

Published in final edited form as:

Antivir Ther. 2008 ; 13(6): 799–807.

Highly Active Antiretroviral Therapy (HAART) versus Zidovudine/Nevirapine Effects on Early Breast Milk HIV-1 RNA: A Phase II Randomized Clinical Trial

Michael H. Chung, MD, MPH¹, James N. Kiarie, MBChB, MMed, MPH⁷, Barbra A. Richardson, PhD^{3,5}, Dara A. Lehman, MHS^{4,6}, Julie Overbaugh, PhD^{5,6}, John Kinuthia, MBChB, MMed⁷, Francis Njiri, BSc⁷, and Grace C. John-Stewart, MD, PhD^{1,2}

¹Department of Medicine, University of Washington, Seattle, USA

²Department of Epidemiology, University of Washington, Seattle, USA

³Department of Biostatistics, University of Washington, Seattle, USA

⁴Department of Molecular and Cellular Biology, University of Washington, Seattle, USA

⁵Divisions of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, USA

⁶Human Biology, Fred Hutchinson Cancer Research Center, Seattle, USA

⁷Department of Obstetrics and Gynaecology, University of Nairobi, Kenya

Abstract

Background—Defining the effect of antiretroviral regimens on breast milk HIV-1 levels is useful to inform the rational design of strategies to decrease perinatal HIV-1 transmission.

Methods—Pregnant HIV-1 seropositive women (CD4 >250 and <500 cells/mm³) electing to breastfeed in Nairobi, Kenya were randomized to HAART (zidovudine, lamivudine, and nevirapine) during pregnancy and 6 months postpartum or to short-course zidovudine plus single-dose nevirapine (ZDV/NVP). Breast milk samples were collected 2-3 times per week in the first month postpartum.

Findings—Between November 2003 and April 2006, 444 breast milk samples were collected from 58 randomized women during the first month after delivery. Between 3 and 14 days postpartum, women in the HAART and ZDV/NVP arms had a similar prevalence of undetectable breast milk HIV-1 RNA. From 15 to 28 days postpartum, women in the HAART arm had significantly lower levels of breast milk HIV-1 RNA than women randomized to ZDV/NVP (1.7 log₁₀ copies/ml (limit of detection) vs. > 2.10 log₁₀ copies/ml, P < 0.001). In contrast to breast milk HIV-1 RNA, suppression of plasma HIV-1 RNA during the neonatal period was consistently several log₁₀ greater in the HAART arm compared to the ZDV/NVP arm.

Conclusions—HAART resulted in lower breast milk HIV-1 RNA than ZDV/NVP, however, ZDV/NVP yielded comparable breast milk HIV-1 RNA levels in the first 2 weeks postpartum. Breast milk HIV-1 RNA remained suppressed in the ZDV/NVP arm despite increased plasma HIV-1 levels, which may reflect local drug-effects or compartmentalization.

Address correspondence and requests for reprints to: Dr. Michael Chung, 325 Ninth Ave, Box 359909, University of Washington, Seattle, Washington 98104, USA, Phone: 206-543-4278, Fax: 206-543-4818, mhchung@u.washington.edu.

None of the authors has a major conflict of interest in this study.

Keywords

breast milk; Africa; prevention of HIV-1 perinatal transmission; nevirapine; zidovudine; HAART

Introduction

Breast milk transmission of HIV-1 contributes to an estimated 30 – 50% of infant HIV-1 infections in Africa [1]. Avoidance of breastfeeding may not be a feasible option for HIV-infected mothers in resource-poor settings due to limited access to potable water, expense, and potential for increased infant mortality and compromised growth [2-4]. Administration of antiretroviral medications to mothers during lactation may be one way to decrease breast milk transmission of HIV-1 while retaining the benefits of breastfeeding.

Antiretrovirals may prevent breast milk HIV-1 transmission through suppression of HIV-1 in breast milk, by providing exposure prophylaxis to the infant, or via combination of these effects [5]. Decreasing perinatal transmission of HIV-1 by antiretrovirals may be accomplished by suppressing levels of HIV-1 RNA in breast milk, which are significantly correlated with vertical HIV-1 transmission risk [6,7]. Post-exposure prophylaxis has been demonstrated to decrease HIV-1 transmission significantly in infants receiving antiretroviral medications without maternal dosing [8,9], and could potentially occur following infant ingestion of antiretrovirals in breast milk [10].

Current World Health Organization (WHO) guidelines recommend combination short-course antiretroviral medications (zidovudine/nevirapine) to pregnant HIV-positive women unless they are severely immunosuppressed (CD4 count < 200 cells/mm³), at which point they should receive highly active antiretroviral therapy (HAART) [11]. The majority of pregnant HIV-1 infected women in high HIV-1 prevalence regions are not severely immunosuppressed, and therefore do not receive HAART. While some suggest HAART for all pregnant women [12], administering perinatal HAART may present challenges including consistent long-term adherence to medications and adverse effects. It is therefore important to determine the benefits of postpartum HAART versus the current recommended perinatal regimen in decreasing breast milk HIV-1 transmission.

We conducted a Phase II randomized clinical trial that compared the effect of antenatal and postpartum administration of HAART to antenatal zidovudine plus single-dose nevirapine (ZDV/NVP) on breast milk HIV-1 RNA levels in the first month postpartum and compared HIV-1 transmission among infants in the two arms at 12 months postpartum.

Materials and methods

Study Population

The institutional review boards (IRB) at the University of Washington, USA and Kenyatta National Hospital, Kenya approved the study protocol. This analysis focuses on comparison of serial HIV-1 RNA levels in plasma and breast milk in the early postpartum period. The pre-specified primary outcomes for the overall clinical trial included breast milk HIV-1 RNA, breast milk HIV-1 DNA, and HIV-1 specific immune responses in breast milk and in infants. The ClinicalTrials.gov identifier was NCT00167674.

The targeted sample size was 80 women, 40 in each trial arm, estimating undetectable breast milk HIV-1 RNA levels in the HAART arm and median breast milk HIV-1 RNA levels of 3.0 log₁₀ in women receiving ZDV/NVP. Pregnant women were recruited from the Mathare North City Council Clinic in Nairobi, Kenya. Women were offered HIV-1 serological

testing. HIV-1 seropositive women were educated on mother-to-child transmission of HIV-1 and the risks and benefits of breastfeeding versus formula feeding. Women who elected to breastfeed and were ≤ 32 weeks gestation were referred to the research clinic. Women with hemoglobin ≥ 8 g/dl and no previous exposure to antiretrovirals, who were ≥ 18 years, agreed to home visits, and resided in the clinic catchment area were eligible to enroll.

On January 19, 2005, the U.S. Food and Drug Administration released an advisory that recommended against prolonged nevirapine use by women with CD4 counts ≥ 250 cells/mm³ due to evidence of increased hepatotoxicity [13]. Further enrollment of mother-infant pairs was stopped at this point after preliminary analyses indicated the size of the cohort was adequate to detect significant differences in breast milk viral load between the two arms. Four women, who were enrolled in the study but not randomized to either arm, received routine ZDV/NVP per national guidelines and were excluded. One woman in the HAART arm had nevirapine replaced with nelfinavir 8 days after delivery and was included in the analysis.

Enrollment and Randomization

At enrollment, written informed consent was administered and CD4 cell count was determined. If the CD4 count was < 200 cells/mm³ or > 500 cells/mm³, the subject was ineligible for randomization and was referred respectively to a treatment center for free HAART or to the antenatal clinic for standard HIV-1 perinatal care. At 32 weeks gestation, blood was taken for CD4 cell count, HIV-1 RNA levels, liver function tests, urea and electrolytes, and complete blood count. A study physician performed a physical examination and obtained sociodemographic and risk behavior information. Peer counselors accompanied subjects to their homes and mapped home locations for breast milk collection visits.

Women were randomized at 34 weeks gestation to one of two arms. In the HAART arm, 300 mg of zidovudine (ZDV), 150 mg of lamivudine, and 200 mg nevirapine (NVP) was given twice daily from 34 weeks gestation until six months after delivery. In the ZDV/NVP arm, 300 mg of ZDV was given twice daily from 34 weeks gestation until labor then every 3 hours until delivery; 200 mg of NVP was given as a single oral dose at the onset of labor; and a single 2 mg/kg (6 mg if birthweight > 2.5 kg) oral dose of NVP suspension was administered to the infant within 72 hours of delivery.

Randomization was performed using computer-generated block randomization. Study investigators and participants were not blinded to the interventions. After randomization, participants were followed weekly in clinic until delivery, and those assigned to HAART had blood drawn to assess hepatic or hematologic toxicity at 35 and 37 weeks gestation.

Delivery, Breast Milk Collection, and Follow-up

At or within two days of delivery, maternal blood was drawn for CD4 cell count, HIV-1 RNA levels, liver function tests, and complete blood count. Infant blood was collected for plasma HIV-1 RNA and filter paper HIV-1 DNA testing.

Breast milk was collected through home visits 1 to 3 times per week up to 10 times over the first month. Peer counselors obtained 5-40 mls of breast milk by observing mothers manually express milk from a single breast into a sterile container. At each home visit, peer counselors gathered information on breastfeeding practices and the condition of the breast at the time of collection. Peer counselors were also instructed to obtain breast milk from the same breast for each mother and to support exclusive breastfeeding practices.

Mothers and their infants attended study clinic at 2 weeks and 1 month postpartum and then every 3 months after delivery until 12 months postpartum. At these visits mothers had blood and breast milk collected, and those assigned to HAART had liver function tests and complete blood counts checked through 6 months postpartum. Infants had blood collected at all of these visits.

Laboratory Methods

Breast milk samples were centrifuged, the lipid layer was discarded, and the supernatant was aspirated [14]. Cryopreserved breast milk supernatants were shipped to Seattle, Washington. HIV-1 RNA levels were measured by the Gen-Probe HIV-1 viral load assay (Gen-Probe Incorporated, San Diego, California, USA) [15,16]. The lower limit of detection for the Gen-Probe assay was 10 copies/assay [17]. One hundred μ l of breast milk supernatant or 50 μ l of maternal plasma was tested, and therefore the lower limit of detection of HIV-1 RNA in breast milk or plasma was defined to be 100 copies/ml or 200 copies/ml respectively. CD4 cell counts were determined using flow cytometry (FACScan, Becton Dickinson, Franklin Lakes, New Jersey, USA). Infant HIV-1 filter paper polymerase chain reaction (PCR) for HIV-1 DNA was conducted as previously described [18].

Statistical Methods

All analyses were intent-to-treat and were performed using SPSS version 14.0 (SPSS Inc, Chicago, IL) or S-Plus 2000 (Insightful Inc, Seattle, WA). Comparison of baseline characteristics for the randomization groups was done using the Mann-Whitney U test for continuous variables and Pearson's Chi-square test for binary variables. Viral load data were \log_{10} transformed. Breast milk HIV-1 RNA viral load levels below the lower limit of detection (100 copies/ml) were assigned a value at the midpoint between the lower limit of detection and zero (50 copies/ml). Analyses included grouping breast milk samples by weekly postpartum intervals. If multiple samples were available from a woman within a particular time interval, then the average of these samples was used in analyses. Comparisons of median \log_{10} HIV-1 RNA viral load across treatment arms were performed using the Mann-Whitney U test. Chi-square tests were used to compare the proportion of women with undetectable HIV-1 RNA levels in breast milk for the different time periods. Finally, graphical assessment of the differences in viral loads in the two groups was done using Loess curves.

Results

Maternal Population

Enrollment began on 3 November 2003 and ended on 11 March 2005. The last date of study follow-up was 20 April 2006. During enrollment, 4,429 pregnant women were offered HIV-1 testing and 3,643 (82%) accepted. Of these, 533 (15%) were HIV-1 seropositive and 162 consented to participate in the study. Three hundred and seventy-one HIV-1 positive women did not participate either because they did not return to be informed of the study; were ineligible due to a decision to formula feed, age, illness, previous exposure to antiretroviral medications, or plans to move outside Nairobi; were too late in gestation; did not receive test results; or were uninterested in joining the study.

Of the 162 enrolled women, 84 were ineligible due to CD4 count, 2 were ineligible due to illness, 10 were lost to follow-up, 4 were withdrawn due to change in study protocol, 3 delivered, and 1 declined before randomization at 34 weeks gestation. At 34 weeks gestation, 30 women were randomized to the HAART regimen and 28 women were randomized to the ZDV/NVP regimen. Of the 58 randomized women, 4 mothers were lost to follow-up prior to delivery (2 in the HAART arm and 2 in the ZDV/NVP arm), 3 women

had stillbirths (2 in the HAART arm and 1 in the ZDV/NVP arm), and 51 mothers gave birth: 26 mothers in the HAART arm and 25 mothers in the ZDV/NVP arm (figure 1). Mothers had a median age of 25 years (IQR, 22 – 30). Median CD4 count was 321 (IQR, 272 – 421). The median gestational age at delivery was 38 weeks (IQR, 38 – 40) and most (98%) infants were vaginally delivered. At enrollment, the characteristics of these women who delivered were comparable between study arms (table 1). One mother in the ZDV/NVP arm who delivered did not contribute breast milk and plasma postpartum but had follow-up infant transmission data collected at 12 months postpartum. Twenty-four (92%) mothers in the HAART arm and 24 (86%) mothers in the ZDV/NVP arm completed 12 month follow-up.

Adverse Events and Adherence

Two adverse events were reported among women receiving HAART. One woman developed a diffuse rash on the arms and trunk prior to delivery that resolved after discontinuation of HAART and was attributed to nevirapine. The second woman presented 2 months postpartum with anemia which resolved after blood transfusion and discontinuation of HAART. In addition, 5 women assigned to the HAART arm prematurely discontinued HAART: two elected to switch to formula feeding at 3 months postpartum; and three stopped HAART at least 2 months early but continued to breastfeed off HAART. Of those who remained on antiretroviral medications between 34 weeks gestation and six months postpartum, overall adherence to the HAART regimen was 96% by pill count (range, 89-100%).

Breast Milk HIV-1 RNA Levels

There was frequent breast milk sampling in the first month postpartum; 444 specimens were collected: 230 samples from 26 women in the HAART arm and 214 samples from 24 women in the ZDV/NVP arm. The mean number of breast milk samples collected per woman was 9 (range, 4-10) during the first 28 days postpartum. Ninety-eight percent of the women had all breast milk samples collected from the same breast. Of the one woman who had milk collected from both breasts, 9 samples were collected from the left breast and 1 sample was collected from the right breast; each was collected at a different time point and all were included in the analysis. A total of 4 samples (0.1%) were collected from two women (4%) in the HAART arm who had breasts that exhibited signs of mastitis at the time of milk collection. In an analysis excluding these 4 samples, the results remained unchanged. No woman exhibited signs of nipple bleeding or breast abscess. All women reported exclusive breastfeeding during the first 6 months postpartum.

In the first 2 days postpartum, median breast milk HIV-1 RNA levels in mothers randomized to HAART arm were significantly lower than levels in mothers randomized to the ZDV/NVP arm (median log₁₀ HIV-1 RNA, 2.03 versus 2.74, $P = 0.02$) (table 2). Between days 3 and 7 postpartum, HIV-1 RNA levels in breast milk obtained from women randomized to HAART remained significantly lower than from those who received ZDV/NVP, however the magnitude of median breast milk HIV-1 RNA levels was at the lower limit of detection for both arms (median log₁₀ HIV-1 RNA, 1.70 versus 1.70, $P = 0.04$). In the second week postpartum, levels of breast milk HIV-1 RNA remained similar between the two arms (median log₁₀ HIV-1 RNA, 1.70 versus 1.70, $P = 0.10$). By the third (median log₁₀ HIV-1 RNA, 1.70 versus 2.22, $P < 0.001$) and fourth weeks postpartum (median log₁₀ HIV-1 RNA, 1.70 versus 2.11, $P < 0.001$), breast milk levels of HIV-1 RNA among those who received HAART were significantly lower than those who received ZDV/NVP (table 2). Similar patterns were seen when assessing the proportion of women with undetectable HIV-1 RNA in breast milk: less difference was noted between the two arms from days 3 to 7 (proportion undetectable HIV-1 RNA, 77% versus 55%, $P = 0.1$) and 8 to 14 days postpartum

(proportion undetectable HIV-1 RNA, 81% versus 64%, $P = 0.2$) than after 14 days (figure 2).

Plasma HIV-1 RNA levels

In contrast to breast milk HIV-1 RNA, suppression of plasma HIV-1 RNA during the neonatal period was consistently several \log_{10} greater in the HAART arm compared to the ZDV/NVP arm (figure 3). From 0 to 2 days postpartum, plasma HIV-1 RNA levels were significantly lower in the HAART arm compared to the ZDV/NVP arm (median \log_{10} HIV-1 RNA, 3.66 versus 4.37, $P = 0.01$) (table 2), and remained significantly lower from days 8 to 14 (median \log_{10} HIV-1 RNA, 2.49 versus 5.00, $P = 0.001$) and days 22 to 28 (median \log_{10} HIV-1 RNA, 1.70 versus 4.39, $P = 0.02$). At delivery, 7 (28%) women in the HAART arm had plasma HIV-1 RNA levels that were undetectable.

Infant Outcomes including HIV-1 Transmission, Mortality and Growth

Neonates whose mothers received ZDV/NVP had a higher median birth weight than those whose mothers received HAART (median birth weight, 3 kg versus 2.8 kg, $P = 0.02$) (table 1). There was no statistically significant difference in gestational age at delivery between the two arms ($P = 0.6$). There were 3 stillbirths, two in the HAART arm and one in the ZDV/NVP arm.

Forty-five infants reached the 12-month study endpoint, 24 in the HAART arm and 21 in the ZDV/NVP arm. There were no infant mortalities in the HAART arm and 3 infant deaths in the ZDV/NVP arm. One infant died from sepsis at 2 weeks postpartum and 2 infants, who had been weaned at 6 months postpartum, died following diarrhea and dehydration at 7 and 11 months after delivery.

Three infants tested positive for HIV-1, 2 of whom had HIV-1 DNA detected at < 48 hours of age and were born to mothers randomized to HAART. The third HIV-infected infant, who had been randomized to ZDV/NVP, was negative at birth, had HIV-1 detected at 6 months, and died at 7 months postpartum as described above. All other infants, including the other two infants who died, tested negative for HIV-1 at last visit.

Discussion

In this Phase II randomized clinical trial, we serially compared early postpartum breast milk HIV-1 RNA levels of women randomized to HAART for 6 weeks prior to delivery and for 6 months postpartum to those who received 6 weeks of antenatal ZDV plus single-dose nevirapine (SD-NVP) during labor and delivery. Combined antenatal ZDV and SD-NVP is recommended in non-immunosuppressed HIV-1 infected women to prevent infant HIV-1 per current WHO guidelines, while HAART is under consideration [2]. This study demonstrated that HAART significantly suppressed breast milk HIV-1 RNA compared to ZDV/NVP particularly before 3 days and after 14 days postpartum. However, between 3 and 14 days postpartum, the difference in breast milk HIV-1 RNA suppression by the ZDV/NVP regimen versus HAART was modest, and there was a similar proportion of women with undetectable breast milk HIV-1 RNA levels in the two arms. The durable effect of ZDV/NVP on suppression of HIV-1 RNA in breast milk, despite no antiretroviral doses beyond labor/delivery, derives from SD-NVP which has been demonstrated to result in prolonged breast milk HIV-1 RNA suppression [17,19].

During the first 2 weeks, the modest relative effect of HAART versus ZDV/NVP on breast milk HIV-1 RNA contrasted with a much larger relative effect on plasma HIV-1 RNA. HAART reduced plasma HIV-1 RNA levels several \log_{10} -fold lower compared to ZDV/NVP. In contrast to plasma, breast milk levels of HIV-1 RNA differed much less between

study arms (figure 3). This may be due to compartmental effects of drug or viral replication. In untreated HIV-1 infected women, breast milk HIV-1 RNA levels are $\sim 1 \log_{10}$ lower than plasma [7,20]. Thus, because of lower baseline levels, antiretroviral medications such as SD-NVP may more readily suppress HIV-1 RNA to levels below detection in breast milk in contrast to plasma. For breast milk HIV-1 transmission, infant exposure to breast milk HIV-1 is the more relevant parameter of transmission than plasma HIV-1 RNA. While SD-NVP cannot suppress maternal plasma HIV-1 RNA to undetectable levels, the effects in breast milk may be sufficient to decrease infectivity and transmission significantly.

We observed that only 28% of women randomized to the HAART arm in our study had undetectable plasma HIV-1 RNA at delivery. This contrasts with a recent European study which found that 73% of women initiating HAART during pregnancy had undetectable plasma viral levels at delivery, and may be due to the fact that many of these women initiated HAART around 22 weeks gestation compared to 34 weeks gestation in our study [21]. Current WHO recommendations to start HAART at 28 weeks gestation would be expected to result in greater plasma HIV-1 suppression by delivery and fewer in-utero and intrapartum transmissions [11].

In previous studies, elevated levels of both HIV-1 RNA and proviral DNA in breast milk were associated with increased mother-to-child transmission rates [22,23]. In contrast to the effect of HAART on breast milk HIV-1 RNA that was demonstrated here, HAART did not have a significant effect on HIV-1 proviral DNA obtained in a subset of breast milk samples from this trial [24], and this is similar to results from other studies [25]. The fact that HIV-1 RNA, but not HIV-1 DNA, was suppressed by treatments shown to reduce mother-to-child transmission suggests that these treatments may act both by suppressing HIV-1 RNA in maternal breast milk as well by prophylaxis to the breastfeeding infant.

Infants in the HAART arm of this study had significantly lower birthweight than those in the ZDV/NVP arm. There is conflicting evidence regarding the association between HAART and low birthweight, and the effect has been ascribed to metabolic consequences of protease-inhibitors (PI) [26-33]. In our study, low birthweight due to HAART was seen in the absence of PIs or a significant difference in gestational age and would require alternative mechanisms to explain [34]. Though this observation is intriguing, the study was small and was not primarily designed to address this outcome. Because the study was designed to be a Phase II clinical trial there was insufficient power to compare HIV-1 transmission rates. There were 3 infant HIV-1 infections, 2 of which occurred in the HAART arm. Among 26 women randomized to HAART in this study, 7 women prematurely stopped HAART either due to adverse events or inconvenience. These rates of early cessation are consistent with other studies and may compromise long-term efficacy of HAART for prevention of breast milk HIV-1 transmission [35,36].

Given comparable early breast milk HIV suppression between the ZDV/NVP and HAART arms, the incremental effectiveness of 6-month HAART in reducing breast milk transmission compared to SD-NVP would be expected to occur between 3 weeks and 6 months postpartum, a period in which breast milk HIV-1 transmission risk has been noted to be relatively low [37]. Further studies to compare transmission risk between these regimens will be important to determine whether 6-month HAART results in additional benefit to decrease HIV-1 transmission compared to combined ZDV/NVP. Meanwhile, this study points to the surprising durability of SD-NVP on early breast milk HIV-1 RNA shedding compared to HAART and may help explain its ability to reduce mother-to-child transmission in the early postpartum period. This finding contrasts with the significant differences in plasma HIV-1 RNA suppression between the two arms, and may demonstrate

that an undetectable plasma viral load is not necessary to reduce breast milk HIV-1 RNA to non-transmissible levels.

Acknowledgments

We would like to acknowledge the contributions of the research personnel, laboratory staff, and data management teams in Nairobi, Kenya and Seattle, Washington; the Mathare North City Council Clinic for their participation and cooperation; the Divisions of Obstetrics and Gynaecology and Paediatrics at Kenyatta National Hospital for providing facilities for laboratory and data analysis. Most of all, we thank the mothers and children who participated in the study.

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or in the writing of the report.

This work was supported by the Elizabeth Glaser Pediatric AIDS Foundation (#311-03) and AI 38518. Michael H. Chung was a scholar in the International AIDS Research and Training Program and is supported by the Fogarty International Center, National Institutes of Health (D43-TW00007). Dara A. Lehman was supported by a Hearst Fellowship. Grace John-Stewart is an Elizabeth Glaser Pediatric AIDS Foundation (EGPAF) Scientist.

References

1. Nduati R, John G, Mbori-Ngacha D, Richardson B, Overbaugh J, Mwatha A, et al. Effect of breastfeeding and formula feeding on transmission of HIV-1: a randomized clinical trial. *JAMA* 2000;283:1167–1174. [PubMed: 10703779]
2. WHO. HIV and infant feeding technical consultation, October 25-27, 2006. Geneva: World Health Organization; 2006.
3. Sinkala, M.; Kuhn, L.; Kankasa, C.; Kasonde, P.; Vwalika, C.; Mwiya, M., et al. No Benefit of Early Cessation of Breastfeeding at 4 Months on HIV-free Survival of Infants Born to HIV-infected Mothers in Zambia: The Zambia Exclusive Breastfeeding Study. Fourteenth Conference on Retroviruses and Opportunistic Infections; Los Angeles. 2007.
4. Thior I, Lockman S, Smeaton LM, Shapiro RL, Wester C, Heymann SJ, et al. Breastfeeding plus infant zidovudine prophylaxis for 6 months vs formula feeding plus infant zidovudine for 1 month to reduce mother-to-child HIV transmission in Botswana: a randomized trial: the Mashi Study. *JAMA* 2006;296:794–805. [PubMed: 16905785]
5. John-Stewart G, Mbori-Ngacha D, Ekpin R, Janoff EN, Nkengasong J, Read JS, et al. Breast-feeding and Transmission of HIV-1. *J Acquir Immune Defic Syndr* 2004;35:196–202. [PubMed: 14722454]
6. Manigart O, Crepin M, Leroy V, Meda N, Valea D, Janoff EN, et al. Effect of perinatal zidovudine prophylaxis on the evolution of cell-free HIV-1 RNA in Breast Milk and on postnatal transmission. *J Infect Dis* 2004;190:1422–1428. [PubMed: 15378434]
7. Rousseau CM, Nduati RW, Richardson BA, Steele MS, John-Stewart GC, Mbori-Ngacha DA, et al. Longitudinal analysis of human immunodeficiency virus type 1 RNA in breast milk and of its relationship to infant infection and maternal disease. *J Infect Dis* 2003;187:741–747. [PubMed: 12599047]
8. Gray GE, Urban M, Chersich MF, Bolton C, van Niekerk R, Violari A, et al. A randomized trial of two postexposure prophylaxis regimens to reduce mother-to-child HIV-1 transmission in infants of untreated mothers. *AIDS* 2005;19:1289–1297. [PubMed: 16052084]
9. Taha TE, Kumwenda NI, Gibbons A, Broadhead RL, Fiscus S, Lema V, et al. Short postexposure prophylaxis in newborn babies to reduce mother-to-child transmission of HIV-1: NVAZ randomised clinical trial. *Lancet* 2003;362:1171–1177. [PubMed: 14568737]
10. Shapiro RL, Holland DT, Capparelli E, Lockman S, Thior I, Wester C, et al. Antiretroviral concentrations in breast-feeding infants of women in Botswana receiving antiretroviral treatment. *J Infect Dis* 2005;192:720–727. [PubMed: 16088821]
11. Antiretroviral Drugs for Treating Pregnant Women and Preventing HIV Infection in Infants, towards Universal Access: Recommendations for a Public Health Approach. World Health Organization; Geneva: WHO Press; 2006.

12. McIntyre J. Strategies to prevent mother-to-child transmission of HIV. *Curr Opin Infect Dis* 2006;19:33–38. [PubMed: 16374215]
13. US Food and Drug Administration. FDA Public Health Advisory for Nevirapine (Viramune). 2005
14. Lewis P, Nduati R, Kreiss JK, John GC, Richardson BA, Mbori-Ngacha D, et al. Cell-free human immunodeficiency virus type 1 in breast milk. *J Infect Dis* 1998;177:34–39. [PubMed: 9419167]
15. DeVange Panteleeff D, Emery S, Richardson BA, Rousseau C, Benki S, Bodrug S, et al. Validation of performance of the gen-probe human immunodeficiency virus type 1 viral load assay with genital swabs and breast milk samples. *J Clin Microbiol* 2002;40:3929–3937. [PubMed: 12409354]
16. Emery S, Bodrug S, Richardson BA, Giachetti C, Bott MA, Panteleeff D, et al. Evaluation of performance of the Gen-Probe human immunodeficiency virus type 1 viral load assay using primary subtype A, C, and D isolates from Kenya. *J Clin Microbiol* 2000;38:2688–2695. [PubMed: 10878065]
17. Chung MH, Kiarie JN, Richardson BA, Lehman DA, Overbaugh J, John-Stewart GC. Breast milk HIV-1 suppression and decreased transmission: a randomized trial comparing HIVNET 012 nevirapine versus short-course zidovudine. *AIDS* 2005;19:1415–1422. [PubMed: 16103773]
18. Panteleeff DD, John G, Nduati R, Mbori-Ngacha D, Richardson B, Kreiss J, Overbaugh J. Rapid method for screening dried blood samples on filter paper for human immunodeficiency virus type 1 DNA. *J Clin Microbiol* 1999;37:350–353. [PubMed: 9889216]
19. Mirochnick M, Capparelli E. Pharmacokinetics of antiretrovirals in pregnant women. *Clin Pharmacokinet* 2004;43:1071–1087. [PubMed: 15568888]
20. Giuliano M, Guidotti G, Andreotti M, Pirillo MF, Villani P, Liotta G, et al. Triple Antiretroviral Prophylaxis Administered During Pregnancy and After Delivery Significantly Reduces Breast Milk Viral Load: A Study Within the Drug Resource Enhancement Against AIDS and Malnutrition Program. *J Acquir Immune Defic Syndr*. 2006
21. Patel D, Cortina-Borja M, Thorne C, Newell ML. Time to undetectable viral load after highly active antiretroviral therapy initiation among HIV-infected pregnant women. *Clin Infect Dis* 2007;44:1647–1656. [PubMed: 17516411]
22. Koulinska IN, Villamor E, Chaplin B, Msamanga G, Fawzi W, Renjifo B, Essex M. Transmission of cell-free and cell-associated HIV-1 through breast-feeding. *J Acquir Immune Defic Syndr* 2006;41:93–99. [PubMed: 16340480]
23. Rousseau CM, Nduati RW, Richardson BA, John-Stewart GC, Mbori-Ngacha DA, Kreiss JK, Overbaugh J. Association of levels of HIV-1-infected breast milk cells and risk of mother-to-child transmission. *J Infect Dis* 2004;190:1880–1888. [PubMed: 15499546]
24. Lehman DA, Chung MH, John-Stewart GC, Richardson BA, Kiarie JN, Kinuthia J, Overbaugh JM. HIV-1 persists in breast milk cells despite antiretroviral treatment to prevent mother-to-child transmission. *AIDS*. 2008 In Press.
25. Shapiro RL, Ndung'u T, Lockman S, Smeaton LM, Thior I, Wester C, et al. Highly active antiretroviral therapy started during pregnancy or postpartum suppresses HIV-1 RNA, but not DNA, in breast milk. *J Infect Dis* 2005;192:713–719. [PubMed: 16088820]
26. Combination antiretroviral therapy and duration of pregnancy. *Aids* 2000;14:2913–2920. [PubMed: 11398741]
27. Boer K, Nellen JF, Patel D, Timmermans S, Tempelman C, Wibaut M, et al. The AmRo study: pregnancy outcome in HIV-1-infected women under effective highly active antiretroviral therapy and a policy of vaginal delivery. *Bjog* 2007;114:148–155. [PubMed: 17305888]
28. Cotter AM, Garcia AG, Duthely ML, Luke B, O'Sullivan MJ. Is antiretroviral therapy during pregnancy associated with an increased risk of preterm delivery, low birth weight, or stillbirth? *J Infect Dis* 2006;193:1195–1201. [PubMed: 16586354]
29. Schulte J, Dominguez K, Sukalac T, Bohannon B, Fowler MG. Declines in low birth weight and preterm birth among infants who were born to HIV-infected women during an era of increased use of maternal antiretroviral drugs: Pediatric Spectrum of HIV Disease, 1989–2004. *Pediatrics* 2007;119:e900–906. [PubMed: 17353299]

30. Szyld EG, Warley EM, Freimanis L, Gonin R, Cahn PE, Calvet GA, et al. Maternal antiretroviral drugs during pregnancy and infant low birth weight and preterm birth. *AIDS* 2006;20:2345–2353. [PubMed: 17117021]
31. Thorne C, Patel D, Newell ML. Increased risk of adverse pregnancy outcomes in HIV-infected women treated with highly active antiretroviral therapy in Europe. *AIDS* 2004;18:2337–2339. [PubMed: 15577551]
32. Tuomala RE, Shapiro DE, Mofenson LM, Bryson Y, Culnane M, Hughes MD, et al. Antiretroviral therapy during pregnancy and the risk of an adverse outcome. *N Engl J Med* 2002;346:1863–1870. [PubMed: 12063370]
33. Tuomala RE, Watts DH, Li D, Vajaranant M, Pitt J, Hammill H, et al. Improved obstetric outcomes and few maternal toxicities are associated with antiretroviral therapy, including highly active antiretroviral therapy during pregnancy. *J Acquir Immune Defic Syndr* 2005;38:449–473. [PubMed: 15764963]
34. Martin F, Taylor GP. Increased Rates of Preterm Delivery Are Associated with the Initiation of Highly Active Antiretroviral Therapy during Pregnancy: A Single-Center Cohort Study. *J Infect Dis* 2007;196:558–561. [PubMed: 17624841]
35. Marazzi MC, Germano P, Liotta G, Guidotti G, Loureiro S, da Cruz Gomes A, et al. Safety of nevirapine-containing antiretroviral triple therapy regimens to prevent vertical transmission in an African cohort of HIV-1-infected pregnant women. *HIV Med* 2006;7:338–344. [PubMed: 16945080]
36. Jamisse L, Balkus J, Hitti J, Gloyd S, Manuel R, Osman N, et al. Antiretroviral-associated toxicity among HIV-1-seropositive pregnant women in Mozambique receiving nevirapine-based regimens. *J Acquir Immune Defic Syndr* 2007;44:371–376. [PubMed: 17259905]
37. Taha TE, Hoover DR, Kumwenda NI, Fiscus SA, Kafulafula G, Nkhoma C, et al. Late Postnatal Transmission of HIV-1 and Associated Factors. *J Infect Dis* 2007;196:10–14. [PubMed: 17538877]

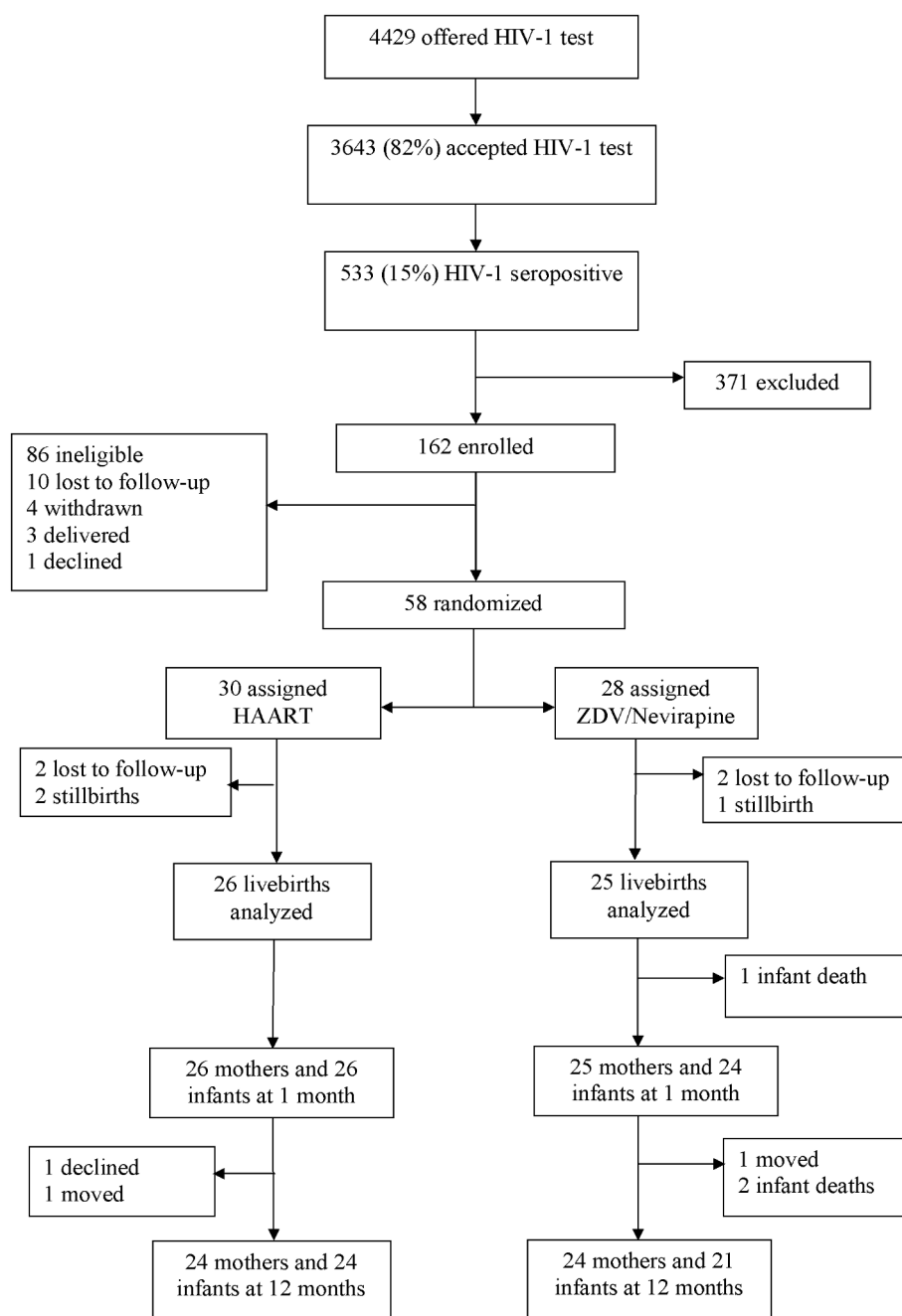


Figure 1. Trial Profile

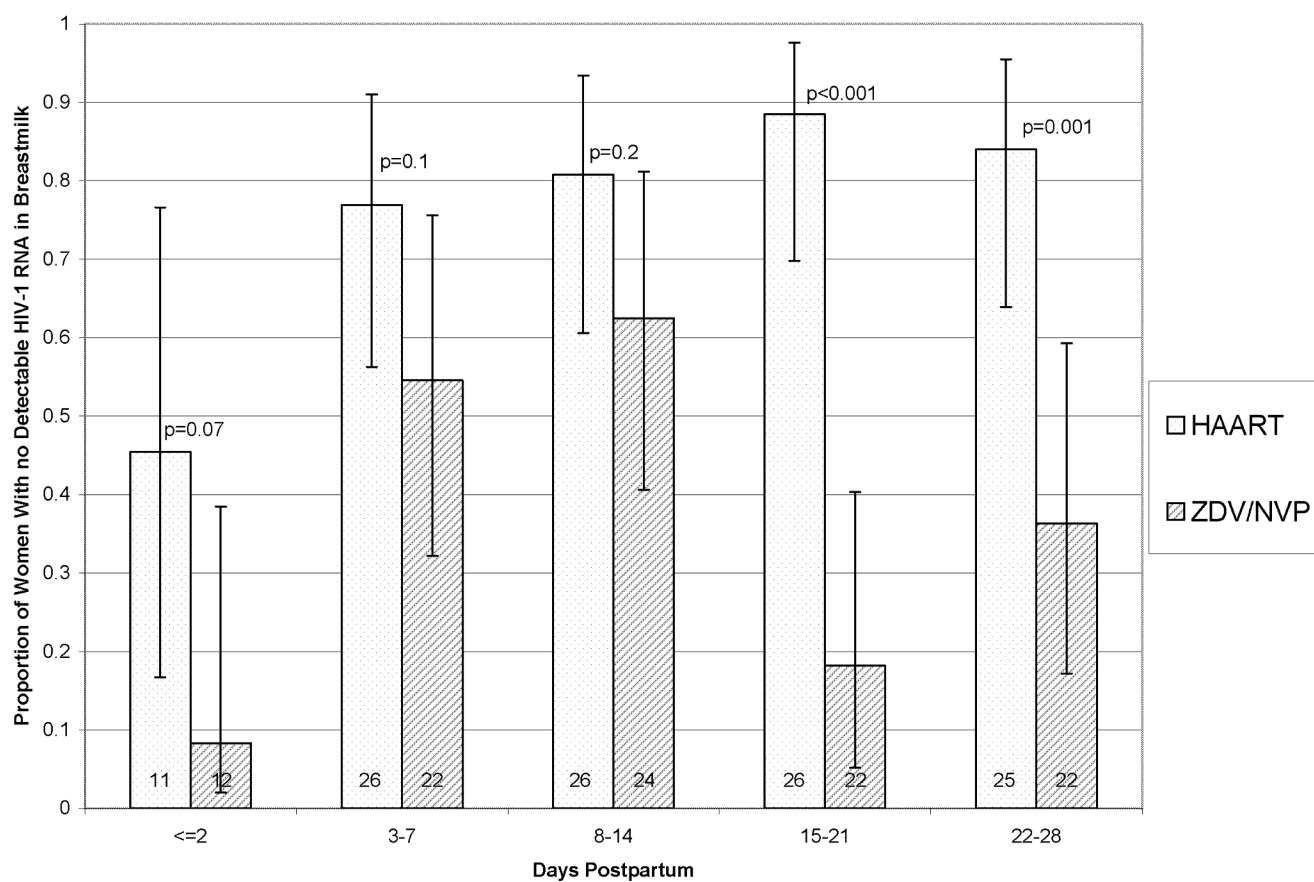


Figure 2. Proportion of women with undetectable HIV-1 RNA in breast milk

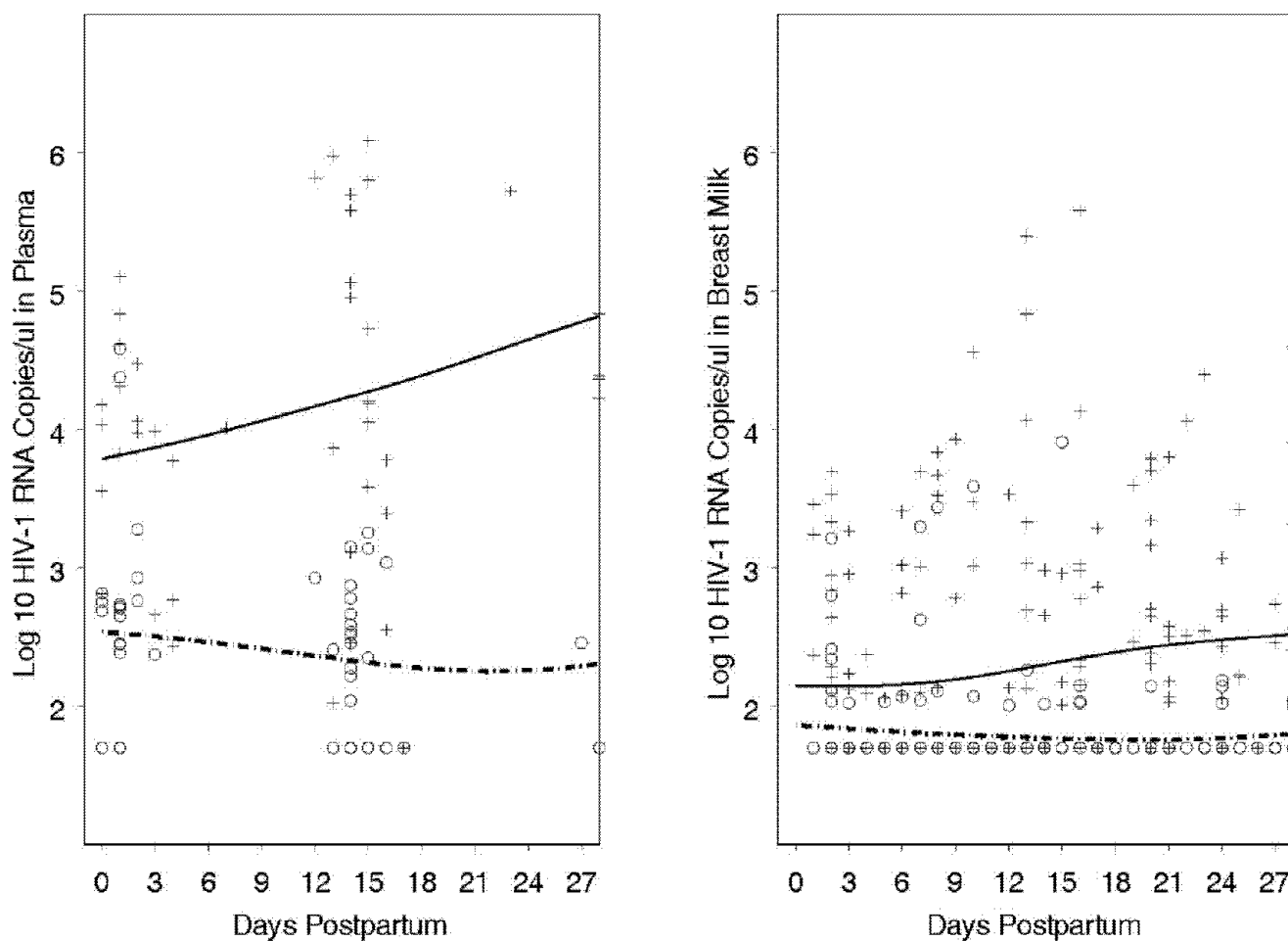


Figure 3. Mean log₁₀ HIV-1 RNA copies in breast milk and plasma over days postpartum
Solid lines and crosses = AZT/NVP arm. Dashed lines and circles = HAART arm.

Table 1
Characteristics of women who gave birth and neonates by treatment arm

Characteristic	HAART		ZDV/NVP		p-value
	n	Median (IQR) or %	n	Median (IQR) or %	
Women who had live birth					
Total	26		25		
Age (years)	26	26 (24 – 29)	25	24 (20 – 31)	0.5
Schooling (years)	26	8 (7 – 12)	25	8 (8 – 10)	0.3
Age at first intercourse	26	16 (15 – 18)	25	17 (15 – 18)	0.6
Number of lifetime sexual partners	26	3 (2 – 4)	24	3 (2 – 5)	0.7
CD4-cell count (cells/ μ L) at 32 weeks gestation	26	314 (260 – 421)	25	333 (299 – 431)	0.3
HIV-1 RNA (log ₁₀ copies/mL) at 32 weeks gestation	25	4.87 (4.51 – 5.05)	22	4.52 (4.16 – 5.28)	0.4
Caesarean section	26	4%	25	0%	
Neonates					
Birthweight (g)	25	2800 (2500 – 3150)	21	3000 (2950 – 3500)	0.02
Birthweight < 2500 g	25	20%		0%	0.05
Gestational age at birth (weeks)	25	38 (37 – 39)	21	37 (38 – 39)	0.6

Table 2
Median of mean log₁₀ copies in breast milk and plasma during the first month postpartum follow-up

Breast milk						
Weeks postpartum	N	HAART median viral load (IQR)	N	ZDV/NVP median viral load (IQR)	p-value	
Week 0 (0 to 2 days)	11	2.03 (1.70, 2.41)	12	2.74 (2.23, 3.43)	0.02	
Week 1 (3 to 7 days)	26	1.70 (1.70, 1.73)	22	1.70 (1.70, 2.35)	0.04	
Week 2 (8 to 14 days)	26	1.70 (1.70, 1.70)	24	1.70 (1.70, 2.14)	0.10	
Week 3 (15 to 21 days)	26	1.70 (1.70, 1.70)	22	2.22 (1.91, 3.12)	<0.001	
Week 4 (22 to 28 days)	25	1.70 (1.70, 1.70)	22	2.11 (1.70, 3.07)	<0.001	
Plasma						
Week 0 (0 to 2 days)	22	2.67 (1.70, 2.78)	14	4.04 (3.37, 2.78)	<0.001	
Week 2 (8 to 14 days)	16	2.49 (2.08, 2.75)	10	5.00 (2.95, 5.72)	0.001	
Week 4 (22 to 28 days)	4	1.70 (1.70, 2.27)	5	4.39 (4.29, 5.28)	0.02	