**Supplemental Digital Content to**

**Association Of Prophylactic Antiretroviral Regimen With Time To Positive HIV-1 DNA PCR In Infants Infected With Non-B Subtype HIV-1**

Raji Balasubramanian, Mary Glenn Fowler, Ken Dominguez, Shahin Lockman, Pat Tookey, Nicole Ngo Giang Huong, Steve Nesheim, Michael Hughes, Marc Lallemant, Jennifer Tosswill, Nathan Shaffer, Gayle Sherman, Paul Palumbo, David E. Shapiro

In this Supplemental Digital Content, we present the details regarding each of the following:

1. Cohorts included in the analysis.
2. PCR specimens and assays
3. Distribution of DNA PCR test times (ages) by each primary covariate [maternal ARV regimen, infant ARV regimen, timing of maternal ARV initiation]
4. Definition of HIV infection status
5. Ascertainment of infant feeding [breast fed or formula fed]
6. Covariates: Maternal HIV-1 viral load and CD4+ cell count, Mode of delivery, Timing of maternal HIV infection diagnosis, Gestational age, Infant birth weight
7. Other related variables
8. Statistical methods
9. Subgroup analysis: Data from studies conducted in Thailand (n=299).
10. References for items 1 and 8 above.

We also present Tables S1-S3 and Figures S1-S3

1. **Details on each cohort included in the analysis**:

**Botswana.** This 2x2 factorial randomized clinical trial (‘MASHI’) enrolled in Botswana between 2001 and 2003[1], where subtype C HIV-1 infection is prevalent. Infants born to HIV-infected mothers were randomized to sdNVP or placebo and to either formula feeding or to breastfeeding with extended infant zidovudine prophylaxis. The majority of mothers received zidovudine during pregnancy with or without sdNVP during labor, and a small fraction started cART during pregnancy. Data comprising 91 DNA PCR test results from 32 HIV-infected infants born to mother-infant pairs randomized to the formula-feeding arm of the trial were included.

**Thailand (CDC).** Data from two perinatal studies,[2-4] one a natural history study and one a randomized controlled trial of short-course zidovudine vs. placebo, conducted by this collaboration between 1992 and 1998 were included in this analysis. The studies were conducted in Thailand, where subtype E HIV-1 infection is prevalent. The analysis included 370 DNA PCR test results on 122 HIV-infected non-breastfed infants. The majority of the mothers enrolled in both studies did not receive ARV, with a small fraction in the later study receiving single NRTI.

**Thailand - Program for HIV Prevention and Treatment (PHPT).** Data on 678 DNA PCR test results from 177 HIV-infected non-breastfed infants from two studies conducted in Thailand between 1997 and 2003 were included in this analysis.[5, 6] Subtype E HIV-1 infection is prevalent in the region. The majority of mothers received single NRTI with or without sdNVP during labor, and a small number receiving no ARV.

**United Kingdom - National Study of HIV in Pregnancy and Childhood/Health Protection Agency Collaboration (NSHPC/HPA).** This ongoing reporting scheme in the UK and Ireland has tracked pregnancies in HIV-infected women, infants born to HIV-infected women, and other children living with HIV infection and AIDS since 1990[7-9]. Data comprising 181 DNA PCR test results from 74 HIV-infected non-breastfed infants collected during the period 2000-2009 were analyzed. All 74 HIV-positive mothers were known to have acquired HIV infection in countries with predominantly non-B subtype virus. Maternal ARV regimen was varied with 46% (n=34) on cART, 41% (n=30) receiving no ARV, 11% (n=8) on single NRTI and 2% (n=2) on single NRTI with sdNVP at labor.

1. **PCR assays/specimens**: Qualitative DNA PCR assays were performed on either blood specimen or dried blood spots. DNA PCR testing was carried out using Roche Amplicor 1.5 HIV-1 DNA PCR assays. Tests done after 2004 in the NSHPC/HPA dataset were based on an in-house DNA PCR assay.
2. **Age at first DNA PCR tests**: Visual inspection of the histograms of age at first DNA PCR testing by maternal ARV, infant ARV and timing of maternal ARV initiation showed no marked differences between groups (See Supplemental Figures 1 – 3, below).
3. **Definition of HIV infection status:**

**Botswana:** Infants were considered infected at time of earliest positive test result which was subsequently confirmed, or if they were lost/died after one positive PCR test result.

**Thailand (CDC)**: Before 2 months of age, infants were considered HIV-infected with just one positive DNA PCR test, but after 2 months infants were not considered infected if their most recent DNA PCR test was negative.

**Thailand (PHPT):** Infants were considered HIV-infected if there were two separate occasions of positive DNA PCR tests.

**United Kingdom (NSHPC/HPA):** As in Thailand (PHPT)**.**

1. **Feeding status (not breastfeeding):** was based on maternal self-report with the exception of NSHPC/HPA, which was based on clinician report.
2. **Covariates:**

**Maternal HIV-1 viral load and CD4+ cell count:** from the closest specimen obtained before delivery or within three days after delivery were included. Maternal viral load and CD4+ cell count were analyzed as categorical covariates according the categories in Table 1 (Appendix).

**Mode of delivery:** was categorized according to three groups, namely (1) Vaginal; (2) Cesarean section (C-section) before the onset of labor or membrane rupture; (3) C-section after the onset of membrane rupture or labor.

**Timing of maternal HIV infection diagnosis**: was categorized according to five groups, namely (1) before current pregnancy; (2) during current pregnancy; (3) during labor; (4) after delivery (all cohorts except UK); and (5) within two days following birth (UK only).

**Gestational age at birth:** was categorized as (1) less than 37 weeks (preterm) or (2) greater than or equal to 37 weeks.

**Infant birth weight:** was categorized as (1) less than 2500 g (low birth weight) or (2) greater than or equal to 2500 g.

1. **Other variables**: The following maternal characteristics were obtained from each study: age at delivery, race/ethnicity, country of birth and country where HIV infection was acquired. Information on HIV subtype was inferred based on the most prevalent subtype in the country where mother’s HIV infection was acquired. Data on the following infant characteristics were also obtained from each study: calendar year of birth, race/ethnicity, AIDS defining condition diagnosed prior to 18 months of age, CD4% closest to birth, whether ARV was initiated following HIV diagnosis and age of ARV initiation for treatment of HIV infection.
2. **Statistical Methods**

Time to first positive HIV-1 DNA PCR test among all non-breastfed HIV-infected infants was estimated using parametric models appropriate for interval-censored outcomes. The data are interval-censored because for each infant, the true (unobserved) time when the HIV-1 DNA PCR test would first be positive is only known to have occurred in the interval from the time of the last negative test to the time of the first positive test result. We assume that after an infant has his/her first positive HIV test result, all subsequent tests would be positive. The models provide estimates of the probabilities of testing positive by HIV-1 DNA PCR among HIV-infected, non-breastfed infants, by age of infant.

Time to first positive HIV-1 DNA PCR was estimated according to three primary variables - maternal or infant ARV regimen category and timing of maternal ARV initiation. These variables could not be modeled jointly due to their high concordance (Table 1 of Main paper). Parametric estimates based on the Weibull assumption were compared graphically to non-parametric estimates using the non-parametric maximum likelihood estimate (NPMLE) algorithm described by Turnbull (1976) [10], and were qualitatively similar (results available upon request). Stratified Weibull models were fit to evaluate the association of each variable (maternal/infant ARV regimen, timing of maternal ARV initiation) with time to first positive HIV-1 DNA PCR[11] . In these models, the distribution of the time to first positive HIV-1 DNA PCR was assumed to follow an independent Weibull distribution within each ARV regimen or timing of initiation category. Assuming proportional hazards (PH), models were adjusted for other covariates including maternal CD4+ cell count and viral load closest to the time of delivery, mode of delivery, gestational age, and infant birth weight. Statistical significance of the association of each variable with time to first positive HIV-1 DNA PCR was assessed using a two-sided likelihood ratio test. Pair wise comparisons of the rates of test positivity at specific times between groups (e.g. categories of timing of maternal ARV initiation or maternal/infant ARV regimen) were made by comparing the point estimates and their corresponding 95% confidence intervals. Stratified Weibull models[11] were also used to model the association of time to first positive HIV-1 DNA PCR with each of the covariates maternal CD4+ cell count and viral load closest to the time of delivery, mode of delivery, gestational age and infant birth weight.

Analyses were conducted using the R software[12], version 2.8.0.

1. Subgroup analysis: Data from studies in Thailand (n=299).

This analysis is based on data from the Thailand-PHPT and Thailand-CDC studies. See Table S2 for the distribution of the number of infants according to maternal, infant ARV regimen and timing of maternal ARV initiation. In this subgroup of 299 HIV -1 infected infants, none of the infants or their HIV-1 positive mothers were exposed to cART.

We evaluated the association of each of the key exposures (maternal/infant ARV regimen, timing of ARV initiation) with time to first positive HIV-1 DNA PCR in univariate and multivariable models. Maternal ARV regimen was significantly associated with time to first positive HIV-1 DNA PCR in a univariate model (p < 0.0001) and remained significant after adjusting for potential confounders including CD4+ cell count and viral load closest to delivery, mode of delivery, gestational age and birth weight (p < 0.0001). Similar results were observed for the association of infant ARV regimen and timing of maternal ARV initiation with time to first positive HIV-1 DNA PCR. See Table S3 for the probabilities of a positive HIV-1 DNA PCR test (95% confidence interval) at 1, 14, 28 and 60 days after birth, according to Maternal ARV regimen; Infant ARV regimen; and Timing of maternal ARV initiation, obtained in univariate models. The results from this subgroup analysis show trends consistent with that reported in the primary analyses combining all cohorts.

1. References

1. Thior I, Lockman S, Smeaton LM, Shapiro RL, Wester C, Heymann SJ, Gilbert PB, Stevens L, Peter T, Kim S *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**(7):794-805.

2. Shaffer N, Chuachoowong R, Mock PA, Bhadrakom C, Siriwasin W, Young NL, Chotpitayasunondh T, Chearskul S, Roongpisuthipong A, Chinayon P *et al*: **Short-course zidovudine for perinatal HIV-1 transmission in Bangkok, Thailand: a randomised controlled trial. Bangkok Collaborative Perinatal HIV Transmission Study Group**. *Lancet* 1999, **353**(9155):773-780.

3. Shaffer N, Roongpisuthipong A, Siriwasin W, Chotpitayasunondh T, Chearskul S, Young NL, Parekh B, Mock PA, Bhadrakom C, Chinayon P *et al*: **Maternal virus load and perinatal human immunodeficiency virus type 1 subtype E transmission, Thailand. Bangkok Collaborative Perinatal HIV Transmission Study Group**. *J Infect Dis* 1999, **179**(3):590-599.

4. Young NL, Shaffer N, Chaowanachan T, Chotpitayasunondh T, Vanparapar N, Mock PA, Waranawat N, Chokephaibulkit K, Chuachoowong R, Wasinrapee P *et al*: **Early diagnosis of HIV-1-infected infants in Thailand using RNA and DNA PCR assays sensitive to non-B subtypes**. *J Acquir Immune Defic Syndr* 2000, **24**(5):401-407.

5. Lallemant M, Jourdain G, Le Coeur S, Kim S, Koetsawang S, Comeau AM, Phoolcharoen W, Essex M, McIntosh K, Vithayasai V: **A trial of shortened zidovudine regimens to prevent mother-to-child transmission of human immunodeficiency virus type 1. Perinatal HIV Prevention Trial (Thailand) Investigators**. *N Engl J Med* 2000, **343**(14):982-991.

6. Lallemant M, Jourdain G, Le Coeur S, Mary JY, Ngo-Giang-Huong N, Koetsawang S, Kanshana S, McIntosh K, Thaineua V: **Single-dose perinatal nevirapine plus standard zidovudine to prevent mother-to-child transmission of HIV-1 in Thailand**. *N Engl J Med* 2004, **351**(3):217-228.

7. Judd A, Ferrand RA, Jungmann E, Foster C, Masters J, Rice B, Lyall H, Tookey PA, Prime K: **Vertically acquired HIV diagnosed in adolescence and early adulthood in the United Kingdom and Ireland: findings from national surveillance**. *HIV Med* 2009, **10**(4):253-256.

8. Townsend CL, Byrne L, Cortina-Borja M, Thorne C, de Ruiter A, Lyall H, Taylor GP, Peckham CS, Tookey PA: **Earlier initiation of ART and further decline in mother-to-child HIV transmission rates, 2000-2011**. *AIDS* 2014, **28**(7):1049-1057.

9. Townsend CL, Cortina-Borja M, Peckham CS, de Ruiter A, Lyall H, Tookey PA: **Low rates of mother-to-child transmission of HIV following effective pregnancy interventions in the United Kingdom and Ireland, 2000-2006**. *AIDS* 2008, **22**(8):973-981.

10. Turnbull BW: **The Empirical Distribution Function with Arbitrarily Grouped, Censored and Truncated Data**. *Journal of the Royal Statistical Society Series B (Methodological)* 1976, **38**(3):290-295.

11. Xiangdong Gu DS, Michael D. Hughes, Raji Balasubramanian: **Stratified Weibull Regression Model for Interval-Censored Data**. *R Journal* 2014, **6**(1):10.

12. R-Core-Team: **R: A Language and Environment for Statistical Computing**. 2012.

Table S1: Maternal and infant characteristics by maternal ARV regimen

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Characteristic** | **No ARV**  **% (n)** | **Single NRTI**  **% (n)** | **sdNVP + ZDV**  **% (n)** | **cART**  **% (n)** | **P value** |
| **Cohort:** |  |  |  |  | **0.0005** |
| Botswana (MASHI) | 0.0%  (0) | 9.7% (16) | 21.2% (14) | 5.6%  (2) |  |
| Thailand - CDC | 75.4% (104) | 10.9% (18) | 0.0%  (0) | 0.0%  (0) |  |
| Thailand - PHPT | 2.9%  (4) | 74.5% (123) | 75.8% (50) | 0.0%  (0) |  |
| United Kingdom (NSHPC) | 21.7% (30) | 4.8%  (8) | 3.0%  (2) | 94.4% (34) |  |
| **Enrollment years:** |  |  |  |  | **0.0005** |
| 1992-1994 | 48.6% (67) | 0.0%  (0) | 0.0%  (0) | 0.0%  (0) |  |
| 1996-1999 | 27.5% (38) | 67.9% (112) | 0.0%  (0) | 0.0%  (0) |  |
| 2000-2003 | 14.5% (20) | 29.1% (48) | 98.5% (65) | 36.1% (13) |  |
| 2004-2008 | 9.4% (13) | 3.0%  (5) | 1.5%  (1) | 63.9% (23) |  |
| **Maternal CD4+ cell count, cells/ul:** |  |  |  |  | **0.002** |
| < 200 | 12.6% (14) | 26.5% (36) | 31.7% (20) | 39.4% (13) |  |
| [200 - 350) | 27.0% (30) | 35.3% (48) | 28.6% (18) | 30.3% (10) |  |
| [350 - 500) | 27.0% (30) | 24.3% (33) | 22.2% (14) | 18.2% (6) |  |
| >= 500 | 33.3% (37) | 14.0% (19) | 17.5% (11) | 12.1% (4) |  |
| **Maternal viral load, copies/ml:** |  |  |  |  | **0.0005** |
| < 400 | 0.0%  (0) | 8.0%  (9) | 1.6%  (1) | 33.3% (11) |  |
| 400 - 999 | 0.9%  (1) | 14.3% (16) | 4.7%  (3) | 15.2% (5) |  |
| 1,000 - 9,999 | 8.0%  (9) | 34.8% (39) | 18.8% (12) | 9.1%  (3) |  |
| 10,000 - 99,999 | 54.5% (61) | 72.3% (81) | 53.1% (34) | 30.3% (10) |  |
| >= 100,000 | 36.6% (41) | 14.3% (16) | 21.9% (14) | 12.1% (4) |  |
| **Mode of delivery:** |  |  |  |  | **0.0001** |
| Vaginal | 91.2% (124) | 77.6% (128) | 75.8% (50) | 22.2% (8) |  |
| C-section before onset of labor and membrane rupture | 2.2%  (3) | 10.3% (17) | 4.5%  (3) | 44.4% (16) |  |
| C-section after onset of labor or membrane rupture | 6.6%  (9) | 12.1% (20) | 19.7% (13) | 33.3% (12) |  |
| **Gestational age** |  |  |  |  | **0.34** |
| < 37 weeks | 10.7% (14) | 14.1% (23) | 13.6% (9) | 22.2% (8) |  |
| >= 37 weeks | 89.3% (117) | 85.9% (140) | 86.4% (57) | 77.8% (28) |  |
| **Birth weight:** |  |  |  |  | **0.77** |
| < 2500 grams | 16.2% (21) | 19.5% (32) | 18.5% (12) | 23.5% (8) |  |
| >= 2500 grams | 83.8% (109) | 80.5% (132) | 81.5% (53) | 76.5% (26) |  |
| **Timing of maternal HIV diagnosis** |  |  |  |  | **0.0001** |
| Before pregnancy | 14.7% (5) | 95.4% (125) | 96.2% (50) | 23.5% (8) |  |
| During pregnancy | 11.8% (4) | 3.1%  (4) | 1.9%  (1) | 70.6% (24) |  |
| After delivery | 52.9% (18) | 0.0%  (0) | 0.0%  (0) | 0.0%  (0) |  |
| During labor | 0.0%  (0) | 1.5%  (2) | 1.9%  (1) | 5.9%  (2) |  |
| Within 2 days following labor | 20.6% (7) | 0.0%  (0) | 0.0%  (0) | 0.0%  (0) |  |
| **HIV subtype:** |  |  |  |  | **0.0005** |
| A | 1.5%  (2) | 0.0%  (0) | 0.0%  (0) | 2.9%  (1) |  |
| AG | 0.7%  (1) | 0.0%  (0) | 0.0%  (0) | 2.9%  (1) |  |
| C | 10.2% (14) | 12.7% (21) | 24.2% (16) | 48.6% (17) |  |
| E | 79.6% (109) | 85.5% (141) | 75.8% (50) | 2.9%  (1) |  |
| Mixed | 8.0% (11) | 1.8%  (3) | 0.0%  (0) | 42.9% (15) |  |

Table S2: Thai subset analysis: Infants classified by Maternal ARV regimen and either infant ARV regimen, or timing of maternal ARV initiation (n=299).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Infant ARV** | **Maternal ARV** | | | |
|  | No ARV | Single NRTI | sdNVP + ZDV | cART |
| No ARV | 104 | 18 | 0 | 0 |
| Single NRTI | 1 | 120 | 20 | 0 |
| sdNVP+ZDV | 3 | 3 | 30 | 0 |
| cART | 0 | 0 | 0 | 0 |
| **Timing of maternal ARV initiation** |  | | | |
| No ARV | 108 | 0 | 0 | 0 |
| Labor and delivery | 0 | 7 | 9 | 0 |
| During trimester of delivery | 0 | 131 | 38 | 0 |
| Prior to trimester of delivery | 0 | 3 | 3 | 0 |
| Abbreviations: Abbreviations: ARV = antiretroviral; cART = combination antiretroviral therapy; NRTI = nucleoside reverse transcriptase inhibitor; sdNVP = single-dose nevirapine; ZDV = zidovudine | | | | |

Table S3: Analysis of Thai Cohorts: Probability of a positive HIV-1 DNA PCR test (95% confidence interval) among HIV-infected non-breastfed infants at 1, 14, 28 and 60 days after birth, according to Maternal ARV regimen; Infant ARV regimen; and Timing of maternal ARV initiation (n=299).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Age at positive DNA PCR test**  **(days since birth)** | | | |
| **Maternal**  **ARV category** | **1 day**  **Probability**  **(95% CI)** | **14 days**  **Probability**  **(95% CI)** | **28 days**  **Probability**  **(95% CI)** | **60 days**  **Probability**  **(95% CI)** |
| No ARV  (n=108) | 47%  (37% - 59%) | 79%  (72% - 86%) | 86%  (80% - 92%) | 92%  (87% - 96%) |
| Single NRTI  (n=141) | 86%  (80% - 91%) | 92%  (88%-96%) | 94%  (89%-96%) | 95%  (91%-97%) |
| sdNVP + ZDV  (n=50) | 76%  (64% - 86%) | 88%  (79% - 95%) | 91%  (83% - 96%) | 93%  (85% - 98%) |
| **Infant**  **ARV category** |  | | | |
| No ARV  (n=122) | 58%  (48% - 67%) | 82%  (76% - 88%) | 88%  (82% - 92%) | 92%  (87% - 96%) |
| Single NRTI  (n=141) | 83%  (76%-88%) | 91%  (86%-94%) | 92%  (88%-96%) | 94%  (90%-97%) |
| sdNVP + ZDV  (n=36) | 80%  (67%-91%) | 90%  (79%-96%) | 91%  (82%-97%) | 93%  (84%-98%) |
| **Timing of Maternal ARV Initiation** |  | | | |
| No ARV  (n=108) | 47%  (37%-59%) | 79%  (72%-86%) | 86%  (80%-92%) | 92%  (87%-96%) |
| Labor and delivery  (n=16) | 92%  (75%-99%) | 95%  (80%-100%) | 96%  (81%-100%) | 97%  (82%-100%) |
| During trimester of delivery  (n=169) | 83%  (78%-88%) | 91%  (87%-94%) | 93%  (89%-96%) | 94%  (90%-97%) |
| Prior to trimester of delivery  (n=6) | 29%  (6%-85%) | 81%  (48%-99%) | 92%  (60%-100%) | 98%  (68%-100%) |
| Abbreviations: Abbreviations: ARV = antiretroviral; cART = combination antiretroviral therapy; CI = confidence interval; DNA = deoxyribonucleic acid; NRTI = nucleoside reverse transcriptase inhibitor; PCR = polymerase chain reaction; sdNVP = single-dose nevirapine; ZDV = zidovudine | | | | |



Figure S1: Distribution of HIV-1 DNA PCR test times (ages), by Maternal ARV regimen.



Figure S2: Distribution of HIV-1 DNA PCR test times (ages), by Infant ARV Regimen.



Figure S3: Distribution of HIV-1 DNA PCR test times (ages), by timing of maternal ARV initiation.