

# 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 single dose NVP: HIVNET 012

S Eshleman<sup>1\*</sup>, D Nissley<sup>2,3</sup>, C Claasen<sup>3</sup>, D Jones<sup>1</sup>, C Shi<sup>1</sup>, L Guay<sup>1</sup>, P Musoke<sup>4</sup>, F Mmimo<sup>4</sup>, J Strathern<sup>3</sup>, J Jackson<sup>1</sup>, J Eshleman<sup>1</sup>, and T Flys<sup>1</sup>

<sup>1</sup>The Johns Hopkins Medical Institutions, Baltimore, MD, USA, <sup>2</sup>Basic Research Program, SAIC-Frederick, <sup>3</sup>National Cancer Institute-Frederick, Frederick MD, USA, <sup>4</sup>Makerere Univ, Kampala, Uganda

## ABSTRACT

**Background:** The HIVNET 012 trial showed that single dose (SD) nevirapine (NVP) can prevent HIV-1 mother-to-child transmission. However, NVP-resistant variants are selected in some women and infants who receive this regimen. We tested whether variants with the K103N mutation could persist at low levels in women and infants for a year or more after SD NVP exposure using a sensitive point-mutation assay.

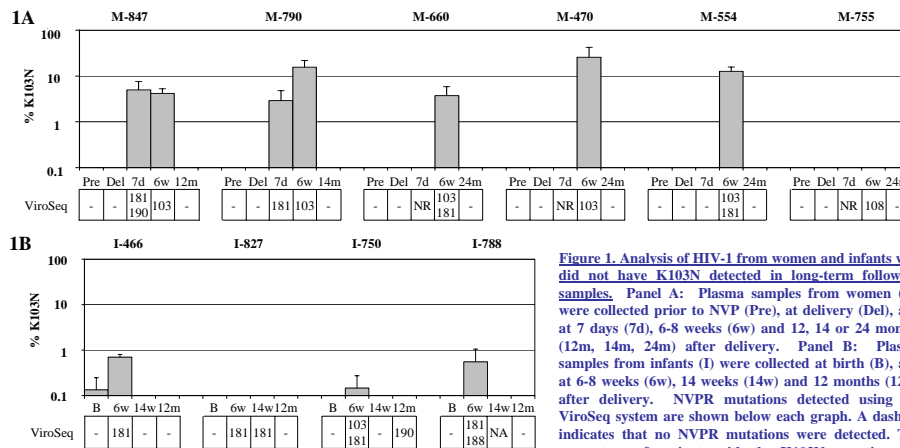
**Methods:** Plasma collected before and up to 12-24 months after SD NVP was available from nine women and five infants in the HIVNET 012 cohort. Samples were genotyped using the ViroSeq system, and the level of K103N-containing variants was quantified using a sensitive resistance assay, LigAmp, which has a lower limit of detection of 0.08%. In the LigAmp assay, a mutation-specific ligation step is followed by a universal real-time PCR detection step. Selected samples were also analyzed with a yeast-based, phenotypic resistance assay, TyHRT. Introduction of HIV-1 reverse transcriptase (RT) domains into hybrid Ty1/HIV retrotransposons *in vivo* independent of conventional cloning facilitates the assembly of large RT libraries. RT isolates are screened in the presence and absence of NVP to provide a measure of phenotypic NVP susceptibility. The HIV-1 RT region from selected NVP-resistant colonies is sequenced to confirm the presence of NVP resistance mutations.

**Results:** At 6-8 weeks after NVP, K103N was detected by ViroSeq in 8/9 women and 2/5 infants, and was detected by LigAmp at a level above 0.1% in 8/9 women (mean=14%) and 4/5 infants (mean=12%). The two infants who had the mutation detected by LigAmp only had levels of 0.6% and 0.7%. At 12-24 months, K103N was not detected by ViroSeq in any of the samples, but was detected by LigAmp above pre-NVP levels in 3/9 women (at 0.8%, 1.3%, and 3.5%) and 1/5 infants (at 1.5%). Persistence of K103N in the 12-24 month samples was confirmed using the TyHRT assay.

**Conclusions:** K103N-containing HIV-1 variants can persist in some women and infants for a year or more after SD NVP. Assays that can quantify HIV-1 variants at low levels may provide new insight into the impact of antiretroviral drug exposure on HIV-1 evolution. Further studies are needed to determine the clinical significance of minority variants with NVP resistance mutations.

## INTRODUCTION

Approximately 800,000 children are infected with HIV-1 world-wide, most in resource-poor settings that have limited access to antiretroviral drugs for treatment and prevention of HIV-1 infection. Short regimens of antiretroviral prophylaxis can reduce the risk of HIV-1 mother-to-child transmission. The HIVNET 012 regimen, which consists of a single dose of nevirapine (NVP) to women in labor and a single dose of NVP to infants shortly after birth, is simple, safe, inexpensive and effective for prevention of mother-to-child transmission (pMTCT). A potential disadvantage of the HIVNET 012 regimen is the emergence of NVP resistance (NVPR) in some women and infants after NVP administration. Selection of NVPR in this setting is concerning, since it could potentially reduce the efficacy of NVP-containing regimens for pMTCT in subsequent pregnancies, or the efficacy of non-nucleoside reverse transcriptase (RT) inhibitor (NNRTI)-containing regimens for future treatment of HIV-1 infection in women and infants who received NVP prophylaxis. We used the LigAmp assay to evaluate how long and at what levels NVP-resistant HIV-1 variants persist in women and infants after single dose NVP (SD NVP).



## METHODS

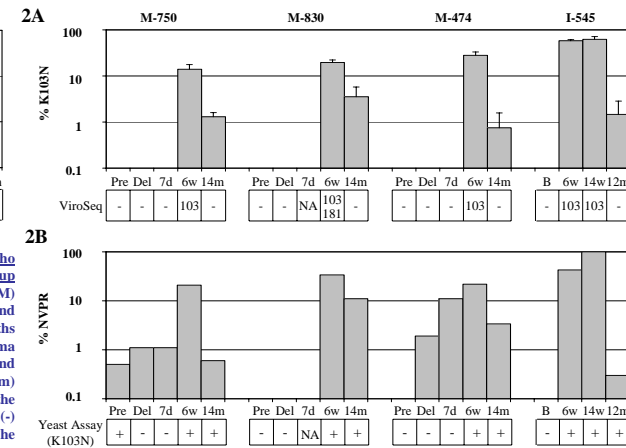
**Samples used for analysis:** Plasma samples were obtained from nine women and five infants enrolled in HIVNET 012 who had NVPR mutations detected using the ViroSeq assay 6-8 weeks after SD NVP and who also had pre-NVP samples and follow-up samples (collected 12-24 months after NVP administration) available for analysis. The samples were genotyped using the FDA-cleared ViroSeq™ HIV-1 Genotyping System. *Pol* region subtypes were determined by phylogenetic analysis.

**Detection of the K103N mutation using the LigAmp assay:** For analysis of plasma HIV-1, we used PCR products produced in the ViroSeq system. PCR products from each sample were analyzed using LigAmp in three independent experiments. For each experiment, a standard curve (e.g. control dilution curve derived from one woman with the same HIV-1 subtype) was included as an internal control and was used to quantify the percentage of K103N in each sample. For details on the LigAmp assay, refer to poster #723.

**Yeast TyHRT assay:** Samples with K103N detected by LigAmp a year or more after SD NVP were also analyzed with a yeast-based, phenotypic resistance assay, TyHRT. HIV-1 RT domains were introduced into hybrid Ty1/HIV retrotransposons *in vivo* to assemble large RT libraries. RT isolates were screened in the presence and absence of NVP to measure phenotypic NVP susceptibility. The RT region from selected NVP-resistant colonies was sequenced to confirm the presence of NVPR mutations.

## RESULTS

**Analysis of K103N in maternal samples:** In most women, the % K103N in samples collected prior to NVP exposure or at the time of delivery was less than 0.1%. Selection of the K103N mutation was evident in 8/9 women at 7 days and/or 6-8 weeks after NVP. In the 6-8 week samples, the mean % K103N among the eight women was 13.9% (Figures 1A and 2A). In six women, the K103N mutation faded below 0.1% by 12-24 months after delivery (Figure 1A). However, in three women, K103N was detected above the pre-NVP level (at 0.8%, 1.3%, and 3.5%) a year or more after NVP administration (Figure 2A).



**Figure 1. Analysis of HIV-1 from women and infants who did not have K103N detected in long-term follow-up samples.** Panel A: Plasma samples from women (M) were collected prior to NVP (Pre), at delivery (Del), and at 7 days (7d), 6-8 weeks (6w) and 12, 14 or 24 months (12m, 14m, 24m) after delivery. Panel B: Plasma samples from infants (I) were collected at birth (B), and at 6 weeks (6w), 14 weeks (14w) and 12 months (12m) after delivery. NVPR mutations detected using the ViroSeq system are shown below each graph. A dash (-) indicates that no NVPR mutations were detected. The percentage of variants with the K103N mutation was determined in triplicate for each sample using the LigAmp assay (% K103N). The mean and standard deviation from the three experiments are shown. In the 6-week sample from infant 750, ViroSeq detected the K103N mutation encoded by AAT not by AAC, which was probed in the LigAmp assay.

**Analysis of K103N in infant samples:** At birth, the % K103N was less than 0.1% in two of the infants. In the other three infants, K103N was detected at a low level in at least one of the triplicate runs. At 6-8 weeks, the K103N mutation was detected above 0.1% in 4/5 infants. The level of K103N remained high in one infant at the 14-16 week visit. In two infants, the mutation faded below 0.1% by 14-16 weeks. The fourth infant who had K103N detected at 6-8 weeks did not have a 14-16 week sample (Figure 1B). In the infant who had high levels of K103N at the 6-8 and 14-16 week visits (I-545), K103N was detected at a level of 1.5% a year after SD NVP (Figure 2A).

**Comparison of results from the LigAmp and TyHRT assays:** Results from the LigAmp assay (% K103N) and the TyHRT assay (% NVPR) are shown in Figure 2B. Results from the two assays were consistent. Clones with phenotypic NVPR were isolated in the TyHRT assay from all of the samples that had K103N detected by the LigAmp assay. K103N was detected among the resistant clones in all of those samples. In five samples, phenotypic NVPR was detected in the TyHRT assay, but K103N was not detected in the LigAmp assay. NVP-resistant clones from those samples (2-8 clones per sample) isolated in the TyHRT assay did not have the K103N mutation (AAC).

In the four long-term follow-up samples, K103N was detected by the LigAmp assay at 1.3%, 3.5%, 0.8%, and 1.5% (M-750, M-830, and M-474 and I-545, respectively). The % NVPR detected in the TyHRT assay was 0.6%, 11%, 3.4%, and 0.3% for those samples, reflecting isolation of 2, 33, 7, and 1 NVP-resistant clones, some or all of which had the K103N mutation. Even though relatively few NVP-resistant clones were isolated, K103N was detected among the NVP-resistant clones from all four samples.

In contrast, long-term follow-up samples from women and infants who did not have K103N detected by the LigAmp assay (Figure 1), had relatively few NVP-resistant clones isolated in the TyHRT assay, and none of the clones that were sequenced had K103N (data not shown).

## CONCLUSIONS

Analysis of plasma HIV-1 using the LigAmp assay reveals persistence of the K103N mutation above pre-NVP levels in 3/9 women and 1/5 infants a year or more after SD NVP administration. The ability of HIV-1 variants with K103N to persist for a year or more after SD NVP is consistent with data from *in vitro* studies that demonstrate that the K103N mutation confers a relatively small fitness cost. Variants with the K103N mutation have also been shown to persist for years in the absence of antiretroviral exposure in some patients who are infected with resistant strains. Further studies are needed to determine whether persistence of NVPR mutations at low levels after SD NVP prophylaxis compromises efficacy of NNRTI-containing regimens for HIV-1 treatment or pMTCT in subsequent pregnancies, especially years after NVP exposure.