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Antiretroviral drug concentrations in breastmilk, maternal HIV viral load, and HIV transmission to the infant: results from the BAN study

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Abstract

Background: Concentration of antiretroviral (ARV) drug found in plasma, and amounts of drug excreted into breastmilk, may affect HIV viral load and potentially perinatal HIV transmission.

Methods: In this cohort study with two-phase sampling, we included mothers randomized to postpartum maternal ARVs or daily infant nevirapine during 28 weeks of breastfeeding in the Breastfeeding, Antiretrovirals and Nutrition (BAN) study. Among these, we included all mothers who transmitted HIV to their infants between 2-28 weeks and 15% of mothers who did not (n=27 and 227, respectively). Spearman correlation coefficients (r2) were used to assess correlation between maternal plasma and breastmilk ARV concentration. Associations between the median effective drug concentration (EC50) and detectable maternal viral load (plasma: >40 copies/ml, breastmilk: >56 copies/ml) were assessed using mixed effects models. Cox models were used to estimate the association between maternal or infant plasma drug concentration and breastmilk HIV transmission from 2-28 weeks.

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Results: All ARV compounds exhibited substantial correlations between maternal plasma and breastmilk concentrations (r2: 0.85-0.98, p-value <0.0001). Having plasma drug concentration above the EC50 was associated with lower odds of having detectable HIV RNA (maternal plasma OR 0.64, 95%CI 0.45-0.91; breastmilk OR 0.22, 95% CI 0.14-0.35) and a reduced rate of breastmilk HIV transmission (HR 0.40, 95% CI 0.18-0.93). Having breastmilk drug concentration above the EC50 was also associated with lower odds of having detectable maternal HIV RNA (plasma OR 0.62, 95%CI 0.45-0.85; breastmilk OR 0.42, 95% CI 0.29-0.59).

Conclusion: Ensuring adequate drug concentration is important for viral suppression and preventing breastmilk HIV transmission.

Introduction

The concentration of antiretroviral (ARV) drug found in plasma as well as the amount of drug excreted into breastmilk may affect the rate at which ARVs begin to suppress viral replication, and/or the duration of the effect on viral replication. Higher HIV viral loads in plasma have been associated with higher risk of HIV transmission from mother to infant.[1] Drug disposition during the postpartum period can be affected by the physiologic changes that take place during pregnancy, and the intra-subject variability in the time it takes for these changes to revert back to pre-pregnancy levels.[2] In addition, two potential mechanisms are thought to be at play regarding virus populations in breastmilk. The first, which is thought to constitute the largest proportion, is by continual trafficking from blood into breastmilk of cell-associated virus which resides inside the cell (measured as HIV-DNA), or cell-free virions (measured as HIV-RNA).[3] The second is by local production of virus in the breastmilk.[3, 4] To further add to the complexity, there are differential levels of antiretroviral exposure in breastmilk and infant plasma both within and between classes of drugs.[2] Only ZDV and 3TC have been shown to concentrate to any real extent in breastmilk, among the ARVs that have been evaluated to date.[4] In addition, ART (antiretroviral therapy) has been shown to suppress HIV RNA in plasma and breastmilk, while HIV DNA in breastmilk persisted.[4] These challenges have resulted in incomplete knowledge regarding the antiretroviral therapeutic levels that are needed to prevent breastmilk HIV transmission. To help fill this knowledge gap, we explored associations between antiretroviral pharmacokinetics and HIV viral load in plasma and breastmilk, the relationship between plasma and breastmilk concentrations of ARVs, and subsequent infant HIV infection, in order to inform future prevention efforts.

Methods

Parent study and sample collection

A cohort study with two-phase sampling[5] was conducted using data from the Breastfeeding, Antiretrovirals and Nutrition (BAN) study. BAN was a randomized clinical trial conducted in Lilongwe, Malawi between 2004 and 2010 to assess the benefit and safety of maternal or infant antiretrovirals to prevent HIV transmission during breastfeeding.[6, 7] Details of the BAN study have been previously described.[8] Briefly, all mothers were HIV-infected with no previous antiretroviral use, and had CD4+ T cell counts ≥250 cells/mm^3 (≥200 cells/mm^3 before July 24, 2006) at enrollment. A single peripartum dose of nevirapine

(NVP) was given to each mother along with a twice daily dose of zidovudine (ZDV) 300mg and lamivudine (3TC) 150 mg during labor and for 7 days postpartum. Mothers randomized to the maternal ARV arm received combination therapy with three drugs including ZDV +3TC (Combivir®) and either lopinavir 200 mg + ritonavir 50 mg (LPV/r, 1 combination tablet, Aluvia®), nelfinavir (NFV) 1250 mg, or NVP 200 mg twice a day until breastfeeding cessation or 28 weeks postpartum, with NVP dose escalation during the first two weeks. All infants received a single dose of NVP (2 mg/kg) after delivery along with ZDV (12 mg) plus 3TC (6 mg) twice daily for 7 days. Infants randomized to the infant NVP arm also received NVP daily for up to 28 weeks (doses ranging from 6 mg to 26 mg, increasing as the infant aged).

Each mother was scheduled to return to the clinic at weeks 2, 6, 12, 18 and 24 postpartum. Mothers were instructed to refrain from taking their morning antiretroviral dose until after the sample collection; 5mL of whole blood and 10mL of breast milk were collected from the mothers as well as 3mL of whole blood from the infant. Breast milk was collected from the right breast unless clinically contraindicated at which time it was noted whether the milk was collected from the right or left breast.

Study population

We included mothers randomized to postpartum maternal ARVs or daily infant nevirapine during 28 weeks of breastfeeding who had ≥1 plasma or breastmilk (maternal ARV arm only) specimen available 2-24 weeks postpartum. Among these, we included all mothers who transmitted HIV to their infants between 2-28 weeks and 15% of mothers who did not; sampling was based primarily on stored specimen availability. Breastmilk HIV transmission to the infant was determined by Roche Amplicor 1.5 DNA PCR (Roche Molecular Systems, Pleasanton, CA, USA) at 2, 12, 28, and 48 weeks to indicate infant HIV status. PCR-positive results were confirmed by testing an additional blood specimen. The window of infection was narrowed by testing stored dried blood-spot specimens collected at 4, 6, 8, 18, 24, 32, and 36 weeks.

Sample storage and analysis

All whole blood samples were collected in Vacutainer tubes containing K3-EDTA (Fisher Scientific, Hampton, New Hampshire, USA) and centrifuged at 2600 rpm at 4°C for 10 minutes. Plasma was removed and aliquoted to 2 mL cryovials and stored at −70°C until shipped to the UNC Center for AIDS Research Clinical Pharmacology and Analytical Chemistry lab for analysis. Drug concentrations for zidovudine, lamivudine, lopinavir and ritonavir in plasma and breast milk were analyzed using a validated HPLC-MS/MS method. [9] Briefly, drugs were extracted from 50-100 μL of plasma and 200μL of whole breast milk. A solid-phase extraction procedure (BOND ELUT-C18, Harbor City, California, USA) was used with a 90:10 methanol:water solution as the eluent. Eluted samples were dried at 40°C under a gentle steam of nitrogen and reconstituted in 100 μL of mobile phase before using a LEAP HTC Pal thermostated autosampler to inject the samples onto an Applied Biosystems API 4000 triple quadruple mass spectrometer. Antiretrovirals were separated using an Aquasil C18 column (Thermo-Electron, San Jose, CA). Analyst 1.3.1 software was used for data collection. The concentration range for all ARVs was 10-10,000 ng/mL, with an intra-

and inter-day precision and accuracy falling within 15%. The laboratory participates in two external proficiency testing programs twice annually: the Division of AIDS Clinical Pharmacology Quality Assurance Program and the Dutch Association for Quality Assessment in TDM and Clinical Toxicology (KKGT) International and Interlaboratory QC Program.[10, 11]

HIV RNA was quantified from maternal plasma and whole breastmilk at enrollment, 2, 6, 12, 18, and 24 weeks postpartum/postnatally. HIV RNA was also measured in breastmilk at 4 and 8 weeks postpartum/postnatally. Maternal plasma viral load was quantified using the Abbott RealTime HIV-1 assay (Abbott Molecular, Des Plaines, IL) according to the package insert instructions (lower limit of quantitation 40 copies/mL). Breastmilk viral load was quantified from 0.6 mL of whole breastmilk pretreated with 209 μL Abbott RNA sample prep lysis buffer and 60 μL Abbott Proteinase K (53°C incubation for 20 minutes), using the Abbott RealTime HIV assay (lower limit of quantitation 56 copies/mL).

Statistical analysis

In this secondary analysis of BAN data, we sought to 1) examine correlation between maternal plasma and breastmilk ARV drug concentration, 2) estimate associations between maternal ARV drug concentration and HIV viral load in maternal plasma and breastmilk, and 3) estimate association of rate of breastmilk HIV transmission with breastmilk and maternal or infant plasma drug concentration status. All drug concentrations were treated as continuous variables when assessing correlations between maternal plasma and breastmilk ARV concentrations. Plasma and breastmilk drug concentrations for NVP, NFV, and LPV were treated as binary variables using the median effective concentration (EC50) as a cutoff (>EC50 vs ≤EC50) in all regression models. The following EC50 values were used: NVP 23.97 ng/ml, NFV 25.55 ng/ml, and LPV 110 ng/ml.[12-14] Maternal plasma and breastmilk viral load were dichotomized as detectable (plasma: >40 copies/ml, breastmilk: >56 copies/ml) or undetectable. Breastmilk transmission was defined as first detection of HIV infection by PCR in infant blood between 2-28 weeks of age.

Spearman correlation coefficients were used to assess correlation between maternal plasma and breastmilk ARV concentrations among women randomized to maternal ARVs. Associations between maternal drug concentrations and maternal viral load were assessed using generalized linear mixed models with a logit link, binomial distribution, and random intercept. Cox models with robust variance estimators were used to estimate the association between plasma and breastmilk drug concentrations and breastmilk HIV transmission between 2-28 weeks. When assessing the association between plasma drug concentrations and HIV transmission, maternal plasma drug concentrations were used for mother-infant pairs randomized to maternal ARVs and infant NVP plasma concentrations were used for mother-infant pairs randomized to infant NVP; only mothers randomized to maternal ARVs were included when assessing the association between breastmilk drug concentrations and HIV transmission. Mother-infant pairs were right-censored at the first occurrence of the following when assessing time until infant HIV infection: time of infant death, time of infant's last PCR negative HIV test if lost to follow-up or at 28 weeks if the infant remained HIV-uninfected, or at reported cessation of breastfeeding unless HIV transmission occurred

within 30 days of reported cessation.[7] The reciprocal of the study inclusion probability was used as a sampling weight in all regression analyses to account for the two-phase sampling.[15]

Effect measure modification was assessed by comparing unadjusted and adjusted estimates and 95% confidence intervals using an interaction term between the exposure and variable of interest. No effect measure modification was identified. A directed acyclic graph was used to identify potential confounders and a minimally sufficient adjustment set.[16] Potential confounding variables consisted of nutritional randomization assignment, demographic characteristics, and health status information collected at enrollment (hereafter referred to as baseline). All data analyses were conducted using SAS version 9.3 (SAS Institute, Cary, North Carolina, USA).

Results

Characteristics of mother-infant pairs

A total of 254 mother-infant pairs were included; 27 transmitted HIV to their infant between 2-28 weeks (82% of all 2-28 week transmission events), and 227 did not (15% of all mothers randomized to maternal ARVs or infant NVP with an HIV-uninfected infant). Of the 254 included mother-infant pairs, 48% (n=122) were randomized to maternal ARVs and 52% (n=132) were randomized to infant NVP (Table 1). A total of 16 and 11 infant HIV infections occurred between 2-28 weeks among mother-infant pairs randomized to maternal ARVs and infant NVP, respectively. Among mothers randomized to maternal ARVs, 68% received a boosted protease inhibitor regimen of zidovudine, lamivudine, and lopinavir-ritonavir; 27% received zidovudine, lamivudine, and nelfinavir, and 5% received zidovudine, lamivudine, and nevirapine. The majority of mothers were between the ages of 15 and 25, were married, and only had a primary school or no education. At baseline, 67% of mothers had a CD4+ count <500 cells/mm^3 and 70% had a plasma viral load >10,000 copies/ml.

Comparing ARV concentrations among infant HIV transmissions and non-transmissions

Among mothers in the maternal ARV arm, 56% of mothers who transmitted HIV to their infant between 2-28 weeks had no plasma drug concentrations >EC50 at any measured time point, compared to 24% of mothers who did not transmit (Table 2). Percentages were similar for breastmilk drug concentrations, with 54% and 25% of transmitting and non-transmitting mothers having no concentrations >EC50 at any measured time point, respectively. Among infants randomized to infant NVP, 9% (n=1) of HIV-infected infants and 9% (n=11) of HIV-uninfected infants had no plasma NVP concentrations >EC50 at any measured time points.

Correlations between plasma and breastmilk ARV concentrations

3TC and ZDV exposures in the breastmilk of mothers randomized to the maternal ARV arm were higher than the exposures in plasma, with mean breastmilk to plasma ratios of 2.73 and 1.26, respectively (Table 3). In contrast, breastmilk exposures were lower than those in plasma for all remaining drugs tested, with breastmilk to plasma ratios ranging from 0.15 for lopinavir to 0.61 for nevirapine. All ARV compounds exhibited substantial correlations

between maternal plasma and breastmilk concentrations, with correlation coefficients ranging from 0.85 to 0.98 ($p<0.0001$ for all) (Figure 1).

Associations between plasma and breastmilk ARV concentrations and plasma and breastmilk HIV viral load

Having maternal plasma drug concentrations ≥EC50 between 2-24 weeks postpartum was associated with lower odds of having detectable HIV RNA in both maternal plasma [odds ratio (OR) 0.64, 95% confidence interval (CI) 0.45-0.91] and breastmilk (OR 0.22, 95% CI 0.14-0.35), compared with having drug concentrations <EC50 (Table 4). Having breastmilk drug concentrations ≥EC50 resulted in an analogous association with having detectable HIV RNA in maternal plasma (OR 0.62, 95% CI 0.45-0.85), and a similar though somewhat attenuated association with having detectable HIV RNA in breastmilk (OR 0.42, 95% CI 0.29-0.59). Adjusting for nutritional randomization, maternal age, baseline maternal HIV viral load, and baseline maternal CD4+ count resulted in similar findings.

Associations between plasma and breastmilk ARV concentrations and breastmilk HIV transmission

Maternal plasma specimens were missing for 1 instance of HIV transmission to the infant in the maternal ARV arm, and breastmilk specimens were missing for 3 instances of infant HIV transmission in the maternal ARV arm. Therefore limiting the number of transmissions to 26 when assessing the relationship between plasma drug concentrations and HIV transmission, and 13 when assessing the association between breastmilk drug concentrations and HIV transmission. Having maternal or infant plasma drug concentrations ≥EC50 between 2-24 weeks postpartum was also associated with a 60% reduction in the rate of breastmilk HIV transmission between 2-28 weeks postpartum (hazard ratio (HR) 0.40, 95% CI 0.18-0.93), compared with having maternal or infant plasma drug concentrations <EC50 (Table 5). Having breastmilk drug concentrations ≥EC50 between 2-24 weeks postpartum also appeared to be associated with a reduced rate of breastmilk HIV transmission, though the result did not reach statistical significance (HR 0.31, 95% CI 0.08-1.17).

Discussion

We showed in a subset of participants from the Breastfeeding, Antiretrovirals and Nutrition (BAN) study that lower concentrations of ARVs in maternal plasma and breastmilk were associated with detectable maternal viral loads, that higher maternal and breastmilk ARV drug levels were associated with lower viral load detectability in each matrix, and that higher maternal and infant ARV plasma levels were associated with a reduced rate of breastmilk HIV transmission. The lack of a statistically significant association between breastmilk drug concentrations and HIV transmission was likely due to the limited number of transmission events that occurred in the maternal ARV arm, as BAN was not powered to compare transmission by study ARV arm. These findings further validate our previous reports that 1) having ≥90% adherence measured by pill count was associated with reduced log10 plasma VL, reduced odds of detectable breastmilk VL, as well as a reduced rate of HIV transmission to the infant, and 2) having detectable breastmilk viral load was associated with an increased rate of HIV transmission.[17, 18]

Similar to previous reports, higher exposures of ZDV and 3TC and lower exposures of NVP, NFV, and LPV were found in breastmilk compared with maternal plasma.[19-21] Breastmilk-plasma ratios for ZDV and LPV were similar to ratios we have reported previously from an intensive PK study on a subset of this study population.[21] As previously reported, the finding of nucleoside reverse transcriptase inhibitors concentrating in breastmilk while the non-nucleoside reverse transcriptase inhibitors and protease inhibitors achieve low exposure in breast milk, suggests that multiple characteristics including degree of plasma protein binding likely play a role.[21-23]

Due to the limited number of transmission events, we were not able to adjust for multiple factors when assessing associations between drug concentrations and HIV transmission. Therefore, it is possible that residual confounding or confounding from unmeasured factors is present even after adjusting for baseline maternal plasma viral load. Missing breastmilk viral load and drug concentration data were mainly due to protocol amendments made in 2006 and 2007 aimed at decreasing study costs and staff workload. Protocol amendments included reducing the number of visits at which breastmilk specimens were collected.

We did not have data on the exact timing of the last antiretroviral dose taken before each collection and therefore were only able to assess random drug concentrations. The addition of accurate dosing time would allow for a more comprehensive look at the relationships between plasma and breastmilk concentrations. For example, accurate data on dosing time could allow the drugs to be modeled and describe the extent of each ARV being excreted into the breastmilk. With a stable PK model, simulations could be conducted to examine the time each subject's plasma and/or breastmilk concentration was above the EC50 as another parameter to compare against HIV transmission and/or plasma/breastmilk viral load. In all regression models, we used the third drug (LPV, NFV, or NVP) to determine whether the drug concentration was above or below the EC50 value. However, ARV drugs may be taken differentially. Ideally, there will be a more optimal way to obtain a measure for the pharmacokinetics of the entire regimen in the future.

In conclusion, we have shown that ensuring adequate drug concentrations in plasma and breastmilk is important for reaching and maintaining viral suppression. In addition, ensuring adequate drug concentrations in plasma, and potentially in breastmilk, is important for preventing breastmilk HIV transmission. Efforts to develop and evaluate tools to assist patients with adhering to antiretroviral medications throughout pregnancy and breastfeeding remain of utmost importance.

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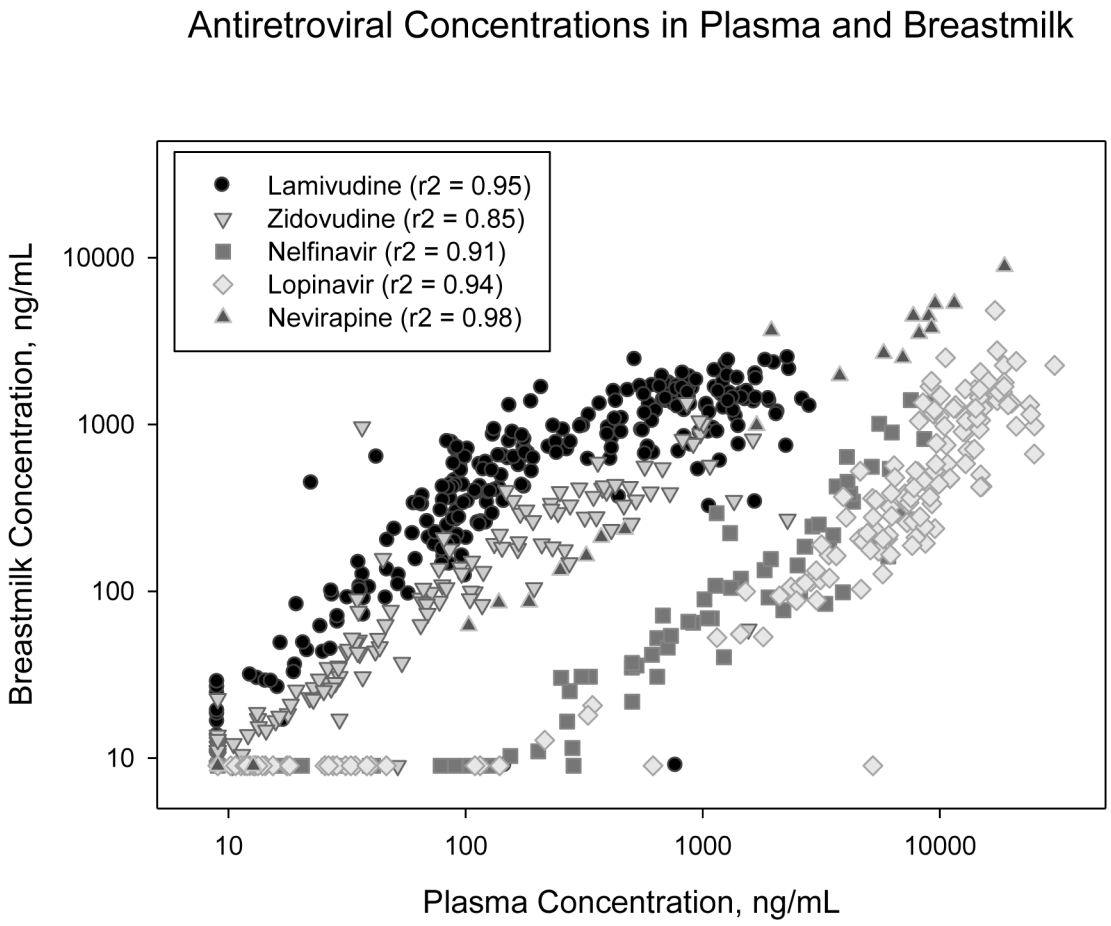

Figure 1.
Correlation (r2) between maternal plasma and breastmilk antiretroviral drug concentrations among 122 Malawian postpartum women, the Breastfeeding, Antiretrovirals, and Nutrition study.

Table 1.

Baseline characteristics of 254 Malawian mother-infant pairs, the Breastfeeding, Antiretrovirals, and Nutrition study.

	Total*	
	N	(%)
Antiretroviral randomization		
Maternal antiretroviral	122	48
Infant nevirapine	132	52
Nutritional randomization		
No supplement	122	48
Received supplement	132	52
Mothers:		
Age (years)		
15-25	139	55
26-35	101	40
36-45	13	5
Education (none or primary school only)	167	66
Married	233	92
CD4+ count per mm^3		
200-349	89	35
350-499	80	31
≥500	85	33
Plasma viral load copies/ml		
≤ 1,000	20	8
1,001-10,000	56	22
> 10,000	176	70
Hemoglobin <11 g/dl	157	62
Infants:		
Sex (female)	120	47
Birth weight <2.5 kg	24	9

*Maternal age is missing for 1 mother, baseline plasma viral load is missing for 2 mothers

Table 2.

Summary of antiretroviral regimens and distribution of mother-infant pairs who never, intermittently, or always had drug concentrations >EC50, by compartment and infant HIV status.

	HIV-infected infants (N=27)		HIV-uninfected infants (N=227)	
	n	**%**	**n**	**%**
Treatment Arm:				
Maternal antiretroviral arm *	16	59	106	47
Lopinavir-based	12		71	
Nelfinavir-based	4		29	
Nevirapine-based	0		6	
Infant nevirapine arm	11	41	121	53
PK Concentrations:				
Plasma				
Maternal antiretroviral arm (based on 3rd drug):				
None >EC50 at any time point	9	56	26	25
Intermittently >EC50	2	13	49	47
All time points >EC50	5	31	29	28
Infant nevirapine arm:				
None >EC50 at any time point	1	9	11	9
Intermittently >EC50	5	45	30	25
All time points >EC50	5	45	80	66
Breastmilk				
Maternal antiretroviral arm (based on 3rd drug):				
None >EC50 at any time point	7	54	27	25
Intermittently >EC50	3	23	59	56
All time points >EC50	3	23	20	19

EC50=median effective concentration, PK=pharmacokinetic

* Note: Reported regimen at first specimen collection. Plasma specimens missing for 2 mothers assigned to the maternal ARV arm, but breastmilk specimens were tested. Breastmilk specimens missing for 3 HIV-infected mothers assigned to maternal ARV arm.

Table 3.

Ratio of breastmilk concentration to maternal plasma concentration, maternal arm only

		Breastmilk/plasma ratio	
	# specimens	Mean	Median
LPV	165	0.15	0.07
NFV	84	0.21	0.08
NVP	21	0.61	0.52
3TC	258	2.73	2.24
AZT	188	1.26	1.00

Table 4.

Association between maternal drug concentrations, by compartment, and detectable maternal plasma and breastmilk viral load between 2-24 weeks postpartum; maternal arm only

	Detectable plasma VL				*Detectable breastmilk VL*			
	Unadjusted		**Adjusted**		**Unadjusted**		**Adjusted**	
	OR	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**
Plasma drug concentration								
>EC50 vs ≤EC50	0.64	(0.45, 0.91)	0.66	(0.47, 0.93)	0.22	(0.14, 0.35)	0.22	(0.14, 0.34)
Breastmilk drug concentration								
>EC50 vs ≤EC50	0.62	(0.45, 0.85)	0.63	(0.46, 0.87)	0.42	(0.29, 0.59)	0.41	(0.29, 0.59)

CI=confidence interval, EC50=median effective concentration, OR=odds ratio, VL=viral load

*Adjusted for nutritional randomization, maternal age, baseline viral load, and baseline CD4

Table 5.

Association between drug concentrations, by compartment, and breastmilk HIV transmission between 2-28 weeks

	Unadjusted			Adjusted*		
	# transmissions	HR	(95% CI)	# transmissions	HR	(95% CI)
Plasma drug concentration **						
>EC50 vs ≤EC50	26	0.40	(0.18, 0.93)	25	0.43	(0.18, 0.99)
Breastmilk drug concentration **						
>EC50 vs ≤EC50	13	0.31	(0.08, 1.17)	13	0.31	(0.09, 1.09)

HR=hazard ratio, CI=confidence interval, EC50=median effective concentration

*Adjusted for baseline maternal viral load; baseline viral load was missing for 1 mother

**Maternal plasma drug concentrations were used for mother-infant pairs randomized to maternal ARVs and infant NVP plasma concentrations were used for mother-infant pairs randomized to infant NVP; only mothers randomized to maternal ARVs were included when assessing the association between breastmilk drug concentrations and HIV transmission