



Predictors of nevirapine resistance in Ugandan infants who were HIV-infected despite single dose nevirapine prophylaxis

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INTRODUCTION

Single-dose nevirapine (sdNVP) is used to prevent mother-to-child transmission of HIV (MTCT) in resource-limited settings. Unfortunately, NVP resistance emerges in many infants who are HIV-infected despite sdNVP prophylaxis. In women, emergence of NVP resistance after sdNVP has been associated with high viral load, low CD4 cell count, HIV subtype (C>D>A), and increased pharmacokinetic NVP exposure. In previous studies, emergence of NVP resistance in infants was associated with high maternal viral load and HIV subtype (C > A and D combined).

Analysis of factors associated with NVP resistance in sdNVP-exposed infants is often limited by the small number of infants who are HIV-infected in a single study. In this report, we pooled data from four clinical studies conducted in Kampala, Uganda to analyze emergence and persistence of NVP resistance in sdNVP-exposed infants who were HIV-infected by 6-8 weeks of age.

METHODS

In the four studies analyzed, 109 infants who received sdNVP were diagnosed with HIV infection by 6-8 weeks of age. Eighty-two of the 109 infants had a plasma sample available for resistance studies. HIV genotyping was performed with the ViroSeq HIV Genotyping System v2.6 and HIV subtypes were determined by phylogenetic analysis of HIV *pol* sequences.

Independent sample chi-square tests and Fisher's exact tests were used to evaluate the association of NVP resistance at 6-8 weeks with *in utero* HIV infection (diagnosis of HIV infection at birth), infant viral load at 6-8 weeks, infant gender, HIV subtype, pre-NVP maternal viral load and CD4 cell count. Odds ratio (OR) estimates and 95% confidence intervals (CI) for these variables were obtained using logistic regression.

RESULTS

ViroSeq results were obtained for 80 (97.5%) of the 82 infants who had plasma samples available from 6-8 weeks of age. Thirty-six (45.0%) of the 80 infants had at least one NVP resistance mutation detected (Figure); the mutations identified were Y181C (n=28), K103N (n=9), Y188C (n=3), G190A (n=3), V106A (n=2), V106M (n=2), and K101E (n=1); 10 infants had two or more NVP resistance mutations detected. The mean pre-NVP maternal \log_{10} viral load and mean pre-NVP maternal CD4 cell count were similar for the 80 women whose infants had ViroSeq resistance results vs. the 29 women whose infants did not (p=0.45 and p=0.96).

For samples with subtype A or D HIV, PCR products produced in the ViroSeq system were also tested using the LigAmp assay (assay cutoffs for mutation detection: 0.5% for K103N, 1.0% for Y181C, 0.5% for G190A). LigAmp results were obtained for 72 (90%) of the 80 infants who had ViroSeq results (Figure). The proportion of infants who had K103N, Y181C, or G190A detected by LigAmp (33/72=45.8%) was similar to the proportion of infants who had resistance detected by ViroSeq (36/80=45.0%, p=0.563). The two assays detected Y181C in a similar proportion of infants (LigAmp: 40.3%, ViroSeq: 35.0%, p=0.157). In contrast, LigAmp detected K103N and G190A in a higher proportion of infants than ViroSeq (K103N: 23.6% vs. 11.3%, p=0.021; G190A: 20.8% vs. 3.8%, p=0.0003).

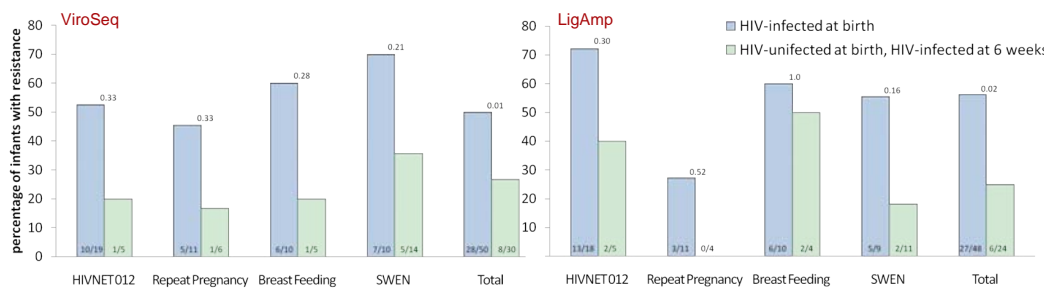


Figure. Detection of NVP resistance by ViroSeq and LigAmp at 6-8 weeks among infants who were HIV-infected *in utero* vs. infants who were diagnosed with HIV infection at 6 weeks. Four studies, Uganda.

Infants were enrolled in four studies; dates of enrollment are shown: HIVNET 012 (1997-1999, sdNVP arm only) ¹, the Repeat Pregnancy study (2004-2006, prospective portion only) ², the Pathophysiology of Breast Milk study (2003-2004), and the Ugandan component of the SWEN study (2004-2006, sdNVP arm only) ³. P-values for comparison of proportions are shown above the bars. Resistance detected by ViroSeq indicates detection of one or more NVP resistance mutation (A98G, L100I, K101E/P, K103N/S, V106A/M, Y181C//V, Y188C/H/L, G190A/S/C/E/Q/T/V, M230L, K103R+V179D). LigAmp testing was performed to detect K103N, Y181C, and G190A; resistance detected by LigAmp indicates detection of one or more of these three mutations.

Predictor Variable	Resistance detected by ViroSeq			Resistance detected by LigAmp		
	N	Odds Ratio (95% CI)	P value	N	Odds Ratio (95% CI)	P value
Maternal pre-NVP viral load (per \log_{10} increase in HIV RNA)	71	1.06 (0.5 - 2.3)	0.89	64	0.98 (0.4 - 2.2)	0.95
Maternal pre-NVP CD4 cell count (per decrease of 100 cells/ul)	77	1.15 (0.9 - 1.4)	0.20	69	1.15 (0.9 - 1.4)	0.21
Infant viral load at 6-8 weeks (per \log_{10} increase in HIV RNA) ^a	41	1.65 (0.7 - 4.1)	0.28	37	1.49 (0.6 - 4.7)	0.21
HIV subtype (D vs. A) ^b	65	1.51 (0.6 - 4.2)	0.42	62	1.15 (0.4 - 3.2)	0.79
HIV subtype (D vs. non-D) ^b	80	1.70 (0.7 - 4.4)	0.28	72	1.45 (0.5 - 3.9)	0.46
Infant gender (male vs. female)	80	0.50 (0.2 - 1.2)	0.13	72	0.65 (0.3-1.7)	0.37
Diagnosed with HIV infection at birth (yes/no)	80	3.50 (1.3 - 9.4)	0.013	72	3.90 (1.3 - 11.4)	0.015

Table. Analysis of factors associated with detection of NVP resistance in infants at 6-8 weeks of age^a. Four studies, Uganda.

^a Univariate logistic regression models were used for analysis.

^b Viral load testing was performed at 6-8 weeks for infants in HIVNET 012, the Breast Feeding study, and the SWEN study; viral load testing was not performed at 6-8 weeks for infants in the Repeat Pregnancy study.

^c Among the 80 infants who had HIV subtype data, 41 had subtype A, 4 had subtype C, 24 had subtype D, and 11 had inter-subtype recombinant HIV.

A higher proportion of infants who were infected *in utero* had resistance at 6-8 weeks, compared to infants who were diagnosed with HIV infection after birth by 6-8 weeks of age (OR=3.5 [95% CI: 1.3-9.4], p=0.01 for ViroSeq; OR=3.9 [95% CI: 1.3-11.4], p=0.01 for LigAmp) (Table). We did not see an association of NVP resistance with maternal pre-NVP viral load or pre-NVP CD4 cell count, infant viral load at 6-8 weeks, HIV subtype (for A vs. D or D vs. non-D), or infant gender (Table).

Overall, 43 infants who were diagnosed with HIV infection by 6-8 weeks of age had NVP resistance detected by ViroSeq and/or LigAmp at 6-8 weeks. Thirty-four of those infants had a plasma sample collected at either 6 months of age (in the Repeat Pregnancy study, Breast Feeding study, and the SWEN study) or at 12 months of age (in HIVNET 012). We analyzed persistence of NVP resistance in 27 of the 34 infants (19 infants at 6 months, and eight additional infants at 12 months); seven infants were excluded from this analysis because they were started on ARV therapy before the 6-month study visit. At 6 months, NVP resistance was detected by either ViroSeq or LigAmp in 12 (63.2%) of 19 infants tested. Eight infants had mutations detected by ViroSeq [Y181C (n=4), K103N (n=1), V106M (n=1), Y188C (n=1), and V179D+K103R (n=1)], and four infants had resistance detected by LigAmp only (all with Y181C, at 1.2%, 1.4%, 3.5%, and 7.8%, one infant also had G190A at 1.9%). At 12 months, NVP resistance was detected in four (50%) of eight infants tested. Two (25%) infants had resistance detected by both ViroSeq and LigAmp. Most of the infants who had resistance detected at 6 or 12 months had Y181C.

CONCLUSIONS

NVP resistance was detected in 45-46% of HIV-infected infants 6-8 weeks after sdNVP. NVP resistance was associated with *in utero* HIV infection, but not with HIV subtype, maternal or infant viral load, maternal CD4 cell count, or infant gender. Among infants who had NVP resistance at 6-8 weeks of age, NVP resistance was still detected in 16 (59.3%) of 27 infants at 6-12 months. Further studies are needed to evaluate the relationship between the timing of HIV MTCT, the emergence and persistence of NVP resistance, and ARV treatment response.

REFERENCES

- Guay LA, Musoke P, Fleming T, et al. Lancet 1999;354:795-802.
- McConnell M, Bakaki B, Eure C, et al. J Acquir Immune Defic Syndr 2007;291-296.
- Six Week Extended-Dose Nevirapine (SWEN) Study Team, Bedri A, Gudetta B, et al. Lancet 2008;372:300-313.