

## Supplementary Material

### Predictors of cell-associated HIV-1 DNA over one year in very early treated infants

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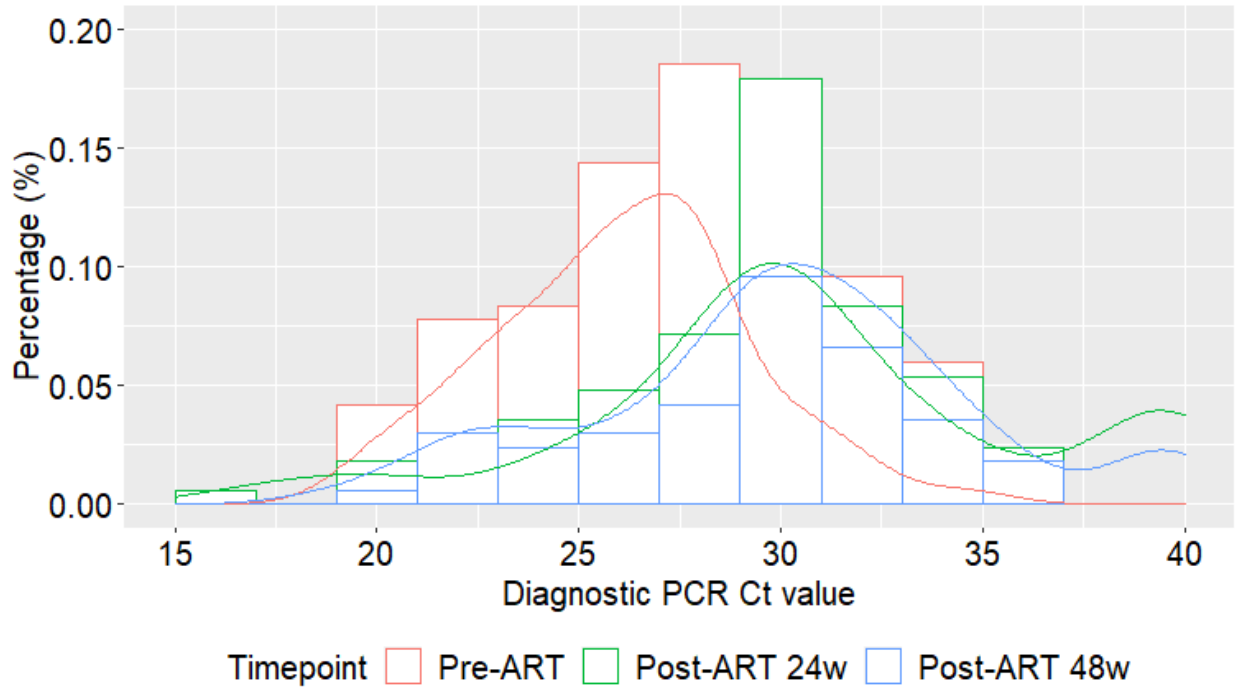
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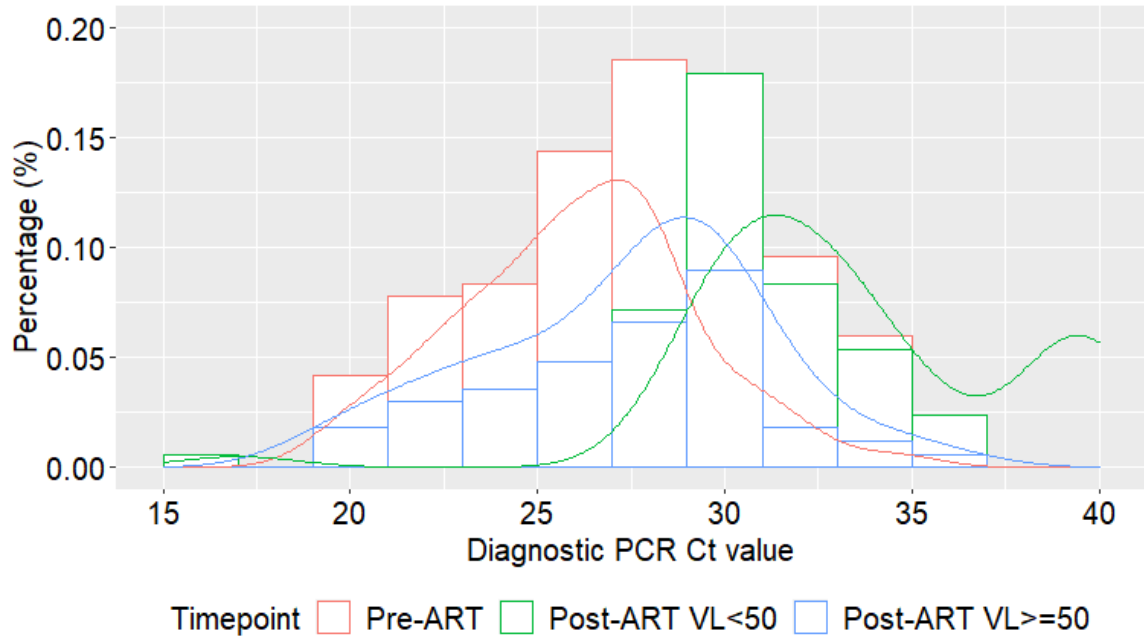
**Sequences of primers and probes in the assay:**

Sequences of primers and probes were as follows, *integrase-1*: first-round conventional PCR, forward primer 5'-TGG CAG TRT TCA TTC ACA ATT TTA-3', reverse primer 5'-ACT ACT GCY CCT TCA CCT TTC CA-3'; second-round qPCR, forward primer 5'-CGG GTT TAT TAC AGR GAC AGC AGA G-3', reverse primer 5'-ACT ACT GCY CCT TCA CCT TTC CA-3', probe 5'-6FAM-TTG GCT GGT CCT TTC CA-MGB-3'; *integrase-2*: first-round conventional PCR, forward primer 5'-AAA GGT GAA GGR GCA GTA GTA ATA CAA-3', reverse primer 5'-TCA TCC TGT CTA CYT GCC ACA CAA-3'; second-round qPCR, forward primer 5'-AAA GGT GAA GGR GCA GTA GTA ATA CAA-3', reverse primer 5'-TCC TGT CYA CYT GCC ACA CAA TCA-3', probe 5'-6FAM-TGC CAT CTG TTT TCC ATA-MGB-3'.

Supplementary Figure 1: Distribution of the cycle threshold (Ct) values from the diagnostic HIV-1 PCR tests pre-ART and at 24 and 48 weeks post-ART initiation (Panel A) and pre-ART and post-ART time points stratified by viral load below or above 50 copies/ml of plasma (Panel B).

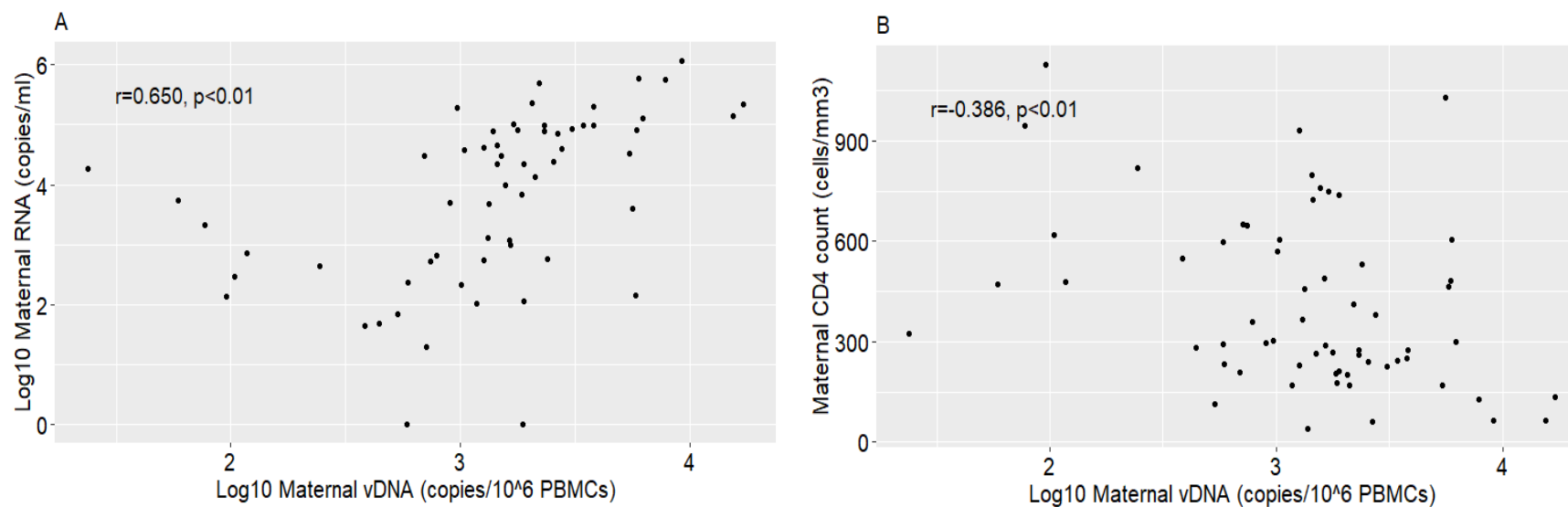


	Pre-ART	24 weeks	48 weeks
N	63	41	63
PCR Ct Median (IQR)	26.1 (23.7, 28.0)	30.2 (28.1, 33.6)	30.1 (27.7, 32.5)
Mean (std)	26.1 (3.2)	30.6 (5.5)	30.0 (4.7)
Min, Max	19.5 , 34.6	17.0 , 39.5	19.1 , 39.5
# negative PCR	0/63	7/41	4/63



	Pre-ART	Post-ART vl<50	Post-ART vl≥50
N	63	50	54
PCR Ct Median (IQR)	26.1 (23.7, 28.0)	32.5 (30.5, 35.7)	28.1 (24.5, 29.8)
Mean (std)	26.1 (3.2)	33.4 (4.4)	27.4 (3.8)
Min, Max	19.5 , 34.6	17.0 , 39.5	19.1 , 36.0
# negative PCR	0/63	11/50	0/54

**Supplementary Figure 2: Association between maternal levels of HIV-1 total DNA / 10<sup>6</sup> PBMC equivalents and concurrent HIV-1 RNA copies/ml plasma (Panel A) and CD4+ T cells /mm<sup>3</sup> (Panel B) among 62 mothers of the infants included in this analysis**



C: Univariate linear regression predicting maternal HIV-1 DNA (log copies/10<sup>6</sup> cells) among 62 mothers by baseline characteristics

	Univariate linear regression predicting maternal HIV-1 DNA (log copies/10 <sup>6</sup> cells)		
	Coefficient	95% Confidence Interval	P-value
Mother had no ART prior to delivery (ref: Had ART)	-0.188	-0.516, 0.140	0.256
Maternal VL (per log10 copies/ml)	0.180	0.093, 0.266	<b>&lt;0.0001</b>
Maternal CD4 count ≤ 350 cells/mm <sup>3</sup> (ref: > 350 cells/mm <sup>3</sup> )	0.311	0.032, 0.591	<b>0.030</b>
Maternal CD4 count cells (per 100 cells/mm <sup>3</sup> )	-0.080	-0.131, -0.029	<b>0.003</b>

Supplementary Table 1: Multivariable associations between maternal and infant characteristics and infant HIV-1 DNA in the first 48 weeks of antiretroviral therapy among the subset of infant with plasma RNA viral load below 50 copies/ml (30 infants; 43 measurements)

	Generalized estimating equation (GEE) analysis predicting post-ART vDNA in log copies/10 <sup>6</sup> PBMC equivalents		
	Parameter co-efficient	95% Confidence Interval	P-value
<b>Maternal Characteristics</b>			
Mother had no ART prior to delivery (ref: Had ART)	-0.6751	-1.0691, -0.2811	<b>0.0008</b>
<b>Infant Characteristics</b>			
Start ART < 48 hours after birth (ref: >14 days)	-0.2868	-0.6968, 0.1233	<b>0.1705</b>
Start ART 49 hours to 14 days (ref >14 days)	-0.3768	-0.7624, 0.0089	<b>0.0555</b>
<b>Pre-ART infant disease severity markers</b>			
Infant Pre-ART CD4 percent	-0.0503	-0.0654, -0.0352	<b>&lt;0.0001</b>
<b>Post-natal</b>			
Ever BF (ref: never BF)	-0.6045	-0.9613, -0.2477	<b>0.0009</b>

Tabular data pertaining to Figure 1: Box plot of maternal HIV-1 DNA levels and infant HIV-1 DNA levels by time in weeks from initiation of antiretroviral therapy.

	Pre-ART*	0-18 weeks	19-35weeks	36 weeks52 weeks	Maternal DNA
Number of samples	13	60	36	37	62
Log <sub>10</sub> vDNA copies/10 <sup>6</sup> PBMC equivalents					
Median	3.21	2.62	2.40	2.36	3.20
IQR (Q1 , Q3)	2.67, 3.76	1.58, 3.13	1.44, 2.73	1.70, 2.95	2.87, 3.42
Min , Max	0 , 4.04	0 , 4.55	0 , 4.13	0 , 4.61	1.38 , 4.23
vDNA copies/10 <sup>6</sup> PBMC equivalents in categories N (%)					
Undetectable	1 (7.7)	6 (10.0)	3 (8.3)	5 (13.5)	0
1-10	0	3 (5.0)	1 (2.8)	1 (2.7)	0
11-100	0	10 (16.7)	7 (19.4)	7 (18.9)	4 (6.5)
101-400	2 (15.4)	10 (16.7)	12 (33.3)	10 (27.0)	4 (6.5)
401-1000	2 (15.4)	9 (15.0)	8 (22.2)	6 (16.2)	11 (17.7)
>1000	8 (61.5)	22 (36.7)	5 (13.9)	8 (21.6)	43 (69.4)

\*Pre-treatment samples were collected from 7 infants on day 1, 3 infants on day 2 of life and one infant at each of day 6, 22 and 60.

Tabular data pertaining to Figure 2: Cross-tabulation between infant cell-associated HIV-1 DNA and infant HIV-1 RNA in plasma (viral load) in categories over the first 48 weeks of antiretroviral therapy.

	Viral load HIV-1 RNA copies/ml plasma					
	Target Not Detected	Detected but <50	50-1000	1001-10,000	>10,000	Total
<b>vDNA copies/10<sup>6</sup> PBMC equivalents</b>	<b>N (column percentage)</b>					
Undetectable	6 (23.1)	3 (17.7)	5 (10.6)	0	0	14 (10.6)
1-10	3 (11.5)	0	1 (2.1)	1 (6.7)	0	5 (3.8)
11-100	7 (26.9)	6 (35.3)	9 (19.2)	1 (6.7)	0	23 (17.4)
101-400	7 (26.9)	4 (23.5)	14 (29.8)	5 (33.3)	3 (7.4)	32 (24.2)
401-1000	3 (11.5)	3 (17.7)	12 (25.5)	1 (6.7)	4 (14.8)	23 (17.4)
>1000	0	1 (5.9)	6 (12.8)	7 (46.7)	21 (77.8)	35 (26.5)
Total	26 (19.7)	17 (12.9)	47 (35.6)	15 (11.4)	27 (20.5)	132