



# Clonal Analysis of Drug Resistant Mutations in Plasma and Breast-Milk Following Single Dose Nevirapine

E Lee<sup>1\*</sup>, R Kantor<sup>1</sup>, E Johnston<sup>1</sup>, S Kassaye<sup>1</sup>, L Zijenah<sup>2</sup>, D Katzenstein<sup>1N</sup>

<sup>1</sup>Stanford University, Stanford, CA, <sup>2</sup>University of Zimbabwe, Harare, Zimbabwe

Esther J. Lee, Seble Kassaye  
300 Pasteur Drive, S-146  
Stanford, CA 94306  
estherL@gmail.com  
skassaye@stanford.edu  
650-723-8291 (work)



## INTRODUCTION

Single-dose nevirapine (SD-NVP) reduces mother-to-child transmission (MTCT) of HIV-1, but selects for drug resistance mutations (DRM) in the reverse transcriptase (RT) of Plasma and Breast-milk (BM) HIV-1 RNA. NNRTI drug resistance is identified by population based sequencing, however, it is unknown if this method gives an accurate picture of the individual subtype C HIV-1 viral genomes post SD NVP. Population sequencing, sensitive to DRM at >20% may underestimate their dynamics after SD NVP.

The objective of this study was to determine the relationships between population-based and clonal sequences of plasma and BM HIV-1 RNA shedding among women receiving SD NVP in the HPTN023 clinical trial.

## METHODS

Women who were part of the HPTN 023 study from Chitungwiza, Zimbabwe received SD NVP at labor. HIV-1 RNA from P and BM, from one woman in HPTN 023 study, were quantified by ultra-sensitive Roche Amplicor 1.5, was subject to population sequencing by Trugene 1.5 (Bayer) and Stanford ABI. Population RT sequences were compared to >50 RT clones from plasma at 0, 2, 8 and 24 weeks (wks), and right (R) & left (L) BM at 2 and 8 wks, a total of ~ 400 RT clones from 8 samples. DRM were scored for RT codons 98, 100, 101, 103, 106, 108, 179, 181, 188, 190, 225, 227 and 230. Statistical comparisons of P and BM DRM were performed as differences in proportions (Proc Freq) in SAS.



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## RESULTS

Figure 1. Plasma clones with NNRTI-associated mutations at 0, 2, 8, 24 week post SD NVP.

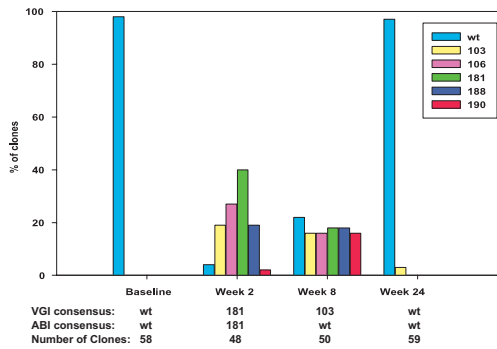


Figure 2. Left and Right Breast-milk clones with NNRTI-associated mutations at 2 and 8 weeks post SD NVP

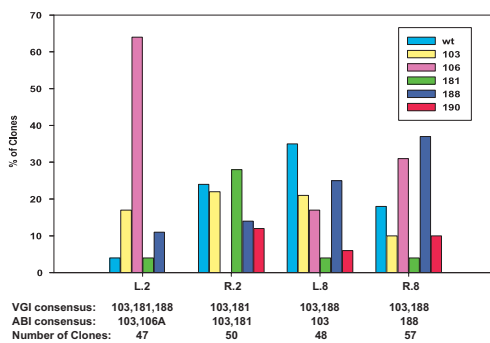
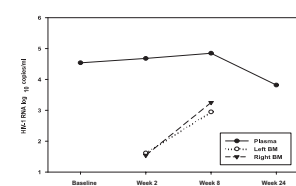


Figure 3. Phylogenetic tree of RT Plasma and Breast-milk clones



Figure 4. Plasma and Breast-milk Viral Loads at 0, 2, 8, and 24 week post SD NVP



- \* HIV-1 RNA levels in Plasma ranged from 3.82 to 4.85 log<sub>10</sub> copies/ml at 0 to 24 wks. BM HIV-1 RNA at 2 wk from left (L) and right (R) BM (1.62 and 1.54 log<sub>10</sub> copies/ml) were lower than 8 wk values (L BM 3.25 and R BM 4.85 log<sub>10</sub> copies/ml). (Figure 4)
- \* Population sequencing (Trugene) in Plasma identified Y181YC and K103KN at 2 and 8 wks, respectively. (Figure 1)
- \* Analysis of ~ 50 unique RT sequences (clones) from Plasma demonstrated DRM in 3/64 (5%) at 0 wk, 46/48 (97%) at 2 wks, 41/50 (80%) at 8 wks, and 3/59 (5%) at 24 wks (p<0.01). (Figure 1)
- \* The proportions of DRM in Plasma clones at 2 wks (40% Y181C, 19% K103N, 27% V106AM, 19% Y188C) was significantly different from wk 8 where DRM at 103, 106, 181, 188, 190 were each ~16% (p<0.01). (Figure 1)
- \* BM population sequencing at 2 wks showed L K103KN, Y181YC, Y188YC vs R K103KN, Y181YC and at 8 wks; L K103KN, Y188YC and R K103KN, Y188YC. (Figure 2)
- \* Phylogenetic analysis showed all sequences (clonal and consensus) were closely related, but not identical subtype C RT. The distribution of clonal DRM between L and R BM samples at 2 and 8 wks, and between Plasma and BM were significantly different (p<0.05). (Figure 3)

## CONCLUSIONS

- \* Rapid selection of NVP resistance mutations following SD NVP in P and BM HIV-1 RNA may be underestimated by population sequencing methods.
- \* Both VGI and ABI consensus sequences were insensitive to the diversity found in clones, where a majority of viruses had at least one DRM at 2 and 8 wks, although most of DRM existed in < 20% of clones (below the threshold of detection).
- \* Baseline Subtype C HIV-1 viral genome may naturally contain NNRTI resistance at around 5%. After SD NVP, virus with mutations gets rapidly selected for with up to 97% of virus in P and 96% in BM containing an NNRTI-mutation by week 2. By 24 weeks, majority of virus reverts back to WT and baseline mutation levels of 5%.
- \* Different patterns of DRM in P and BM clones provide evidence for differential independent inter and intra compartmental selection and expression of NVP resistance after SD NVP. 97% of virus in P and 96% in BM containing an NNRTI-mutation by week 2.

## ACKNOWLEDGEMENTS

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