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Postpartum Antiretroviral Drug Resistance in HIV-1-infected Women Receiving Pregnancy-Limited Antiretroviral Therapy

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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 mothers.

Methods: We evaluated antiretroviral-naïve, HIV-1-infected pregnant women who received PLAT between 1998 and 2005, and had 2- or 6-month postpartum plasma samples available with HIV-1 RNA levels (VL) >500 copies/mL. Postpartum DRM rates were assessed blindly using population sequencing (PS) and allele-specific PCR (ASPCR) of the M184V, K103N and D30N mutations. Factors associated with selection of DRM were investigated.

Results: 146 women were included. All women received zidovudine+lamivudine during pregnancy; 76% also received nelfinavir and 8.2% nevirapine. Resistance data were available from 114 women (78%). Postpartum rates of single-, dual-, and triple-class resistance were, respectively, 43.0%, 6.1% and 0% (63.2%, 10.5% and 1.7% by ASPCR). In women receiving dual or triple PLAT, respectively, postpartum M184V/I rates were 65.0% (95.0% by ASPCR) and 28.7% (51.6% by ASPCR), respectively ($p<0.01$). Postpartum NNRTI resistance rates among women receiving nevirapine were 25% for K103N (37.5% by ASPCR) and 12.5% for Y188C. PI resistance rates in women receiving nelfinavir were: 1.1% for D30N (1.1% by ASPCR) and 1.1% for L90M. Dual versus triple PLAT and prolonged zidovudine exposure were associated with selection of M184V. Nevirapine use and length of zidovudine plus lamivudine exposure were associated with selection of K103N.

Conclusions: PLAT is associated with frequent selection of resistance to drugs with low-genetic barrier. Triple-drug PLAT decreases the odds for M184V selection. Routine postpartum genotypic resistance testing may be useful to guide future treatment decisions in mothers.

Keywords

HIV-1; resistance; pregnancy; mother-to-child transmission; allele-specific PCR; minor variants

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ROLE OF THE AUTHORS IN THE STUDY

Roger Paredes performed the population and allele-specific resistance testing, drafted initial manuscript and participated in its discussion and review. Irene Cheng performed the statistical analyses and participated drafting, discussing and reviewing the manuscript. Daniel R. Kuritzkes and Ruth E Tuomala conceived and coordinated the study; reviewed the study results and drafted, discussed and reviewed the manuscript. The Women and Infant's Transmission Study group contributed to the study design and logistics and reviewed, discussed and helped drafting the manuscript.

INTRODUCTION

Programs to prevent mother-to-child HIV-1 transmission (MTCT) have averted thousands of new HIV-1 infections worldwide. Present rates of vertical transmission in women with sustained access to triple-drug antiretroviral therapy (ART), perinatal zidovudine treatment, elective C-sections, and alternatives to breast-feeding are extremely low.[1-5] Even a simplified approach based on the intrapartum and neonatal administration of a single dose of nevirapine with or without zidovudine, lamivudine or tenofovir has reduced MTCT in resource-poor areas. [6,7] In spite of these successes, pre-existence of transmitted drug resistant viruses [8,9] or selection of antiretroviral drug resistance during MTCT prophylaxis may hinder the long-term efficacy of MTCT preventive programs as well as severely constrain future treatment options for both mother and child.[7,10-15]

A suitable way of investigating how often antiretroviral MTCT prophylaxis selects for drug-resistant HIV-1 in mothers is to assess the postpartum rates of resistant viruses in antiretroviral-naïve pregnant women who receive pregnancy-limited antiretroviral therapy (PLAT). Selection of resistant viruses during PLAT, even as minority variants, could impair the virological outcome when women subsequently initiate long-term ART,[16,17] particularly if treatment is started within 6-12 months after delivery.[18,19]

This study sought to investigate post-partum drug resistance in ARV-naïve women who received PLAT in the US between 1998 and 2005, using population-based sequencing of plasma virus and allele-specific PCR testing for the M184V, K103N and D30N mutations, associated with high-level resistance to lamivudine and emtricitabine, nevirapine and efavirenz, and nelfinavir, respectively. We also examined factors associated with an increased risk for selecting resistant viruses during pregnancy.

METHODS

Study design

This was a substudy utilizing specimens obtained from participants in the Women and Infants Transmission Study (WITS).[20] The 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 and Worcester MA; Houston, TX; Chicago, IL; and Puerto Rico.

Subjects

Women enrolled in WITS between June 1, 1998 and December 31, 2004, were evaluated for eligibility for this study. Study participants included HIV-1-infected pregnant women who had never received antiretroviral therapy before pregnancy, initiated PLAT consisting of zidovudine plus lamivudine either solely or in addition to either nevirapine or nelfinavir, stopped therapy postpartum, and had specimens available from 2- or 6-month postpartum visits during which the presence plasma virus by RNA-PCR testing had previously been documented. A single laboratory, blinded as to PLAT received by participants, analyzed all specimens. Specimens collected within the first 14 days of antiretroviral therapy were also analyzed post-hoc, when available. However as most PLAT initiation occurred prior to WITS enrollment, these specimens were lacking for the majority of women.

Detection of resistance mutations

Population sequencing of plasma viruses—HIV-1 RNA was extracted from 500 µL EDTA-anticoagulated plasma using the QIAamp® Viral RNA MiniKit (QIAGEN Sciences, Maryland, USA) after centrifugation at 24000g for 1 hour at 4°C. Part of each RNA sample

was used for cDNA synthesis immediately after extraction, and the remainder was stored at -80°C .

The extracted RNA was transcribed to cDNA and amplified by PCR in a one-step process (Superscript III One-step RT-PCR with Platinum Taq Kit, Invitrogen™, Carlsbad, CA, USA) following the manufacturer's instructions. Cycling conditions included an initial cDNA synthesis step at 55°C for 25 min followed by a denaturation step at 94°C for 2 min; 30 cycles of PCR amplification (94°C for 40 sec, 60°C for 40 sec, 68°C for 1 min and 20 sec); and a final 5 min extension step at 68°C . The PCR mix contained 25 μL of 2X Reaction Mix (including 0.4 mmol/L of each dNTP and 3.2 mmol/L of MgCl_2), 0.2 mmol/L of each primer OOPF (HXB2:2211-2232) [5'-GAAGCAGGAGCCGATAGACAAG-3'] and OOR2 (HXB2:3466-3444) [5'-TTTTCTGCCAGTTCTAGCTCTGC-3'], 15 μL of extracted RNA as template and nuclease-free H_2O to a final volume of 50 μL .

The resulting PCR product was purified using the QIAquick® PCR Purification Kit (QIAquick® PCR Purification Kit, QIAGEN Sciences, Maryland, USA) and used as the starting template for a 30-cycle nested PCR amplification (High Fidelity Platinum Taq, Invitrogen Corp., Carlsbad, CA, USA) using primers OOPF2 (HXB2:2218-2241) [5'-GAGCCGATAGACAAGGAAGTGTAT-3'] and OOR3 (HXB2:3457-3432) [5'-AGTTCTAGCTCTGCTTCTTCAGTTAG-3'].

The nested PCR product was purified and sequenced (3730XL DNA Analyzer, Applied Biosystems, Foster City, CA, USA). Resistance mutations and polymorphisms were defined according to the International AIDS-Society-USA Panel (Spring 2008 Update). [21] Standard phylogenetic analyses ruled out sequence contamination.

Allele-specific PCR—To quantify the proportion of mutant sequences contained within each specimen, 5 μL of RT-PCR product were added to the real-time PCR together with selective or nonselective primers. When the initial DNA copy number was lower than 10^6 , the nested DNA product was used. PCR conditions have been previously published.[8,22]

Mutant-specific primers ([HXB2:2319-2340] 5'-CTATTAGATACAGGAGCAAATA-3' for D30N; [HXB2:3078-3098] 5'-GACATAGTTATCTATCAATICG-3' for M184V; [HXB2:2884-2858] 5'-CCCACATCCAGTACTGTTACTGATTGG-3' for the K103N AAC allele; and [HXB2:2884-2858] 5'-CCACATCCAGTACTGTTACTGATTCA-3' for the K103N AAT allele) included the target codon in its 3' end and an intentional mismatch at positions -3 or -2 , relative to the 3' end. Non-specific primers ([HXB2:2319-2339] 5'-CTATTAGATACAGGAGCAGAT-3' for D30N; [HXB2:3078-3098] 5'-GACATAGTTATCTATCAATAC-3' for M184V; and [HXB2:2884-2859] 5'-CCCACATCCAGTACTGTTACTGATTT-3' for both AAC and AAT K103N alleles) were similar to the former, but ended just before the target base pair and did not include intentional mismatches. The antiparallel primer ([HXB2:2592-2571] 5'-CTGGCTTTAATTTTACTGGTAC-3' for D30N; [HXB2:3277-3258] 5'-GGCTGTACTGTCCATTTATC-3' for M184V; and [HXB2:2757-2785] 5'-AAATGGAGAAAATTAGTAGATTCAGAGA-3' for both AAC and AAT K103N alleles) was common for each pair of mutant-specific and non-specific reactions.

The different ASPCR assays were performed separately. Within each ASPCR assay, nonselective and selective amplifications were always performed in parallel. All reactions were performed in duplicate, and the mean of the two values was used for calculation. The percentage of viral sequences containing each mutation was calculated as follows: % mutant sequences = [(quantity of mutant sequences in the sample)/(quantity of total viral sequences in the sample)] $\times 100$.

In addition to the sensitivity threshold for each ASPCR assay, we calculated a specific detection threshold per each sample, defined as the minimum proportion of variants that could be detected based on the specimen's HIV-1 RNA level (pVL), the volume of plasma used in the RNA extraction (V), the fraction of the RNA elution volume used for cDNA synthesis (fe), and the assumed efficiencies of the RNA extraction (E_{RNA}) and cDNA synthesis (E_{cDNA}). The sample specific detection threshold was calculated as $1/N_{RNA}$, where N_{RNA} was the number of viral RNA copies that were effectively sampled after RNA extraction and reverse transcription. N_{RNA} was calculated as: $N_{RNA} = pVL \times V \times fe \times E_{RNA} \times E_{cDNA}$. We assumed an E_{RNA} of 0.96 and an E_{cDNA} of 0.7.[23,24] Allele-specific PCR values between the ASPCR assay sensitivity threshold and the sample-specific detection threshold were considered undetectable.

Statistical analysis

Subjects' characteristics and postpartum rates of resistance mutations were described using standard descriptive methods. 95% confidence intervals for resistance mutation rates were generated using the Exact Binominal Test. Variables associated with the selection of M184V, K103N and D30N mutations were investigated using Chi-square, Fisher's exact test or F-test, as needed. Odds of developing M184V or K103N were evaluated using the Generalized Estimating Equations model (SAS procedure GENMOD).

Alpha was set at 0.05 for determining statistical significance in all univariate and multivariate analyses. For M184V and K103N mutations, the multivariate analysis models were built using forward selection technique. The multivariate analysis model only included characteristics that achieved statistical significance in the univariate analysis. All analyses were intention-to-treat.

RESULTS

Performance of the ASPCR assays

Using the mean plus 3 standard deviations of 20 negative control repeats (wild-type laboratory viral constructs), the detection threshold of the ASPCR was calculated at: 0.1%, 0.4% and 0.003% for the D30N, M184V and K103N ASPCR assays, respectively. Delta Cts between mutant and wild-type DNA equivalents were > 10 cycles for the D30N and M184V assays and > 17 cycles for the K103N assays. Proportion measurements were linear down to at least 1% for the D30N and M184V assays and to 0.01% for the K103N ASPCR assays.[17,24]

Subject's characteristics

1328 women were enrolled in the WITS Study between June 1, 1998 and December 31, 2004; 636 of them were ART-naïve prior to current pregnancy; 315 women started and received one of the three targeted regimens (zidovudine + lamivudine; zidovudine + lamivudine + nevirapine; or zidovudine + lamivudine + nelfinavir) during more than 28 days during the index pregnancy. Of them, 146 women stopped therapy postpartum and had plasma specimens available from 2- or 6-month postpartum visits with HIV-1 RNA levels ≥ 500 copies/mL, thereby being included in the present study.

The mean age of the 146 women fulfilling the inclusion criteria was 27 years, most were African-American or Hispanic, the majority were CDC disease category A, and almost 30% had used hard drugs and/or had a known history of alcohol use prior to delivery (Table 1). The median gestational age was 29.3 years (IQR: 22.4 – 36.3 years). The median first CD4 count available during pregnancy was 455 cells/mm³ (IQR: 316 – 690 cells/mm³); being ≥ 200 cells/mm³ in more than 90% of women. The median time of blood sample collection was 2.2 months postpartum (IQR: 1.9-4.6 months), with 111 samples (76%) collected at the 2-month postpartum visit [median 2.0 months (IQR: 1.8-2.5 months)]; the remaining 35 samples (24%)

were collected at the 6-month postpartum visit [median 6.2 months (IQR: 5.9 – 6.9 months)]. Median postpartum CD4+ counts were 575 cells/mm³ (IQR: 397-767), and median postpartum HIV-1 RNA levels were 4780 copies/mL (IQR: 1352-18121 copies/mL). Seventy women (48%) maintained HIV-1 RNA levels <400 copies/mL at all timepoints during PLAT after their enrollment in WITS; 40 (27%) had all HIV-1 RNA levels >400 copies/mL during PLAT, and the 33 (23%) alternated viremic with aviremic periods during PLAT. All women initiated PLAT including zidovudine + lamivudine; 93 (64%) also started nelfinavir and 10 (7%) nevirapine. Some women switched between dual- and triple-PLAT during pregnancy. Of the 146 women, 18 (12%) switched from dual- to triple-PLAT (1 added nevirapine and 17 added nelfinavir). Another 2 women switched between triple-PLAT with nevirapine and triple-PLAT with nelfinavir. The duration of exposure to zidovudine, lamivudine, nelfinavir and nevirapine are shown in Table 1.

Postpartum rates of antiretroviral resistance

Post-partum resistance data were available from 114 women (78%). The virological, immunological and clinical characteristics of this subset of women did not differ from those fulfilling the study inclusion criteria. Pre-treatment resistance data was available from 25 of these 114 women (22%). Twenty women (18%) remained on dual-PLAT through delivery whereas 94 (82%) received triple-PLAT during pregnancy.

Overall, 49 women (43.0%) had at least 1 resistance mutation detected by the population-based sequencing analysis postpartum; 7 women (6.1%) had dual-class resistance after delivery. Three (2.6%) had resistance to NRTIs and PIs, 3 (2.6%) had resistance to NRTIs and NNRTIs and 1 (0.9%) had resistance to NNRTIs and PIs. When including the results from the ASPCR analysis for D30N, M184V and K103N mutations, 72 women (63.2%) had at least 1 resistance mutation detected postpartum, 12 women (10.5%) had dual-class resistance, and 2 (1.7%) women had triple-class resistance after delivery. Five women with dual-class resistance (4.4%) had resistance to NRTIs and PIs, whereas 7 (6.1%) had resistance to NRTIs and NNRTIs.

NRTI resistance—Using population sequencing of plasma viruses, the M184V/I mutation was detected postpartum in 65.0% of women receiving dual PLAT throughout pregnancy compared to 28.7% of women treated with 3 drugs ($p=0.004$). (Table 2) Using ASPCR, this mutation was detected in 95.0% of women who had received dual PLAT and in 51.6% of those having received triple-drug PLAT. ($p<0.001$). Using ASPCR, the M184V mutation was detected postpartum in 2/5 (40%) women with M184V present by population sequencing at baseline and in 15/20 (75%) women who did not have this mutation before treatment.

Mutations associated with resistance to nucleoside analogues (NAMs) were also more frequent among women exposed only to dual-drug PLAT (M41L, 5.0%; D67N, 5.0%; K70R, 10.0; and T215Y, 5.0%) than in those treated with 3 drugs (M41L, 1.1%; D67N, 1.1%; K70R, 1.1%; L210F, 1.1%; K219Q, 1.1%). Such differences were, at most, marginally significant.

NNRTI resistance—Using population sequencing of plasma viruses, the postpartum rates of NNRTI resistance among the eight women receiving nevirapine were: 25% [95% CI: 3.2%-65.1%] for K103N (2 cases), and 12.5% [95% CI: 0.3%-52.7%] for Y188C (1 case). Using ASPCR, the K103N mutation was detected in three out of eight women exposed to nevirapine (37.5% [95% CI: 8.5%-75.5%]), including the two cases detected through population sequencing, and in 8 out of 106 women not exposed to nevirapine (7.5%) ($p=0.029$). Using ASPCR, the K103N mutation was detected postpartum in 1 woman harboring this mutation before treatment and in 2/24 (8.3%) women who did not have this mutation before starting PLAT.

PI resistance—Using population sequencing, each of the D30N and L90M mutations were detected in one out of 87 women receiving nelfinavir during PLAT (1.1% [95% CI: 0.03%-6.24%] for each mutation). The ASPCR testing confirmed the detection of the D30N mutation in one out of 87 women exposed to nelfinavir (1.1% [95% CI: 0.03%-6.24%]) and allowed detection of this mutation in two women out of 27 (7.4%) that did not receive nelfinavir during PLAT. One D30N mutation detected at baseline was not found post-partum.

Factors associated with selection of post-partum antiretroviral resistance

In the univariate analysis (Table 3), postpartum detection of the M184V mutation was more likely in women who had all HIV-1 RNA levels > 400 copies/mL during PLAT (OR= 2.65, 95%CI=1.02-6.87, p=0.05) relative to those who remaining aviremic through delivery, and in those with longer exposure to zidovudine (OR=1.25, 95%CI=1.01-1.54, p=0.04, per each additional month). The M184V mutation, conversely, was less likely to be observed in women who received triple-drug PLAT including either nelfinavir or nevirapine (OR=0.06, 95% CI=0.007-0.44, p=0.006), relative to dual therapy only; in those treated with triple-drug PLAT including nelfinavir (OR=0.06, 95% CI=0.008-0.46, p=0.007) or nevirapine (OR=0.04, 95% CI=0.003-0.48, p=0.01), relative to dual therapy alone; and in those treated with triple-drug PLAT including nelfinavir, relative to any other regimen (OR=0.25, 95%CI=1.01-1.54, p=0.04). Univariate factors associated with postpartum detection of K103N were nevirapine exposure (OR=7.35, 95%CI=1.48-36.5, p=0.01), and longer exposure to zidovudine (OR=1.40, 95%CI=1.02-1.91, p=0.04) or to the combination of zidovudine and lamivudine (OR=1.42, 95%CI=1.04-1.94, p=0.03).

In the multivariate analysis (Table 4), the variables significantly associated with selection of M184V were exposure to dual versus triple-drug PLAT (OR=19.64, 95%CI=2.47-156.25, p<0.01), and duration of zidovudine exposure (OR =1.29, 95%CI=1.03-1.63, p=0.03, per additional month). Variables associated with selection of K103N were nevirapine use during pregnancy (OR=9.75, 95%CI=1.62-58.84, p=0.01) and length of dual-PLAT zidovudine + lamivudine exposure (OR =1.46, 95%CI=1.05- 2.02, p=0.02, per additional month).

Of note, other factors such as presence of resistance mutations before starting PLAT, the first available CD4+ count during pregnancy, use of hard drugs before delivery, alcoholism before delivery, ethnicity, or age at delivery, were not associated with the postpartum detection of the M184V or K103N mutations.

DISCUSSION

This study showed high post-partum antiretroviral resistance rates in antiretroviral-naïve women receiving PLAT. The main determinants of resistance selection during pregnancy were the characteristics of the antiretroviral regimen chosen to prevent MTCT.

Virtually all women receiving dual therapy developed the M184V mutation. This mutation confers high-level resistance to lamivudine and emtricitabine[25,26] and is associated with an increased risk of virological failure of treatment combinations including these drugs.[18] Thymidine analogue resistance mutations (TAMs) were detected in few women but were also more frequent in those receiving dual therapy. A high proportion of women receiving triple-drug therapy also selected resistance mutations during pregnancy. Based on allele-specific PCR testing, 50% of women treated with three drugs developed the M184V mutation. Moreover, although few women received nevirapine in this study, almost 40% of these women had non-nucleoside analogue (NNRTI) resistance mutations detected at the postpartum visit. On the contrary, selection of PI resistance was rare in women treated with nelfinavir.

Use of dual therapy and duration of zidovudine exposure, which reflects the overall duration of antiretroviral therapy, were the only two variables that were independently associated with an increased risk of M184V detection after delivery. Similarly, the post-partum detection of the K103N mutation was independently associated with exposure to nevirapine and duration of exposure to zidovudine and lamivudine. These findings strongly argue against using dual therapy to prevent MTCT whenever triple therapy is available.

The fact that resistance mutations were so frequently detected among women receiving triple therapy contrasts with previous estimates from Latin America and Caribbean countries,[27] and suggests that PLAT was less effective than expected at continuously suppressing HIV-1 RNA levels. In the univariate risk factor analysis, women who had all HIV-1 RNA levels above 400 copies/mL during the study were 2.7 times more likely to have the M184V mutation detected postpartum than those remaining aviremic through delivery. Similarly, subjects receiving nelfinavir were 4 times less likely to develop postpartum M184V than those not receiving this drug. These findings suggest that women treated with drugs with high genetic barrier to attain resistance are less likely to develop the M184V mutation. None of these variables, however, remained independently associated with risk of postpartum resistance in the multivariate analyses.

The pre-existence of primary resistance mutations could also have explained the high frequency of postpartum resistance found in this study. The prevalence of primary resistance increased during the last decade in pregnant women in the US.[28] Using allele-specific PCR in women enrolled in WITS with similar characteristics to those included in this study, we previously reported a 9.4% prevalence of primary lamivudine and emtricitabine resistance, and a 6.3% prevalence of nelfinavir resistance between 1998 and 2004.[8] As most women included in this analysis started antiretroviral therapy before WITS enrolment, pre-treatment resistance data was only available from one third of women. Based on this limited number of subjects, we did not observe an association between pre-existing resistance and the postpartum selection of M184V or K103N.

We did not evaluate treatment adherence; therefore we cannot rule out an association between suboptimal adherence and the observed rates of postpartum resistance. Ethnicity and hard drug or alcohol consumption have been previously associated with lower adherence to antiretroviral therapy and worse virological outcomes,[29-31] however, these factors were not associated with postpartum resistance in this study.

Finally, altered drug pharmacokinetics due to physiological changes occurring in women during pregnancy could have favored the existence of suboptimal drug levels during pregnancy or prolonged drug elimination in the postpartum period. Exposure to most PIs, including nelfinavir, is reduced in HIV-1-infected women during pregnancy due to increased intestinal and/or hepatic CYP3A activity. [32-36] Pregnant women, as well, have increased nevirapine clearance and lower plasma concentrations than non-pregnant women, although plasma levels are largely influenced by body weight [37]. Chaix et al[38] found that selection of nevirapine-resistance strongly correlated with higher median nevirapine plasma concentration. Prolonged nevirapine elimination after delivery in subjects with higher plasma levels could have allowed viral replication in the presence of suboptimal nevirapine levels after delivery.

In concordance with previous studies,[8,17,39] allele-specific PCR increased the frequency of detection of key resistance mutations relative to population sequencing of plasma viruses. Several studies have shown that pre-treatment detection of minority NNRTI-resistant variants more than triples the risk of virological failure to subsequent NNRTI-based therapy.[17,40] Therefore, the results of this study have important clinical implications for women receiving PLAT during pregnancy. It is well established that women selecting lamivudine, emtricitabine

or NNRTI-resistant mutants during PLAT are at a higher risk of failing subsequent NNRTI-based antiretroviral therapy, particularly if treatment is started within 6 to 12 months after delivery.[18,19]

Whereas ASPCR is several orders of magnitude more sensitive than viral population sequencing, it has a number of limitations that currently prevent its routine clinical application, i.e.: a) ASPCR interrogates only one codon per experiment; b) proportion measurements might be biased in the presence of polymorphisms at primer sites, and c) minority variant cut-offs that predict antiretroviral therapy outcomes with high sensitivity and specificity have not been established.

In spite of the limited numbers in some of the categories assessed and the limited pre-treatment resistance data available, our findings suggest that triple-drug therapy should be the preferred MTCT prevention approach to preserve future treatment options for mothers. When possible, antiretroviral regimens to prevent MTCT should include drugs with high genetic barrier. Nelfinavir-based therapy is no longer a preferred regimen for MTCT,[41] but the findings of this study likely apply to other PIs.[27] In women treated with nevirapine-based regimens the optimal timing of nevirapine interruption and length of continuation of other concomitant agents merits further inquiry to avoid active viral replication in the presence of suboptimal nevirapine levels. All efforts should be undertaken to ensure optimal adherence to antiretroviral therapy during pregnancy. Lastly, given that resistance mutations selected during pregnancy will wane after PLAT interruption, performing postpartum genotypic resistance testing within 1 to 2 months after delivery would be highly informative for designing future treatment regimens for women exposed to PLAT and may be useful in guiding the choice of antiretroviral regimen postpartum.

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Table 1**Subject characteristics**

Number of subjects evaluated	146
Age (Mean $\pm$ SD)	26.7 $\pm$ 5.2
Ethnicity, n (%)	
White	4 (2.7)
Black	84 (57.5)
Hispanic	52 (35.6)
Other/unknown	6 (4.1)
CDC category, n (%)	
A	124 (84.9)
B	19 (13.0)
C	3 (2.1)
Ever used hard drug before delivery, n (%)	
Yes	40 (27.4)
No	105 (71.9)
Unknown	1 (0.7)
Alcoholism, n (%)	
Yes	42 (28.8)
No	101 (69.2)
Unknown	3 (2.0)
First available CD4+ cell count during pregnancy, n (%)	
<200 cells/mm ³	9 (6.2)
200-350 cells/mm ³	33 (22.6)
>350 cells/mm ³	100 (68.5)
Unknown	4 (2.7)
Postpartum CD4+ cell counts (median, Q1-Q3)	
Absolute (cells/mm ³)	575 (397-767)
Percent (%)	29 (22-34)
HIV-1 RNA during PLAT, n (%)	
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 (2.1)
Postpartum HIV-1 RNA copies (median, Q1-Q3) (copies/mL)	4780 (1352- 18121)
Timing of blood sample collection (months since delivery, median, (Q1-Q3))	
Overall, n=146	2.2 (1.9-4.6)
Samples collected at the 2-month postpartum visit, n=111	2.0 (2.5-1.8)
Samples collected at the 6-month postpartum visit, n=35	6.2 (5.9-6.9)
Samples tested, n (%)	146 (100%)
Samples with no amplification, n (%)	32 (21.9%)
Initial PLAT, n (%) ^b	

AZT-3TC	43 (29.4)
AZT-3TC-Nelfinavir	93 (63.7)
AZT-3TC-Nevirapine	10 (6.8)
Length of exposure to AZT (days, up to delivery) (mean $\pm$ SD) [range]	122.5 $\pm$ 61.3 [2 , 309]
Length of exposure to AZT-3TC (days, up to delivery) (mean $\pm$ SD) [range]	118.2 $\pm$ 60.0 [1 , 270]
Nelfinavir exposure during PLAT, n (%)	
Yes	111 (76.0)
No	35 (24.0)
Length of exposure to Nelfinavir ^c (days, up to delivery) (mean $\pm$ SD) [range]	109.9 $\pm$ 58.9 [1 , 270]
Nevirapine exposure during PLAT, n (%)	
Yes	12 (8.2)
No	134 (91.8)
Length of exposure to Nevirapine ^d (days, up to delivery) (mean $\pm$ SD) [range]	68.1 $\pm$ 61.3 [1 , 177]

^a PLAT, pregnancy-limited antiretroviral therapy; AZT, zidovudine; 3TC, lamivudine

^b Refers to initial treatment; some subjects changed from dual-PLAT to triple-PLAT, and vice-versa.

^c For 111 exposed subjects

^d For 12 exposed subjects

Table 2Postpartum Rates of Resistance to Nucleoside Reverse Transcriptase Inhibitors.^{a,b}

Resistance Mutation	Overall	Women exposed to dual-PLAT through delivery	Women receiving any triple-PLAT during pregnancy	Fisher's exact test, p-value
Population-based sequencing	n=114 (% , CI)	n=20 (% , CI)	n=94 (% , CI)	
M41L	2 (1.8%, 0.2%-6.2%)	1 (5.0%, 0.1%-24.9%)	1 (1.1%, 0.03%-5.8%)	0.321
D67N	2 (1.8%, 0.2%-6.2%)	1 (5.0%, 0.1%-24.9%)	1 (1.1%, 0.03%-5.8%)	0.321
K70R	3 (2.6%, 0.6%-7.5%)	2 (10%, 1.2%-31.7%)	1 (1.1%, 0.03%-5.8%)	0.079
M184V/I	40 (35.1%, 26.4%-44.6%)	13 (65.0%, 40.8%-84.6%)	27 (28.7%, 19.9%-39.0%)	0.004
L210F	1 (0.9%, 0.02%-4.8%)	0 (0%, N/A)	1 (1.1%, 0.03%-5.8%)	1.000
T215Y	1 (0.9%, 0.02%-4.8%)	1 (5.0%, 0.1%-24.9%)	0 (0%, N/A)	0.175
K219Q	1 (0.9%, 0.02%-4.8%)	0 (0%, N/A)	1 (1.1%, 0.03%-5.8%)	1.000
Allele-specific PCR ^c	n=111	n=20	n=91	
M184V	66 (59.5%, 50.3%-68.6%)	19 (95%, 75.1%-99.9%)	47 (51.6%, 40.9%-62.3%)	<0.001

^a Mutation frequencies and rates correspond to women with available genotypic resistance data^b CI, 95% confidence intervals, Fisher's exact test.^c Three specimens with population-based sequencing available did not amplify by ASPCR.

Table 3

Factors associated with selection of the M184V and the K103N mutation during pregnancy-limited antiretroviral therapy. Univariate analysis.

Characteristic	M184V			K103N		
	OR	CI	p-value	OR	CI	p-value
HIV RNA viral load during PLAT^a						
All VLs >400 vs. all VLs < 400	2.65	1.02-6.87	0.05	1.39	0.34-5.63	0.64
Mixed < 400 and > 400 vs. all VLs < 400	2.57	0.95-6.96	0.06	0.72	0.13-3.99	0.71
Resistance mutations before PLAT^b						
Yes vs. No	0.22	0.03-1.74	0.15	N/A	N/A	1.00
First available CD4 count during pregnancy (cells/mm³)						
< 200 vs. > 350	3.73	0.43-32.66	0.23	1.64	0.17-15.67	0.67
200 – 350 vs. > 350	0.58	0.24-1.41	0.23	0.21	0.29-5.15	0.77
Ever used hard drug before delivery^c						
Yes vs. No	3.39	1.31-8.76	0.01	2.20	0.62-7.80	0.22
Alcoholism^d						
Yes vs. No	1.20	0.52-2.76	0.66	0.85	0.21-3.41	0.81
Ethnicity						
White vs. Black	0.77	0.10-5.83	0.80	3.87	0.34-44.39	0.28
Hispanic vs. Black	1.21	0.54-2.69	0.65	1.19	0.30-4.71	0.80
Age at delivery (per additional year)	1.03	0.96-1.11	0.42	1.05	0.94-1.19	0.39
ART exposure during pregnancy						
Triple therapy (with NFV or NVP) vs. Dual therapy only	0.06	0.007-0.44	0.006	0.95	0.19-4.79	0.95
Triple therapy with NFV vs. Dual therapy only	0.06	0.008-0.46	0.007	0.68	0.13-3.62	0.65
Triple therapy with NVP vs. Dual therapy only	0.04	0.003-0.48	0.01	6.75	0.83-54.66	0.07
NFV vs. No NFV	0.25	0.09-0.72	0.01	0.33	0.09-1.17	0.09
NVP vs. No NVP	0.38	0.09-1.68	0.20	7.35	1.48-36.50	0.01
Length of NFV exposure (per additional month)	0.95	0.80-1.13	0.59	0.91	0.68-1.23	0.55
Length of NVP exposure (per additional month)	0.68	0.38-1.23	0.20	1.06	0.50-2.22	0.88
Length of AZT/3TC combination (per additional month)	1.21	0.98-1.48	0.07	1.42	1.04-1.94	0.03
Length of AZT exposure (per additional month)	1.25	1.01-1.54	0.04	1.40	1.02-1.91	0.04

Characteristic	M184V			K103N		
	OR	CI	p-value	OR	CI	p-value
Length of 3TC exposure (per additional month)	1.21	0.98-1.48	0.07	1.42	1.04-1.94	0.03

^aPLAT, pregnancy-limited antiretroviral therapy; AZT, zidovudine; 3TC, lamivudine; NFV, nelfinavir; NVP, nevirapine

^bAvailable from 25 out of 114 women with postpartum resistance data available.

^cIncludes crack, cocaine, heroin, methadone, and any intravenous drug.

^dDefined as “Ever got drunk or have been admitted to alcohol detoxification center before delivery”

Table 4

Factors associated with selection of the M184V and the K103N mutation during pregnancy-limited antiretroviral therapy. Multivariate analysis.^a

Characteristic	M184V			K103N		
	OR	CI	p-value	OR	CI	p-value
ART exposure during pregnancy						
Triple therapy (with NFV or NVP) vs. Dual therapy only	19.64	2.47-156.25	<0.001	-	-	-
Length of AZT (per additional month)	1.29	1.03-1.63	0.03	-	-	-
NVP vs. No NVP	-	-	-	9.75	1.62-58.84	0.01
Length of AZT/3TC combination (per additional month)	-	-	-	1.46	1.05-2.02	0.02

^aThe multivariate analysis only included characteristics that achieved statistical significance in the univariate analysis.