HIV-1 reservoir size after neonatal antiretroviral therapy and the potential to evaluate antiretroviral-therapy-free remission (IMPAACT P1115): a phase 1/2 proof-of-concept study

Deborah Persaud, Yvonne Bryson, Bryan S Nelson, Camlin Tierney, Mark F Cotton, Anne Coletti, Jennifer Jao, Stephen A Spector, Mark Mirochnick, Edmund V Capparelli, Diane Costello, Joseph Szewczyk, Nicol Nicodimus, Lynda Stranix-Chibanda, Adeodata R Kekitiinwa, Violet Korutaro, Christina Reding, Mary N Carrington, Sai Majji, Dwight E Yin, Patrick Jean-Philippe, Ellen G Chadwick

Summary

Background Infants born with HIV-1 require lifelong antiretroviral therapy (ART). We aimed to assess whether very early ART in neonates might restrict HIV-1 reservoirs, an important step towards ART-free remission.

Methods IMPAACT P1115 is an ongoing, phase 1/2, proof-of-concept study in which infants were enrolled at 30 research clinics in 11 countries (Brazil, Haiti, Kenya, Malawi, South Africa, Tanzania, Thailand, Uganda, the USA, Zambia, and Zimbabwe) into two cohorts. Infants at least 34 weeks' gestational age at high risk for in-utero HIV-1 with either untreated maternal HIV-1 (cohort 1) or who were receiving pre-emptive triple antiretroviral prophylaxis outside of the study (maternal ART permissible; cohort 2) were included. All infants initiated treatment within 48 h of life. Cohort 1 initiated three-drug nevirapine-based ART, and cohort 2 initiated three-drug nevirapine-based prophylaxis then three-drug nevirapine-based ART following HIV diagnosis by age 10 days. We added twice-daily coformulated oral ritonavir 75 mg/m² and lopinavir 300 mg/m² from 14 days of life and 42 weeks postmenstrual age. We discontinued nevirapine 12 weeks after two consecutive plasma HIV-1 RNA levels below limit of detection. We tracked virological suppression, safety outcomes, and meeting a predetermined biomarker profile at age 2 years (undetectable RNA since week 48, HIV-1 antibody-negative, HIV-1 DNA not detected, and normal CD4 count and CD4 percentage) to assess qualification for analytical treatment interruption. This study is registered with ClinicalTrials.gov, NCT02140255.

Findings Between Jan 23, 2015, and Dec 14, 2017, 440 infants were included in cohort 1 and 20 were included in cohort 2. 54 of these infants (34 from cohort 1 and 20 from cohort 2) had confirmed in-utero HIV-1 and were enrolled to receive study ART. 33 (61%) of 54 infants were female and 21 (39%) were male. The estimated probability of maintaining undetectable plasma RNA through to 2 years was 33% (95% CI 17–49) in cohort 1 and 57% (28–78) in cohort 2. Among infants maintaining protocol-defined virological control criteria through to study week 108, seven of 11 (64%, 95% CI 31–89) in cohort 1 and five of seven (71%, 29–96) in cohort 2 had no detected HIV-1 DNA. Ten of 12 (83%, 52–100) in cohort 1 and all seven (100%, 59–100) in cohort 2 tested HIV-1 antibody-negative at week 108. Among 54 infants initiated on very early ART, ten (19%; six in cohort 1 and four in cohort 2) met all criteria for possible analytical treatment interruption. Reversible grade 3 or 4 adverse events occurred in 15 (44%) of 34 infants in cohort 1 and seven (35%) of 20 infants in cohort 2.

Interpretation Very early ART for in-utero HIV-1 can achieve sustained virological suppression in association with biomarkers indicating restricted HIV-1 reservoirs by age 2 years, which might enable potential ART-free remission.

Funding National Institute of Allergy and Infectious Diseases, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, and the National Institute of Mental Health.

Copyright © 2023 Elsevier Ltd. All rights reserved.

Introduction

Annually, 1·3 million women living with HIV-1 become pregnant worldwide. Babies born to mothers with HIV-1 not receiving antiretroviral therapy (ART) are at high risk of vertical transmission.[1] Babies acquiring HIV-1 need lifelong ART because HIV-1 latency is established early in resting memory CD4 T cells.[2] If ART is interrupted, HIV-1 viraemia typically returns within 2–4 weeks.[2] New treatment approaches prolonging ART-free remission would advance HIV-1 therapies for children.

The case of the so-called Mississippi baby, who initiated ART at age 30 h and was treated for 18 months before achieving 27 months of ART-free remission (aviraemia and HIV-1 seronegative status), suggests that very early

Lancet HIV 2023

Published **Online** December 4, 2023 https://doi.org/10.1016/S2352-3018(23)00236-9

See **Online/Comment** https://doi.org/10.1016/S2352-3018(23)00260-6

Johns Hopkins University School of Medicine, Baltimore, MD, USA (D Persaud MD, J Szewczyk BS); **University of California Los Angeles, Los Angeles, CA, USA** (Y Bryson MD, D Costello BS); **Harvard T H Chan School of Public Health, Boston, MA, USA** (B S Nelson SM, C Tierney PhD); **Stellenbosch University, Tygerberg, South Africa** (M F Cotton PhD); **FHI 360, Durham, NC, USA** (A Coletti MS); **Northwestern University Feinberg School of Medicine, Chicago, IL, USA** (E G Chadwick MD, J Jao MD); **University of California San Diego School of Medicine, La Jolla, CA, USA** (E V Capparelli PharmD, S A Spector MD); **Boston University School of Medicine, Boston, MA, USA** (M Mirochnick MD); **University of Zimbabwe, Clinical Trials Research Centre, Harare, Zimbabwe** (N Nicodimus BSc, L Stranix-Chibanda MBChB); **Baylor College of Medicine Children's Foundation, Kampala, Uganda** (A R Kekitiinwa MMed, V Korutaro MBChB); **Frontier Science and Technology Research Foundation, Amherst, NY, USA** (C Reding MPH); **Frederick National Laboratory for Cancer Research and Laboratory of Integrative Cancer Immunology National Cancer Institute, MD, USA** (M N Carrington PhD); **Eunice Kennedy Shriver National Institute of Child**

Health and Human Development, National Institutes of Health, Bethesda, MD, USA (S Majji PhD)**; National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA** (D E Yin MD, P Jean-Philippe MD)

Correspondence to: Deborah Persaud, Johns Hopkins University School of Medicine, Baltimore, MD, USA
dpers@jhmi.edu

Research in context

Evidence before this study

Antiretroviral therapy (ART)-free remission of in-utero infection would save a child from a lifetime of ART and advance strategies to restrict and control neonatal HIV reservoirs. We searched PubMed from Jan 1, 1994, to May 1, 2013, for "pediatric HIV", "perinatal HIV", "in-utero infection", "very early ART", "treatment interruption", "ART-free remission", "functional cure", and "HIV clearance" in any language. We found one case report of clearance of HIV infection in a perinatally infected infant. We repeated the search on July 15, 2023, and found three studies on the very early treatment of neonates to achieve ART-free remission. The studies show the feasibility of test and treat for neonates with in-utero HIV-1 and the challenges of adherence to daily ART in infants.

Added value of this study

This study contributes novel data on HIV reservoirs in children who initiated very early ART and informs the feasibility of very early treatment of newborn infants in multiple countries, especially in sub-Saharan Africa, where the burden of new perinatal HIV infections predominates. The long-term safety and tolerability of this very early four-drug ART regimen is vital to the field. The study adds to the body of knowledge of the potential effects of very early or pre-emptive treatment of in-utero HIV in newborn infants, including limiting the establishment of HIV reservoirs. The study findings provide information about the virological and immunological effects of very early treatment approaches in neonates and biomarkers of ART-free remission once the primary objective of the study is evaluated through analytical treatment interruption.

Implications of all the available evidence

The study highlights the challenge of sustained virological suppression with current neonatal nevirapine and ritonavir-boosted lopinavir-based ART regimens, emphasising the need for improved antiretroviral drugs to enhance viral control in infancy. Ongoing clinical trials will continue to inform remission and cure as outcomes of HIV therapeutics for this population.

ART might restrict formation of latent reservoirs.[3,4] Although viraemic rebound occurred in that infant, the 27 months of aviraemia off ART with non-detectable HIV-1 DNA and seronegative status remains notable for HIV-1 remission and cure therapeutics.

HIV-1 seronegative status and non-detectable HIV-1 DNA with early (by age 2–3 months) treatment of perinatal transmission do not indicate HIV-1 remission.[5,6] The International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) study P1115 is an ongoing phase 1/2 proof-of-concept trial aiming to evaluate whether ART-free remission of in-utero HIV-1 can be induced by ART initiation within 48 h of life in neonates at high risk for HIV-1 acquisition.[7] We hypothesised that this approach could reduce HIV-1 reservoirs to levels enabling potential ART-free remission. Here, we report 2-year virological and safety outcomes from IMPAACT P1115, as well as factors associated with maintaining strict virological control through to age 2 years, for possible analytic treatment interruption to assess remission.

Methods

Study design and participants

IMPAACT P1115 is an ongoing, phase 1/2, proof-of-concept study in which infants were enrolled at 30 research clinics in 11 countries (Brazil, Haiti, Kenya, Malawi, South Africa, Tanzania, Thailand, Uganda, the USA, Zambia, and Zimbabwe) into two cohorts. Infants at least 34 weeks' gestational age at high risk for in-utero HIV-1 (untreated maternal HIV-1) were included in cohort 1. Infants at least 34 weeks' gestational age at high risk for in-utero HIV-1 who were receiving pre-emptive triple antiretroviral prophylaxis outside of the study (maternal ART permissible) were included in cohort 2.

We obtained written informed consent from each study participant's mother or legal guardian. Each local institutional review board or ethics committee provided oversight and approval for the study.

Procedures

For infants in cohort 1, within 48 h of birth before HIV-1 was confirmed we pre-emptively initiated ART with a study regimen of two nucleoside reverse transcriptase inhibitors (NRTIs; for infants weighing 2 to <3 kg: zidovudine at 1 mL of a 10 mg/mL liquid formulation twice daily or abacavir at 0·4 mL of a 20 mg/mL liquid formulation twice daily, plus lamivudine at 0·5 mL of a 10 mg/mL liquid formulation twice daily; for infants weighing 3 to <4 kg: zidovudine at 1·5 mL of a 10 mg/mL liquid formulation twice daily or abacavir at 0·5 mL of a 20 mg/mL liquid formulation twice daily, plus lamivudine at 0·8 mL of a 10 mg/mL liquid formulation twice daily) plus oral nevirapine, dosed 6 mg/kg twice daily in term neonates (≥37 weeks' gestational age) or 4 mg/kg twice daily for 1 week, followed by 6 mg/kg twice daily thereafter for preterm neonates (34 to <37 weeks' gestational age).[7] Infants testing negative for HIV-1 discontinued study ART and transitioned to local antiretroviral prophylaxis; infants testing positive for HIV-1 continued with study ART. Infants in cohort 2 were receiving three-drug nevirapine-based prophylaxis (zidovudine or abacavir plus lamivudine, plus nevirapine) as part of clinical care within 48 h of birth before study enrolment. They enrolled after having at least one positive nucleic acid test for HIV-1 before study entry and

initiated the study ART regimen (as described for cohort 1) within 10 days of life.

For both cohorts, because a four-drug protease-inhibitor-based regimen has the potential to increase virological suppression,[8] we added ritonavir-boosted lopinavir (twice-daily coformulated oral ritonavir 75 mg/m² and lopinavir 300 mg/m²) when age-appropriate (after age 14 days and 42 weeks' postmenstrual age). We stopped nevirapine 12 weeks after the first confirmed viral load below the limit of detection and followed up all infants through to week 24. Thereafter, infants needed to maintain a viral load less than the limit of detection using the Roche COBAS TaqMan HIV-1 RNA assay (Roche Molecular Systems, Branchburg, NJ, USA; 20 copies per mL) or the Abbott RealTime HIV-1 RNA assay (Abbott Molecular, Des Plaines, IL, USA; 40 copies per mL) at week 24 or 36 (later amended to viral load <200 copies per mL). Infants also needed to maintain a viral load less than the limit of detection with target not detected from week 48 onwards. Infants were discontinued from the study if confirmed plasma viral load (two consecutive viral loads ≤3 weeks apart) did not meet these criteria.

Plasma viral load was collected at study entry (within 48 h of life for cohort 1 and within 10 days for cohort 2), by 2 weeks of life (cohort 1), every 4 weeks through to week 24, and every 12 weeks thereafter (figure 1A). HIV-1 DNA was collected at study entry (within 48 h of life for cohort 1 and within 10 days for cohort 2), by 2 weeks of life (cohort 1), and every 12 weeks thereafter and HIV-1 antibody testing was done every 12 weeks from week 84 onwards. CD4 and CD8 cell counts and percentages were obtained after confirmation of HIV-1 infection, at weeks 2 and 12, and every 12 weeks thereafter. Safety monitoring laboratory tests (haematology and chemistry) were obtained for each cohort at study entry and at weeks 2, 4, 8, 16, and 24, every 12 weeks from week 24 to 84, and at week 108. Dried blood spots for pharmacokinetics were collected with each safety monitoring test with additional pharmacokinetic collections at weeks 12 and 20. We collected whole venous blood by venipuncture and dried blood spot by finger or heel prick. We quantified HIV-1 DNA with a single-amplicon HIV-1 DNA droplet digital PCR assay validated across HIV-1 subtypes and certified under the Clinical Laboratory Improvement Amendments (sensitivity 1·25 copies per 10^6 peripheral blood mononuclear cells [PBMCs], limit of detection 4·09 copies per 10^6 PBMCs on analyses of 800 000 cells).[9] We measured lopinavir concentrations in dried blood spot samples collected randomly in 48 infants between weeks 4 and 60, modifying a previously published method for liquid-chromatography mass-spectrometry.[10]

Infants who maintained a viral load less than the limit of detection with target not detected from study week 48 to week 108 and had discontinued breastfeeding became eligible for biomarker testing to assess whether they met study criteria for initiating analytical treatment interruption. Criteria included two sequential paired HIV-1

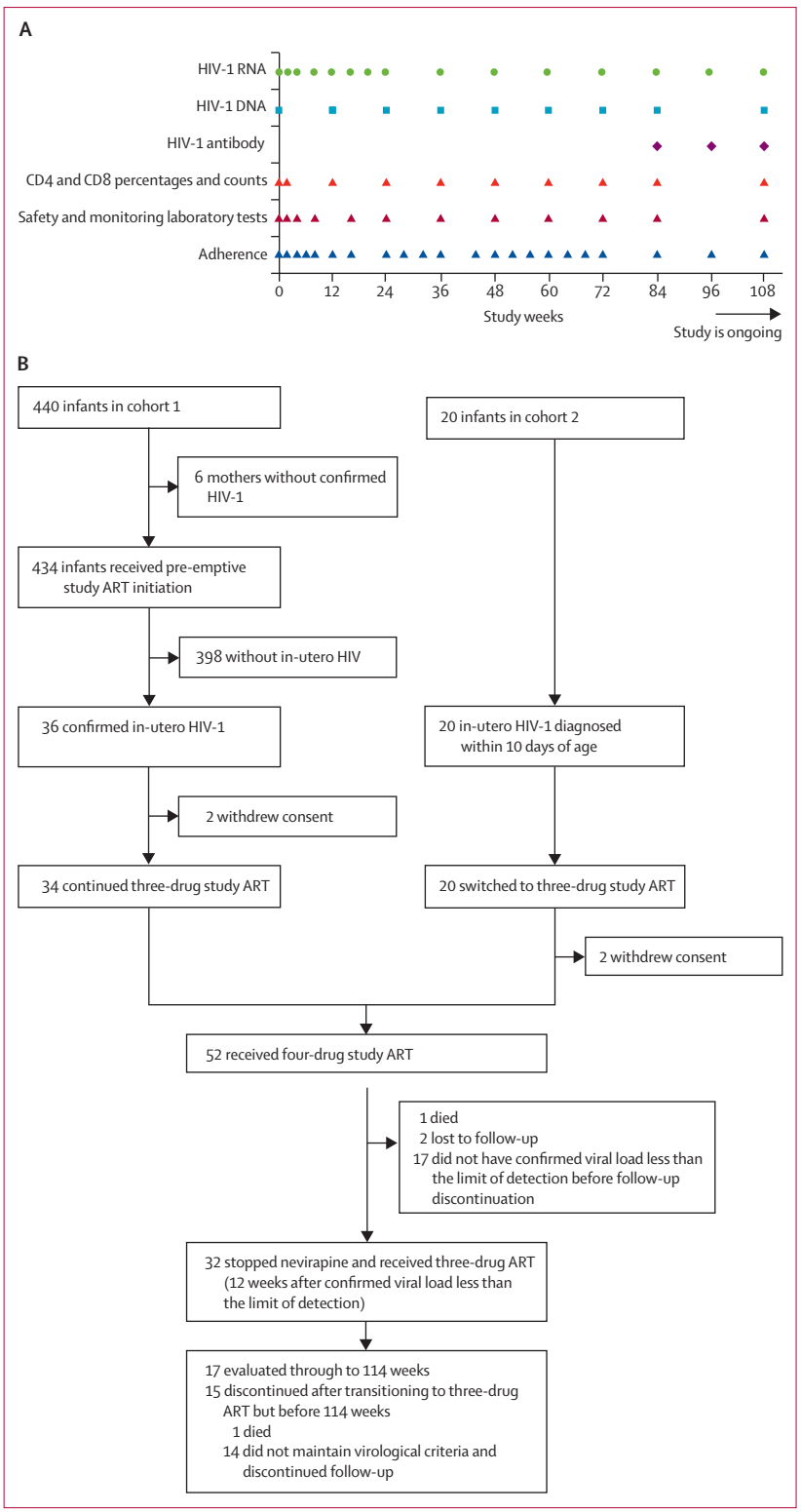


Figure 1: **Schedule of evaluations and study profile**
(A) Schedule of evaluations. Study weeks correspond approximately to infant age due to enrolment within 48 h of age for cohort 1 and 10 days of age for cohort 2. (B) Study profile. ART=antiretroviral therapy.

	Cohort 1 (n=34)	Cohort 2 (n=20)
Region		
Africa	33 (97%)	14 (70%)
Asia	1 (3%)	0
North America	0	2 (10%)
South America	0	4 (20%)
Gestational age		
34 to <37 weeks	4 (12%)	2 (11%)
≥37 weeks	30 (88%)	17 (89%)
Mode of delivery		
Spontaneous vaginal delivery	31 (91%)	14 (70%)
Caesarean	3 (9%)	6 (30%)
Twin gestation	0	2 (10%)
Sex		
Female	23 (68%)	10 (50%)
Male	11 (32%)	10 (50%)
Age at study entry		
Median	22·2 h (12·6–32·6)	8 days (5·5–8·0)
Mean	24·4 h (13·3)	7·2 days (1·9)
Age at first ART, h†	7·3 (1·8–21·0)	32·8 (1·1–40·1)
Earliest measured bodyweight, kg‡		
Median	2·7 (2·5–3·0)	2·7 (2·3–3·3)
Mean	2·8 (0·4)	2·8 (0·6)
Earliest HIV-1 plasma viral load, log_{10} copies per mL§		
Median	4·9 (4·0–5·3)	4·1 (3·2–5·2)
Mean	4·6 (1·1)	4·2 (1·3)
<10 000 copies per mL	25 (74%)	11 (55%)
≥10 000 copies per mL	9 (26%)	9 (45%)
Earliest HIV-1 DNA load, log_{10} copies per 10^6 PBMCs¶		
Median	2·4 (1·7–3·0)	2·8 (1·8–3·3)
Mean	2·3 (1·0)	2·4 (1·1)
Earliest CD4 count, cells per μL‖		
Median	2458·5 (2080·0–2789·5)	2330·0 (1923·0–2654·0)
Mean	2480·9 (889·6)	2560·3 (1149·0)
Earliest CD4 percentage‖		
Median	50·4% (42·0–57·5)	53·5% (45·0–59·0)
Mean	48·7% (13·1)	50·8% (12·2)
Breastfed	33 (97%)	13 (65%)
Maternal age, years**		
Median	23·5 (20·5–29·0)	26·5 (23·9–30·2)
Mean	25·2 (5·7)	27·4 (5·3)
Maternal ART exposure before pregnancy**	0	4 (21%)
Maternal ART exposure during pregnancy and delivery**		
During pregnancy and delivery	0	7 (37%)
During pregnancy only	0	2 (11%)
During delivery only	16 (47%)	3 (16%)
None	18 (53%)	7 (37%)
Maternal HIV-1 plasma viral load at enrolment, log_{10} copies per mL**		
Median	4·7 (4·1–5·3)	4·3 (3·0–4·8)
Mean	4·6 (1·0)	4·0 (1·0)
Below limit of detection (undetectable)	0	1 (5%)
Above limit of detection to <10 000	6 (18%)	8 (42%)
10 000 to <100 000	16 (47%)	6 (32%)
≥100 000	12 (35%)	4 (21%)
Maternal HIV-1 subtype††		
A1	1 (4%)	3 (23%)
B	0	5 (39%)
C	20 (87%)	2 (15%)
D	2 (9%)	2 (15%)
F1	0	1 (8%)
Maternal NNRTI resistance††		
Lys103Lys or Lys103Asn	1 (4%)	0
Lys103Lys or Lys103Asn, Gly190Ser, Lys65Arg	0	1 (8%)
Lys103Asn	1 (4%)	2 (15%)
Thr69Ser or Thr69Thr	0	1 (8%)
Potentially protective infant HLA-B allele‡‡		
*57	4 (12%)	0
58:01	3 (9%)	2 (10%)
81:01	3 (9%)	2 (10%)
*27	0	1 (5%)
At least one allele	10 (30%)	5 (25%)

Data are median (IQR), mean (SD), or n (%). Mean (SD) reported for continuous measures with reasonably symmetrical distributions. ART=antiretroviral therapy. NNRTI=non-nucleoside reverse transcriptase inhibitor. PBMC=peripheral blood mononuclear cell. †n=17 in cohort 2. ‡Taken at a median of 1·0 days (IQR 0·0–1·0) in cohort 1 and 8·0 days (5·5–8·0) in cohort 2. §Taken at a median of 1·0 days (IQR 0·0–1·0) in cohort 1 and 6·5 days (2·0–8·0) in cohort 2. ¶Taken at a median of 1 day (0–1) in cohort 1 and 8 days (5–8) in cohort 2 (cohort 2 n=19). ‖Taken at a median of 15 days (IQR 13–16) in cohort 1 and 8 days (5–8) in cohort 2 (cohort 1 n=32; cohort 2 n=18). **Cohort 1 $N_{mothers}$=34; cohort 2 $N_{mothers}$=19 (one set of twins). ††Cohort 1 n=23; cohort 2 n=13. Entry samples were analysed in batch after 2 years of follow-up. Ten maternal samples from cohort 1 and two maternal samples from cohort 2 were tested with no result; three additional cohort 2 results excluded from analysis per team decision. ‡‡Cohort 1 n=33; cohort 2 n=20.

Table 1: **Participant characteristics**

DNA and antibody tests at least 8 weeks apart, showing HIV-1 DNA not detected (minimum of 850 000 cells analysed), normal CD4 count and percentage, and negative HIV-1 antibody results. HIV-1 antibody testing was done in a central laboratory (Quest Diagnostics, Baltimore, MD, USA) with a fourth-generation HIV-1/HIV-2 chemiluminescent microparticle immunoassay (Abbott Architect fourth generation HIV Ag/Ab Combo, Abbott Diagnostics, Scarborough, ME, USA), beginning at week 84 and every 12 weeks thereafter.

Relationships between adverse events and study treatment were based on site reports and assessment by the study team's Clinical Management Committee, per the Division of AIDS grading system (version 1.0).[11]

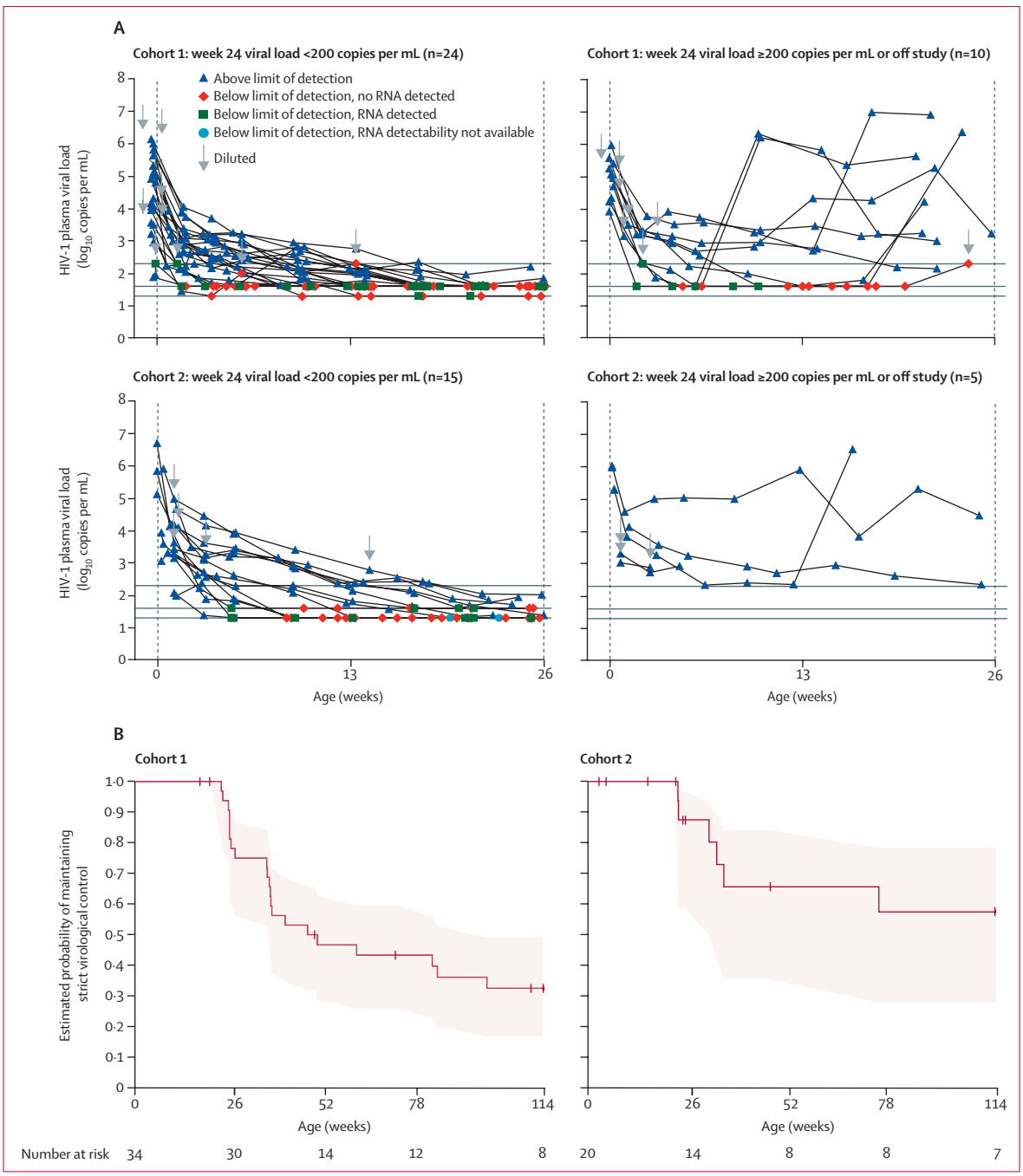


Figure 2: **HIV-1 plasma viral load**

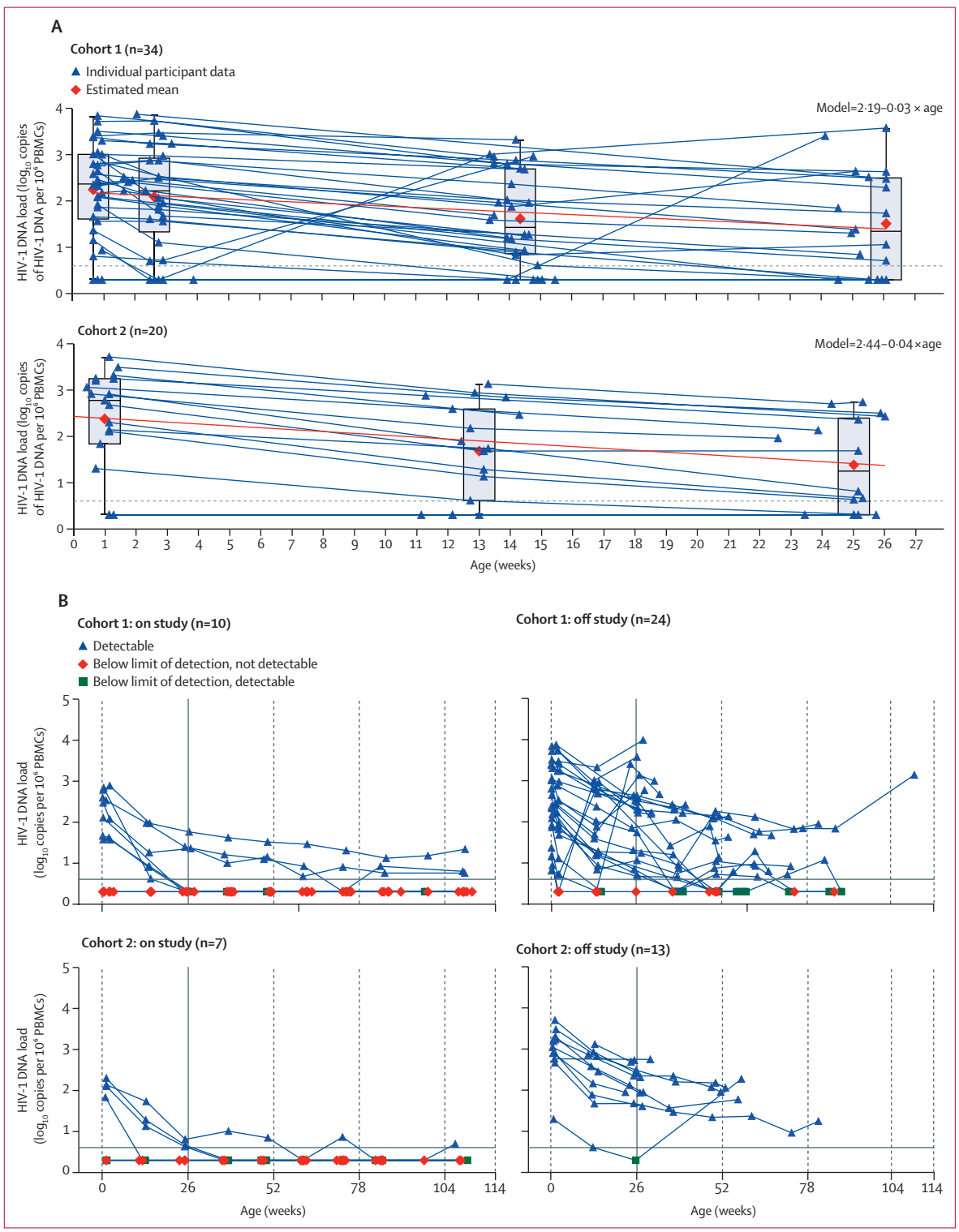
(A) Plasma HIV-1 RNA levels (log_{10} copies per mL) in the first 26 weeks of life by cohort and by reaching study-defined criteria to stay in study follow-up. The first, second, and third horizontal lines correspond to plasma HIV-1 RNA of 200 copies per mL (log_{10} HIV-1 RNA of 2·3), 40 copies per mL (log_{10} HIV-1 RNA of 1·6), and 20 copies per mL (log_{10} HIV-1 RNA of 1·3). The downward grey arrows indicate samples that required dilution for viral load testing due to low plasma volume. (B) Kaplan-Meier plot showing the estimated probability of maintaining strict virological control (plasma HIV-1 RNA <200 copies per mL at study week 24 or no confirmed detectable plasma HIV-1 RNA after that) through to 114 weeks of age. In cohort 1, there were 21 total events and in cohort 2, there were six total events. 95% CIs are shown in the shaded red areas. Tick marks indicate a participant was censored.

Statistical analysis

Target enrolment was 54 infants with in-utero HIV-1: approximately 32 in cohort 1 among 440 enrolled at high-risk of in-utero HIV-1 acquisition, and approximately 22 in cohort 2, to allow 80% or higher probability of observing events of interest that occur with frequencies of at least one in 100 neonates, such as eligibility for analytical treatment interruption. We did not design the

study to compare cohorts because they had different entry criteria.

We calculated point estimates and 95% Clopper-Pearson exact CIs for events chosen a priori (having plasma HIV RNA <200 copies per mL at week 24, having HIV-1 DNA not detected at week 108, and being antibody negative at week 108). We estimated survival probabilities by the Kaplan-Meier method, with the



estimate through to age 2 years taken from the end of the week 108 study visit window (age 114 weeks). Samples with HIV-1 DNA less than the limit of detection were set to half the limit of detection (2·045 copies per 10^6 PBMCs). Linear mixed-effects regressions on HIV-1 DNA (copies per 10^6 PBMCs) through to 26 weeks of life fit age as a linear, fixed effect with a random intercept assuming a normal distribution and autoregressive covariance structure (model selected based on minimising the Akaike Information Criterion).

We defined strict virological control as a viral load of fewer than 200 copies per mL at week 24 and no confirmed detectable (target not detected) plasma HIV-1 RNA thereafter. Univariable covariate association analyses applied Cox proportional hazards regression for time to loss of strict virological control, from birth to the initial sample date without strict virological control, censored at the earlier of last HIV-1 RNA sample date or 114 weeks, using exact ties. We aimed to identify virological and immunological characteristics associated with strict virological control, such as initial plasma viral load, HIV-1 DNA load, and CD4 percentage (full list in appendix p 6). We conducted analyses in SAS (version 9.4). This study is registered with ClinicalTrials.gov, NCT02140255.

Role of the funding source

The funders of the study contributed to the study design but had no role in data collection, data analysis, data interpretation, or writing of the report.

Results

Between Jan 23, 2015, and Dec 14, 2017, 440 infants were included in cohort 1 and 20 were included in cohort 2. 54 of these infants (34 from cohort 1 and 20 from cohort 2) had confirmed in-utero HIV-1 and continued or switched to receive study ART (table 1 and figure 1B). Most participating infants were from sub-Saharan Africa, born by spontaneous vaginal delivery, and were breastfed (table 1). 33 (61%) of 54 infants were female and 21 (39%) were male. We initiated ART for cohort 1 at a median age of 7·3 h (IQR 1·8–21·0) and triple antiretroviral nevirapine-based prophylaxis for cohort 2 at a median age of 32·8 h (1·1–40·1). We added ritonavir-boosted lopinavir at median ages of 4·1 weeks in cohort 1 and 3·0 weeks in cohort 2 and discontinued nevirapine at a median age of 29·6 weeks in cohort 1 and 25·7 weeks in cohort 2. Among mothers with available specimens, two in cohort 1 and three in cohort 2 had Lys103Asn non-nucleoside reverse transcriptase (NNRTI)-resistance mutations (table 1). About a third of infants in cohort 1 and a quarter in cohort 2 had potentially protective HLA-B alleles (table 1).

Participants were followed up over 2484 person-weeks (median 66·4 weeks of age [IQR 40·0–114·0]) in cohort 1 and 1291 person-weeks (median 54·9 weeks of age [27·9–114·0]) in cohort 2. 24 (71%) of 34 participants in cohort 1 and 13 (65%) of 20 in cohort 2 were discontinued from follow-up before age 2 years. Of those who discontinued, 21 (88%) of 24 in cohort 1 and ten (77%) of 13 in cohort 2 did not meet protocol-defined virological criteria for continued study follow-up from week 24 onwards. Two infants died (unrelated to HIV, both in cohort 1), two were lost to follow-up (one in each cohort), and two (twins in cohort 2) had withdrawal of parental consent for continued follow-up at 59 days of age.

The median earliest viral load for cohort 1 was 4·9 $\log_{10}$ copies per mL (IQR 4·0–5·3), measured at median 1·0 days of age (IQR 0·0–1·0). The median earliest viral load for cohort 2 infants was 4·1 $\log_{10}$ copies per mL (IQR 3·2–5·2), measured at medians 6·5 days of age (IQR 2·0–8·0). In cohort 1, viral load decreased by a median of 1·80 $\log_{10}$ copies per mL (IQR 1·33–2·22) by week 2, 1·93 $\log_{10}$ copies per mL (1·78–2·58) by week 4, and 2·62 $\log_{10}$ copies per mL by week 8 (1·56–3·08; figure 2A). At week 24, 75% (95% CI 57–89; 24 of 32) in cohort 1 and 88% (64–99; 15 of 17) in cohort 2 had a viral load less than 200 copies per mL. The estimated probability of maintaining strict virological control through to 2 years of age was 33% (95% CI 17–49) in cohort 1 and 57% (28–78) in cohort 2 (figure 2B).

The median earliest HIV-1 DNA loads for cohort 1 was 2·4 $\log_{10}$ copies per 10^6 PBMCs (IQR 1·7–3·0), obtained at median 1 day of age (IQR 0–1). The median earliest HIV-1 DNA loads for cohort 2 was 2·8 $\log_{10}$ copies per 10^6 PBMCs (IQR 1·8–3·3), obtained at median 8 days of age (IQR 5–8). Through to 26 weeks of age, HIV-1 DNA declined from the first tested timepoint by a median of 1·0 $\log_{10}$ copies per 10^6 PBMCs (IQR 0·0–1·3) in cohort 1 and 1·0 $\log_{10}$ copies per 10^6 PBMCs (0·9–1·3) in cohort 2 (figure 3A). Four infants with no HIV-1 DNA detected at each timepoint to week 108 had HIV-1 RNA detected in peripheral blood. In cohort 1, one female infant had an initial plasma viral load of 76 copies per mL and no HIV-1 DNA detected in

Figure 3: **HIV-1 DNA load**

(A) Boxplots of HIV-1 infected cell concentrations in the first 26 weeks of life by cohort with repeated measures regression slopes. Outliers are defined as points more than 1·5 × IQR above Q3 or 1·5 × IQR below Q1. Whiskers correspond to the lesser of 1·5 × IQR or most extreme observed value. The horizontal grey line shows the limit of detection of the HIV-1 DNA assay at 0·612 $\log_{10}$ copies per 10^6 PBMCs. Levels lower than the limit of detection were set to ½ (limit of detection). The blue triangles and blue lines represent the data from each participant throughout very early ART. Regressions treated time as a linear, fixed effect. Splines were evaluated at ages 2 weeks (cohort 1, corresponding to HIV-1 infection confirmation) and 13 weeks (cohorts 1 and 2, corresponding to the week 12 visit). Akaike Information Criterion was minimised for random intercept and fixed slope (eg, the model for each cohort fit best without splines). (B) HIV-1 DNA through to 114 weeks of age, by cohort and by study status (on or off study). The horizontal grey line shows the limit of detection of the HIV-1 DNA assay at 0·612 $\log_{10}$ copies per 10^6 PBMCs. Only infants meeting study-defined virological control criteria stayed in the study past week 24 (corresponding to 26 weeks of age). ART=antiretroviral therapy. PBMC=peripheral blood mononuclear cell.

	Cohort 1	Cohort 2
Covariates at study entry		
Earliest* CD4 percentage (per 10% higher)	21; 0·86 (0·59–1·24)	6; 0·42 (0·18–1·00)
Earliest* CD8 percentage (per 10% lower)	19; 0·73 (0·48–1·12)	..
Earliest* CD4 percentage to CD8 percentage ratio	19; 0·83 (0·58–1·19)	..
Earliest† plasma HIV-1 viral load <10 000 copies per mL (reference ≥10 000 copies per mL)	21; 0·26 (0·08–0·91)	6; 0·22 (0·03–1·88)
Earliest‡ HIV-1 DNA load (per 1 $\log_{10}$ copies per 10^6 PBMCs lower)	21; 0·60 (0·36–1·01)	6; 0·15 (0·03–0·76)
Male sex (reference female)	21; 0·31 (0·11–0·94)	6; 1·47 (0·29–7·36)
ART initiation 24–48 h of age (reference 0–24 h of age)	21; 0·12 (0·02–0·87)	6; 0·21 (0·04–1·24)
Weight appropriate for gestational age for gestational age (reference small for gestational age§)	21; 0·57 (0·21–1·56)	..
No maternal ART during pregnancy	..	6; 0·90 (0·18–4·55)
Maternal plasma HIV-1 RNA at study entry (per 1 $\log_{10}$ copies per mL lower)	21; 0·83 (0·53–1·30)	6; 0·95 (0·41–2·22)
No maternal NNRTI or NRTI resistance¶	13; 0·89 (0·11–7·14)	4; 0·88 (0·10–5·00)
No maternal NNRTI resistance¶	13; 0·89 (0·11–7·14)	4; 0·40 (0·06–2·86)
Potentially protective HLA-B allele	20; 0·54 (0·19–1·50)	6; 0·55 (0·06–4·79)
Covariates after study entry		
Nevirapine dried blood spot concentration above 3 µg/mL at week 1	16; 0·84 (0·19–3·72)	..
Age at ritonavir-boosted lopinavir initiation (per 1 week lower)	21; 0·93 (0·70–1·25)	6; 0·48 (0·25–0·93)
Never reported missing a dose through to week 24 visit (reference reported missing at least one dose)	21; 0·92 (0·37–2·27)	6; 0·82 (0·17–4·11)
Time to confirmed HIV-1 RNA less than the limit of detection (target not detected; censored at 26 weeks of age; per 1 week shorter)	21; 0·88 (0·78–0·97)	6; 0·60 (0·27–1·32)
Week 24 CD4 percentage (per 10% higher)	21; 0·75 (0·43–1·32)	6; 0·92 (0·35–2·46)
Change in CD4 percentage from baseline to week 24 (per 10% lower)	21; 0·98 (0·68–1·43)	6; 0·20 (0·04–0·96)
Week 24 CD8 percentage (per 10% lower)	19; 0·83 (0·53–1·28)	..
Change in CD8 percentage from baseline to week 24 (per 10% higher)	19; 0·91 (0·46–1·78)	..
Week 24 CD4 percentage to CD8 percentage ratio	19; 0·98 (0·68–1·41)	..
Change in CD4 percentage to CD8 percentage ratio from baseline to week 24	19; 1·12 (0·79–1·58)	..
Week 12 plasma HIV-1 viral load fewer than 200 copies per mL (reference ≥200 copies per mL)	21; 0·22 (0·08–0·59)	6; 0·06 (0·01–0·58)
Week 12 HIV-1 DNA load (per 1 $\log_{10}$ copies per 10^6 PBMCs lower)	19; 0·46 (0·27–0·79)	5; 0·08 (0·01–0·78)
Week 24 HIV-1 DNA load (per 1 $\log_{10}$ copies per 10^6 PBMCs lower)	19; 0·43 (0·26–0·70)	6; 0·05 (0·004–0·58)

Data are n; hazard ratio (95% CI). We defined strict virological control as plasma HIV-1 RNA <200 copies per mL at study week 24 and no confirmed detectable plasma HIV-1 RNA thereafter. .. indicates data not collected. ART=antiretroviral therapy. NRTI=nucleotide reverse transcriptase inhibitor. NNRTI=non-nucleoside reverse transcriptase inhibitor. PBMC=peripheral blood mononuclear cell. *Taken at a median of 15·0 days (IQR 13·0–16·0) in cohort 1 and 8·0 days (5·0–8·0) in cohort 2. †Taken at a median of 1·0 days (0·0–1·0) in cohort 1 and 6·5 days (2·0–8·0) in cohort 2. ‡Taken at a median of 1·0 days (0·0–1·0) in cohort 1 and 8·0 days (5·0–8·0) in cohort 2. §INTERGROWTH score. ¶Entry samples analysed in batch after 2 years of follow-up. Ten maternal samples from cohort 1 and two maternal samples from cohort 2 were tested with no result; three additional results from cohort 2 were excluded from analysis per team decision.

***Table 2*: Univariable Cox proportional hazards regressions of time to loss of strict virological control within 114 weeks of age**

See Online for appendix

1 042 400 PBMCs; one female infant had an initial plasma viral load of 200 copies per mL and no HIV-1 DNA detected in 307 200 PBMCs; and one male infant had an initial plasma viral load of 1634 copies per mL and no HIV-1 DNA detected in 646 400 PBMCs. In cohort 2, one male infant had an initial plasma viral load of 1969 copies per mL, and no HIV-1 DNA detected in 1 024 800 PBMCs; no HIV-1 DNA was detected at all subsequent timepoints, except for a single positive at week 48, where we detected a single copy of HIV-1 DNA on analysis of 981 000 PBMCs. Maternal samples were available for three of these infants, and all three had detectable HIV-1 DNA.

In linear mixed-effects regressions, the estimated mean decline per week was 0·03 $\log_{10}$ copies per 10^6 PBMCs (95% CI 0·02–0·04) for cohort 1 and 0·04 $\log_{10}$ copies per 10^6 PBMCs (0·03–0·05) for cohort 2. At week 24, six (19%) of 31 infants in cohort 1 and four (22%) of 18 infants in cohort 2 had no detected HIV-1 DNA. Among infants maintaining protocol-defined virological control criteria through to study week 108, seven of 11 (64%, 95% CI 31–89) in cohort 1 and five of seven (71%, 29–96) in cohort 2 had no detected HIV-1 DNA; ten of 12 (83%, 52–100) in cohort 1 and all seven (100%, 59–100) in cohort 2 tested HIV-1 antibody-negative at week 108 (figure 3B). Across all visits, the median number of cells analysed when DNA was undetected was 872 400 (range 307 200–1 625 600).

In cohorts 1 and 2, we observed longer maintenance of strict virological control in participants with an earliest viral load of fewer than 10 000 copies per mL than in participants with an earliest viral load of 10 000 copies per mL or more, in participants with a lower earliest HIV-1 DNA load, and in participants with a higher earliest CD4 cell percentage, although the 95% CIs for these hazard ratios (HRs) were wide and some were compatible with no difference (HR=1; table 2). In addition to earliest HIV-1 DNA load, lower measurements of HIV-1 DNA at weeks 12 and 24 were associated with longer duration of maintaining strict virological control in each cohort (table 2).

In cohort 1, male sex was associated with longer duration of maintaining strict virological control (table 2). The estimated probability of maintaining strict virological control through to age 2 years was 64% (95% CI 30–85) for male infants and 19% (6–38) for female infants. Male infants also had lower median earliest viral load (4·3 $\log_{10}$ copies per mL [IQR 3·6–5·1] *vs* 5·0 $\log_{10}$ copies per mL [4·0–5·6]), lower HIV-1 DNA load (2·1 $\log_{10}$ copies per 10^6 PBMCs [1·6–2·5] *vs* 2·8 $\log_{10}$ copies per 10^6 PBMCs [1·9–3·3]), and earlier ART initiation (3·2 h [0·9–9·5] *vs* 15·8 h [2·3–27·3]) than did female infants.

Division of AIDS grade 3 or 4 events related to the ART regimen occurred in 15 (44%) of 34 infants (13 in the first 6 months) in cohort 1 and seven (35%) of 20 infants (six in the first 6 months) in cohort 2 (appendix pp 1–5). Most were haematological (21 [95%] of 22 had neutropenia or anaemia), which reversed after switching from zidovudine to abacavir. One infant in cohort 2 developed asymptomatic grade 3 elevated lipase.

22 infants discontinued one of the initial antiretroviral drugs in the regimen due to toxicity: 19 discontinued zidovudine (17 of 19 with grade 3 or 4 neutropenia or anaemia), one discontinued nevirapine (grade 4 neutropenia), one discontinued abacavir plus lamivudine (grade 1 vomiting), and one discontinued ritonavir-boosted lopinavir (grade 2 precocious pubarche and thelarche, resolved after switching to nevirapine). Two infants in cohort 1 died: one from probable bacterial pneumonia at 19·4 weeks of age and another from poisoning at age 79·9 weeks of age. Neither death was deemed related to study treatment by the study's clinical monitoring committee; both participants had achieved a viral load lower than the limit of detection with target not detected at the last time tested (age 18·1 weeks and 72·7 weeks).

Lopinavir concentrations varied substantially, with many participants having concentrations of 3000–10 000 ng/mL, the typical values seen in adults and older children. However, we observed a high frequency (128 [38%] of 334) of low concentration values, less than 1000 ng/mL (most less than 50 ng/mL), below the target trough lopinavir concentration of more than 1000 ng/mL,[12,13] suggesting non-adherence or poor absorption.

Infants with sustained viral loads lower than the limit of detection with target not detected since week 48 became eligible for analytical treatment interruption starting at age 2 years if they have ceased breastfeeding and their most recent biomarker profile includes a negative HIV-1 antibody, HIV-1 DNA not detected, CD4 percentage of 25% or greater, and CD4 count normal-for-age. At age 2 years, ten (19%) of 54 infants who were initiated on very early ART (six in cohort 1 and four in cohort 2) met all criteria for eligibility to undergo analytical treatment interruption. However, analytical treatment interruption was postponed during the COVID-19 pandemic. Data collection for analytical treatment interruption is ongoing and will be reported elsewhere.

Discussion

Despite substantial advances in preventing perinatal HIV-1 transmission, infants remain at high risk for HIV-1 due to undiagnosed or untreated maternal HIV-1. ART-free remission could avert long-term toxicities from antiretroviral drugs and combat stigma and adherence challenges. In IMPAACT P1115, among infants with in-utero HIV-1 receiving pre-emptive treatment or triple-drug antiretroviral prophylaxis within 48 h of life, the estimated probabilities of reaching and maintaining strict virological control through to age 2 years were 33% (95% CI 17–49) in cohort 1 and 57% (28–78) in cohort 2. Sustained virological control is the first step in eligibility criteria for analytical treatment interruption, offering hope of achieving low reservoir size, with HIV-1 DNA not detected in peripheral blood cells in early infancy.

Two studies, the LEOPARD trial[14] in South Africa and the Early Infant Treatment (EIT) study[15] in Botswana, also studied very early treatment of neonates. These studies initiated ART after confirmed HIV-1 acquisition and used three-drug ART regimens (nevirapine switched to ritonavir-boosted lopinavir when age appropriate). In IMPAACT P1115, infants were pre-emptively treated with three-drug ART (cohort 1) or received three-drug prophylaxis within 48 h of life with direct transition to study ART (cohort 2), and a fourth drug (ritonavir-boosted lopinavir) was added when age appropriate. The LEOPARD trial did not implement analytical treatment interruption, as too few participants met predefined week 48 virological criteria (viral load <50 copies per mL) and immunological criteria (CD4 cell percentage >30%).[14]

Without established biomarkers to predict ART-free remission,[2] we chose HIV-1 seronegative status combined with non-detectable HIV-1 DNA as the main criteria for analytical treatment interruption eligibility in IMPAACT P1115. Across our two cohorts, ten (19%) of 54 infants met these eligibility criteria at age 2 years. In the Botswana EIT study, among the 16 very early treated infants with viral load suppression from 24–84 weeks, 88% tested HIV-1 seronegative at week 84, and 69% tested negative for HIV-1 DNA; analytical treatment interruption was not part of the EIT study design.[16]

ART-free remission is particularly important in low-resource settings, where access to care and treatment is fragile. All three studies described above involved nevirapine and ritonavir-boosted lopinavir-based ART, the only antiretroviral drugs with pharmacokinetic data for treatment in neonates at the time. Ritonavir-boosted lopinavir-based ART is particularly challenging for children due to liquid formulations with poor palatability and variable absorption from infancy to adulthood.[17]

In a study of ritonavir-boosted lopinavir-based ART in infants younger than 6 months, 19 (66%) of 29 participants had durable virological suppression to fewer than 400 copies per mL through to a median of 123 weeks, which was highly correlated with pre-dose lopinavir concentrations exceeding 100 ng/mL.[18] Here, we observed low lopinavir concentrations in a substantial proportion of infants, probably owing to the known drawbacks,[17] thereby possibly contributing to the poorer virological response we observed. More robust and tolerable very early ART regimens, including integrase inhibitors and long-acting immunotherapeutics such as broadly neutralising antibodies, might improve the likelihood of early, durable virological control in infants. Given the high proportion of unintegrated HIV-1 DNA during untreated infection that can promote productive infection if the infected cell becomes activated,[19] treatment with integrase inhibitors might further restrict HIV-1 reservoirs early in infancy. Version 2.0 of IMPAACT P1115 (NCT02140255) is examining such regimens.

Point-of-care assays for HIV-1 diagnosis in neonates at high risk could provide pre-emptive therapy pending rapid testing results and more focused and rapid ART initiation in neonates.[20] Maximising birth testing is imperative, as HIV-1 RNA and DNA diagnostic testing results can be challenging with point-of-care assays or after triple antiretroviral drugs begin in neonates. Here, the effective enrolment and high adherence to study visits with minimal loss to follow-up showed parental acceptance and feasibility of very early infant diagnosis and treatment in the context of high HIV-1 burden. Birth diagnosis and earlier ART initiation were associated with reduced infant mortality[21] and CD4 T-cell preservation.[22] We identified infants at high risk across multiple clinical research sites and pre-emptively treated and followed them up longitudinally. The findings support global strategies to identify and treat neonates at high risk of HIV-1 acquisition.

Among infants maintaining protocol-defined virological control criteria through to study week 108, 12 (67%) of 18 had no detected HIV-1 DNA and four (22%) had fewer than 1·0 $\log_{10}$ copies DNA per 10^6 PBMCs. Notably, as most (>90%) HIV-1 DNA is defective and incapable of replication,[23] fewer HIV-1 DNA copies probably reflects low reservoir size. Four infants with no HIV-1 DNA detected at study entry continued to test negative through to age 2 years. Maternal samples were available for three of these infants, and all had detectable HIV-1 DNA, excluding defective primer binding. These infants could reflect in-utero transmission near birth before establishment of substantial HIV-1 reservoirs.

Lower HIV-1 DNA at weeks 12 and 24 was associated with maintaining strict virological control in both cohorts. Early assessment of HIV-1 DNA could be a biomarker for the effect of very early ART on the proviral reservoir and ART-free remission, especially considering recent efforts to develop cross-subtype intact proviral DNA assays that would afford quantification of clinically relevant intact proviruses.[24,25]

In infants in cohort 1, male sex and lower earliest plasma viral load were associated with longer-term virological suppression. In a South African study of in-utero HIV-1, male sex was also associated with lower baseline viral loads and a higher likelihood of virological suppression.[26] As in our study, girls had higher levels of HIV-1 DNA. Both findings are relevant to potential sex differences in paediatric HIV-1 remission and cure therapeutics.[26] Female infants might have responded less completely to treatment due to higher viraemia, a poorly understood occurrence, with interferon-resistant HIV-1 noted in one study.[26] In African populations, *HLA-B*57*, *HLA-B*58:01* and *HLA-B*81:01* were associated with low viral setpoints in untreated adults with HIV-1. Here, we did not observe an association between a protective HLA-B allele and maintaining strict virological control, although our sample size was small.

Although the study regimen was generally safe, 15 (44%) of 34 infants in cohort 1 and seven (35%) of 20 infants in cohort 2 had grade 3 or 4 adverse events related to the study regimen, primarily reversible haematological toxicity due to zidovudine. These findings support routine haematological monitoring during very early ART in infants where feasible.

Our small sample size precluded multivariable association analyses; univariable estimates should be considered carefully for sparse data bias,[27] particularly for the small event number in cohort 2, uncontrolled confounding, and built-in selection bias in HRs.[28] In cohort 2, our earliest RNA, DNA, and CD4 measurements were retrospective and not always available before ART initiation. Per study design, we only followed up infants meeting and maintaining protocol-defined long-term virological suppression criteria after week 24. This limited the evaluation of very early treatment for time to virological suppression and HIV-1 DNA decline to the first 6 months of life.

The ART regimen might not have been optimal in cases of pre-existing resistance, although maternal NNRTI resistance was low. Due to limitations on withdrawn blood volume, infant HIV-1 resistance testing was not possible to determine NNRTI resistance as a contributor to reaching or maintaining strict virological control on initial nevirapine-based ART. Safety and pharmacokinetic data for neonatal ART regimens beyond the nevirapine-based regimen (such as integrase inhibitors[29] or broadly neutralising antibodies[30]) were unavailable when we developed the study protocol.

Our research protocol rigorously identified in-utero HIV-1 transmission, administered pre-emptive ART within a strict time window, and monitored virological response at frequent, regular intervals, which is not always feasible in standard clinical care settings globally. This limitation might reduce the clinical generalisability of our study.

Pre-emptive ART within 48 h of life or triple antiretroviral drug prophylaxis with direct transition to ART within 10 days of life in neonates with in-utero HIV-1 was well tolerated over the first 2 years of life. A subset of neonates initiating very early ART can sustain virological control through to age 2 years, the majority of whom reach non-detectable HIV-1 DNA and seronegative status. The extent to which this biomarker profile is sufficient to allow drug-free HIV-1 remission will be tested by analytical treatment interruptions as planned.

Our study and previous studies, such as LEOPARD and EIT, show that neonatal ART following in-utero HIV-1 transmission is feasible and can reduce reservoir size. This strategy holds promise for ART-free remission as paediatric cure research advances. Although 33% in cohort 1 and 57% in cohort 2 achieved and maintained strict viral control to qualify for possible analytical treatment interruption, future studies using more potent regimens might increase this proportion.

Contributors

DP, YB, EGC, JJ, BSN, and CT conceptualised and led the writing of the manuscript. BSN and CT led statistical analysis. DP, JS, DC, and MNC oversaw laboratory assays. BSN, CT, JS, CR, and MNC verified the data. LS-C, ARK, and VK led participant recruitment. All authors contributed to the protocol development, data collection with full access to all the study data, and manuscript drafting. All authors had final responsibility for the decision to submit for publication.

Declaration of interests

MM receives research support from Gilead Sciences, ViiV Healthcare, and Merck. EVC serves as a consultant to Melinta Pharmaceuticals. EGC's spouse holds an equity interest in AbbVie. All other authors declare no competing interests.

Data sharing

The data cannot be made publicly available because of the restrictions in the study's informed consent documents and the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network's approved human participants protection plan; public availability might compromise participant confidentiality. However, data are available to all interested researchers upon request to the IMPAACT Statistical and Data Management Center's data access committee (sdac.data@fstrf.org) with the agreement of the IMPAACT Network.

Acknowledgments

Overall support for IMPAACT was provided by the National Institute of Allergy and Infectious Diseases with co-funding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institute of Mental Health, all components of the National Institutes of Health (NIH), under award numbers UM1AI068632 (IMPAACT LOC), UM1AI068616 (IMPAACT SDMC), and UM1AI106716 (IMPAACT LC), and by NICHD contract number HHSN275201800001I. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The project has also been funded in part with federal funds from the Frederick National Laboratory for Cancer Research under contract number 75N91019D00024. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organisations imply endorsement by the US Government. This research was supported in part by the Intramural Research Program of the NIH, Frederick National Laboratory, Center for Cancer Research. DP was also supported by an R01AI150412 from the NIH, the PAVE Martin Delaney Collaboratory (UM1AI164566), the IMPAACT Center subspecialty laboratory (5UM1AI106716), and the Johns Hopkins Center for AIDS Research (P30 AI094189). PJ-P, SM, and DEY are employees of the NIH. We thank and acknowledge the IMPAACT P1115 study team, the study participants, the site investigators, site staff, collaborating institutions, and the local and IMPAACT community advisory board members who supported this study.

References

1 WHO. Mother-to-child transmission of HIV. https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/prevention/mother-to-child-transmission-of-hiv (accessed March 14, 2023).
2 Deeks SG, Archin N, Cannon P, et al. Research priorities for an HIV cure: International AIDS Society Global Scientific Strategy 2021. *Nat Med* 2021; **27:** 2085–98.
3 Persaud D, Gay H, Ziemniak C, et al. Absence of detectable HIV-1 viremia after treatment cessation in an infant. *N Engl J Med* 2013; **369:** 1828–35.
4 Luzuriaga K, Gay H, Ziemniak C, et al. Viremic relapse after HIV-1 remission in a perinatally infected child. *N Engl J Med* 2015; **372:** 786–88.
5 Butler KM, Gavin P, Coughlan S, et al. Rapid viral rebound after 4 years of suppressive therapy in a seronegative HIV-1 infected infant treated from birth. *Pediatr Infect Dis J* 2015; **34:** e48–51.
6 Giacomet V, Trabattoni D, Zanchetta N, et al. No cure of HIV infection in a child despite early treatment and apparent viral clearance. *Lancet* 2014; **384:** 1320.
7 Ruel TD, Capparelli EV, Tierney C, et al. Pharmacokinetics and safety of early nevirapine-based antiretroviral therapy for neonates at high risk for perinatal HIV infection: a phase 1/2 proof of concept study. *Lancet HIV* 2021; **8:** e149–57.
8 Luzuriaga K, McManus M, Mofenson L, Britto P, Graham B, Sullivan JL. A trial of three antiretroviral regimens in HIV-1-infected children. *N Engl J Med* 2004; **350:** 2471–80.
9 Powell L, Dhummakupt A, Siems L, et al. Clinical validation of a quantitative HIV-1 DNA droplet digital PCR assay: applications for detecting occult HIV-1 infection and monitoring cell-associated HIV-1 dynamics across different subtypes in HIV-1 prevention and cure trials. *J Clin Virol* 2021; **139:** 104822.
10 Zheng JH, Rower C, McAllister K, et al. Application of an intracellular assay for determination of tenofovir–diphosphate and emtricitabine–triphosphate from erythrocytes using dried blood spots. *J Pharm Biomed Anal* 2016; **122:** 16–20.
11 Division of AIDS, US Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases. Division of AIDS table for grading the severity of adult and pediatric adverse events, version 1.0. https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables (accessed Nov 21, 2023).
12 Chadwick EG, Capparelli EV, Yogev R, et al. Pharmacokinetics, safety and efficacy of lopinavir/ritonavir in infants less than 6 months of age: 24 week results. *AIDS* 2008; **22:** 249–55.
13 Waalewijn H, Turkova A, Rakhmanina N, et al. Optimizing pediatric dosing recommendations and treatment management of antiretroviral drugs using therapeutic drug monitoring data in children living with HIV. *Ther Drug Monit* 2019; **41:** 431–43.
14 Kuhn L, Strehlau R, Shiau S, et al. Early antiretroviral treatment of infants to attain HIV remission. *EClinicalMedicine* 2020; **18:** 100241.
15 Garcia-Broncano P, Maddali S, Einkauf KB, et al. Early antiretroviral therapy in neonates with HIV-1 infection restricts viral reservoir size and induces a distinct innate immune profile. *Sci Transl Med* 2019; **11:** eaax7350.
16 Ajibola G, Garcia-Broncano P, Maswabi K, et al. Viral reservoir in early-treated human immunodeficiency virus-infected children and markers for sustained viral suppression. *Clin Infect Dis* 2021; **73:** e997–1003.
17 Yang J, Nikanjam M, Best BM, et al. Population pharmacokinetics of lopinavir/ritonavir: changes across formulations and human development from infancy through adulthood. *J Clin Pharmacol* 2018; **58:** 1604–17.
18 Chadwick EG, Yogev R, Alvero CG, et al. Long-term outcomes for HIV-infected infants less than 6 months of age at initiation of lopinavir/ritonavir combination antiretroviral therapy. *AIDS* 2011; **25:** 643–49.
19 Persaud D, Pierson T, Ruff C, et al. A stable latent reservoir for HIV-1 in resting CD4(+) T lymphocytes in infected children. *J Clin Invest* 2000; **105:** 995–1003.
20 Ochodo EA, Guleid F, Deeks JJ, Mallett S. Point-of-care tests detecting HIV nucleic acids for diagnosis of HIV-1 or HIV-2 infection in infants and children aged 18 months or less. *Cochrane Database Syst Rev* 2021; **8:** CD013207.
21 Lilian RR, Kalk E, Technau KG, Sherman GG. Birth diagnosis of HIV infection in infants to reduce infant mortality and monitor for elimination of mother-to-child transmission. *Pediatr Infect Dis J* 2013; **32:** 1080–85.
22 Nelson BS, Tierney C, Persaud D, et al. Infants receiving very early antiretroviral therapy have high CD4 counts in the first year of life. *Clin Infect Dis* 2023; **76:** e744–47.
23 Uprety P, Chadwick EG, Rainwater-Lovett K, et al. Cell-associated HIV-1 DNA and RNA decay dynamics during early combination antiretroviral therapy in HIV-1-infected infants. *Clin Infect Dis* 2015; **61:** 1862–70.
24 Bruner KM, Wang Z, Simonetti FR, et al. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature* 2019; **566:** 120–25.
25 Cassidy NAJ, Fish CS, Levy CN, et al. HIV reservoir quantification using cross-subtype multiplex ddPCR. *iScience* 2021; **25:** 103615.
26 Adland E, Millar J, Bengu N, et al. Sex-specific innate immune selection of HIV-1 in utero is associated with increased female susceptibility to infection. *Nat Commun* 2020; **11:** 1767.

27 Greenland S, Mansournia MA, Altman DG. Sparse data bias: a problem hiding in plain sight. *BMJ* 2016; **352:** i1981.

28 Hernán MA. The hazards of hazard ratios. *Epidemiology* 2010; **21:** 13–15.

29 Clarke DF, Acosta EP, Cababasay M, et al. Raltegravir (RAL) in neonates: dosing, pharmacokinetics (PK), and safety in HIV-1-exposed neonates at risk of infection (IMPAACT P1110). *J Acquir Immune Defic Syndr* 2020; **84:** 70–77.

30 Cunningham CK, McFarland EJ, Morrison RL, et al. Safety, tolerability, and pharmacokinetics of the broadly neutralizing human immunodeficiency virus (HIV)-1 monoclonal antibody VRC01 in HIV-exposed newborn infants. *J Infect Dis* 2020; **222:** 628–36.