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Addition of 7 Days of Zidovudine plus Lamivudine to Peripartum Single-Dose Nevirapine Effectively Reduces Nevirapine Resistance Postpartum in HIV-Infected Mothers in Malawi

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Abstract

Background—We assessed whether 7 days of zidovudine+lamivudine postpartum with single-dose nevirapine at labor decreases nevirapine resistance in HIV-infected women in Malawi.

Methods—HIV-infected pregnant women receiving intrapartum single-dose nevirapine and 7 days of zidovudine+lamivudine (n=132), and women receiving intrapartum single-dose nevirapine alone (n=66) were followed from an antenatal visit through 6 weeks postpartum. Plasma specimens at 2 and 6 weeks postpartum were tested for genotypic resistance to nevirapine by population sequencing and sensitive real-time PCR. Poisson regression was used to determine predictors of postpartum nevirapine resistance.

Results—Median HIV RNA was similar at entry (4.27 log vs. 4.35 log, p=0.87), differed at 2 weeks postpartum (2.67 log vs. 3.58 log, p<0.0001), but not at 6 weeks postpartum (4.49 log vs. 4.40 log, p=0.79), between single-dose nevirapine/zidovudine+lamivudine and single-dose nevirapine groups, respectively. Nevirapine resistance, measured by population sequencing and sensitive real-time PCR, was significantly less common in those receiving single-dose nevirapine/zidovudine+lamivudine compared to single-dose nevirapine, respectively, at 2 weeks (10% (4/40) vs. 74% (31/42), p<0.0001) and 6 weeks postpartum [10% (11/115) vs. 64% (41/64), p<0.0001; adjusted relative risk=0.18, 95% confidence interval (0.10–0.34)].

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Preliminary results on 6 week plasma specimens were presented at the Conference on Retroviral and Opportunistic Infections, February 2009, in Montreal, Quebec, Canada (Abstract 958b).

Conclusions—The significant decrease in nevirapine resistance conferred by one week of zidovudine+lamivudine should help policymakers optimize peripartum HIV prophylaxis recommendations.

INTRODUCTION

Providing a single-dose of nevirapine, a non-nucleoside reverse transcriptase inhibitor (NNRTI), to HIV-infected women during labor and to their infants within 72 hours of birth greatly reduces perinatal HIV transmission 1›2. However, nevirapine's low threshold to resistance and long half-life allow viruses with NNRTI resistance mutations to emerge 3⁻5. Importantly, a single mutation may confer resistance to the two most commonly used NNRTIs, nevirapine and efavirenz.

A recent meta-analysis found that at 6 to 8 weeks postpartum, nevirapine mutations may occur in 36% (95% confidence interval (CI) 23–51%) of women who receive single-dose nevirapine at labor and in 53% (95% CI 38%–67%) of their infants who become HIV-infected 6. Use of more sensitive assays has demonstrated an even higher proportion of women with resistant variants, which can persist at very low levels in a small proportion of women for up to 5 years 5. In the absence of continued use of antiretroviral drugs, these mutations fade but remain archived in the latent reservoir 7 and may increase the risk of failure to respond to subsequent therapy 8⁻13.

Efficacious, simple, cost-effective strategies to decrease the selection of nevirapine resistant variants are needed. We took advantage of two ongoing projects in Lilongwe, Malawi, to assess whether adding zidovudine+lamivudine for 7 days to single-dose nevirapine at labor would decrease postpartum emergence of nevirapine viral resistance in HIV-infected women and their infants.

METHODS

Women attending antenatal clinics in Lilongwe are tested for HIV infection at their first antenatal visit through a program to reduce perinatal HIV transmission administered by the University of North Carolina (UNC Project), together with the Malawi Ministry of Health. Women found to be HIV-1 infected who have a CD4 cell count ≥ 250 cells/mm^3 are offered single-dose nevirapine14. Women with < 250 cells/mm^3 are started on combination antiretroviral therapy (ART) 15.

The Breastfeeding, Antiretrovirals, Nutrition (BAN) Study (www.thebanstudy.org), conducted in Lilongwe by UNC Project and sponsored by the U.S. Centers for Disease Control and Prevention (CDC), is a randomized clinical trial to assess the benefit and safety of antiretroviral medications given to infants or their mothers during breastfeeding (ClinicalTrials.gov Identifier: NCT00164736) 16. From April, 2004 to September, 2008, HIV-1 infected women who met eligibility criteria were offered participation in the BAN study as an alternative to the standard regimen. All women and infants in the BAN study received single-dose nevirapine at labor and an additional 7 days of twice daily zidovudine +lamivudine. Mother-infant pairs were randomized to receive 28 weeks of maternal or infant daily antiretroviral prophylaxis or no ART during the breastfeeding period.

Women were eligible for the BAN study if they met the following criteria at enrollment: HIV-1 infected, ≤30 weeks gestation, ≥18 years of age (≥14 years of age if married), hemoglobin >7 g/dL, CD4 lymphocyte count ≥ 200 cells/mm^3 (later raised to 250 cells/mm^3), no prior history of antiretroviral use, normal liver function tests (alanine

aminotransferase <2.5 times the upper limit of normal), and no serious complications of pregnancy.

Based on sample size calculations, we selected 132 BAN study participants for the current study from women who were randomized to the arm in which neither mother nor child received antiretrovirals after the 7 days of zidovudine+lamivudine (referred to hereafter as the nevirapine/zidovudine+lamivudine group). The first 132 consecutive women with available specimens were included, and women with HIV-infected infants were over-sampled. The comparison group in the current analysis (the nevirapine group) consisted of 110 consecutively presenting HIV-infected pregnant women receiving single-dose nevirapine through the UNC Project public program at a Lilongwe clinic not participating in the BAN study. Eligibility for the nevirapine group was similar to that in the BAN Study in terms of age, hemoglobin level, CD4 count, history of ARV use and liver function. Study visits relevant for this analysis occurred approximately one week after the first antenatal clinic visit, 2 weeks after the first antenatal visit, at delivery, and at 2 and 6 weeks postpartum. All women gave written informed consent for study participation.

All mothers' antenatal plasma specimens were tested for HIV viral load, hemoglobin level, CD4 lymphocyte count, and liver function. Drug concentrations were measured at delivery to assess compliance with drug regimens. At 2 and 6 weeks postpartum, all plasma and breastmilk specimens were tested for nevirapine, zidovudine and lamivudine concentrations and resistance mutations using population sequencing and real time PCR. Mutations were classified based on information in the Stanford University Drug Resistance Database (http://hivdb.stanford.edu/index.html). If any resistance mutation was detected postpartum, an antenatal plasma specimen was tested for preexisting resistance mutations. All plasma from HIV-infected infants was tested for nevirapine, zidovudine and lamivudine resistance mutations using population sequencing and real time PCR.

All blood plasma specimens collected at 2 and 6 weeks postpartum were evaluated for drug resistance mutations through population sequencing and real-time PCR, regardless of viral load level. Population sequencing cannot generally detect mutant virus at levels lower than 20%, while real-time PCR can detect mutant virus at levels as low as 0.5 to 1.0%17. HIV-1 RNA was quantified from blood plasma using the Roche Amplicor Monitor v1.5 kit (Pleasanton, CA; LLQ = 400 copies/mL). HIV-1 RNA was quantified from 0.6 mL whole breastmilk pre-treated with 209ul Abbott RNA sample prep lysis buffer and 60ul Abbott Proteinase K (53°C incubation for 20 min) using the Abbott RealTime HIV-1 assay (Abbott Molecular, Des Plaines, IL; LLQ = 40 copies/ml). For population sequencing, the reverse transcriptase gene was amplified using an in-house RT-PCR assay from plasma or breastmilk viral RNA derived from Roche or Abbott RNA isolation, or after extraction using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). Primers for cDNA synthesis and nested PCR were designed from the subtype C consensus (http://www.hiv.lanl.gov) and the final PCR product corresponded to HXB2 nt 2564–3682. PCR products were sequenced using the ABI PRISM BigDye v1.1 Terminator Cycle Sequencing kit. Each product was sequenced with the second round PCR primers and two internal primers (5'-ATATATGGATGACTTGTATG-3' and 5'-TTGTCTGGTGTGGTAAATCC-3'). After alignment using Sequencher 4.5, resistance mutations were determined as described above. A few blood plasma RNAs were sequenced using TruGene HIV-1 Genotyping Kit (Siemens Healthcare Diagnostics, Tarrytown, NY). Sample integrity was checked by aligning the sequences using CLC Sequence Viewer (CLC bio A/S) and Treemaker (http://www.hiv.lanl.gov/content/sequence/TREEMAKER/TreeMaker.html).

For the sensitive real-time PCR resistance assays, HIV-1 genomic RNA from the blood plasma samples was extracted using Qiagen BioRobot M48 from 200 μL patient plasma. A

region of the HIV-1 template that included nucleotide 58 to nucleotide 777 of RT was RT-PCR amplified as previously described 17,18. This RT-PCR-amplified template was used in the real-time PCR testing for drug resistance mutations. For each mutation, we had established assay cut-offs (ΔCTs) equivalent to 0.5%–1% mutant virus, which are above the background reactivity observed when testing wild type virus, pre-nevirapine rollout samples from South Africa 17. The RNA samples were tested for five mutations: RT K103N, Y181C, and T215F/Y (ΔCT 10 cycles), V106M and M184V (ΔCT 8.5 cycles). Sensitive testing for codon 215 Y and F mutations was performed to rule out evidence of primary resistance because population sequence-detectible polymorphisms were present at that position. To verify samples positive for drug resistance mutations by real-time PCR, we analyzed a 575 bp region of RT (nt. 133–708 of RT) amplified from the primary RT-PCR of the specimen and also the mutation-positive amplicon derived from the real-time PCR test. All templates underwent double-strand chain-termination sequencing (Big Dye kit v1.1, Applied Biosystems). The real-time PCR amplicon sequences were evaluated for insertions and deletions to verify that the virus sequence was intact. We also examined for the presence of other mutations linked on the mutation-targeted amplicons generated by the real-time PCRs 18.

Drug concentrations in blood plasma and breastmilk were measured using validated high performance liquid chromatography (HPLC)/ultraviolet (UV) methods as previously described 19–21. For blood plasma, the method was validated over the range of 10–10,000 ng/ml for all analytes. Intra- and interday precision was within 5.1% and 5.6%, respectively, and intra- and interday accuracy (average percent deviation from nominal) was between 97% and 105%. For breastmilk, the method was validated over the range of 20 to 20,000 ng/mL. Intra- and interday precision was within 3.6% and 7.5%, respectively, and intra- and interday accuracy was within 99.8% and 98.7%, respectively. Drug exposure was calculated as area under the concentration-time curve (AUC) from delivery to 6 weeks postpartum using non-compartmental methods and the linear/log trapezoidal rule in WinNonlin® Pro version 4.0.1 (Pharsight Corp, Mountain View, CA, USA).

We compared characteristics between treatment groups using Pearson's chi-square and the Wilcoxon rank sum tests. Cumulative and type-specific nevirapine mutations assessed through population-sequencing and real-time PCR testing at 2 and 6 weeks postpartum in plasma and breastmilk were compared by treatment group. A relative risk and 95% confidence interval for nevirapine resistance comparing women receiving nevirapine/zidovudine+lamivudine to women receiving nevirapine alone was estimated in a modified multivariable Poisson regression model using robust error variances 22. Drug resistance refers to combined results of population sequencing and/or real-time PCR testing, unless otherwise stated. Unamplifiable specimens and those with viral load levels too low to detect minority variants were excluded from analyses (2 weeks postpartum: n=54 in sdNVP/ZDV+3TC group and n=19 in sdNVP group (p=0.001); 6 weeks postpartum: n=15 in sdNVP/ZDV+3TC group and n=2 in sdNVP group (p=0.05). Statistical analyses were performed using SAS version 9.1.

The current study was approved by the Malawi National Health Science Research Council and the institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention (ClinicalTrials.gov Identifier NCT00164762). The institutions' human experimentation guidelines were followed.

RESULTS

Demographic and Obstetric Characteristics

Of the 486 women in the sdNVP+ZDV/3TC arm of the BAN study who had completed 6 weeks postpartum as of July 2007, the first 132 (27%) with available specimens were included in the analysis. Demographic characteristics, CD4 count, hemoglobin levels, and obstetric complications did not differ between those included and excluded. Of the 110 women enrolled and eligible for the nevirapine group, 66 (60%) had the necessary specimens available and were included in analyses. No significant differences in loss to follow-up rates between BAN women and women in the public PMTCT program were detected at 2 (p=0.34) or 6 (p=0.50) weeks postpartum.

Maternal age, gestational age at entry, parity, viral load, CD4 count, and hemoglobin did not differ between groups (Table 1). The median BMI at 2 weeks postpartum was lower (22.7 kg/m^2 vs. 24.5 kg/m^2, p<0.001) and the prevalence of electricity in the home was higher (23.8% vs. 3.2%, p<0.001) in the nevirapine/zidovudine+lamivudine group compared to the nevirapine group, respectively.

Obstetric outcomes did not differ between groups (Table 1). There were no statistically significant group differences for self-reported nevirapine ingestion, where the women took nevirapine (data not shown), percent redosed, or time between ingestion and delivery. Among women taking zidovudine+lamivudine, 89.7% reported taking all doses.

Nevirapine compliance, as measured at delivery by self-report (97% and 100%, p=0.15) (Table 1), median concentration (1301.6 and 1319.4, p=0.999) and percent of women with detectable nevirapine concentrations (90% and 93%, p=0.47) (Figure 1) did not differ between groups.

Maternal drug resistance in plasma

At two weeks postpartum, median viral load (2.7 and 3.6, p<0.0001) and percent of women with detectable nevirapine levels (47% and 82%, p<0.0001) were lower in the nevirapine/zidovudine+lamivudine compared to the nevirapine group, respectively, (MFigure 1). No differences between groups were detected in antenatal or 6 week postpartum viral load or percent with detectable nevirapine concentrations.

At two weeks postpartum, 10% (4/40) of women in the nevirapine/zidovudine+lamivudine group and 74% (31/42) of women in the nevirapine group had nevirapine resistance mutations detected through population sequencing or real-time PCR (p<0.0001) (Figure 2). At 6 weeks postpartum, 10% (4/40) of women in the nevirapine/zidovudine+lamivudine group had nevirapine resistance mutations detected through population sequencing or real-time PCR, compared to 64% (41/64) of women in the nevirapine group (p<0.0001). Two women in the nevirapine/zidovudine+lamivudine group had nevirapine resistance mutations detected in their antenatal plasma sample and were excluded from analyses.

In the multivariable model predicting nevirapine viral resistance at 6 weeks postpartum detected through population sequencing or real-time PCR, the addition of zidovudine +lamivudine to single-dose nevirapine was associated with significant reductions in the risk of viral resistance to nevirapine in plasma (RR=0.18 95% CI: 0.10–0.34) (Table 2). A higher nevirapine AUC was also associated with increased risk of nevirapine resistance, while an association between BMI and nevirapine resistance was of borderline significance. In a similar model examining nevirapine resistance at 2 weeks postpartum, only drug regimen predicted viral resistance to nevirapine (RR=0.14, 95% CI 0.05–0.35) (data not shown). Relative risk estimates did not differ when specimens that could not be sequenced were

considered wild type, nor when specimens with viral load levels <2000 cp/ml were excluded.

The most prevalent nevirapine resistance mutations detected through population sequencing or real-time PCR in maternal plasma were K103N/S, V106A/M, Y181C/I and Y188C/L/H (Table 3). At 6 weeks postpartum, 2 women in the nevirapine/zidovudine+lamivudine group and 1 woman in the nevirapine group (the latter who, to our knowledge, had not received any lamivudine) had newly-emerged lamivudine mutations (M184V).

Forty women in the nevirapine/zidovudine+lamivudine group and 42 women in the nevirapine group had testable plasma specimens at both 2 and 6 weeks postpartum. Comparing the nevirapine/zidovudine+lamivudine group to the nevirapine group, respectively, 10% and 12% had detectable nevirapine resistance at 2 weeks postpartum only, 12.5% and 7% had nevirapine resistance mutations at 6 weeks postpartum only; and 0% and 70% had nevirapine resistance mutations at both 2 and 6 weeks postpartum.

Maternal drug resistance in breastmilk

Seven women had sufficient viral loads in breastmilk to test for viral resistance to nevirapine. In the nevirapine/zidovudine+lamivudine group, 0/4 women had nevirapine resistance mutations in breastmilk. In the nevirapine group, 3/3 women had at least one nevirapine resistance mutation.

Infant Drug Resistance

Mother to child transmission of HIV at 6 weeks in the 515 infants in the BAN study arm receiving nevirapine/zidovudine+lamivudine and no further ART [7.1% (95% CI: 5.1–9.1%)] was approximately half that of the 66 infants in the Lilongwe program who received nevirapine alone [13.9% (6.5–24.7%)] (p=0.06). Among 14 HIV-infected infants in the current study, one of six (17%) who received nevirapine/zidovudine+lamivudine and three of eight (38%) who received nevirapine alone had nevirapine resistance mutations.

The detection of maternal nevirapine resistance mutations in plasma or breastmilk did not always correspond with detection of nevirapine resistance mutations in the 14 HIV-infected infants. In 4 infants, nevirapine resistance mutations were detected that were not found in the mother. In another 4 infants, nevirapine resistance mutations were detected in the mother, but not in the infant. In the 6 remaining infants, three from each group, no nevirapine resistance mutations were detected in the mother or infant.

Maternal Drug Concentrations

At delivery and 6 weeks postpartum, the percentages of women with detectable nevirapine concentration in plasma and breastmilk did not vary by drug regimen, antenatal CD4 count, or antenatal viral load. However, at 2 weeks postpartum, a lower percentage of women in the nevirapine/zidovudine+lamivudine group, compared to the nevirapine group, respectively, had detectable nevirapine in plasma (46.9% and 82.0%, p<0.0001, Figure 1) and breastmilk (78.9% and 90.5%, p<0.05). Additionally, among women taking nevirapine/zidovudine+lamivudine, we found no association between zidovudine or lamivudine concentrations at labor and delivery and nevirapine concentration or emergence of nevirapine viral resistance mutations at 2 or 6 weeks postpartum.

DISCUSSION

The addition of seven days of postpartum zidovudine+lamivudine after single-dose nevirapine was independently associated with an over 80% reduction in women's risk of

nevirapine resistance mutations at 2 and 6 weeks postpartum. Additionally, the 7-day tail of zidovudine+lamivudine for mothers and infants was associated with a nearly 50% reduction in vertical HIV transmission at 6 weeks. The finding on vertical transmission of HIV should be replicated, since our results did not reach statistical significance, we were unable to examine separately in utero, intrapartum, and postnatal transmission, and another study found no decrease in perinatal transmission of HIV with the same drug regimen 23. We also found that in infants who become HIV-infected, nevirapine resistance mutations occur independently of their mothers, as has also been shown previously24. In addition, mutations were more prevalent in maternal plasma than breastmilk, suggesting that breast milk is a protected reservoir for virus 25,26.

The mechanism by which zidovudine+lamivudine reduces nevirapine viral resistance is unknown, but may be due to its suppressive effects on viral replication. We found lower levels of viral load at 2 weeks postpartum among women who took zidovudine+lamivudine, compared to those who did not. Of interest, we also found lower nevirapine concentrations at 2 weeks postpartum among women who took zidovudine+lamivudine. No differences were seen in nevirapine compliance between groups. Zidovudine and lamivudine are not known to have a pharmacologic effect on nevirapine concentration. Whether the finding of lower nevirapine concentration among women taking zidovudine+lamivudine may be due to chance or unknown factors is unclear.

One previous study assessed nevirapine resistance with the addition of 4 and 7 days of zidovudine+lamivudine to single-dose nevirapine at labor compared to single-dose nevirapine alone among South African women and their infants with HIV-1 subtype C 23. By 6 weeks postpartum, nevirapine resistance, assessed through population sequencing, was reduced from 59% in the single-dose nevirapine arm to 12% and 7% in the 4- and 7-day zidovudine+lamivudine arms, respectively. Of 11 infants with nevirapine resistance mutations, seven were from the single-dose nevirapine arm and four were from the 4-day zidovudine+lamivudine arm 23. However, this study did not use real-time PCR testing and prevalence of resistance mutations may be underestimated; and no information was provided on drug concentrations over time, limiting inference into biological mechanism. In addition, the study excluded women with viral loads <2,000 cp/ml. In the current study, specimens from all HIV-infected women and infants were tested for drug resistance and five women with viral load levels <2000 cp/ml at enrollment had detectable drug resistance mutations. Additionally, of the 148 resistance mutations detected in this study, over 54% were detected through real-time PCR testing only.

Four additional studies assessed the effect of different postpartum short-course drug regimens on nevirapine resistance after antepartum short-course zidovudine and single-dose nevirapine at labor and delivery 27–30. All found a reduction in viral resistance to nevirapine between 2 and 6 weeks postpartum with postpartum short-course ART. However, these studies were conducted among women who used antepartum short-course zidovudine, which may not be available in many African settings, and three were among women with HIV subtypes different from those in much of sub-Saharan Africa 27,29,30. These factors limit generalizability to HIV-infected women in the least developed countries of sub-Saharan Africa. The current analysis is among women with HIV-1 subtype C, which has been reported to have a greater propensity for selecting nevirapine resistance mutations than other subtypes 3.

The current study is not a randomized clinical trial and some differences between study groups existed. However, no measured variables substantially confounded the strong association between drug regimen and nevirapine resistance.

Several studies show that use of single-dose nevirapine increases the risk of virologic failure in women and infants when nevirapine-based ART is begun within 6 to 24 months of receiving single-dose nevirapine [8,9,11,13,31]. However, this report shows that the addition of 7-days of zidovudine+lamivudine postpartum to single-dose nevirapine at labor and delivery significantly decreased emergence of viral resistance to nevirapine and may have implications for the success of future NNRTI-based ART. Use of this short-course therapy should be considered in areas where longer, more intense antenatal antiretroviral prophylaxis is not feasible.

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Appendix

The **BAN Study Team** at University of North Carolina Chapel Hill, U.S. Centers for Disease Control and Prevention, Atlanta, and UNC Project in Lilongwe including: Linda Adair, Yusuf Ahmed, Mounir Ait-Khaled, Sandra Albrecht, Shrikant Bangdiwala, Ronald Bayer, Margaret Bentley, Brian Bramson, Emily Bobrow, Nicola Boyle, Sal Butera, Charles Chasela, Charity Chavula, Joseph Chimerang'ambe, Maggie Chigwenembe, Maria Chikasema, Norah Chikhungu, David Chilongozi, Grace Chiudzu, Lenesi Chome, Anne Cole, Amanda Corbett, Amy Corneli, Ann Duerr, Henry Eliya, Sascha Ellington, Joseph Eron, Sherry Farr, Yvonne Owens Ferguson, Susan Fiscus, Shannon Galvin, Laura Guay, Chad Heilig, Irving Hoffman, Elizabeth Hooten, Mina Hosseinipour, Michael Hudgens, Stacy Hurst, Lisa Hyde, Denise Jamieson, George Joaki (deceased), David Jones, Zebrone Kacheche, Esmie Kamanga, Gift Kamanga, Coxcilly Kampani, Portia Kamthunzi, Deborah Kamwendo, Cecilia Kanyama, Angela Kashuba, Damson Kathyola, Dumbani Kayira, Peter Kazembe, Caroline King, Rodney Knight, Athena P. Kourtis, Robert Krysiak, Jacob Kumwenda, Edde Loeliger, Misheck Luhanga, Victor Madhlopa, Maganizo Majawa, Alice Maida, Cheryl Marcus, Francis Martinson, Navdeep Thoofer, Chrissie Matiki (deceased), Douglas Mayers, Isabel Mayuni, Marita McDonough, Joyce Meme, Ceppie Merry, Khama Mita, Chimwemwe Mkomawanthu, Gertrude Mndala, Ibrahim Mndala, Agnes Moses, Albans Msika, Wezi Msungama, Beatrice Mtimuni, Jane Muita, Noel Mumba, Bonface Musis, Charles Mwansambo, Gerald Mwapasa, Jacqueline Nkhoma, Richard Pendame, Ellen Piwoz, Byron Raines, Zane Ramdas, John Rublein, Mairin Ryan, Ian Sanne, Christopher Sellers, Diane Shugars, Dorothy Sichali, Wendy Snowden, Alice Soko, Allison Spensley, Jean-Marc Steens, Gerald Tegha, Martin Tembo, Roshan Thomas, Hsiao-Chuan Tien, Beth Tohill, Charles van der Horst, Esther Waalberg, Jeffrey Wiener, Cathy Wilfert, Patricia Wiyo, Innocent Zgambo, Chifundo Zimba. Finally and most especially, all the women and infants that have agreed to participate in the study.

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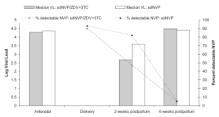

Figure 1. Median viral load† and percent of women with detectable nevirapine‡ (NVP) by drug regimen among HIV-infected mothers*, Lilongwe, Malawi

*Among women with testable plasma specimens at 2 weeks (sdNVP: n=45; sdNVP/ZDV +3TC: n=53) and 6 weeks postpartum (sdNVP: n=65; sdNVP/ZDV+3TC: n=120);

†Chi square p-value for viral load: antenatal: p=0.87; 2 weeks postpartum: p<0.0001, 6 weeks postpartum: p=0.79

‡Chi square p-value for NVP concentration: delivery: p=0.47; 2 weeks postpartum: p<0.0001; 6 weeks postpartum: p=0.79

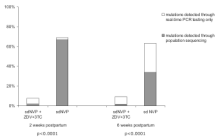

Figure 2. Prevalence of NVP resistance mutations by drug regimen among HIV-infected mothers*, Lilongwe, Malawi

*Among women with testable plasma specimens at 2 weeks (sdNVP: n=45; sdNVP/ZDV +3TC: n=53) and 6 weeks postpartum (sdNVP: n=65; sdNVP/ZDV+3TC: n=120) and no NVP resistance at an antenatal visit

Table 1

Baseline Characteristics of Enrolled Women

	Single-dose nevirapine/ zidovudine+lamivudine		**Single-dose nevirapine alone**		**p-value***
Total	N=132		N=66		
	N	n (%) Median (range)	N	n (%) Median (range)	
Age (years)	128	26 (17 – 44)	62	25 (19 – 37)	0.45
Gestational age at enrollment (weeks)	69	27 (14 – 40)	35	27 (16 – 37)	0.94
Parity (births)	131	3 (1 – 11)	59	3 (1 – 6)	0.43
BMI 2 weeks postpartum (kg/m^2):	128	22.7 (17.9 – 31.8)	63	24.5 (16.9 – 33.3)	<0.001
< 18.5		2 (1.6%)		1 (1.6%)	0.01
18.5 – 24.9		99 (77.3%)		35 (55.6%)	
25 – 29.9		22 (17.2%)		23 (36.5%)	
≥ 30		5 (3.9%)		4 (6.4%)	
Marital status	131		63		0.08
Married		117 (89.3%)		61 (96.8%)	
Other†		14 (10.7%)		2 (3.2%)	
Maternal education	131		63		0.46
None		16 (12.2%)		11 (17.5%)	
Primary		75 (57.3%)		37 (58.7%)	
Secondary		40 (30.5%)		15 (23.8%)	
Electricity in home	101		63		< 0.001
Yes		24 (23.8%)		2 (3.2%)	
No		77 (76.2%)		61 (96.8%)	
Pregnancy Outcome					
Singleton birth	129	128 (99.2%)	65	64 (98.5%)	1.0
Twin birth		1 (0.8%)		1 (1.5%)	

	Single-dose nevirapine/ zidovudine+lamivudine		Single-dose nevirapine alone		p-value*
Vaginal birth	130		66		0.72
Yes		123 (94.6%)		64 (97.0%)	
No		7 (5.4%)		2 (3.0%)	
Obstetric/delivery complication	130		66		0.75
Yes		9 (6.9%)		3 (4.5%)	
No		121 (93.1%)		63 (95.5%)	
Infant outcome					
Infant birth Weight	132	3050 (2200 – 4300)	66	3000 (1700 – 4500)	0.87
Infant ever breastfed	117		66		0.36
Yes		117 (100%)		65 (98.5%)	
No		0		1 (1.5%)	
Antenatal laboratory measures					
Viral load	130	19,575 (400 – 479,286)	66	22,519 (313 – 390,421)	0.94
CD4 count (cells per μL)	132	437 (210 – 2,000)	63	419 (202 – 1,329)	0.72
200 – 350		36 (27.3%)		20 (31.8%)	0.61
351 – 500		47 (35.6%)		18 (28.6%)	
> 500		49 (37.1%)		25 (39.7%)	
Hemoglobin (g/dL)	132	10.9 (7.9 – 13.3)	63	10.6 (8.2 – 13.1)	0.29
Grade 0		94 (71.2%)		44 (69.8%)	0.94
Grade 1		35 (26.5%)		18 (28.6%)	
Grade 2		3 (2.3%)		1 (1.6%)	
Drug characteristics					
Nevirapine ingestion	130		66		0.15
Yes		126 (96.9%)		66 (100%)	
No		4 (3.1%)		0 (0%)	

	Single-dose nevirapine/ zidovudine+lamivudine		Single-dose nevirapine alone		p-value*
Redosed with nevirapine	131		66		0.40
Yes		3 (2.3%)		3 (4.6%)	
No		128 (97.7%)		63 (95.4%)	
Time between nevirapine ingestion and delivery‡ (hours)	121	6.9 (0.7 – 694)	65	6.4 (0.6 – 1095)	0.61
Zidovudine+lamivudine	126			N/A	
All doses taken		113 (89.7%)			
Some doses taken		13 (10.3%)			

*Wilcoxon rank-sum tests to compare medians; Pearson chi-square tests to compare frequencies

‡Six out of 7 women with values >80 hours were redosed with NVP

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Table 2

Predictors of HIV resistance* to nevirapine in plasma among HIV-infected mothers at 6 weeks' postpartum

	Unadjusted RR (95% CI)	Adjusted RR (95% CI)¶
Total		
Drug Regimen		
Single-dose nevirapine/ zidovudine+lamivudine	0.15 (0.08 – 0.27)	0.18 (0.10 – 0.34)
Single-dose nevirapine	1.0	1.0
Age	0.98 (0.93 – 1.02)	†
Log10 (Antenatal Viral load)	0.80 (0.56 – 1.15)	†
Antenatal CD4 count	0.999 (0.998 – 1.000)	†
Antenatal hemoglobin	0.89 (0.72 – 1.09)	†
Nevirapine concentration at delivery	1.000 (1.000 – 1.001)	†
Nevirapine concentration at 2 weeks postpartum	1.005 (1.002 – 1.007)	†
Nevirapine concentration at 6 weeks postpartum	1.002 (1.002 – 1.003)	†
Log (Nevirapine Area Under the Curve)	1.48 (1.11 – 1.97)	1.47 (1.16–1.87)
BMI (kg/m^2) at 2 weeks postpartum	1.17 (1.11 – 1.23)	1.06 (1.00 – 1.13)

RR=Relative risk; CI=confidence interval

*Detected through population-sequencing or real-time PCR

†Not included in multivariable model

¶Includes 153 women with no missing data; 98 in the nevirapine/zidovudine+lamivudine group and 55 in the nevirapine group

Table 3

Nevirapine and lamivudine resistance mutations in plasma of HIV-infected mothers

	Nevirapine/zidovudine+lamivudine				Nevirapine alone			
	2 weeks postpartum		6 weeks postpartum		2 weeks postpartum		6 weeks postpartum	
	Population-detected	Real-time PCR[†] only	Population-detected	Real-time PCR only	Population-detected	Real-time PCR only	Population-detected	Real-time PCR only
	n	n	n	n	n	n	n	n
L100I	0	ND	1	ND	0	ND	0	ND
K103N/S	0	0	0	5	9	5	13	17
V106A/M	0	1	0	6	5	8	1	17
V118C	0	ND	0	ND	1	ND	1	ND
Y181C/I	0	2	0	3	18	3	9	11
Y188C/L/H	1	ND	0	ND	4	ND	1	ND
G190A	0	ND	0	ND	0	ND	2	ND
M230L	0	ND	1	ND	0	ND	0	ND
M184V	0	0	0	2	0	0	0	1
Total	1	3	2	16	37	16	27	46

ND=not done

No Zidovudine resistance mutations detected