

# Dynamics of Nevirapine (NVP)-resistant HIV-1 following Single-dose (sd) NVP Assessed in Plasma and Cells by Consensus Sequencing (CSeq) and Oligonucleotide Ligation Assay (OLA)

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## ABSTRACT:

**Background:** sd-NVP for the prevention of HIV-1 mother-to-child transmission (MTCT) has been associated with higher rates of virologic failure in women subsequently treated with NVP based ART<sup>3,5</sup>. Detection of NVP resistance by CSeq of plasma virus at 10 days (d) postpartum was not predictive of virologic failure in multivariate analysis<sup>3</sup>. A sensitive predictor of an increased risk of failing NVP-ART might prove useful for the management of women following sdNVP. We analyzed NVP resistance longitudinally in a group of women who had selection of NVP-resistant HIV-1 after sdNVP, and assessed the rate of NVP-mutants detectable in plasma and peripheral blood cells at 3 time-points by CSeq and an OLA<sup>1</sup>.

**Methods:** Specimens from women enrolled in PHPT2 who received sdNVP and started NVP-ART 4-18 months later were screened for NVP resistance. Those with K103N, Y181C or G190A by CSeq of plasma from 10 days(d) after sdNVP or by OLA of cells at 6 weeks (w) or later were selected to for further analysis. A minimum of 150 copies of HIV-1 RNA or DNA from plasma and cells, respectively, from 10d, 6w and at the initiation of ART was PCR-amplified. The amplicons were analyzed for NVP resistance by CSeq and OLA. OLA with optical densities >5% mutant control were classified as positive.

**Results:** 65 women initiated NVP-ART 4-18 months after sdNVP and had longitudinal samples collected. 32 had longitudinal samples evaluated, and 19 had resistance detected at more than one timepoint. ART was started a median of 258d (range 120 - 527d) after sdNVP in these 19 women. Specimens had one or more NVP mutations detected as follows:  
CSeq of plasma / cells: 10d - 63%/23%; 6w - 50%/50%; ART start 13%/11%  
OLA of plasma / cells: 10d - 94%/93%; 6w - 75%/94%; ART start 13%/23%  
79% (15/19) of women had multiple mutations. Mutants persisting at ART start by OLA of cells: 2/14 (14%) K103N, 1/8 (13%) Y181C; and 5/19 (26%) G190A.

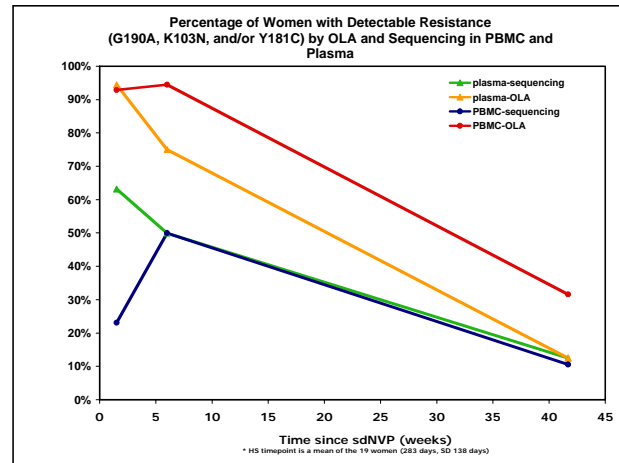
**Conclusions:** Selection of NVP-resistant HIV-1 mutants following sdNVP occurred more rapidly in plasma compared to cells. The concentration of mutant virus began to decay before 6w postpartum. Mutants remained detectable at ART start in a subset of women. Decay of mutants in cells appeared slower compared to plasma when evaluated by OLA. OLA on HIV-1 DNA from cells appeared more sensitive than CSeq, or testing of plasma RNA by either method, to detect the persistence of NVP-resistant mutants. Additional studies are needed to determine the clinical significance of the low-level NVP-resistant HIV-1 persisting in cells

## RESISTANCE DETECTED IN EACH WOMAN (color coded by codon):

Participant ID (Days until HS)	Sample	Method	10d	6wk	HS
282	plasma	seq			
(120)	PBMC	seq			
317	plasma	seq			
(130)	PBMC	seq			
407	plasma	seq			
(504)	PBMC	seq			
652	plasma	seq			
(419)	PBMC	seq			
673	plasma	seq			
(391)	PBMC	seq			
692	plasma	seq			
(185)	PBMC	seq			
724	plasma	seq			
(354)	PBMC	seq			
826	plasma	seq			
(284)	PBMC	seq			
887	plasma	seq			
(527)	PBMC	seq			
905	plasma	seq			
(341)	PBMC	seq			
922	plasma	seq			
(349)	PBMC	seq			
991	plasma	seq			
(453)	PBMC	seq			
1062	plasma	seq			
(212)	PBMC	seq			
1064	plasma	seq			
(232)	PBMC	seq			
1091	plasma	seq			
(212)	PBMC	seq			
1200	plasma	seq			
(164)	PBMC	seq			
3705	plasma	seq			
(136)	PBMC	seq			
3717	plasma	seq			
(135)	PBMC	seq			
3925	plasma	seq			
(131)	PBMC	seq			

Average time to HS: 283 days since sdNVP (SD: 138 days)

## OVERALL SUMMARY OF DATA:



## DISCUSSION:

This study was restricted to a small cohort of women (n = 19) exposed to sdNVP and selected based on the presence of detectable NVP resistance.

**The data confirmed previous studies by Flys, et al<sup>6</sup> and Loubser, et al<sup>8</sup>:**

- K103N decays slowly over time:
  - 2 out of 13 women (15%) still had detectable K103N at the time they started ART, at 131 and 164 days following sdNVP
- Consensus sequencing has limited sensitivity to detect resistance mutations:
  - OLA designed to detect greater than 5% mutant was able to detect roughly twice as much NVP resistance as consensus sequencing

**These data suggests several new findings:**

- G190A and Y181C also decay slowly over time
- Many of these women had more than one detectable NVP resistance codon (79%)
- G190A seems to present at the highest percentages and decay the most quickly in the plasma
- Y181C seems to emerge in the PBMC the most slowly
- Some of these findings may be unique to HIV-1 CRF01\_AE which predominates in Thailand
- Analysis of three separate resistance mutations provides a more complete description to the resistance patterns detected after sdNVP
- This study quantified the number HIV-1 DNA copies used as input for OLA, and therefore allows a direct comparison of the rates drug resistance in HIV-1 RNA and DNA

## CONCLUSIONS:

Dynamics of HIV-1 drug resistance after sdNVP:

- at 10 days K103N and Y181C are more likely to be detected in the plasma than PBMC
- at 10days G190A detection is comparable in plasma and PBMC
- between 10days and 6 weeks, for each codon analyzed:
  - in PBMC, the percentage of women with detectable resistance and the estimated percentage of drug resistance variants increased
  - in Plasma, the percentage of women with detectable resistance and the estimated percentage of drug resistance variants decreased
- after 6 weeks, resistance more likely to be detected in the PBMC
- there may be differences in the rates at which different resistance mutations decay
- these findings may help guide the approach to all clinical HIV resistance testing, especially in situations when there is a history of ARV exposure

## REFERENCES:

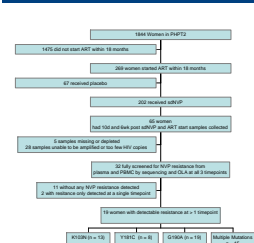
- Edelstein RE, Nickerson DA, Tobe VO, Manos-Arciniegua LA, Frenkel LM. Oligonucleotide ligation assay for detecting mutations in the human immunodeficiency virus type 1 pol gene that are associated with resistance to zidovudine, didanosine, and lamivudine. J Clin Microbiol. 1998 Feb;36(2):569-72.
- Flys TS, Donnell D, Mwamba A, Nakabito C, Musoke P, Mmro F, et al. Persistence of K103N-containing HIV-1 variants after single-dose nevirapine for prevention of HIV-1 mother-to-child transmission. J Infect Dis. 2007 Mar 1;195(5):711-5.
- Jourdain G, Ngo-Giang-Huong N, La Coeur S, Bowonwatanuwong C, Kantipong P, Leechachadri P, et al. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. N Engl J Med. 2004 Jul 15;351(3):229-40.
- Lallemand M, Jourdain G, La Coeur S, Mary JY, Ngo-Giang-Huong N, Keetawang 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 Jul 15;351(3):217-28.
- Lockman S, Shapiro R, Smeaton L, Wester C, Thor L, Stevens L, et al. Response to Antiretroviral Therapy after a Single, Partipartum Dose of Nevirapine. N Engl J Med. 2007 Jan 11;356(2):136-47.
- Loubser S, Balle P, Sherman G, Hamner 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 Apr 24;20(7):995-1002.

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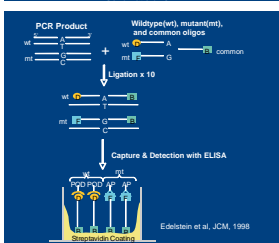
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Schematic Diagram of the Selection of the 19 Women Studied:

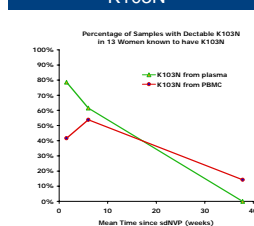


Schematic of OLA:

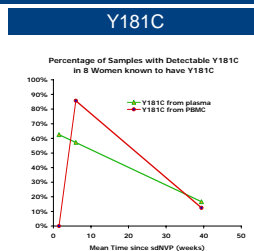


## BY CODON

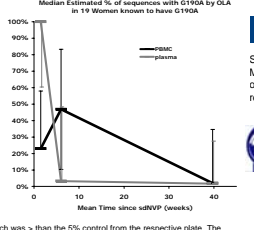
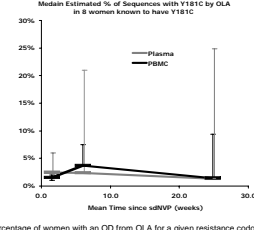
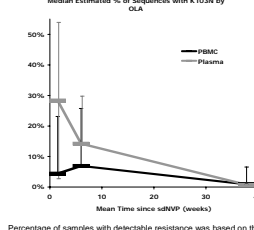
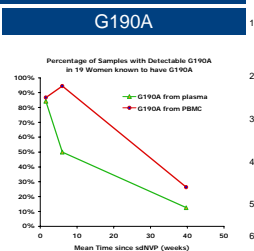
### K103N



### Y181C



### G190A



Percentage of samples with detectable resistance was based on the percentage of women with an OD from OLA for a given resistance codon which was > than the 5% control from the respective plate. The estimated % mutant was calculated based on a logarithmic curve fit to the mutant dilution curve. SD was calculated based on the estimated % mutant of all women at a given codon and timepoint. One SD indicated on graphs, unless that lead to a value < 0% or > 100% mutant.