

# Frequent emergence of lamivudine and NNRTI drug-resistance during pregnancy-limited antiretroviral therapy in the US. Results from the Women and Infants Transmission Study

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## Abstract

**Background:** Pregnancy-limited antiretroviral therapy (PLAT) drastically reduces HIV-1 transmission to the newborn, but may select for antiretroviral drug resistance mutations (DRM) in exposed women.

**Methods:** We evaluated antiretroviral-naïve, HIV-1-infected pregnant women enrolled in the WITS cohort who received PLAT between 1998 and 2004, and had 2- or 6-month postpartum plasma samples available with HIV-1 RNA levels (VL) >500 copies/mL. Postpartum rates of DRM were assessed blindly using bulk sequencing (BSQ) of protease (PR) and reverse transcriptase (RT), and allele-specific PCR (ASPCR) of the M184V, K103N and D30N mutations (detection threshold 0.4%, 0.003% and 0.1%, respectively). Factors associated with the emergence of M184V, K103N and D30N were investigated using Chi-square, Fisher's exact test or F-test, as needed. Multivariate odds of developing M184V or K103N were evaluated using a standardized linear model (SAS procedure GENMOD).

**Results:** 146 women fulfilled the inclusion criteria. The mean age was 26.7 (SD=5.23) years. 57.5% of women were black and 35.6% hispanic. 27.4% had history of illicit drug use. Postpartum median CD4+ counts were 575 (IQR 397-767) cells/mm<sup>3</sup>, median VL was 4780 (IQR: 1352-18121) copies/mL. All women received, at least, AZT-3TC. 76% also received nevirapine (NFV) and 8.2% nevirapine (NVP). DRM data were available from 114 women (78%). By BSQ, NRTI-DRM rates in women receiving only AZT/3TC were: M41L=5.0%, D67N=5.0%, K70R=10.0%, M184V/I=65.0% (95.0% by ASPCR), T215Y=5.0%. In women receiving 3 drugs, rates were: M41L=1.1%, D67N=1.1%, K70R=1.1%, M184V/I=28.7% (51.6% by ASPCR), K219Q=1.1%. NNRTI-DRM rates among women receiving NVP were: K103N=25% (37.5% by ASPCR), Y188C=12.5%. PI-DRM rates in those receiving NFV were: D30N=1.1% (1.1% by ASPCR), L90M=1.1%. In the multivariate analysis, variables associated with emergence of M184V were triple vs dual PLAT (OR=0.05, 95%CI=0.01-0.40, p<0.01), and prolonged exposure to AZT (OR per additional month=1.29, 95%CI=1.02-1.62, p=0.03). Variables associated with emergence of K103N were NVP use (OR=9.75, 95%CI=1.61-58.33, p=0.01) and length of AZT/3TC (OR per additional month=1.46, 95%CI=1.05-2.02, p=0.02).

**Conclusions:** Selection of 3TC and NNRTI resistance is frequent during PLAT, but selection of PI-resistance is rare. Triple-drug PLAT decreases the odds for M184V selection. Routine postpartum genotypic resistance testing may be useful to guide future treatment decisions in mothers.

## Background

Pregnancy-limited antiretroviral therapy (PLAT) drastically reduces HIV-1 transmission to the newborn, but may select for antiretroviral drug resistance mutations (DRM) in exposed women. This may potentially hinder the efficacy of MTCT prophylaxis and limit future ARV treatment options for both mother and child. We previously demonstrated a high prevalence of primary lamivudine and nevirapine resistance among antiretroviral-naïve HIV-1-infected pregnant women in the US.<sup>1</sup> The aim of this study was to assess the post-partum rate of drug resistance mutations in previously ARV-naïve women who received PLAT. We also sought to investigate factors associated with an increased risk for selecting particularly relevant resistance mutations (M184V and K103N).

## Methods

### SUBJECTS

The Women and Infants Transmission Study (WITS) is a multi-site observational study designed to examine the impact of HIV infection on HIV infected women and their infants. WITS sites are located in Brooklyn, NY, New York City, NY, Boston, MA, Houston, TX, Chicago, IL and Puerto Rico. (<http://www.niaid.nih.gov/daids/wits.htm>)

In early 2005, 1323 women enrolled in WITS III and WITS IV between June 1, 1998 and December 31, 2004, were evaluated for eligibility for this study. Study participants were HIV-1-infected pregnant women who had never received antiretroviral therapy before and initiated PLAT to prevent vertical transmission. Blinded plasma specimens were collected between 1998 and 2004 and were analyzed in 2007 in a single laboratory.

### DETECTION OF RESISTANCE MUTATIONS

HIV RNA was extracted from plasma, reverse-transcribed to cDNA and PCR-amplified for 30 cycles in a One-Step reaction. After DNA purification, K103N, M184V and D30N-specific and non-specific Real-Time PCR amplifications were performed, in duplicate and in separate wells, using the mutant-specific and non-specific primers as upstream primers, and an downstream primer common for both specific and non-specific reactions. Cts were interpolated onto the standard curve. Proportions of mutant variants were calculated as: mutant-specific copies / non-specific copies ("mutants" / "all others").

The PCR product of the One-Step RT-PCR reaction was used as the template for a nested PCR reaction. Nested PCR products including the complete protease (PR)-coding region and the 1<sup>st</sup> half of the reverse transcriptase (RT)-coding region of *pol* were purified and sequenced using a fluorescently labeled dideoxy-nucleotide chain termination method (TaQ DyeDeoxy Terminator cycle sequencing kit, Applied Biosystems, Foster City, CA, USA)

### STATISTICAL ANALYSIS

Subjects characteristics and postpartum rates of resistance mutations were described using standard descriptive methods. Variables associated with the emergence of M184V, K103N and D30N mutations were investigated using Chi-square, Fisher's exact test or F-test, as needed. Multivariate odds of developing M184V or K103N were evaluated using the General Estimating Equations linear model (SAS procedure GENMOD).

## Results

### ALLELE-SPECIFIC PCR PERFORMANCE<sup>1,2</sup>

The sensitivity thresholds of each ASPCR assay were defined as the mean plus 3 standard deviations of 20 measurements of negative controls (i.e. wild-type laboratory viral constructs), at the following proportions:

D30N	0.1%
K103N (AAT and AAC alleles)	0.003%
M184V	0.4%

Table 1. Subjects characteristics

Number of subjects evaluated	146	Timing of blood sample collection (months since delivery, median, (Q1-Q3))	
Age (Mean ± SD)	26.74 ± 5.23	Overall, n=146	2.22 (1.87-4.60)
Ethnicity		Samples collected at the 2-month postpartum visit, n=111	2.00 (2.50-1.77)
White	4 (2.7)	Samples collected at the 6-month postpartum visit, n=35	6.24 (5.88-6.86)
Black	84 (57.5)	Samples tested (n, %)	146 (100%)
Hispanic	52 (35.6)	CDC category (n, %)	
Other/unknown	6 (4.1)	A	124 (84.93)
Ever used hard drug before delivery*, n (%)		B	19 (13.01)
Yes	40 (27.4)	C	3 (2.02)
No	105 (71.9)	Initial PLAT (n, %) <sup>†</sup>	
Unknown	1 (0.7)	AZT-3TC	43 (29.4)
Alcoholism, n (%)		AZT-3TC-Nevirapine	93 (63.7)
Yes	42 (28.8)	AZT-3TC-Nevirapine	10 (6.8)
No	101 (69.2)	Length of exposure to AZT (days, up to delivery) (mean ± SD) [range]	122.5 ± 61.3 [2, 309]
Unknown	3 (2.0)	Length of exposure to AZT-3TC (days, up to delivery) (mean ± SD) [range]	118.2 ± 60.0 [1, 270]
First available CD4+ cell count during pregnancy, n (%)		Nefirapine exposure during PLAT (n, %)	
<200 cells/mm <sup>3</sup>	9 (6.2)	Yes	111 (76.0)
200-350 cells/mm <sup>3</sup>	33 (22.6)	No	35 (24.0)
<200 cells/mm <sup>3</sup>	100 (68.5)	Length of exposure to Nevirapine for 111 exposed subjects (days, up to delivery) (mean ± SD) [range]	109.9 ± 58.9 [1, 270]
Unknown	4 (2.7)	Nevirapine exposure during PLAT (n, %)	
Postpartum CD4+ cell counts (median, Q1-Q3)		Yes	12 (8.2)
Absolute (cells/mm <sup>3</sup> )	575 (397-767)	No	134 (91.8)
Percent (%)	29 (22-34)	Length of exposure to Nevirapine for 12 exposed subjects (days, up to delivery) (mean ± SD) [range]	66.04 ± 61.3 [1, 177]
HIV-1 RNA during PLAT, n (%)		* Refers to the initial treatment. Some subjects changed from dual PLAT to triple PLAT and vice-versa	
Always > 400 copies/mL	40 (27.4)		
Mixed <400 and > 400 copies/mL	33 (22.6)		
Always < 400 copies/mL	70 (47.9)		
Unknown	3 (2.0)		
Postpartum HIV-1 RNA copies (median, Q1-Q3) (copies/mL)	4780 (1352-18121)		

## Conclusions

- Pregnancy-limited antiretroviral therapy (PLAT) is associated with frequent selection of viruses with high-level resistance to lamivudine and emtricitabine. Although the total number of women receiving nevirapine in this study was small, our data suggest that 3-drug, nevirapine-based PLAT is also associated with frequent selection of viruses with high-level, cross-resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs). Overall postpartum rates of thymidine analogue resistance mutations (TAMs) are low after PLAT, although tend to be higher in subjects receiving 2-drug PLAT than in those treated with 3 drugs. Finally, selection of protease-inhibitor-resistant viruses during nevirapine-based 3-drug PLAT is infrequent.
- In concordance with previous studies,<sup>1,2</sup> allele-specific PCR increases the frequency of detection of key resistance mutations relative to population-based sequencing of plasma viruses. Using ASPCR, M184V mutants can be detected in virtually all women receiving 2-drug PLAT and in more than 60% of those treated with 3 drugs, whereas K103N mutants are found in one third of all women receiving nevirapine during PLAT. Given that pretreatment detection of minority NNRTI-resistant variants more than triples the risk of virological failure to subsequent NNRTI-based therapy,<sup>3,4</sup> these results have relevant clinical implications. Women selecting NNRTI-resistant mutants during PLAT may be at a higher risk of failing subsequent NNRTI-based antiretroviral therapy.
- Importantly, 2-drug PLAT with AZT-3TC increases the odds for selecting M184V viruses 20-fold, relative to 3-drug PLAT. Duration of AZT exposure is also independently associated with an additional risk of M184V selection. Nevirapine use and duration of AZT-3TC exposure are independently associated with an increased risk of K103N mutant selection during PLAT.
- These results strongly support the need of avoiding 2-drug PLAT and using, when possible, 3-drug PLAT regimens containing drugs with high genetic barrier. All efforts should be undertaken to ensure optimal adherence to PLAT. Given that resistant viruses will likely wane after PLAT interruption, postpartum genotypic resistance testing may be useful to guide future treatment decisions in mothers.

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Figure 1. Post-partum rates of resistance mutations by treatment group

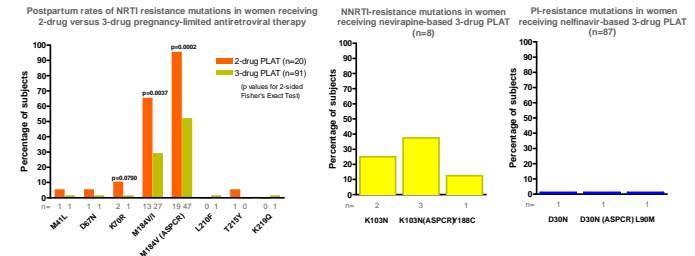
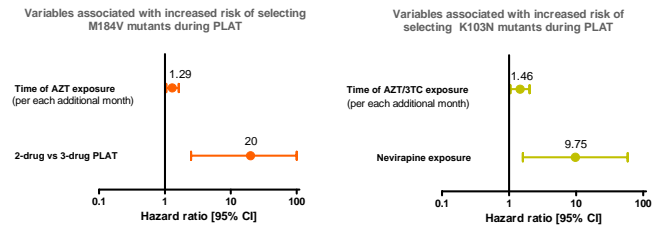


Figure 2. Factors associated with emergence of antiretroviral resistance during pregnancy-limited antiretroviral therapy (multivariate analysis)



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