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Minor resistant variants in nevirapine-exposed infants may predict virologic failure on nevirapine-containing ART

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Abstract

Background—Single-dose nevirapine (sdNVP) is widely used to prevent mother-to-child transmission (PMTCT) of HIV-1. This may result in NVP resistance in both mother and infant. The significance of low levels of NVP resistance mutations in infants treated with NVP-containing antiretroviral treatment (ART) is unknown.

Objectives—To determine the presence of pre-treatment NVP resistance in HIV-infected infants with and without prior NVP exposure.

Study Design—33 HIV-1-infected infants in a PMTCT trial received NVP-containing ART (26 infants with prior NVP exposure). Plasma and buffy coat samples obtained prior to ART initiation were evaluated for drug resistance by bulk sequencing and allele-specific PCR (ASPCR).

Results—ViroSeq™ identified NVP resistance in 3 of 33 infants; all failed first-line therapy. Pre-ART plasma NVP resistance by ASPCR was detected in 9 of 16 children experiencing virologic failure compared to 4 of 17 children without virologic failure (risk ratio 2.4, CI 0.94-7.8, $p=0.08$). Proviral resistance was not associated with virologic failure (risk ratio 1.2, CI 0.8-2.0, $p=0.40$). In the nevirapine-exposed infants, those who started ART before 7 months had higher risk of virologic failure (RR 2.3; CI 0.96-9.2; $p=0.11$).

Conclusions—Low level drug resistance detected in plasma after NVP exposure prior to ART initiation may be associated with virologic failure on ART, while resistance in the DNA reservoir was not predictive of treatment outcome.

Keywords

HIV; minor variant; drug resistance; nevirapine; PMTCT

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Background

Peripartum single-dose (sdNVP), alone or in combination with other antiretroviral medications, is recommended and used globally for the prevention of mother-to-child transmission of HIV-1 (PMTCT) ¹. However, the development of resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) in both the mothers and infected infants after sdNVP exposure raises concerns regarding the effectiveness of future NVP-based antiretroviral treatment (ART) in these individuals.

The generation of resistance with sdNVP is well documented ²⁻⁵, and highly sensitive techniques demonstrate low levels of drug resistance (minor variants) in up to 80% of patients ⁶⁻⁹. Resistance “fades” from detection with increasing time following sdNVP exposure ^{2, 4, 7, 8}. Prior exposure to sdNVP appears to have a negative impact upon the virologic response to subsequent NVP-containing ART among adults who start treatment within 6-12 months of exposure ¹⁰, and perhaps among HIV-infected, sdNVP-exposed children ¹⁰. The pre-treatment presence of minor drug resistant variants has recently been recognized to adversely affect virologic success among adults treated with NVP-containing ART ¹¹, but no data on this topic are available from pediatric populations.

Objectives

To examine the presence and impact (on virologic outcomes) of pre-treatment NVP resistance - using both standard population sequencing and highly sensitive assays in plasma and buffy coat - in HIV-infected infants, with and without prior NVP exposure, who started NVP-based ART in Botswana.

Study Design

Study population

The “Mashi” study (National Clinical Trial 00197587) was designed to compare different chemoprophylaxis and feeding strategies for preventing MTCT of HIV-1 ^{12, 13}. Pregnant, HIV-1-infected women received antenatal zidovudine (AZT) from weeks 34 of gestation through delivery and were randomized to one of two peripartum intervention arms: (1) 200mg sdNVP to the mother during labor with one 6mg dose of NVP administered to the infant within 72 hours of birth, or (2) single-dose placebo to the mother during labor and single-dose placebo to the infant within 72 hours of birth. Both arms were further randomized into two feeding strategies: breast-feeding for 6 months with prophylactic infant AZT for the duration, or formula-feeding with prophylactic infant AZT for one month. Infants were tested for HIV-1 infection at birth and if negative, at multiple time points until 18 months. The study design was adapted in response to data from Thailand ¹⁴, so that all infants born after August 12, 2002 received open-label sdNVP at birth (while mothers continued to be randomized to receive sdNVP). The Botswana Health Research Unit and the Human Subjects’ Committee at the Harvard School of Public Health approved the study..

Procedures

Peripheral blood was obtained from infants at birth, 4 weeks, and then at 4, 7, 9, 12 and 18 months for HIV-1 testing, with a second, confirmatory test among infants with a positive test. HIV acquisition was defined as having occurred *in utero* if the birth PCR was positive; intrapartum (which may also include late *in utero* and early breastfeeding) if the birth PCR was negative but the 4-week PCR was positive; and postpartum (via late breastfeeding) if PCR was first positive at a later date.

Combination ART with NVP, AZT, and lamivudine became available 19 months into the study, and was offered to all HIV-infected infants and to all mothers with CD4 <200 cells/mm³ or AIDS-defining illness. CD4 cell count/percentage and HIV-1 RNA (Roche Amplicor HIV-1 Monitor V1.5, standard) were tested just before treatment initiation and every 3 months on ART. The treating study staff and participants remained blinded to sdNVP vs. placebo receipt. A plasma sample for genotyping was obtained and stored at the treatment initiation visit among children who underwent their confirmatory HIV-1 blood draw at least two months earlier (to minimize phlebotomy), and at the time of virologic failure confirmation (among children with virologic failure).

Virologic End-Points

Virologic failure was defined as confirmed plasma HIV-1 RNA level of at least 400 copies per milliliter at or after the 6-month visit following the initiation of ART.

Plasma RNA and pro-viral DNA genotyping

HIV-1 genotyping of plasma viral RNA was performed using the ViroSeq HIV Genotyping System (version 2.0) following manufacturer's protocol. Genomic DNA was isolated from stored PBMCs using the Qiagen QIAmp DNA Blood mini kit protocol. 100ng of genomic DNA was used in a one-step PCR and sent for sequencing. PCR products generated by ViroSeq, and through amplification of pro-viral DNA, were also analyzed by allele-specific PCR (ASPCR, as described below).

Population sequencing

NNRTI resistance and HIV-1 subtype were determined using the Stanford Genotypic Resistance Interpretation Algorithm (<http://hivdb.stanford.edu>). PCR products from amplified provirus were sequenced and genomic DNA sequences were manually edited and aligned using BioEdit sequence alignment editor (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) to the subtype C consensus sequence from the Los Alamos National Laboratory (<http://www.hiv.lanl.gov>).

ASPCR

An allele-specific PCR assay (ASPCR) was applied to these samples as described previously with respect to the 103 and 181 positions of reverse transcriptase^{15, 16}. The assay uses primers that amplify all four possible nucleotides at the position of interest, and is capable of detecting low level drug resistance at the 103 and 181 positions of reverse transcriptase when only 0.1% of the sample contains resistance. Results using this assay were considered positive if 0.2% or more of the sample contained resistance, a clinically significant threshold seen in our previous work¹⁶ and in a recent published report¹¹.

Statistical methods

Analysis of virologic failure was intent-to-treat. The statistical significance for comparing presence of mutation and treatment outcome was assessed by risk ratios using two-sided Fisher's exact tests. The Mann-Whitney rank sum test was used to compare medians with associations considered statistically significant at $p < 0.05$. All analyses were carried out using the StatXact software (Cytel).

Results

Study population

Of the 92 infants in the Mashhi study who were diagnosed with HIV, 56 started NVP-containing ART during the study period. Pre-treatment samples from 33 of these 56 infants

were available for genotypic analysis. All baseline samples were collected at the same visit as ART initiation or within the preceding 21 days (median=0 days; mean=5 days). Infants with and without baseline samples available shared similar distributions with regards to randomized feeding strategy and sdNVP exposure status. Among the 33 infants with samples available, 16 (48%) were exposed to maternal and infant sdNVP, 8 (24%) were exposed to maternal placebo and infant sdNVP (after the study design was adapted to provide open-label infant sdNVP), and two infants were considered to have had unintended exposure to NVP, outside the study design, through breast milk (as their mothers, in the placebo arm, were taking NVP-containing ART during breastfeeding). Overall, 79% of enrolled infants (26 out of 33) were considered to have had some NVP exposure, with a median age at ART initiation of 6.5 months (IQR: 4.25-9.75).

The clinical characteristics of the 33 infants evaluated in this study are presented in Table 1. There were no significant differences between groups of children with and without prior NVP exposure, in terms of age at HIV diagnosis, age at ART initiation, and CD4 percentage at ART initiation. Near equal proportions of infants in both NVP-exposed and unexposed groups were breastfed (75% vs 76%, respectively). All infants genotyped were infected with HIV-1 subtype C.

A total of 17 (52%) of the 33 infants who started ART maintained adequate virologic response and did not experience virologic failure. All infants with virologic success were followed for at least 24 months. Among the 16 (48%) infants experiencing virologic failure on ART, the median time to failure was 5 months (IQR 3-6 months, range 2-36 months).

Pre-ART resistance mutations in plasma HIV-1 RNA and proviral DNA

ViroSeq detected NNRTI resistance mutations in the plasma of 3 (12%) of 26 NVP-exposed infants before the initiation of ART (Table 2). ViroSeq detected no NNRTI resistance mutations in the 7 infants who had no known NVP exposure.

ASPCR detected NNRTI resistance mutations in the plasma of 12 (46%) of the 26 infants exposed to NVP, and in 1 (14%) of the 7 infants with no known NVP exposure (Tables 2 and 3). ASPCR correlated with ViroSeq population sequencing for both K103N and Y181C mutations; the three samples with ViroSeq resistance had the highest percentages (>19% for a single base pair change conferring resistance) of NNRTI mutations within a sample as tested by ASPCR (Table 3). NNRTI resistance mutations were detectable in the proviral DNA using both bulk sequencing and ASPCR (detectable in 79%) of the infant samples (Table 2).

Virologic Outcome on NVP-containing ART, by Presence of NNRTI Resistance Mutations

All three (NVP-exposed) infants with resistance detectable by ViroSeq failed NVP-containing ART. Detection of resistance by ASPCR in the plasma exhibited a trend toward association with virologic failure: of 16 infants failing first-line ART, 9 (56%) had NNRTI resistance mutations by ASPCR, while only 4 (23%) of the 17 infants maintaining virologic suppression showed NNRTI resistance (RR 2.4; CI: 0.94-7.8; $p=0.08$) (Table 4). Twelve (71%) of the 17 infants without virologic failure had previous exposure to NVP, 4 (33%) of whom had NVP-associated resistance in the viral RNA detected by ASPCR. None of the infants who maintained adequate virologic response and had no known previous exposure to nevirapine had detectable resistance in the plasma using ASPCR.

Only three (19%) of infants who failed and 2 (12%) of those who were successful had detectable proviral resistance by bulk sequencing ($p=0.64$) (Table 4). Detection of resistance by this method was not predictive of virologic failure (RR 1.6; CI: 0.4-8.1; $p=0.64$). NNRTI resistance was found in DNA by ASPCR in 14 (88%) of 16 infants who failed treatment and

12 (71%) of 17 infants who did not fail treatment. Therefore, the finding of resistance by ASPCR in the DNA reservoir was also not predictive of virologic failure (RR 1.2 CI 0.8-2.0; $p=0.40$).

Of the 26 NVP-exposed infants, 16 infants started ART before or at the 7 months visit of whom 11 failed treatment, compared to only 3 of the 10 NVP-exposed infants who started treatment after 7 months of age (RR 2.3; CI 0.96-9.2; $p=0.11$). This is shown graphically in Figure 1.

Post-treatment resistance mutations

Of the 16 infants who experienced virologic failure, samples were available from 11 (69%) from the time of failure. All 11 had detectable NNRTI resistance using ASPCR, with 9 (82%) of those having resistance when tested by ViroSeq (data not shown). The K103N mutation was present in all of the infants who had detectable resistance at virologic failure, with three infants having the additional NRTI mutation M184V.

Maternal factors in development of infant nevirapine resistance

Mothers of infants with NNRTI resistance tended to have a higher enrollment HIV-1 RNA at 34 weeks gestation than mothers without resistance, median viral load of $\log_{10}$ 5.28 copies/mL vs. $\log_{10}$ 4.83 copies/mL ($p=0.02$, Mann-Whitney test) (Figure 2). There was a significant association between maternal enrollment CD4 count and resistance: mothers of infants with NNRTI resistance had a lower median CD4 count (118 cells/ μ L) compared with mothers of infants without resistance (365 cells/ μ L, $p=0.009$, Mann-Whitney test) (Figure 2).

Other possible predictors of virologic outcome and infant nevirapine resistance

Infant HIV-1 RNA data at ART initiation were available for 31 (94%) of the 33 infants. Twenty seven (87%) had a viral load greater than $\log_{10}$ 5.0 RNA copies/mL. There was no association between infant HIV-1 RNA level at treatment initiation and the presence of NNRTI mutations ($p=0.39$; Mann-Whitney test) or likelihood of treatment failure ($p=0.17$; Mann-Whitney test). There was also no association between infant CD4 percentage at the start of treatment and the probability of virologic failure or the presence of NNRTI resistance ($p=1.0$ and $p=0.38$, respectively; Mann-Whitney test).

Discussion

We present evidence that in NVP-exposed children, the detection of pre-treatment NNRTI resistance in plasma HIV-1 by highly sensitive methods, but not by standard bulk sequencing, may correlate with virologic failure on subsequent NVP-containing ART. This finding is similar to a recent study among adults by Coovadia *et al*, which showed that low levels of NVP resistance predicted virologic failure on subsequent NVP-containing ART¹¹. In that study, however, the presence of minor resistant variants was not associated with prior sdNVP exposure, and sdNVP exposure was not in and of itself associated with subsequent virologic failure (and no infants/children were followed). This is in contrast to our findings among children: we found that the presence of NNRTI resistance mutations in plasma using ASPCR was associated with prior infant NVP exposure as 12 of the 13 infants (92%) with resistance had been exposed to nevirapine; and that NNRTI resistance trended toward association with virologic failure (RR 2.4, CI 0.96-9.17, $p=0.08$), although this association failed to reach statistical significance in this small sample set.

The failure to detect resistance by ViroSeq in plasma from 23 infants of the 26 with known exposure to NVP demonstrates the lack of utility of this type of testing pre-ART in the majority of HIV-infected children who were exposed to NVP in the past. The delay in

treatment initiation (average time between NVP exposure and ART initiation was 6.5 months in this cohort) allows resistance to fade as wild-type virus will outgrow the less fit resistant virus. Therefore, the most sensitive methods are more likely to detect resistance at the pre-ART time point.

The analysis of DNA from buffy coat samples (archived reservoir) demonstrated a high degree of resistant virus. However, the presence of resistance in the DNA was distributed fairly evenly between infants who did and did not experience virologic failure, which suggests that DNA analysis of infant samples after sdNVP is not predictive of ART success.

This study also supports previously published results among adults suggesting that individuals whose initiation of NVP-containing ART is delayed post-sdNVP exposure may have a greater chance of virologic success (although among patients with indication for treatment soon after sdNVP exposure, non-NVP-based ART is preferable)^{17, 18}. Failures occurred more frequently in the NVP-exposed infants when treatment was initiated prior to their 7-month visit. The development of resistance in infants was associated with high viral load and low CD4 counts in the mother, but not associated with either viral load or CD4 count in the infant.

This analysis has several limitations. First, the sample size was small, with only 7 infants with no known NVP exposure; we have limited power to explore the effect of minor variants (and of NVP exposure itself) on subsequent ART response. Furthermore, infants were exposed to NVP by several possible mechanisms (maternal/infant sdNVP; infant sdNVP only; and NVP via breast milk alone). Each NVP exposure group is small in number, further limiting our ability to determine if route or degree of NVP exposure had an impact upon virologic treatment outcome in these infants, or if in fact failure with ART initiation before 7 months is multifactorial and related to difficulties administering medications to these infants as well as resistance. Additionally, the ability to check for NVP resistance at a set time point after NVP exposure was not possible, as earlier samples (several months pre-ART) were not available. It would be interesting to perform longitudinal analysis of minor variant resistance as it fades in the plasma after NVP exposure to determine if there is an obvious threshold of percent mutant virus below which clinical success would be predicted. In addition, the children in this study started ART at a relatively young age (6.5 months); these results may not be applicable to children starting ART at an older age (by which time NVP resistance may have declined further), nor to children exposed to NVP alone (without maternal AZT, which may have decreased the rate and amount of infant NVP resistance).

This study demonstrates that resistance after sdNVP exposure is extensive when highly sensitive assays are used to evaluate clinical samples, and likely to be missed if commercial assays are applied pre-ART. The data using highly sensitive methods are also suggestive that minor plasma resistance mutations after sdNVP may have a negative impact on infant virologic treatment outcome on NVP-containing ART. These findings accentuate the importance of implementing MTCT preventions that minimize the development of antiretroviral drug resistance whenever possible; and of making more effective treatment regimens (e.g. protease inhibitor-containing regimens¹⁹) available to NVP-exposed, HIV-1-infected infants globally.

References

1. WHO. Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: towards universal access. Sep 10. 2009 Published Last Modified Date. Accessed Dated Accessed
2. Eshleman SH, Mracna M, Guay LA, Deseyve M, Cunningham S, Mirochnick M, et al. Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). *Aids* 2001;15:1951–1957. [PubMed: 11600822]

3. Eshleman SH, Hoover DR, Chen S, Hudelson SE, Guay LA, Mwatha A, et al. Resistance after single-dose nevirapine prophylaxis emerges in a high proportion of Malawian newborns. *Aids* 2005;19:2167–2169. [PubMed: 16284468]
4. Martinson NA, Morris L, Gray G, Moodley D, Pillay V, Cohen S, et al. Selection and persistence of viral resistance in HIV-infected children after exposure to single-dose nevirapine. *J Acquir Immune Defic Syndr* 2007;44:148–153. [PubMed: 17117145]
5. Jourdain G, Ngo-Giang-Huong N, Le Coeur S, Bowonwatanuwong C, Kantipong P, Leechanachai P, et al. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. *N Engl J Med* 2004;351:229–240. [PubMed: 15247339]
6. Johnson JA, Li JF, Morris L, Martinson N, Gray G, McIntyre J, et al. Emergence of drug-resistant HIV-1 after intrapartum administration of single-dose nevirapine is substantially underestimated. *J Infect Dis* 2005;192:16–23. [PubMed: 15942889]
7. Loubser S, Balfe P, Sherman G, Hammer S, Kuhn L, Morris L. Decay of K103N mutants in cellular DNA and plasma RNA after single-dose nevirapine to reduce mother-to-child HIV transmission. *Aids* 2006;20:995–1002. [PubMed: 16603851]
8. Palmer S, Boltz V, Martinson N, Maldarelli F, Gray G, McIntyre J, et al. Persistence of nevirapine-resistant HIV-1 in women after single-dose nevirapine therapy for prevention of maternal-to-fetal HIV-1 transmission. *Proc Natl Acad Sci U S A* 2006;103:7094–7099. [PubMed: 16641095]
9. Flys T, Nissley DV, Claassen CW, Jones D, Shi C, Guay LA, et al. 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. *J Infect Dis* 2005;192:24–29. [PubMed: 15942890]
10. Lockman S, Shapiro RL, Smeaton LM, Wester C, Thior I, Stevens L, et al. Response to antiretroviral therapy after a single, peripartum dose of nevirapine. *N Engl J Med* 2007;356:135–147. [PubMed: 17215531]
11. Coovadia A, Hunt G, Abrams EJ, Sherman G, Meyers T, Barry G, et al. Persistent Minority K103N Mutations among Women Exposed to Single-Dose Nevirapine and Virologic Response to Nonnucleoside Reverse-Transcriptase Inhibitor-Based Therapy. *Clin Infect Dis*. 2009
12. Shapiro RL, Thior I, Gilbert PB, Lockman S, Wester C, Smeaton LM, et al. Maternal single-dose nevirapine versus placebo as part of an antiretroviral strategy to prevent mother-to-child HIV transmission in Botswana. *Aids* 2006;20:1281–1288. [PubMed: 16816557]
13. Thior I, Lockman S, Smeaton LM, Shapiro RL, Wester C, Heymann SJ, et al. Breastfeeding plus infant zidovudine prophylaxis for 6 months vs formula feeding plus infant zidovudine for 1 month to reduce mother-to-child HIV transmission in Botswana: a randomized trial: the Mashi Study. *JAMA* 2006;296:794–805. [PubMed: 16905785]
14. Lalléant M, Jourdain G, Le Coeur S, Mary JY, Ngo-Giang-Huong N, Koetsawang S, et al. Single-dose perinatal nevirapine plus standard zidovudine to prevent mother-to-child transmission of HIV-1 in Thailand. *N Engl J Med* 2004;351:217–228. [PubMed: 15247338]
15. Rowley CF, Boutwell CL, Lockman S, Essex M. Improvement in allele-specific PCR assay with the use of polymorphism-specific primers for the analysis of minor variant drug resistance in HIV-1 subtype C. *J Virol Methods* 2008;149:69–75. [PubMed: 18295909]
16. Rowley CF, Boutwell CL, Lee E, MacLeod IJ, Ribaudo H, Essex M, et al. Ultrasensitive detection of minor drug resistant variants for HIV after nevirapine exposure using allele-specific PCR: clinical significance. *AIDS Research and Human Retroviruses*. 2009 In Press.
17. Lockman, S.; the A5208/Octane Study Team. Lopinavir/ritonavir+Tenofovir/Emtricitabine Is Superior to Nevirapine+Tenofovir/Emtricitabine for Women with prior Exposure to Single-dose Nevirapine: A5208 (“OCTANE”). Paper presented at: 16th Conference on Retroviruses and Opportunistic Infections; Montreal, Canada. February 8-11, 2009, 8-11 February 2009; (Abstract #94LB)
18. Palumbo, PVA.; Lindsey, J.; Hughes, M.; Jean-Philippe, P.; Mofenson, L.; Purdue, L.; Eshleman, S. IMPAACT P1060 Team Nevirapine (NVP) vs lopinavir-ritonavir (LPV/r)-based antiretroviral therapy (ART) in single dose nevirapine (sdNVP)-exposed HIV-infected infants: preliminary results from the IMPAACT P1060 trial. Paper presented at: 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 19-22 July 2009; (Abstract LBPEB12)

19. Prendergast A, Mphatswe W, Tudor-Williams G, Rakgotho M, Pillay V, Thobakgale C, et al. Early virological suppression with three-class antiretroviral therapy in HIV-infected African infants. *Aids* 2008;22:1333–1343. [PubMed: 18580613]

Figure. 1. NNRTI resistance as detected by plasma ASPCR and treatment outcome in relation to timing ART initiation after NVP exposure

Figure 1 shows that in the group of infants who started treatment before their 7 month visit, more failed treatment with detectable pre-ART resistance. Pre-ART resistance was not detectable in the failure patients to the same degree when ART was started later.

Fig. 2. Antepartum (34 weeks gestation) maternal characteristics among mothers of infants with and without detectable NNRTI resistance mutations

Maternal viral load was increased at 35 weeks' gestation in those infants with NVP resistance ($p=0.02$). Maternal CD4 counts were similarly associated with the development of resistance in infants ($p=0.009$).

Horizontal lines in box plots represent (bottom to top) 10th percentile, 25th percentile, median, 75th percentile, and 90th percentile

Table 1

Clinical characteristics of 33 HIV-infected infants who initiated anti-retroviral treatment.

Characteristic	NVP-exposed infants (n=26)	NVP-unexposed infants (n=7)	p	Overall study population (n=33)
Time from birth to HIV diagnosis, median months (IQR)	0.5 (0-4)	4 (0-9.5)	0.25	1 (0-4)
In utero MTCT	12	4		16
Intrapartum	3	1		4
Late breast-feeding MTCT	11	2		13
Time to ART initiation from HIV diagnosis, median months (IQR)	4 (3-8.75)	6 (4-9.5)	0.50	4 (3-9)
HIV RNA levels at ART initiation, median log ₁₀ copies/ml (IQR)	5.88 (5.54-5.88)	5.68 (5.04-5.88)	0.51	5.88 (5.45-5.88)
CD4 percentage at ART initiation	23 (16-33)	22 (11-23)	0.23	22.50 (14.5-31.5)
Breastfed	20 (77)	5 (71)		25 (75)
Formula-fed	6 (23)	2 (29)		8 (25)
Exposure				
Enrolled in sdNVP arm	16 (62)	-	-	
Born after 12 Aug 2002	8 (31)	-	-	
Other ^a	2 (7)	-	-	
Infants that failed ART	14 (54)	2 (29)	0.40	16 (48)
Time from NVP exposure to HAART initiation, median months (IQR)	6.5 (4.25-9.75)	-	-	

NOTE: Data are no. (%) of subjects, unless otherwise indicated. NVP, nevirapine; N/A, not applicable.^aFirst positive polymerase chain reaction (PCR) result at birth.^bFirst positive polymerase chain reaction (PCR) result at one month.^cFirst positive polymerase chain reaction (PCR) result after one month.^dTwo infants had potential exposure to NVP via maternal breast milk as the mother had initiated a NVP-containing ART regime during the breastfeeding stage.

Table 2
Pre-ART resistance mutations in RNA and DNA as detected by K103N and Y181C
ASPCR among nevirapine-exposed and nevirapine-unexposed infants

ViroSeq and bulk sequencing only detected resistance in 9% and 15% of the 33 samples tested. ASPCR results are subdivided into whether these mutations were associated with clinical failure or not as ASPCR detected resistance in 39% of the plasma samples and in 79% of the proviral DNA samples.

	NVP exposure (n=26)	No NVP exposure (n=7)	Total (n=33)
Plasma RNA			
ViroSeq			
K103N	1	0	1
Y181C	0	0	0
K103N / Y181C	1	0	1
K103N / G190A	1	0	1
ASPCR			
Virologic failure on ART	14	2	16
No NNRTI resistance mutation	6	1	7
K103N	1	0	1
Y181C	4	0	4
K103N and Y181C	3	1	4
No virologic failure on ART	12	5	17
No NNRTI resistance mutation	8	5	13
K103N	1	0	1
Y181C	2	0	2
K103N and Y181C	1	0	1
Proviral DNA			
Bulk Sequencing			
K103N	4	0	4
Y181C	0	0	0
K103N / Y181C	1	0	1
ASPCR			
Virologic failure on ART	14	2	16
No NNRTI resistance mutation	2	0	2
K103N	10	2	12
Y181C	1	0	1
K103N and Y181C	1	0	1
No virologic failure on ART	12	5	17
No NNRTI resistance mutation	2	3	5
K103N	9	2	11
Y181C	0	0	0
K103N and Y181C	1	0	1

* Denotes samples that tested positive by ViroSeq.

** Sample not positive by ViroSeq. At 103 position, this sample had 17.6% nt and 18.3% ntAAT. Both confer resistance but not detected by ViroSeq individually.

Table 3

Pre-ART resistance mutations in RNA and DNA as detected by K103N and Y181C ASPCR among nevirapine-exposed and nevirapine-unexposed infants.

	RNA - ASPCR Result (%)		DNA - ASPCR Result (%)	
	103N	181C	103N	181C
NVP-exposed infants				
Virologic failure	<0.2	<0.2	1.57	<0.2
	<0.2	0.36	0.85	<0.2
	<0.2	<0.2	0.49	<0.2
	19.21 [*]	10.73	44.7	27.28
	<0.2	<0.2	<0.2	<0.2
	<0.2	0.63	<0.2	1.17
	98.61 [*]	<0.2	0.8	<0.2
	<0.2	0.26	0.22	<0.2
	<0.2	<0.2	29.58	<0.2
	<0.2	<0.2	0.71	<0.2
	<0.2	0.27	1.04	<0.2
	<0.2	<0.2	<0.2	<0.2
	49.04 [*]	45.13	0.52	<0.2
	35.87 ^{**}	0.53	34.97	<0.2
	1.42	<0.2	2.46	<0.2
Viral suppression	<0.2	<0.2	<0.2	<0.2
	<0.2	<0.2	14.01	0.53
	<0.2	<0.2	<0.2	<0.2
	<0.2	<0.2	2.78	<0.2
	<0.2	<0.2	13.52	<0.2
	<0.2	<0.2	1.47	<0.2
	<0.2	0.46	1.99	6.04
	<0.2	<0.2	2.48	<0.2
	<0.2	<0.2	28.87	<0.2
	22.17	1.27	33.22	<0.2
NVP-unexposed infants	<0.2	0.34	3.60	<0.2
	<0.2	<0.2	1.58	<0.2
	4.35	0.81	4.34	<0.2
	<0.2	<0.2	<0.2	<0.2
	<0.2	<0.2	<0.2	<0.2
	<0.2	<0.2	<0.2	<0.2
	<0.2	<0.2	0.73	<0.2
	<0.2	<0.2	5.88	<0.2
	<0.2	<0.2	<0.2	<0.2
	<0.2	<0.2	<0.2	<0.2

* Denotes samples that tested positive by ViroSeq.

** Sample not positive by ViroSeq. At 103 position, this sample had 17.6% ntAAC and 18.3% ntAAT. Both confer resistance but not detected by ViroSeq individually.

Risk of virologic failure based on pre-ART NVP resistance detection in both RNA and DNA of the 33 infants who commenced NVP-containing ART.

Table 4

	Virologic failure (n=16)	No virologic failure (n=17)	Relative Risk	p	CI
Plasma (ViroSeq)	3	0	-	-	-
Plasma (ASPCR)	9	4	2.4	0.08	0.96-9.2
DNA (bulk sequencing)	3	2	1.6	0.64	0-8.1
DNA (ASPCR)	14	12	1.2	0.40	0.8-2.0