

# Analysis of NVP Resistance in Ugandan Infants who were HIV-infected Despite Receiving Single Dose (SD) Nevirapine (NVP) vs. SD NVP Plus up to 6-weeks of Daily NVP to Prevent HIV Vertical Transmission



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## INTRODUCTION

The SWEN Study in Uganda, Ethiopia, and India compared a regimen of SD NVP (the HIVNET 012 regimen) to a regimen of SD NVP plus up to 6 weeks of daily NVP (extended NVP arm) to the infant for prevention of HIV transmission in breastfeeding infants [1]. We analyzed HIV genotypic resistance, phenotypic resistance, and HIV replication capacity in Ugandan infants in this study.

## METHODS

Plasma samples were available from 49 (71%) of 69 infants diagnosed with HIV infection by 6 weeks of age (24 in the SD NVP arm; 25 in the extended NVP arm). In the extended NVP arm, the median number of NVP doses received was 14 (range 3-33) in infants diagnosed with HIV infection at birth (n=17), 14 (range 7-26) in infants diagnosed at 2 weeks of age (n=5), and 34 (range 21-35) in infants diagnosed with HIV infection by 6 weeks of age (n=3). Samples were analyzed using the ViroSeq HIV Genotyping system, a quantitative point mutation assay (LigAmp, for K103N, Y181C, and G190A), and the PhenoSense GT assay (Monogram Biosciences).

## RESULTS

Clinical and laboratory variables listed in Table 1 were similar among infants included in this study and infants who had no sample available for analysis. Among the infants with results, clinical and laboratory variables were similar for infants in the two study arms, except for time of HIV diagnosis (Table 1).

### Analysis of NVP Resistance using the ViroSeq System

HIV genotyping results were obtained using the ViroSeq system for all 49 infants who had 6-week samples. We compared the resistance results for infants in the two study arms (Table 2). NVP resistance was detected at 6 weeks in a higher portion of infants in the extended NVP arm compared to the SD NVP arm (21/25=84% vs. 12/24=50%, p=0.01). The higher risk of NVP resistance among infants in the extended NVP arm was only seen among those infants who were diagnosed with HIV infection at 2 or 6 weeks of age, (7/8=89% in the extended NVP arm vs. 5/14=36% in the SD NVP arm, p=0.03). In contrast, among infants who were HIV-infected at birth, the proportion of infants with NVP resistance was similar in the two study arms (14/17=82.3% in the extended NVP arm vs. 7/10=70% in the SD NVP arm, p=0.39). Detection of NVP resistance mutations was not associated with subtype A vs. D (p=1.00).

	SD NVP (n=24)	Extended NVP (n=25)	P value
Median maternal log <sub>10</sub> viral load at enrollment	5.0	5.1	0.53 <sup>a</sup>
Median maternal CD4 cell count at enrollment	299	392	0.53 <sup>a</sup>
Time of diagnosis of HIV infection			
Birth	10 (41.7%)	17 (68%)	0.088 <sup>b</sup>
2 weeks	2 (8.3%)	5 (20%)	0.417 <sup>b</sup>
6 weeks	12 (50%)	3 (12%)	<b>0.005<sup>b</sup></b>
Median number of NVP doses received (range) <sup>c</sup>	--	14 (3-35)	
Median first infant log <sub>10</sub> viral load after diagnosis of HIV infection	5.6	5.3	0.19
HIV subtype			
A	12 (50%)	13 (52%)	0.56 <sup>b</sup>
C	1 (4.2%)	0 (0%)	0.49 <sup>b</sup>
D	8 (33.3%)	5 (20%)	0.23 <sup>b</sup>
Recombinant	3 (12.5%)	7 (28.0%)	

**Table 1. Characteristics of infants in this sub-study**

<sup>a</sup> Mann-Whitney rank sum test; <sup>b</sup> Fisher's exact test; <sup>c</sup> median number of NVP doses for infants in the extended NVP arm only.

In the extended NVP arm, detection of NVP resistance was not associated with the number of NVP doses received (median of 14 doses among infants with resistance vs. 17.5 doses among infants without resistance, p=0.85).

	n	SD NVP	Extended NVP	p
<b>VIROSEQ RESULTS</b>				
≥ 1 NVP resistance mutation	49	24	25	
≥ 2 NVP resistance mutations	12	4 (16.7%)	8 (32%)	0.18 <sup>a</sup>
<b>LIGAMP RESULTS<sup>c</sup></b>				
≥ 1 NVP resistance mutation	44	20	24	
# (%) infants with ≥ 0.5% K103N	26	7 (35%)	19 (79%)	<b>0.004<sup>a</sup></b>
Median % K103N	13	3 (15%)	10 (41.7%)	<b>0.05<sup>a</sup></b>
# (%) infants with ≥ 1% Y181C	4.7	4.2	5.2	0.50 <sup>b</sup>
Median %Y181C	22	7 (35%)	15 (62.5%)	0.06 <sup>a</sup>
# (%) infants with ≥ 0.5% G190A	15.6	10.8	20.5	0.57 <sup>b</sup>
Median % G190A	9	3 (15%)	6 (25%)	0.33 <sup>a</sup>
	3.2	1.6	5.6	0.20 <sup>b</sup>

**Table 2. ViroSeq and LigAmp results for infants in the SD NVP and extended NVP arms at 6 weeks of age**

<sup>a</sup> Fisher's exact test; <sup>b</sup> Mann-Whitney rank sum test; <sup>c</sup> LigAmp results over 100% were adjusted to 100%. Median values were calculated only for samples with the mutation detected.

### Analysis of K103N, Y181C, and G190A using the LigAmp assay

We used a sensitive point mutation assay, LigAmp, to detect and quantify HIV variants with the K103N, Y181C, and G190A NVP resistance mutations. Forty-four (89.7%) of the 49 6-week samples that had ViroSeq results were tested with LigAmp (Table 2).

We compared the portion of infants who had Y181C, K103N, or G190A detected by LigAmp in the two study arms. A higher proportion of infants in the extended NVP arm had one or more of these mutations detected, compared to infants in the SD NVP arm (19/24=79% vs. 7/20=35%, p=0.004). Among infants with resistance detected by LigAmp, there was no difference in the type or level of mutations between arms. In the extended NVP arm, detection of any NVP resistance mutation (K103N, Y181C, or G190A) was not associated with the number of NVP doses received (median of 14 doses among those with at least one mutation detected vs. 21 doses among those with no mutations detected, p=0.14).

### Analysis of NVP Resistance in Infants at 6 Months of Age

We used the ViroSeq system and the LigAmp assay to analyze the persistence of NVP resistance at 6 months of age. A 6-month sample was tested if the infant tested positive for resistance at 6 weeks, did not receive antiretroviral therapy, and had an available sample. Results were obtained for six infants in the SD NVP arm and seven infants in the extended NVP arm. Among the six infants in the SD NVP arm who were tested at 6 months of age, only one had a NVP resistance mutation detected by ViroSeq (Y181C), and only two had a NVP resistance mutation detected by LigAmp only (both with Y181C, at 1.4% and 3.5%). In contrast, all of the seven infants in the extended NVP arm who were tested at 6 months of age had one or more NVP resistance mutation detected by ViroSeq.

### Analysis of Non-nucleoside Reverse Transcriptase (NNRTI) Resistance using a Phenotypic Assay

The 49 6-week samples that had ViroSeq results were also tested for phenotypic drug resistance using a commercial assay (PhenoSense, Monogram Biosciences). Results were obtained for 42 (85.7%) of 49 available samples. Twenty-eight (66.7%) of the 42 infants had decreased susceptibility to NVP, with a fold change in IC50 above the assay cut-off of 4.5.

The portion of infants with phenotypic NVP resistance was higher for infants in the extended NVP arm, than for infants in the SD NVP arm (19/22=86.3% of infants vs. 9/20=45% of infants, respectively, p=0.005). When data from infants in the two study arms were combined, the fold change in IC50 for NVP was higher among infants with two or more mutations detected by ViroSeq, than among infants who had only one mutation detected (155.5 vs. 77, p=0.02). The median HIV replication capacity was similar for infants in the two study arms.

## CONCLUSIONS

Ugandan infants who had were HIV infected despite extended NVP prophylaxis were more likely to have genotypic and phenotypic NVP resistance than those who received only SD NVP. Preliminary evidence suggests that NVP resistance mutations may be more likely to persist in infants who received extended NVP prophylaxis compared to infants who received SD NVP.

## REFERENCES

1. The Six Week Extended Dose Nevirapine (SWEN) Study Team. Extended dose nevirapine to six weeks of age for infants in Ethiopia, India, and Uganda: a randomized trial for prevention of HIV transmission through breastfeeding. In: 15th Conf. on Retroviruses and Opportunistic Infections, Boston, MA. 2008: Abstract # S-195.