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MAJOR ARTICLE

Viral Reservoir in Early-Treated Human Immunodeficiency Virus-Infected Children and Markers for Sustained Viral Suppression

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Background. The impact of very early infant treatment on human immunodeficiency virus (HIV) reservoir, and markers for treatment success, require study.

Methods. The Early Infant Treatment Study (EIT) enrolled 40 children living with HIV started on antiretroviral treatment (ART) at <7 days of age, with 23 who had started treatment between 30–365 days to serve as controls. Quantitative HIV DNA was evaluated every 1–3 months in peripheral blood mononuclear cells. 84-week repeat qualitative whole blood DNA polymerase chain reaction and dual enzyme immunosorbent assay were performed.

Results. Median quantitative cell-associated DNA after at least 84 weeks was significantly lower among the first 27 EIT children tested than among 10 controls (40.8 vs 981.4 copies/million cells; $P < .001$) and correlated with pre-ART DNA. Median DNA after 84 weeks did not differ significantly by negative or positive serostatus at 84 weeks ($P = .94$), and appeared unaffected by periods of unsuppressed plasma RNA from 24–84 weeks ($P = .70$). However, negative 84-week serostatus was 67% predictive for sustained RNA suppression, and positive serostatus was 100% predictive for viremia. Loss of qualitative DNA positivity at 84 weeks was 73% predictive for sustained suppression, and persistent positivity was 77% predictive for viremia.

Conclusions. Lower viral reservoir was associated with starting ART at <1 week. Negative serostatus and qualitative DNA were useful markers of sustained viral suppression from 24–84 weeks.

Keywords. children; early treatment; viral reservoir; Botswana.

Early infant treatment for HIV is associated with improved clinical outcomes [1, 2], but most pediatric HIV diagnosis and treatment programs currently test children at 4–6 weeks of age, to capture both in utero and intrapartum transmission events. Such programs miss the opportunity to begin very early antiretroviral treatment (ART) for children infected in utero. By arresting HIV in the first week of life, only a limited population of susceptible CD4+ cells may become infected [3, 4], and immunologic responses to the virus may be less likely to develop [5, 6]. This strategy may reduce seeding of viral reservoirs [3, 7, 8] and improve both short-term and long-term treatment outcomes. Understanding virologic and immunologic differences between children starting ART at birth or later in life could shed light on critical differences in immune development and the viral reservoir.

The unique nature of very early infant treatment also raises the possibility of using the most basic HIV screening tests—enzyme immunosorbent assay (EIA) and a diagnostic qualitative DNA polymerase chain reaction (PCR) —as longitudinal markers for viral reservoir quantification and for treatment success. Children treated from very early in life may not develop a positive serostatus by EIA [5, 6, 9] and may lose DNA PCR positivity [10–13]. Among children, collection of peripheral blood mononuclear cells (PBMCs) – our current best estimate of HIV viral reservoir—is challenging, and new markers would also facilitate monitoring during interventions aimed at reservoir eradication.

The Early Infant Treatment Study (EIT) in Botswana enrolled children living with HIV with in utero infection who initiated ART < 7 days of life, and control children living with HIV who started ART later in the first year of life. Here we describe qualitative and quantitative HIV DNA testing, HIV RNA testing, and HIV serology among children who reached 84 weeks on ART, and among control children who started ART later in life.

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Nonstandard abbreviations: 3TC, lamivudine; ART, antiretroviral treatment; BHHRL, Botswana-Harvard HIV Reference Laboratory; EIA, enzyme immunosorbent assay; EIT, Early Infant Treatment Study; LPV-r, ritonavir-boosted lopinavir; NIAID, National Institute of Allergy and Infectious Disease; NVP, nevirapine; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; REDCap, Research Electronic Data capture; ZDV, zidovudine.

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METHODS

Trial Design and Study Population

Between April 2015 and July 2018, HIV-exposed children < 96 hours of age were screened for HIV by qualitative DNA PCR in the Gaborone and Francistown regions of Botswana as part of the Botswana-Harvard Partnership EIT Study. Screening was conducted at 5 hospital maternity wards (Princess Marina Hospital in Gaborone, Scottish Livingstone Hospital in Molepolole, Deborah Retief Memorial Hospital in Mochudi, Nyangabgwe Referral Hospital in Francistown, and Selebi Phikwe Government Hospital in Selebi Phikwe), and in surrounding maternity clinics in the greater Gaborone and Francistown areas.

Children found to be HIV-positive were offered enrollment in EIT if mother/guardian was ≥ 18 years of age and able to provide informed consent, gestational age at birth ≥ 35 weeks, birth weight ≥2000 grams, age < 96 hours after birth, ability to initiate antiretroviral therapy (ART) within 7 days after birth, and eligible for ART through the Botswana government program (which provided ART for all citizens living with HIV). Children were excluded from screening if they were hospitalized for severe medical illness or if they had a medical condition making it unlikely that the infant would survive. Early-treated children were followed weekly for 6 weeks, then at 8 and 12 weeks, and 12 weekly thereafter, with HIV RNA testing at each visit. Qualitative DNA PCR was repeated at 84 weeks with serologic testing by EIA. Quantitative DNA PCR testing of PBMCs occurred at enrollment prior to ART initiation and longitudinally post ART initiation. Enrolled children were started on an initial regimen of nevirapine (NVP), zidovudine (ZDV), and lamivudine (3TC) provided from birth until 2 weeks of age (for full-term infants), or until 40 weeks corrected gestational age (for pre-term infants). NVP was switched to ritonavir-boosted lopinavir (LPV/r) at 2 weeks of age (for full-term infants) or 40 weeks corrected gestational age (for pre-term infants) per the Botswana national pediatric HIV treatment guidelines.

Control children living with HIV were recruited from routine health care providers or from existing clinical trials, and at the Botswana-Baylor Children's Clinical Center of Excellence. Controls were eligible if 24–36 months of age, had documented HIV-infection within 42 days after birth, initiated ART within 30–365 days after birth, and had all documented pre-enrollment HIV RNA measurements <400 copies/mL after 24 weeks on ART. All enrolled controls underwent a single study visit at 24–36 months of age during which samples for quantitative DNA PCR testing of PBMCs was collected. For this report, we used results from 10 of 23 enrolled controls whose samples have been tested so far to highlight the observed difference between controls and early treated children.

All mothers of screened and enrolled children signed written consent approved by ethical review boards in Botswana (Health Research Development Committee) and Boston (Harvard T.H. Chan School of Public Health Office of Human Research Administration and Partners HealthCare).

Laboratory Procedures

Samples were tested for HIV DNA utilizing Roche Cobas Ampliprep/Cobas Taqman HIV-1 Qualitative Polymerase Chain Reaction testing (Roche Diagnostics, Mannheim, Germany) on 3 mL whole blood at the Botswana-Harvard HIV Reference Laboratory (BHHRL). The Roche Cobas Ampliprep/Cobas Taqman HIV-1 Qualitative Polymerase Chain Reaction testing has a diagnostic sensitivity of 100% and specificity of 99.9%, and a limit of detection ~ 5 copies/million cells. Plasma RNA was tested by Coulter Abbott m2000sp/m2000rt (Abbott Molecular Inc, Des Plaines, IL, USA). Quantitative cell-associated HIV-1 DNA was by amplification of LTR-gag segment by digital-droplet PCR. EIA HIV antibody testing occurred at the BHHRL and was by Murex HIV-1.2.0 and Biorad GS HIV-1/HIV-2 plus O, in parallel.

Data Management and Statistical Analysis

Data for EIT Study participants were collected using the Research Electronic Data capture (REDCap) tools hosted at Harvard T.H. Chan School of Public Health [14]. Data were analyzed using SPSS version 25. The relationships between RNA suppression from 24 to 84 weeks, HIV serostatus by EIA at 84-week, and qualitative DNA results at 84 weeks were evaluated by using Spearman correlation. Wilcoxon Rank Sum test was used to assess the distributions of cell-associated HIV reservoir among early-treated children based on their HIV serology and sustained RNA suppression status at week 84, while comparison of proportions with change in serostatus and reversion of DNA PCR between early treated children and controls was by Fisher's test. Values of HIV RNA below the limit of detection (<40 copies/mL) were deemed to be that of the lower limit of detection (40 copies/mL) for the assay used.

Role of the Funding Source

The funder of the study (National Institute of Allergy and Infectious Diseases, NIAID), represented by P. J. P, provided technical oversight in the study design but played no direct role in data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had responsibility for the decision to submit for publication.

RESULTS

Between April 2015 and July 2018, the EIT Study enrolled 40 children living with HIV who were initiated on treatment in the first week of life (early-treated children) and 23 children living with HIV who had started treatment between 30–365 days (controls). Median age at enrollment and start of ART for

early-treated children was 2.5 days after birth (range 1–6), while controls had started continuous ART at a median age of 130 days (range 79–350) and were later enrolled at a median age of 25 months (range 24–36).

Of 40 early-treated children, 2 died prior to 24 weeks, and 38 were on ART and available for 84-week evaluation. Median HIV RNA at enrollment was 11 335 copies/mL (range < 40, >10 000 000) and 28 (74%) had HIV RNA < 40 copies/mL at 84 weeks (range < 40, 358 491). Twenty-seven (71%) of 38 early-treated children had quantitative DNA evaluations available from PBMCs from enrollment (prior to ART initiation) and at 84 weeks, of whom 20 (74%) were virally suppressed at 84 weeks.

Of 23 controls enrolled at 24–36 months of age, 19 (83%) had HIV RNA < 40 copies/mL (range < 40, 6420) and 10 (43%) had quantitative DNA evaluation available from PBMCs at the time of this analysis, 9 of whom had HIV RNA < 40 copies/mL and one had HIV RNA of 133 copies/mL. Baseline characteristics for sampled and unsampled children were generally comparable. Table 1 shows baseline characteristics of all early-treated and control children.

Quantitative Cell-Associated HIV DNA for Early-Treated and Control Children, and for Early-Treated Children Over Time

Among 27 early-treated children with quantitative cell-associated DNA testing of PBMCs at 84 weeks, median DNA was 40.8 copies/million cells. In contrast, the median quantitative HIV DNA in 10 controls (after an average of 93 weeks on ART) was 981.4 copies/million PBMCs ($P < .001$) (Figure 1). Among the 27 early-treated children, pre-treatment cell-associated DNA (median 492.1 copies/million cells) correlated with on-treatment cell-associated DNA values measured at 84 weeks (r = 0.43, $P = .03$), whereas pre-treatment HIV RNA (median 3145 copies/mL) was not correlated with on-treatment cell-associated DNA values measured at 84 weeks (r = -0.04, $P = .85$) (Table 2). We found no difference in median cell-associated DNA at 84 weeks among 12 early-treated children with sustained viral suppression from 24–84 weeks (22.9, range: 1.7, 2786.2 copies/million cells) when compared with 15 children with at least 1 viral rebound (51.4, range: 0.5, 5830 copies/million cells) ($P = .70$).

Serostatus and Qualitative DNA PCR as Markers for Viral Reservoir at 84 Weeks

For the 27 very-early-treated children with quantitative cell-associated DNA at 84 weeks, 14 (52%) had a negative serostatus, 10 (37%) had a positive serostatus, and 3 (11%) had an indeterminate serostatus. Whole blood qualitative DNA PCR had reverted to negative for 12 (44%) children and remained positive for the remaining 15 (56%). Median 84-week cell-associated

Table 1. Baseline Characteristics Among Early-Treated Children and Controls

	Early-Treated Infants (n = 38)		Controls (n = 23)	
	Sampled (n = 27)	Not Sampled (n = 11)	Sampled (n = 10)	Not sampled (n = 13)
Female: n (%)	19 (70)	8 (73)	6 (60)	7 (54)
Gestational age at birth, weeks: n (%)	< 37: 9 (33) ≥ 37: 18 (67)	< 37: 3 (27) ≥ 37: 8 (73)	Not available	
Birthweight, kg: median (IQR)	3.0 (2.6, 3.1)	2.6 (2.5, 2.9)	Not available	
Pre-treatment HIV-1 RNA, copies/mL: median (IQR)	3145 (1005, 31 708)	25 507 (381, 224 127)	Not available	
Pre-treatment HIV-1 DNA, copies/million PBMCs: median (IQR)	492.1 (77.8, 1245.8)	Not available	Not available	
Enrollment CD4%: median (IQR)	52 (38, 56)	54 (31, 69)	39 (36, 42)	34 (30, 38)
Median age at first HIV test: days (range)	1 (0, 3)	1 (0, 4)	57 (42, 219)	62 (43, 208)
Median age at ART start: days (range)	2 (1, 5)	2 (1, 6)	132 (79, 341)	125 (85, 350)
Maternal HIV-1 RNA at delivery, copies/mL: median (IQR)	9560 (547, 80 649)	45 283 (692, 83 912)	Not available	
Maternal ART regimen in pregnancy: n (%)	None: 10 (37) EFV/TDF/FTC: 10 (37) DTG/TDF/FTC: 6 (22) Others/Unknown: 1 (4)	None: 5 (45) EFV/TDF/FTC: 0 (0) DTG/TDF/FTC: 5 (45) Others/Unknown: 1 (9)	None: 5 (50) EFV/TDF/FTC: 1 (10) DTG/TDF/FTC: 0 (0) Others/Unknown: 4 (40)	None: 7 (54) EFV/TDF/FTC: 4 (31) DTG/TDF/FTC: 0 (0) Others/Unknown: 2 (15)
Maternal highest level of education attained: n (%)	Primary: 2 (7) Junior Secondary: 16 (59) Senior Secondary: 8 (30) Tertiary: 1 (4)	Primary: 1 (9) Junior Secondary: 6 (55) Senior Secondary: 3 (27) Tertiary: 1 (9)	Primary: 2 (20) Junior Secondary: 6 (60) Senior Secondary: 2 (20) Tertiary: 0 (0)	Primary: 1 (8) Junior Secondary: 7 (54) Senior Secondary: 5 (38) Tertiary: 0 (0)
Maternal employment status: n (%)	Salaried: 4 (15) Self-employed: 4 (15) Domestic work: 0 (0) Unemployed: 19 (70)	Salaried: 0 (0) Self-employed: 1 (9) Domestic work: 1 (9) Unemployed: 9 (82)	Salaried: 3 (30) Self-employed: 2 (20) Domestic work: 1 (10) Unemployed: 4 (40)	Salaried: 3 (23) Self-employed: 5 (38) Domestic work: 1 (8) Unemployed: 4 (31)

Abbreviations: ART, antiretroviral treatment; HIV, human immunodeficiency virus; IQR, interquartile range.

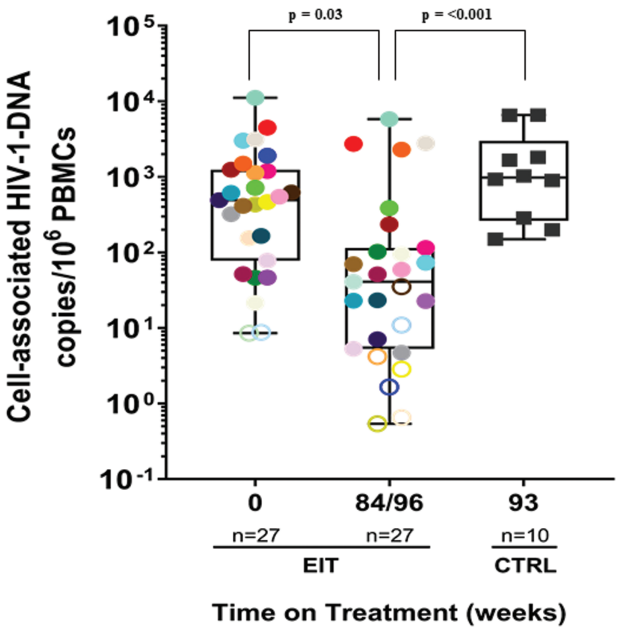

Figure 1. Quantitative cell-associated HIV DNA for early-treated and control children. Abbreviation: HIV, human immunodeficiency virus.

DNA values did not differ among participants with negative or positive serostatus at 84 weeks by EIA (29.1 vs 55.4 copies/million cells; $P = .94$) or by qualitative whole blood DNA-PCR (16.8 vs 70.2 copies/million cells; $P = .06$).

Serostatus and Qualitative DNA PCR as Markers for Sustained Viral Suppression at 84 Weeks

Among all 38 early-treated children who reached 84 weeks, 21 (55%) had a negative serostatus by EIA testing, 14 (37%) were positive, and 3 (8%) were indeterminate. Whole blood qualitative DNA PCR had reverted to negative for 15 (39%) children, remained positive for 22 (58%), and was indeterminate for 1 (3%). In contrast, among the 23 controls, only 2 (9%) had reverted their whole blood qualitative DNA PCR to negative ($P = .01$). From 24 to 84 weeks, 16 (42%) of the 38 early-treated children had sustained HIV RNA < 40 copies/mL at all visits, and 22 (58%) had at least one rebound recorded (Table 2). Among the 16 early-treated children with complete HIV RNA suppression from 24 to 84 weeks, 14 (88%) had negative serostatus by EIA at week 84, and 2 (12%) had an indeterminate serostatus. In contrast, among 22 children with episodes of viremia after 24 weeks, 7 (32%) had negative serostatus, 14 (64%) had positive serostatus, and 1 (4%) was indeterminate (Table 2). The negative predictive value of an 84-week EIA (a negative test indicating sustained viral suppression) was 67% (14/21), and the positive predictive value (a positive test indicating lack of sustained viral suppression) was 100% (14/14) (Table 3). It is also noteworthy that the highest 24–84-week HIV RNA recorded in a child who remained seronegative was 364 copies/mL.

For early-treated children, qualitative DNA PCR was concordant with EIA testing for 71% (24/34) with interpretable results for both tests; 8 children had a positive DNA PCR but a negative EIA, and 2 children had a negative DNA PCR but a positive EIA. Among early-treated children with complete HIV RNA suppression from 24 to 84 weeks, 11 (69%) had negative qualitative DNA PCR at week 84, and 5 (31%) had a positive qualitative DNA PCR. Among 22 children with episodes of viremia after 24 weeks, 4 (18%) had negative qualitative DNA PCR, 17 (77%) had positive qualitative DNA PCR, and 1 (5%) was indeterminate (Table 2). The negative predictive value of an 84-week qualitative DNA PCR (a negative test indicating sustained viral suppression) was 73% (11/15), and the positive predictive value (a positive test indicating lack of sustained viral suppression) was 77% (17/22) (Table 3).

DISCUSSION

In this unique cohort of infants treated from the first week of life, the viral reservoir in PBMCs after 84 weeks of treatment was markedly lower than control infants who started later in the first year of life, and lower than reported cohorts elsewhere [7, 15]. We also found that using readily available diagnostic HIV tests (EIA and the standard infant diagnostic DNA PCR) we could identify children with sustained viral suppression or viral rebound over time.

Although other studies have identified low viral reservoir in very-early-treated infants [16, 17], ours was the first to demonstrate a significant difference from control infants who started treatment later in the first year of life (at a median of 130 days) [3]. Khun et al found that when ART was started < 2 months of age, lower levels of cell-associated HIV-1 DNA were observed compared with starting later in life [7]. Persaud et al found lower T-cell latent reservoir was associated with earlier time to viral suppression among infants started at a median of 8 weeks of life [18]. The low viral reservoir in PBMCs following very early treatment is consistent with the fact that there are differences in the cell types present in early life, and possible functional differences in the early immune system (with a higher proportion of poly-functional HIV-1 specific T cells and an innate immune profile with features of improved antiviral activities), and the early presence of ART may prevent seeding of the cells that become dominant later in life [3]. The difference between reservoir size among our early-treated and control infants suggest that the benefits of ART for reservoir protection may be most critical during the neonatal period.

Viral reservoir prior to ART was a moderate predictor of the PBMC values at 84 weeks. Although the transition of T-cell subsets between the neonatal period and later infancy (towards poly-functional HIV-1 specific T cells later in life) might be expected to de-link the early reservoir from what is apparent later in life [3, 19], this transition may be partial and may not

Table 2. Plasma HIV RNA, Cell-Associated DNA, and Serostatus Trends

	Plasma HIV RNA by Week of ART							Cell-Associated DNA		EIA Antibody	Qualitative DNA
Participant	0	4	12	24	36	60	84	Week 0	Week 84	Week 84	Week 84
A	1661	53	<40	<40	<40	<40	<40	77.80	5.30	-	-
B	17 244	186	<40	11 182	52	<40	<40	320.50	4.70	+	-
C	1636	408	<40	<40	<200	3648	<40	432.60[b]	0.50	+	+
D	1 111 950	1413	42	860	<40	98	49 993	8.50	40.80	+	+
E	1375	161	72	<40	<40	<40	<40	1189.90	115.00[c]	+/-	+
F	>10 000 000	191	<40	<40	<40	<40	<40	1904.80	1.70	-	-
G	<40	<40	<40	<40	<40	<40	<40	8.80	10.90	-	-
H	60 247	307	50	164	46	<40	<40	154.60	0.70	-	-
I	3145	533	<40	<40	<40	<40	<40	46.30	102.30	-	-
J	1005	561	42	<40	<40	<40	<40	465.40	2.90	+/-	-
K	272	6591	<40	735	55	106	339	51.70	51.40	+	+
L	1314	<40	<40	<40	<40	<40	319	1129.30	4.20	+	+
M	23 686	<40	<40	<40	<40	<40	<40	492.10	7.10	-	+
N	20 291	12 315	145 164	1 595 770	133 084	<40	7114	414.60	70.20	+	+
O	<200	<40	<40	<40	<40	<40	<40	615.40	23.00	-	-
P	12 984	<40	<40	<40	<40	<40	<40	46.60	22.80	-	-
Q	315 020	1051	97	300	166	<40	<40	3009.60	73.30	-	+
R	79	66	77	<40	<40	<40	<40	1245.80	236.20	-	+
S	11 677	<40	<40	<40	<40	<40	<40	21.49	95.24	-	-
T	224 127	388	163	<40	<40	<40	<40	[a]	[a]	-	+
U	143 616	46	<40	<40	3966	<40	<40	[a]	[a]	+	-
V	8548	487	<40	<40	<40	<40	<40	[a]	[a]	-	-
W	33 502	628	<40	76	<40	<40	<40	[a]	[a]	-	+/-
X	1837	118	166	5019	15 325	23 868	69 549	1502.73	2304.37	+	+
Y	88 885	456	45	<40	<40	28 822	358 491	4475.52	2734.25	+	+
Z	2279	<40	<40	<40	<40	<40	<40	3141.95	2786.15	-	-
AA	31 708	350	<40	<40	89 356	<40	<40	165.59	23.21	+/-	+
BB	114	105	<40	41	52	<40	<40	11 119.93	5830.03	-	+
CC	276	743	310	255	31 341	<40	<40	[a]	[a]	+	+
DD	292	41	59	<40	<40	364	<40	623.05	35.3	-	-
EE	15 327	9082	93	<40	52 080	713	<40	547.73	59.41	+	+
FF	25 507	42	<40	<40	<40	<40	<40	[a]	[a]	-	+
GG	381	<40	<40	<40	<40	<40	<40	[a]	[a]	-	-
HH	>10 000 000	474	106	<40	17 222	43 871	54 695	719.96	386.47	+	+
II	389 270	1410	632	<40	121	<40	67	[a]	[a]	-	+
JJ	310	271	166	143	55	<40	<40	[a]	[a]	-	+
KK	10 993	445	78	481 050	5630	29 932	71 499	[a]	[a]	+	+
LL	706 316	2252	<40	<40	<40	15 609	1194	[a]	[a]	+	+

Abbreviations: ART, antiretroviral treatment; EIA, enzyme immunosorbent assay; HIV, human immunodeficiency virus.

[a]Sample yet to be tested.

[b]Sample drawn at week 1.

[c]Sample drawn at week 96.

Table 3. Predictive Values for Long-Term Viral Suppression or Rebound From 84-Week HIV Serology and Qualitative DNA Results

	<40 copies/mL 24–84 weeks (sustained)	≥40 copies/mL 24–84 weeks (at least once)	Predictive value for HIV RNA viral suppression from 24–84 weeks
Negative HIV serology	14	7	67% predictive of sustained suppression
Positive HIV serology	0	14	100% predictive of at least one rebound
Indeterminate HIV serology	2	1	–
Negative qualitative DNA	11	4	73% predictive of sustained suppression
Positive qualitative DNA	5	17	77% predictive of at least one rebound
Indeterminate qualitative DNA	0	1	–

Abbreviation: HIV, human immunodeficiency virus.

account for all expansion of viable and nonviable HIV related to the early reservoir [3]. Loss of whole blood qualitative DNA positivity trended toward lower quantitative PBMC values, but direct association could not be established; this finding may be explained by variability in testing methods and in the cellular components included in these different tests. Neither sustained viral RNA suppression from 24–84 weeks, nor negative serostatus at 84 weeks, were predictive for a lower viral reservoir in PBMCs. This finding is consistent with reservoir size being driven by clonally expanded cells [20–22], and suggests that the reservoir may be relatively unaffected by short periods of viremia after 24 weeks. However, over a longer period, we cannot exclude (and might expect) that new seeding of PBMCs and other sanctuary sites would occur. Though our preliminary look at baseline PBMC DNA does not show a correlation with maternal ART regimen or maternal HIV RNA at time of delivery (Supplementary Tables 1 and 2), the impact of more potent ART regimens on reservoir size in infants resulting from in utero and breast milk exposure, as well as its use very early in the neonatal period, is an area that requires further study.

In contrast to viral reservoir, serostatus and whole blood qualitative DNA—both of which are inexpensive and readily available as part of HIV diagnostic efforts—were useful markers to assess sustained viral suppression over time for early-treated children. As such, these easily obtained markers may prove useful for monitoring early-treated children in low-resource settings, in settings where visits may be infrequent or missed, or in clinical research trials. Of note, we observed that all infants with a viral rebound < 400 copies/mL (highest = 364 copies/mL) maintained a negative serostatus, suggesting that lower-level viremia may not result in a sufficiently coordinated immune response for seroconversion.

The strength of this study was close follow-up of a unique cohort of early-treated children. Our study was limited by small numbers, especially for the quantitative DNA testing in PBMCs particularly for the control group. In addition, PBMCs may only represent a small portion of the true viral reservoir (albeit the only portion that we can easily measure), and we cannot exclude much larger reservoir sizes in early-treated infants that are unmeasurable. If the unmeasurable component of the reservoir is large, it might negate the hypothesized benefits of very early ART. Furthermore, our enrollments of controls at a single time point did not allow for in-depth comparison of pre-ART parameters between controls and very-early-treated infants. While it is reassuring that only 2 children (5%) in our cohort have died to date [23], the clinical implication of maintaining a low viral reservoir in these children has not yet been proven.

In conclusion, ART started in the first week of life significantly reduced viral reservoir as compared to starting later in infancy. The size of the viral reservoir after 84 weeks of ART appeared to be moderately correlated with the size at birth, but we were unable to detect an association with intermittent plasma viremia; these findings are consistent with clonal expansion of a limited repertoire of cell types as the dominant factor in reservoir persistence. We also found that basic HIV diagnostic tests (serology and DNA PCR) can reveal long-term patterns of suppression or rebound in early-treated children and may serve as useful clinical markers.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. G. A., P. G. B., and R. S. designed the research question. G. A, K. M., S. M., T. M., M. S, O. B., and R. S. performed the research. G. A., K. B., and R. S. analyzed the data, M. H, P. J. P., S. L., J. M., K. D, M. S., and R. S. provided technical oversight for the study. R. S., D. R. K., and M. L. secured funding and provided oversight for the project overall. G. A., P. G. B., K. B., and R. S. wrote the paper. R. S., S. L., D. R. K., and M. L. edited the final manuscript.

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