1 variants with the K103N mutation, and 1 infant had HIV-1 variants with the Y181C mutation. The K103N mutation was not detected in the samples from infants at 1 year after the administration of SD-NVP. The G190A mutation was detected in a sample obtained from 1 infant at 12 months after the administration of SD-NVP but was not detected in samples obtained from that infant at earlier study visits.

Detection and quantification of variants with the K103N mutation using the LigAmp assay. We used the LigAmp assay to detect and quantify HIV-1 variants with the K103N mutation (figures 3*B* and 4*B*). The K103N mutation was selected for analysis, because K103N was the most common NVPR mutation detected by ViroSeq in women in HIVNET 012 6–8 weeks after exposure to NVP [2] and in a South African cohort 6 months after exposure to NVP [6].

Samples from all 9 women obtained before exposure to NVP and at delivery had frequencies of HIV-1 variants with the K103N mutation below the assay cutoff (< 0.1%) (figure 3*B*). Samples obtained before exposure to NVP from 40 other women in HIVNET 012 were also analyzed. The frequency of HIV-1 variants with the K103N mutation was below the assay cutoff in 36 (90%) of 40 samples. In the remaining 4 samples, HIV-1 variants with the K103N mutation were detected at frequencies of 0.1%, 0.1%, 0.2%, and 0.3% (data not shown). In 8 of 9 women selected for follow-up, HIV-1 variants with the K103N mutation were detected in the samples obtained 6–8 weeks after the administration of SD-NVP. The mean percentage of HIV-1 variants with the K103N mutation in the 8 women was 13.9%. In 6 women, the frequency of HIV-1 variants with the K103N mutation declined to <0.1% by 12–24 months after the administration of SD-NVP. However, in 3 women, HIV-1 variants with the K103N mutation were detected at a frequency of >0.1% (at 0.8%, 1.3%, and 3.5%) 14 months after the administration of SD-NVP (figure 3*B, arrows*).

The frequency of HIV-1 variants with the K103N mutation was below the assay cutoff in 4 of 5 infants at birth (figure 4*B*). In 1 infant, HIV-1 variants with the K103N mutation were detected at a frequency just >0.1%. At 6–8 weeks after the administration of SD-NVP, HIV-1 variants with the K103N mutation were detected at a frequency of >0.1% in 4 of 5 infants, and the percentage of HIV-1 variants with the K103N mutation in 1 infant (I-545) remained elevated at 14–16 weeks after the administration of SD-NVP. In that infant, HIV-1 variants with the K103N mutation were also detected 1 year after the administration of SD-NVP (at a frequency of 1.5%) (figure 4*B, arrow*).

Comparison of results from the LigAmp and TyHRT assays. The samples described above were also analyzed using the yeast-based TyHRT assay to confirm the persistence of the K103N mutation in the samples obtained during long-term follow-up (figures 3*C* and 4*C* and table 2). The TyHRT assay is a genetic assay that allows libraries of HIV-1 RT–containing clones to be screened for RT activity and NVP susceptibility.

Results from the LigAmp assay (measuring the percentage of variants with the K103N mutation) and the TyHRT assay (measuring the percentage of NVP-resistant variants) were consistent. Seventeen of 20 samples that had HIV-1 variants with the K103N mutation detected by the LigAmp assay also had HIV-1 variants with NVPR detected by the TyHRT assay, and the K103N mutation was detected in the NVP-resistant clones. In the 3 remaining samples, the frequency of HIV-1 variants with the K103N mutation detected in the LigAmp assay was <1%. Failure to detect clones with NVPR in those samples in the TyHRT assay may reflect the lower sensitivity of that assay for the detection of mutants (~0.5% mutant). A few samples that did not have HIV-1 variants with the K103N mutation detected by the LigAmp assay had HIV-1 variants with NVPR detected by the TyHRT assay. In those samples, clones isolated in the TyHRT assay did not have the K103N mutation, with the exception of rare clones found in 1 mother-infant pair (1/210 clones from the sample obtained from M-750 before the administration of SD-NVP and 1/190 clones from the sample obtained from I-750 14 weeks after the administration of SD-NVP). In both cases, the K103N mutation was encoded by AAT, which was not probed in the LigAmp assay. The K103N mutation was detected by the LigAmp assay in 4 samples obtained during long-term follow-up, at frequencies of 0.8%, 1.3%, 3.5%, and 1.5% (M-474, M-750, M-830, and I-545, respectively) (figures 3*B* and 4*B, arrows*). The percentage of HIV-1 variants with NVPR detected in the TyHRT assay for those samples was 3.4%, 0.6%, 11%, and 0.3%, respectively. Even though relatively few NVP-resistant clones were isolated from

Downloaded from https://academic.oup.com/jid/article/192/1/24/872933 by guest on 20 August 2022

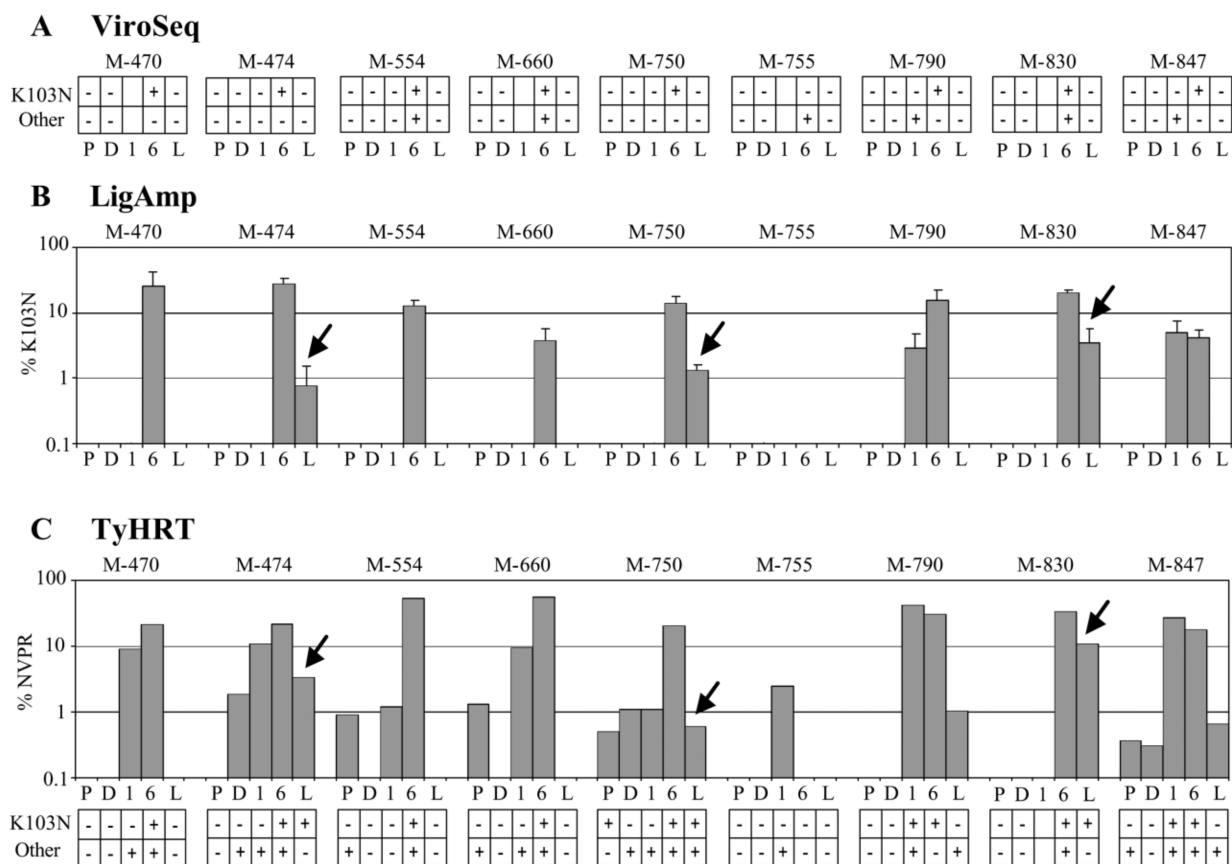

Figure 3. Analysis of HIV-1 in maternal samples. Samples from women (M) were obtained before ("pre"; P) the administration of single-dose nevirapine (SD-NVP), at delivery (D), and at 1 week (1), 6–8 weeks (6), and at 12, 14, or 24 months (during long-term follow-up; L) after the administration of SD-NVP. The samples obtained at 1 week after the administration of SD-NVP from 3 women (M-470, M-660, and M-755) failed to amplify sufficiently for genotyping, and 1 woman (M-830) did not have a sample obtained from that period that was available for analysis. Three women (M-660, M-790, and M-847) had HIV-1 subtype A, and 3 women (M-470, M-554, and M-755) had HIV-1 subtype D. *A,* Analysis of samples with the ViroSeq assay. The presence (+) or absence (-) of the K103N and other NVPR mutations in each sample is indicated. All HIV-1 variants with NVPR mutations were detected as mixtures with wild-type HIV-1. When the K103N mutation was detected, it was exclusively or predominantly encoded by the codon AAC. The alternative codon AAT was present at a low level along with the codon AAC in some samples. *B,* Analysis of samples with the LigAmp assay. The percentage of variants with the K103N mutation was determined for each sample in triplicate using the LigAmp assay. The mean + SD of 3 experiments is shown. Arrows indicate long-term persistence of the K103N mutation above levels measured before the administration of SD-NVP. *C,* Analysis of samples using the TyHRT assay. In the TyHRT assay, HIV-1 *pol* region DNA from each sample is introduced into a yeast element by homologous recombination. Each yeast isolate carries a unique HIV-1 RT domain, and the library of isolates is representative of the RT domains present in the original viral sample. Yeast are cultured in the presence of NVP to isolate NVP-resistant colonies. The percentage of yeast colonies (HIV-1 variants) with NVPR is plotted for each sample. DNA was isolated from individual NVP-resistant colonies and was sequenced to identify NVPR mutations in the HIV-1 RT domain. The presence (+) or absence (-) of K103N and other NVPR mutations in clones isolated from each sample is indicated below the graph. For a more detailed description of this analysis, see the Appendix, which is not provided in the print edition.

those samples, HIV-1 clones with the K103N mutation were detected in all 4 samples. In contrast, samples obtained during long-term follow-up that did not have HIV-1 variants with the K103N mutation detected by the LigAmp assay also did not have HIV-1 variants with the K103N mutation detected by the TyHRT assay. Of note, the only infant sample obtained 12 months after the administration of SD-NVP that had HIV-1 variants with the G190A mutation detected by ViroSeq also had HIV-1 variants with G190A detected by the TyHRT assay. Interestingly, 2 samples (the sample obtained from M-554 1 week after the administration of SD-NVP and the sample obtained from M-847 at delivery) had HIV-1 variants with NVPR detected by the TyHRT assay but did not have known NVPR mutations detected in the NVP-resistant clones. In both cases, substitutions were noted at codon 236 (P236L and P236A, respectively). In HIV-1 subtype B, the P236L mutation is associated with resistance to delavirdine but causes hypersusceptibility (rather than resistance) to NVP.

Downloaded from https://academic.oup.com/jid/article/192/1/24/872933 by guest on 20 August 2022

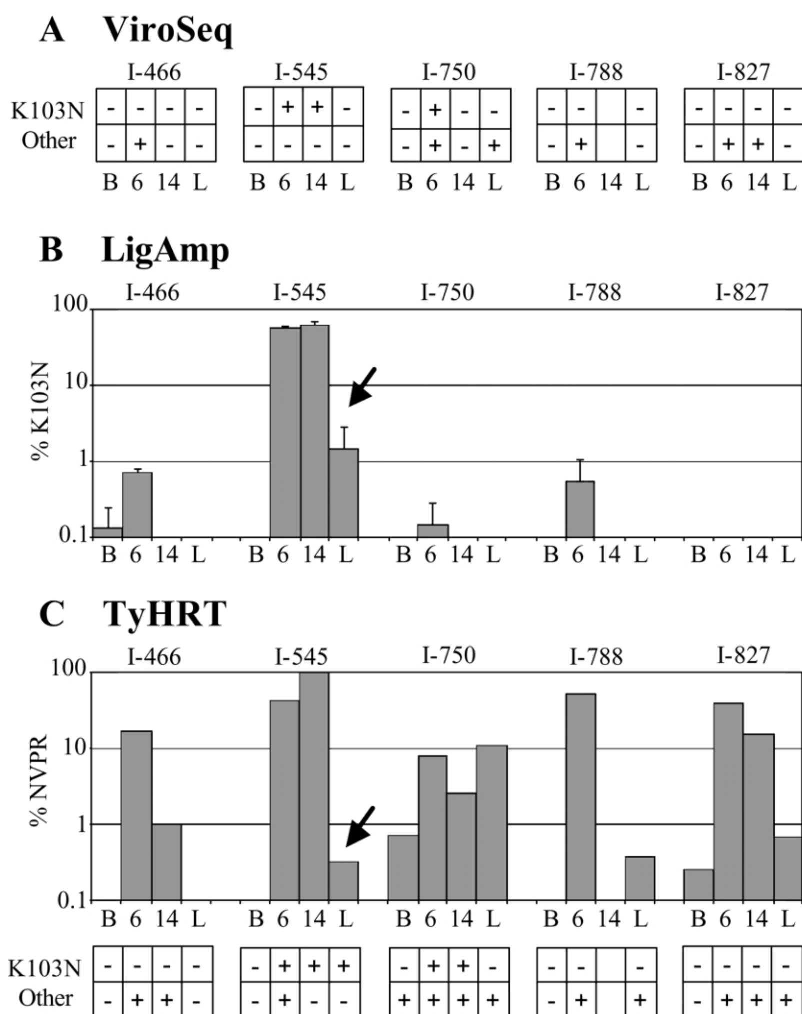

Figure 4. Analysis of HIV-1 in samples from infants. Samples from infants (I) were obtained at birth (B), and at 6–8 weeks (6), 14–16 weeks (14), and 12 months (during long-term follow-up; L) after the administration of single-dose nevirapine (SD-NVP). One infant (I-788) did not have a sample obtained 14-16 weeks after the administration of SD-NVP that was available for analysis. Two infants (I-466 and I-545) had HIV-1 subtype A, and 3 infants (I-827, I-750, and I-788) had HIV-1 subtype D. *A,* Analysis of samples with the ViroSeq assay. The presence (+) or absence (-) of K103N and other NVPR mutations in each sample is indicated. All HIV-1 variants with NVPR mutations were detected as mixtures with wild-type HIV-1, with the exception of the K103N mutation in samples obtained at 6–8 and 14 weeks after the administration of SD-NVP from infant I-545. When the K103N mutation was detected, it was exclusively or predominantly encoded by the codon AAC, with the following exception: K103N was encoded predominantly by AAT in the sample obtained 6–8 weeks after the administration of SD-NVP from infant I-750. *B,* Analysis of samples with the LigAmp assay (for information on the analysis, see the Appendix, which is not provided in the print edition, and figure 1*B*). *C,* Analysis of samples using the TyHRT assay (for information on the analysis, see the Appendix and figure 3*C*).

DISCUSSION

We found that HIV-1 variants with the K103N mutation can persist at frequencies greater than those found before exposure to NVP in some women and infants ⩾1 year after the administration of SD-NVP. This finding is consistent with the results of studies showing that the K103N mutation confers a relatively small fitness cost in vitro [10] and with the results of studies showing persistence of the K103N mutation for years in some patients who are infected with drug-resistant HIV-1 strains [11]. Results of preliminary studies using mutation-specific real-time PCR assays have also shown long-term persistence of NVPR mutations in women with HIV-1 subtype C [12, 13]. It is important to note that the results of our study may not be representative of the entire HIVNET 012 cohort, because the number of women and infants analyzed was small. Furthermore, our study included only women and infants who had HIV-1 variants with NVPR detected by ViroSeq 6–8 weeks after

Table 2. Analysis of samples by use of the ViroSeq and TyHRT assays.

The table is available in its entirety in the online edition of the *Journal of Infectious Diseases.*

Downloaded from https://academic.oup.com/jid/article/192/1/24/872933 by guest on 20 August 2022

the administration of SD-NVP. This approach is likely to bias the results in favor of detection of HIV-1 variants with NVPR in samples obtained during long-term follow-up.

The LigAmp assay was particularly useful for quantification of HIV-1 variants with the K103N mutation in our study. The assay has a broad linear range and can quantify variants present at a frequency of 0.1%. The assay can be performed without patient-specific primers and can be performed using PCR products that remain after routine genotyping. The assay is relatively simple to perform and uses very low concentrations of template. The TyHRT assay also offers unique advantages for the analysis of HIV-1 drug-resistance mutations. That assay provides phenotypic selection of drug-resistant variants, which can be further characterized by DNA sequencing. That approach may be particularly useful for analysis of drug-resistant variants in non-B subtypes, because the genetic correlates of antiretroviral drug resistance in HIV-1 subtypes other than subtype B are not well defined.

Further studies are needed to determine whether persistence of HIV-1 variants with NVPR mutations circulating at low levels after the administration of SD-NVP compromises the efficacy of regimens that contain NNRTIs for HIV-1 treatment or pMTCT in subsequent pregnancies. The emergence of NVPR in women and infants receiving regimens for pMTCT can usually be prevented by providing pregnant women with potent combination antiretroviral therapy. However, in resource-poor countries where there is limited access to antiretroviral drugs, simpler regimens are more likely to be used for pMTCT. Some studies have evaluated NVPR in women who received SD-NVP for pMTCT in combination with other antiretroviral drugs. Unfortunately, NVPR often emerges despite the addition of other drugs to the pMTCT regimen [14–16]. Further studies to evaluate the clinical significance of NVPR in this setting, and the persistence of low levels of HIV-1 variants with NVPR in women and infants after SD-NVP exposure, are needed. Sensitive resistance assays may be useful for the evaluation of the persistence of drug-resistant variants.

Acknowledgments

We thank the HIV Network for Prevention Trials 012 team, for providing the samples; Melissa Allen (clinical trials specialist, Family Health International), for providing assistance; Estelle Piwowar-Manning, Constance Ducar, and the laboratory staff in Uganda, for assisting with the processing of the samples; and Boehringer Ingelheim, for providing nevirapine.

References

1. Guay LA, Musoke P, Fleming T, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-infant transmission of HIV-1 in Kampala, Uganda: HIVNET-012 randomised trial. Lancet **1999**; 354:795–802.
2. Eshleman SH, Guay LA, Mwatha A, et al. Characterization of nevirapine (NVP) resistance mutations in women with subtype A vs. D HIV-1 6–8 weeks after single dose NVP (HIVNET 012). J Acquir Immune Defic Syndr **2004**; 35:126–30.
3. Eshleman SH, Mracna M, Guay LA, et al. Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). AIDS **2001**; 15:1951–7.
4. Jourdain G, Ngo-Giang-Huong N, Le Coeur S, et al. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. N Engl J Med **2004**; 351:229–40.
5. Kantor R, Lee E, Johnston E, et al. Rapid flux in non-nucleoside reverse transcriptase mutations among subtype C HIV-1-infected women after single dose nevirapine. Antivir Ther **2003**; 8:S85.
6. Morris L, Martinson N, Pillay C, et al. Persistence of nevirapine resistance mutations 6 months following single dose nevirapine [abstract ThOrB1353]. In: Program and abstracts of the 15th International AIDS Conference (Bangkok, Thailand, 2004). Vol. 2. Geneva: International AIDS Society, **2004**:325.
7. Eshleman SH, Guay LA, Mwatha A, et al. Comparison of nevirapine (NVP) resistance in Ugandan women 7 days vs. 6–8 weeks after single dose NVP prophylaxis: HIVNET 012. AIDS Res Human Retroviruses **2004**; 20:595–7.
8. Shi C, Eshleman SH, Jones D, et al. LigAmp: sensitive detection of single nucleotide differences. Nature Methods **2004**; 1:141–7.
9. Nissley DV, Boyer PL, Garfinkel DJ, Hughes SH, Strathern JN. Hybrid Ty1/HIV-1 elements used to detect inhibitors and monitor the activity of HIV-1 reverse transcriptase. Proc Natl Acad Sci USA **1998**; 95: 13905–10.
10. Collins JA, Thompson MG, Paintsil E, Ricketts M, Gedzior J, Alexander L. Competitive fitness of nevirapine-resistant human immunodeficiency virus type 1 mutants. J Virol **2004**; 78:603–11.
11. Little SJ, Dawson K, Hellmann NS, Richman DD, Frost SDW. Persistence of transmitted drug-resistant virus among subjects with primary HIV infection deferring therapy. Antivir Ther **2003**; 8:S129.
12. Palmer S, Boltz V, Maldarelli F, et al. Persistence of NNRTI-resistant variants after single-dose nevirapine in HIV-1 subtype C-infected women [abstract 101]. In: Program and abstracts of the 12th Conference on Retroviruses and Opportunistic Infections (Boston, MA, 2005). Alexandria, VA: Foundation for Retrovirology and Human Health, **2005**:101.
13. Loubser S, Balfe P, Sherman G, et al. Sensitive real-time PCR quantification of 103N resistance mutants following single-dose treatment with nevirapine [abstract 102]. In: Program and abstracts of the 12th Conference on Retroviruses and Opportunistic Infections (Boston, MA, 2005). Alexandria, VA: Foundation for Retrovirology and Human Health, **2005**:102.
14. Cunningham CK, Chaix M-L, Rekacewicz C, et al. Development of resistance mutations in women receiving standard antiretroviral therapy who received intrapartum nevirapine to prevent perinatal human immunodeficiency virus type 1 transmission: a substudy of Pediatric AIDS Clinical Trials Group Protocol 316. J Infect Dis **2002**; 186:181–8.
15. Chalermchokcharoenkit A, Asavarpiriyanont S, Teeraratkul A, et al. Combination short-course zidovudine plus 2-dose nevirapine for prevention of mother-to-child transmission: safety, tolerance, transmission, and resistance results [abstract 96]. In: Program and abstracts of the 11th Conference on Retroviruses and Opportunistic Infections (San Francisco, CA, 2004). Alexandria, VA: Foundation for Retrovirology and Human Health, **2004**:111.
16. McIntyre J, Martinson N, Boltz V, et al. Addition of short course combivir (CBV) to single dose viramune (sdNVP) for prevention of mother-to-child transmission (MTCT) of HIV-1 can significantly decrease the subsequent development of maternal NNRTI-resistant virus [abstract LbOrB09]. In: Program and abstracts of the 15th International AIDS Conference (Bangkok, Thailand, 2004). Suppl. Geneva: International AIDS Society, **2004**:32.

Downloaded from https://academic.oup.com/jid/article/192/1/24/872933 by guest on 20 August 2022