Study Title: A prospective, observational, cohort, multicenter study of Early Anti-Retroviral Treatment in HIV- infected Infants.

Short Title: Early Anti-Retroviral Treatment in HIV- infected Children.

Acronym: EARTH

Version 3, Date DECEMBER 2021

Sponsor: Penta (Child Health Research)
Funded by ViiV.

TABLE OF CONTENTS

1 STUDY PERSONNEL

Sponsor Chairs:
Carlo Giaquinto (Penta (Child Health Research), Padova, Italy), carlo.giaquinto@unipid.it, +39 0498213585
Paolo Rossi (Hospital Bambino Gesù, Roma, Italy), paolo.rossi@opbg.net

Clinical Chairs, Protocol Implementation:
Alfredo Tagarro (Hospital 12 de Octubre, Madrid, Spain, Universidad Europea de Madrid), alfredotagarro@gmail.com, +34 606194888
Co-chairs
Pablo Rojo (Hospital 12 de Octubre, Madrid, Spain), pablorojoconejo@aim.com, +34 615513222).
Louise Kuhn (Columbia University, NYC, USA), lk24@cumc.columbia.edu, +1 212 3052398)

Immunology Chair
Paolo Palma
Childrens' Hospital "Bambino Gesu"
P.ZZA Sant'Onofrio 4 00165
Rome, Italy
paolo.palma@opbg.net
Tel: +39 0668592455

Virology Chair
Dr Mathias Lichterfeld
Ragon Institute of MGH, MIT and Harvard
400 Technology Square, Cambridge, MA 02139, USA
Tel: (617) 724-6454

Ethics Chair
Viviana Giannuzzi (Gianni Benzi Pharmacological Research Foundation), vg@benzifoundation.org +393201172913
Alessandra Nardone (Fondazione Penta Onlus) alessandra.nardone@pentafoundation.org
Co-chair: Maria Grazia Lain (F. Ariel Glaser contra o SIDA Pediatrico), mlain@arielglaser.org.mz

In-country responsible of Ethics and Export / Import Permits
South Africa: Shaun Barnabas, barnabas@sun.ac.za, Anita Janse Van Rensburg ajvanr@sun.ac.za
Mozambique: Maria Grazia Lain, mlain@arielglaser.org.mz
Italy: Ilaria Pepponi, ilaria.pepponi@opbg.net
United Kingdom: Moira Spyer, moira.spyer@ucl.ac.uk
Spain: Alfredo Tagarro, alfredotagarro@gmail.com
USA: Louise Kuhn, lk24@cumc.columbia.edu

Person in charge of oversight of sample management and shipping of samples:
Dr Moira Spyer; moira.spyer@ucl.ac.uk (UCL Great Ormond Street Institute of Child Health)

Ilaria Pepponi, OPBG, Rome, ilaria.pepponi@opbg.net

Field Sites Leaders:

1. Tygerberg Children's Hospital (TCH), Stellenbosch University (SU), Cape Town, South Africa.
Clinical Leader: Shaun Barnabas, barnabas@sun.ac.za, Anita Janse Van Rensburg ajvanr@sun.ac.za
Lab leader: Dr. Gert van Zyl; gert guvz@sun.ac.za

2. Perinatal HIV Research Unit, Chris Hani Baragwaneth Hospital, University of the Witwatersrand, Soweto, South Africa.
Clinical leader: Avy Violari, violaria@phru.co.za
Lab leader (subcontracted): Fiona Shahim, Central Laboratory Services (CLS), Johannesburg. fshahim@cls.co.za

3. Africa Health Research Institute , KwaZulu Natal, South Africa
Clinical leader Nigel Klein, n.klein@ucl.ac.uk, Osse Behuhuma Obehuhuma@gmail.com
Lab leader: Nigel Klein, n.klein@ucl.ac.uk,
Thumbi N'dungu: thumbi.ndungu@ahri.org

4. Centro de Investigaçao em Saude de Manhiça (CISM) and Barcelona Institute for Global Health - ISGlobal
Clinical leader: Elisa Lopez Varela, elisa.lopez@isglobal.org and Tacilta Nhampossa, Tacilta.Nhampossa@manhica.net Sheila Fernández, Sheila.fernandeez@isglobal.org
Lab leader: Anelsio Cossa, Anelsio.Cossa@manhica.net

5. Fundação Ariel Glaser contra o SIDA Pediátrico (F.Ariel)
Clinical leader: Maria Grazia Laín, mlain@arielglaser.org.mz, Paula Vaz, pvaz@arielglaser.org.mz
Lab leader: Dulce Bila, dbila@arielglaser.org.mz; Nadia Sitoe (INS-MOH) nadia_sitoe@yahoo.com.br

6. Centre Hospitalier Universitaire Gabriel Touré
Clinical leader: Mariam Sylla dr_mame@yahoo.fr
Laboratory leader: anne-genevieve.marcelin@aphp.fr,
Almoustapha Issiaka: almoustapha@gmail.com, amaiga@icermali.org

Clinical, immunological, virological and statistical co-investigators and advisors:

Dr. Denise Naniche
Barcelona Institute for Global Health – ISGlobal denise.naniche@isglobal.org
Dr. Nicola Cotugno cotugnonicola@yahoo.it
Children' Hospital "Bambino Gesu"
Dr.ssa Paola Zangari paola.zangari@opbg.net
Children' Hospital "Bambino Gesu" Tel: +39 06685925
Kennedy Otwombe, otwombek@phru.co.za

Perinatal HIV Research Unit, Chris Hani Baragwaneth Hospital, University of the Witwatersrand, Soweto, South Africa
Dr. Savita Pahwa,
University of Miami Miller School of Medicine in Miami, USA; spahwa@med.miami.edu
Anne-Genevieve Marcelin; Pierre et Marie Curie University in Paris; anne-genevieve.marcelin@aphp.fr
Dr. Camille Brehin, SERMAS-Fundación 12 de Octubre; brehin.c@chu-toulouse.fr
Dr Moira Spyer; UCL Great Ormond Street Institute of Child Health; moira.spyer@ucl.ac.uk
Dr. Eleni Nastuoli; UCL Great Ormond Street Institute of Child Health; e.nastouli@ucl.ac.uk
Dr Anita de Rossi, University of Padova; anita.derossi@unipd.it

Statistics: Sara Dominguez (Fundación 12 de Octubre), Kennedy Otwombe (Perinatal HIV Research Unit, Chris Hani Baragwaneth Hospital, University of the Witwatersrand, Soweto, South Africa).

Data Management Board:
Andrea Oletto (Penta (Child Health Research), Padova, Italy), andrea.oletto@pentafoundation.org
Sara Domínguez (Hospital Universitario 12 de Octubre, Madrid, Spain); sara.dominguez.r@gmail.com
Kennedy Otwombe (Perinatal HIV Research Unit, Chris Hani Baragwaneth Hospital, University of the Witwatersrand, Soweto, South Africa), otwombek@phru.co.za
Alfredo Tagarro (Hospital Universitario 12 de Octubre, Madrid, Spain), alfredotagarro@gmail.com
Dickman Gareta (AHRI, Kwa-Zulu Natal, ZA), Brian Grey and Lerato Mohale (Tygerberg, Cape Town, ZA), Juan Erasmus (PHRU, Johannesburg, ZA), Josue Togo (CH Bamako, Mali), Aderito magumane, (Manhiça, Mozambique), Loide Cardoso (Ariel Glaser, Maputo, Mozambique).

Social Sciences Substudy Chair and researchers:
Lead: Holly Peay, hpeay@rti.org
RTI International
Miriam Hartmann, mhartmann@rti.org
RTI International
Katherine Porter, kmporter@rti.org
RTI International
Danielle Toth, datoth@rti.org
RTI International
Gail Henderson, gail_henderson@med.unc.edu
University of North Carolina at Chapel Hill
Substudy 2: Strategies to improve viral suppression (SIVS) Substudy leaders
Shaun Barnabas
Tygerberg Children's Hospital (TCH), Stellenbosch University (SU), Cape Town, South Africa.
Clinical Leader: Shaun Barnabas, barnabas@sun.ac.za

Elisa López Varela

Elisa Lopez Varela, elisa.lopez@isglobal.org

Financial management:

Daniel Gomez-Peña (Penta Foundation) daniel.Gomezpena@pentafoundation.org, +39 320322600

2 ABBREVIATIONS

ABV:	Abacavir
AHRI:	African Health Research Institute
AIDS:	Acquired Immunodeficiency Syndrome
ART:	Antiretroviral Therapy
ARV:	Antiretroviral
AUC:	Area Under the Curve
AZT:	Zidovudine
CAF:	Clinical Assessment Form
CCR:	High risk pediatric clinic (from Portuguese: “consulta de criança em risco”)
CISM:	Centro de Investigaçao em Saude de Manhiça
CMV:	Cytomegalovirus
CTZ:	Cotrimoxazole
CRF:	Case Report Form
D4T:	Stavudine
DBS	Dried Blood Spot
DDI:	Didanosine
DNA:	Deoxyribonucleic acid
DSMB:	Data Safety and Monitoring Board
EFV:	Efavirenz
EIA:	Enzyme immunoassay
EPIICAL:	Early treated Perinatally HIV Infected individuals: Improving Children’s Actual Life
F.Ariel:	Fundação Ariel Glaser contra o SIDA Pediátrico
FLIPS:	Full Length Individual Sequencing
FTC:	Emtricitabine
GCP:	Good Clinical Practice
GLP:	Good Laboratory Practice
HF:	Health Facility
HIV:	Human Immunodeficiency Virus
ICH:	International Conference of Harmonization
ICH-UCL:	Institute of Child Health, University College London
ICHT:	Imperial College Healthcare NHS Trust
ICF:	Informed consent form
IEC:	Independent Ethics Committee
INI:	Integrase Inhibitor
IPT:	Isoniazide Preventive Therapy
IQR:	Interquartile Range
IRB:	Institutional Review Board
JCRC	Joint Clinical Research Centre, Kampala
LPV/r:	Lopinavir/ritonavir
MTCT:	Mother-to-child transmission
MRC:	Medical Research Council
NDMTs:	Novel Disease Modifying Therapies
NNRTI:	Non-nucleoside reverse transcriptase inhibitors

NVP:	Nevirapine
PaHIV:	Perinatally acquired HIV
PBMC:	Peripheral Blood Mononuclear Cells
PI:	Protease Inhibitors
PCR:	Polymerase Chain Reaction
PENTA:	Pediatric European Network for Treatment of AIDS
PMTCT:	Prevention of mother-to-child transmission
POC:	Proof of Concept
RNA:	Ribonucleic acid
ROC:	Receiver Operating Characteristics
SAE:	Serious Adverse Event
SD:	Standard Deviation
SMG:	Study Management Group
SOP:	Standard Operating Procedure
SSC:	Study Steering Committee
TNA:	Total DNA
TDF:	Tenofovir
UCL:	University College London
VL:	Viral load
WHO:	World Health Organization
WP:	Work Package
3TC:	Lamivudine

3 SUMMARY

Background: Early antiretroviral therapy (ART) initiation is correlated with a low reservoir (total HIV-DNA) and good immunological outcomes. The EPIICAL (Early treated Perinatally HIV Infected individuals: Improving Children's Actual Life) project is an international consortium whose aim is to select promising HIV therapeutic strategies candidates for use among early-treated perinatally HIV-positive children.

Study Design: Prospective cohort study

Hypothesis: Monitoring HIV-DNA from birth during the first 4 years of age, we can identify and characterize a proportion of early-treated children who will have an excellent control of HIV at 4 years of age in terms of clinical, viral and immunological features, and other who will not have such excellent control. This differentiation will help us to identify which participants are more likely to benefit from which novel therapeutic interventions, depending on the profile of the patient, the intervention and the endpoint.

Study Participants: Perinatally infected infants who begin ART ≤ 90 days after diagnosis. Breastfed infants diagnosed with HIV ≤90 days of age and starting ART ≤ 90 days after diagnosis.

The diagnosis of HIV transmission by breastmilk will be made from infants who:

1. Have a negative birth HIV PCR
2. Are being breastfed
3. Who subsequently have 2 molecular methods positive for HIV. These tests will be conducted selectively among participants with a clinical evaluation suggestive of HIV infection, at the discretion of the attending physician.

Primary aim:

To monitor clinical, virological and immunological features of HIV-positive, early treated children (≤90 days after diagnosis), in order to identify participants with excellent viral and immunological control, and also other without excellent control, in order to stratify potential participants in proof-of-concept trials directed to HIV cure.

Secondary aims

1. To investigate factors associated with mortality, progression to AIDS, suppression (including resistance to ART), adherence and retention, and anthropometric measures.
2. To investigate factors associated with low total HIV-1 DNA in African children with a suppressed viral load, who started ART very early in life (≤6 months of age).
3. To validate models based on from European cohort data predicting which children will have excellent virologic control in limited-resource settings.
4. To study the association of HIV reservoir size with an appropriate immunological response (exhaustion and activation) at age 2 and 4 in limited-resource settings.
5. To evaluate children with unplanned cessation of ART who persist with a low reservoir at age 2 and 4, if any exist.
6. To study potential new virological markers relevant for reservoir, as they arise from ongoing protocols of EPIICAL or another ground-breaking research elsewhere.
7. To study adherence issues and parental expectations from cohort membership and clinical trials, and to ensure acceptable benefit: risk balance in the trials.

8. To know the proportion of infants with HIV that had high-risk of transmission, according to HIV-exposed Infants Programmatic Update 2018, WHO, Geneva.

Planned Sample Size: The size is based on the ability to recruit participants fulfilling the inclusion criteria. We expect to recruit a minimum of 300 participants at all sites during the recruitment period of up to 3 years. Assuming a 30% loss to follow-up during the 2-year follow-up period, we will obtain a sample of 200 participants at the end of the study. Approximately 30% of participants from European Cohorts had a low reservoir (median age, 7 y). Thus, we expect a sample of 60 participants who fulfil the definition of excellent controllers and 140 who do not.

Planned Study Period: Up to 4 years from enrolment from the beginning (began in May 2018), and at least up to 4 years of follow-up for every participant.

Inclusion Criteria

- Perinatally infected infants who start ART at ≤90 days after diagnosis (HIV infection diagnosed and confirmed by molecular methods in 2 different samples, i.e., positive qualitative HIV DNA/RNA test).
- Breastfed-infected children found to be infected ≤ 90 days of age and who started ART at < 90 days after diagnosis. This group will be younger than six months of age at enrolment
- Able to take ART
- Caregivers (mother, if alive and available) able to provide informed consent.

Interventions: Up to 12 visits for all participants (enrolment visit + 11 visits), with 11 blood draws for virological and immunological measurements.

Stratification: Infants will be stratified by study site.

Study procedure: Prospective, cohort study. Newly diagnosed HIV positive children will start early ART as soon as possible, following local standard of care, and will be followed for up to 4 years of age.

Clinical data and blood for viral load, immunology and serology will be collected in the 11 visits. The HIV reservoir size and HIV-specific immune responses will be analyzed at visit 1 and at 1, 2, 3 and 4 years after enrolment. We will identify HIV infected children eligible to participate in proof-of-concept cure/remission trials.

A predictive model developed from European participants' data will help identifying children with a low reservoir. These predictions will then be compared with available data of participants already enrolled with excellent control to refine the model.

Primary outcome variable: Percentage of children with excellent HIV control profile at 2 and 4 years of age. This excellent control profile includes a composite endpoint of:

1) Undetectable HIV RNA viral load in peripheral blood in the last year
2) Reservoir size (Total DNA /million PBMC) below 25th percentile
3) Good immunological control (defined as a CD4 % >30%) during the last year

Treatment: Mother-To-Child Transmission Prophylaxis: according to local guidelines.

Treatment: according to local standard of care: 2 nucleoside reverse transcriptase Inhibitors (NRTIs) + 1 non-nucleoside reverse transcriptase inhibitors (NNRTI) or Protease inhibitor (PI) or 1 Integrase Inhibitor (INI) (according to national guidelines).

Recommended: NVP at birth and LPV/R when >14 days of age.

Assays

In-country

Point of care HIV diagnosis
Qualitative HIV DNA, RNA or total DNA (TNA) by Polymerase Chain Reaction (PCR).
HIV RNA quantification (Viral Load)
CD4 count/percent.
serology - if 4th generation test (AbbotTM) available
Hematology and biochemistry
Malaria slide (where appropriate).
HIV ART resistance testing

AHRI

Total HIV DNA / million PBMC (ddPCR/IPDA)

Ragon Institute

Deep sequencing on selected PBMC (FLIPS)

Stellenbosch

Detailed viral reservoir profiling on selected EARTH samples, using HIV-1 envelope single-genome sequencing (SGS) using Oxford Nanopore Technology (ONT)

Rome (Italy)

a. To identify cytokine profile associated with viral control in the early phase of the infection and longitudinally to define ontology modification of proteomic in children living with HIV
b. To identify cytotoxicity of NK and CD8 in the early phase of the infection and correlate it with disease progression and HIV reservoir in later timepoints.
c. To analyze frequency of ICAM+ Ag specific CD8 cells.
d. Development of neutralizing antibodies and ADCC.
e. To analyze telomere length as a measurement of immunosenescence.
f. To define Ab responses upon routine vaccination in HIV infected children. Final goal: design booster intervention in HIV infected children.
g. Investigate whether the presence of protective HLA allele (HLA-B57, -B27, -B14, -Cw8, and -DRB1*10) interferes with HIV progression. Furthermore, we will define telomer length in association to HIV progression.
h. To predict bNAb susceptibility for characterized bNAb. Ospedale Bambino Gèsu will store plasma samples for susceptibility to bnAbs until the final laboratory to do this test is identified.

Miami (USA).

Transcriptome: RNA sequencing and gene expression profiles on PBMC inhibitory check points evaluation on T cells.

Harvard (USA)
Metabolomics, untargeted proteomics by mass spectrometry

Cape Town (South Africa)
ARV drug levels

Substudies: 2 substudies are proposed:

Substudy 1: Social/Behavioural Research Substudy (SBR)

Background

The social and behavioral aspects of children with HIV are essential areas to take into account for preserving their health and involve the adherence to the drugs and to the programs. We need to explore attitudes and social context of caregivers to understand adherence barriers, expectations of EARTH cohort participation, and attitudes about future research options. This is a first and exploratory step that will inform a larger social/behavioral research study that will develop, adapt, apply, and evaluate supportive interventions (under a separate and later protocol).

Aims

To explore adherence, retention, and attitudes about future clinical research.

Participants:

Biological mothers, legal guardians, and/or other primary caregivers of children enrolled in EARTH.

Specific Aims:

To study caregivers' HIV experiences, ART attitudes, adherence barriers and facilitators and parental expectations from cohort membership and future clinical research.

To compare self-reported and biological measures of adherence in mother and infant.

Sample size:

Investigators will recruit the biological mother or another guardian/caregiver for each infant enrolled in EARTH. Thus, the anticipated sample size is up to the size of the EARTH cohort.

Inclusion criteria:

The substudy will include caregivers (biological mothers and/or other primary caretakers) of infants enrolled in the EARTH study. Eligibility will be dependent on 1) being a self-reported primary caregiver of an infant enrolled in the EARTH study who is aware of the infant's HIV+ status and gives ARVs at least occasionally 2) being aged 18 and above, 3) willingness to provide informed consent, and 4) ability to participate in the study in the local language(s) offered at each site. Only one caregiver will be enrolled in the SBR substudy for each infant enrolled in EARTH.

Intervention:

Data collection will occur via in-depth interviewing and brief questionnaires that may be self- or interviewer-administered.

Data collection will occur via a brief questionnaire including measures of mental health, internalized stigma, social support, and adherence; and an in-depth interview to explore perceptions, attitudes, and social context of caregivers of children enrolled in the EARTH study to understand adherence barriers, expectations of EARTH cohort participation, and attitudes about future research options. The data collection is expected to take 60-90 minutes and may occur at one or two study visits.

Substudy 2: Strategies to improve viral suppression (SIVS)

Background

Early antiretroviral (ARV) initiation is associated with low HIV reservoir and good immunological and virological outcomes in children. The Early Anti-Retroviral Treatment in HIV- infected Children (EARTH) cohort initiates HIV-infected infants on ARV treatment within the first 3 months after diagnosis (always within 6 months of life) and aims to establish a cohort of infants with good immunological and viral markers who may subsequently benefit from novel cure/remission therapeutic intervention trials. However, the poor viral suppression seen in the EARTH cohort, of 36% at 24 weeks across all sites, will likely limit the inclusion of the majority of EARTH participants in these future studies.

Aim

The overarching goal is to contribute to the understanding of the barriers to viral suppression to improve suppression rates in the EARTH study participants

Hypothesis

Low or undetectable ARV concentrations are associated with unsuppressed viral loads

Primary Objective

- To determine exposure to ARV drugs in infants enrolled on EARTH through the quantification of plasma ARV concentrations

Secondary objectives

- To determine exposure to ARV drugs in mothers of infants enrolled on EARTH through the quantification of plasma ARV concentrations

Study Design

Longitudinal study performed at 24, 48, 72 and 96 weeks after treatment initiation in the infant.

Cross-sectional sampling from their mothers at 48 weeks.

Study Participants

All participants and their mothers enrolled in EARTH at TBH-CT and CISM Mozambique sites

Interventions

Adherence assessments in infants:

- Indicators of adherence such as dispensing records, pill counts and self- reported adherence.
- CD4 count and viral loads coupled to adherence measures.
- Plasma concentrations of lopinavir or nevirapine

Adherence assessments in mothers of infants:

- Simultaneous (with the infant) DBS of tenofovir diphosphate (weeks of adherence) as a longer-term marker of adherence (sub-study).

Assays

- DBS of tenofovir diphosphate as a longer-term marker of adherence;
- Plasma nevirapine and/or lopinavir/ritonavir concentrations (above level of detection and above therapeutic minimum

4. BACKGROUND

There is currently no cure for HIV infection. An estimated 3-4 million children are still living with HIV, more than 90% in sub-Saharan Africa. Almost all of these infections are acquired through mother-to-child transmission (MTCT).[1,2] Despite a 52% decline in the number of newly infected children from 2001 to 2012, there are still about 250,000 infants newly infected with HIV every year. [2]

Antiretroviral therapy (ART) is very effective in preventing mortality when initiated early in infancy.[3] Currently, international guidelines recommend initiation of ART in all HIV-positive infants aged < 5 year, regardless of clinical and immunological parameters.[4–6] However, although ART has revolutionized survival, it is unable to cure as latent viral reservoirs are immediately established in HIV-1 infection[7,8.] This latent HIV-1 reservoir in memory $CD4^+$ T cells forms a major barrier to cure by providing a lifetime of viral persistence, necessitating life-long ART.[9]

Lifelong ART has disadvantages. Toxicity is frequent and concerns around the in-utero effects of ART persist including the possible long-term effects on mitochondrial and immunologic function[10]. Lifelong treatment adherence and retention in care can also be challenging. Poor adherence is associated with increased viral resistance to ART and more than 20% of vertically HIV-positive children develop triple-class virological failure after 5 years on the same ART regimen possibly increasing the prevalence of primary drug resistance at treatment initiation.[11–13] Finally, the economic burden for families and countries is significant and concerns exist about the feasibility of treating all participants for life in limited resource settings.[14] Thus, there is an urgent need to define new treatment strategies that provide long-term viral suppression off ART in this population.[15]

Currently, the viral reservoir is considered the main barrier to achieving HIV cure. Initiation of ART soon after infection seems to minimize the size of the long-lived HIV reservoir in resting memory $CD4^+$ T cells and limits the evolution of HIV-1 in viral reservoirs.[16] In most early treated cases that interrupt ART, plasma viral rebound is observed in less than 2-4 weeks.[17,18] However, the Mississippi baby case showed that long-term viral control can be maintained months after ART interruption in newborns after very early ART. This child had an unplanned ART interruption at the age of 18 months followed by 27 months with undetectable plasma HIV RNA, no replication competent virus in CD4+ T cells, with only traces of HIV DNA in peripheral blood cells. This case provides proof-of-concept that limiting the size of the HIV-1 latent reservoir in perinatal infection may allow a subsequent time-limited period of remission off ART. However, at the age of 4 years, the child had a rebound of HIV RNA to nearly 20,000 copies/ml and ART was resumed.[19] This single case is consistent with the notion that targeting acute HIV infection could lead to a significant reduction of HIV reservoirs. [20,21]

Children with prolonged excellent control may be the best candidates to try new treatment strategies that target ART withdrawal and a functional cure of HIV. At the moment, broadly neutralizing antibodies (bNAbs), eCD4-Ig and therapeutic vaccines, among others, are possible future choices for children after ART interruption.

The EPIICAL project is an international consortium whose aim is to select promising HIV therapeutic strategies candidates among early-treated perinatally HIV-positive children.

A previous study from the EPIICAL Group with European cohorts of perinatally infected children demonstrated that early initiation ART of life in limits the size of the viral reservoir.[22] A model is being built to predict which children will have a low reservoir with data from these studies.

Several questions remain. What immunological and virological factors are associated with excellent HIV control? Do factors, such as malaria, cytomegalovirus infection or latent TB infection impact HIV control? Are we able to predict which children will achieve good viral control and a low reservoir? Are models built in high-resource countries applicable to limited-resource settings? How is good immunological and viral response elicited in children with early ART? Are these excellent controller potential participants for proof-of-concept (POC) studies aimed at curing HIV infection?.[21]

Substudies considerations

During the first months of EARTH, some questions became evident:

Substudy 1: Social/Behavioural Research Substudy (SBR)
Are there barriers or facilitators to adherence experienced by caregivers that may be amenable to intervention? To what extent are caregivers willing to enroll their children in POC studies, and what may make them more willing? What do caregivers expect from their children's participation in EARTH?
A social sciences/behavioural substudy was designed to answer these questions.

Substudy 2: Strategies to improve viral suppression (SIVS)
Also, an interim analysis of EARTH patients shows that only 36% reach suppression at 24 weeks after ART, across all sites, which prompted a second substudy proposal to test if low or undetectable ARV concentrations are associated with unsuppressed viral load.

5. RATIONALE

Perinatally HIV-positive individuals represent a unique population to examine how early ART (alone or in combination with new Novel Disease Modifying Therapies [NDMTs]) affect the HIV reservoir and on-treatment long-term viral control.

This protocol is part of the EPIICAL consortium (Early treated Perinatally HIV Infected individuals: Improving Children's Actual Life). EPIICAL is a multi-center, multi-cohort global collaboration coordinated by Penta (Child Health Research).

EPIICAL overall goals are:

1. To characterize the virological, immunological and transcriptomic correlates/profiles of viral control in existing cohorts of early treated HIV-positive children.
2. Using these data, to develop a predictive model for positive response to ART.
3. Conducting proof of concept clinical studies of disease modifying therapies in the setting of early treated HIV-positive children using and validating endpoints derived from the above predictive platform.

The EARTH study is nested in the first and second goals of EPIICAL and intends to prepare the ground for responding to the third.

This protocol intends to develop a multicenter cohort study of children with perinatally acquired HIV infection. We intend to establish a well characterized cohort of newly infected infants who will start on suppressive ART soon after diagnosis, ideally in the first weeks of life. We intend to gain knowledge about factors involved in excellent control (viral, host and external factors). Participants with excellent control may be considered for immunotherapeutic interventions aimed at achieving ART-free HIV control (i.e. functional cure or remission). However, not only participants with excellent control may benefit from future clinical trials. Different participants with varying phenotypes, including those not suppressed, might benefit from different approaches.

More participants than the initially 30 estimated participants may benefit from proof-of-concepts trials, hence the increase in sample size.

Substudies proposals rationale:

Substudy 1: Social/Behavioural Research Substudy (SBR)

The experience to date in EARTH has indicated that adherence to ART is a challenge. In other populations, pediatric adherence has been shown to have a broad range of predictors including characteristics of the child, the caregivers and family, socio-cultural, organizational and community characteristics. Understanding and supporting efforts to improve adherence will advance the health of the child and result in a larger cohort of children who may be eligible for future POC trials. Further, maintaining high retention rates

is a key goal for all cohorts and is especially important and relevant to the EARTH cohort, where maintenance in the cohort may help protect the child's health.

The objective is to explore perceptions, attitudes, and social context of caregivers of children enrolled in the EARTH study to understand adherence barriers, expectations of EARTH cohort participation, and attitudes about future research options. This is the first and exploratory step that will inform a larger social/behavioral research study that will develop or adapt, apply, and evaluate supportive interventions related to adherence, retention, and future clinical research opportunities. Results from this first data collection will be used to determine the longer-term aims and procedures. The larger SBR substudy will be described later in a separate protocol.

Substudy 2: Strategies to improve viral suppression (SIVS)

Early antiretroviral (ARV) initiation is associated with low HIV reservoir and good immunological and virological outcomes in children. The Early Anti-Retroviral Treatment in HIV- infected Children (EARTH) cohort initiates HIV-infected infants on ARV treatment within the first 3 months after diagnosis (always within 6 months of life) and aims to establish a cohort of infants with good immunological and viral markers who may subsequently benefit from novel cure/remission therapeutic intervention trials. However, the poor viral suppression seen in the EARTH cohort, of 36% at 24 weeks across all sites, will likely limit the inclusion of the majority of EARTH participants in these future studies.

The overarching goal of this substudy is to contribute to the understanding of the barriers to viral suppression to improve suppression rates in the EARTH study participants

6. **HYPOTHESIS**

Monitoring HIV-DNA from birth during the first 4 years of age, we can identify and characterize a proportion of early-treated children who will have an excellent control of HIV at 4 years of age in terms of clinical, viral and immunological features, and other who will not have such excellent control. This differentiation will help us to identify which participants are more likely to benefit from which novel therapeutic interventions, depending on the profile of the patient, the intervention and the endpoint.

Substudies hypothesis:

Substudy 1: Social/Behavioural Research Substudy (SBR)

The collection and analysis of study data will generate hypotheses to test in a subsequent study.

Substudy 2: Strategies to improve viral suppression (SIVS)

Hypothesis:

Low or undetectable ARV concentrations are associated with unsuppressed viral loads, even in children with good adherence records.

7. OBJECTIVES

Primary aim

To monitor clinical, virological and immunological features of HIV-positive, early treated children (≤90 days after diagnosis), in order to identify participants with excellent viral and immunological control, and also other without excellent control, in order to stratify potential participants in proof-of-concept trials directed to HIV cure.

Secondary aims

1. To investigate factors associated with mortality, progression to AIDS, suppression, adherence and retention, and anthropometric measures.
2. To investigate factors associated with low total HIV-1 DNA in African children with a suppressed viral load, who started ART very early in life (≤6 months of age).
3. To validate models based on from European cohort data predicting which children will have excellent virologic control in limited-resource settings.
4. To study the association of HIV reservoir size with an appropriate immunological response (exhaustion and activation). Specific immunological aims are:
    a. To identify cytokine profile associated with viral control in the early phase of the infection and longitudinally to define ontology modification of proteomic in children living with HIV
    b. To identify cytotoxicity of NK and CD8 in the early phase of the infection and correlate it with disease progression and HIV reservoir in later timepoints.
    c. To analyze frequency of ICAM+ Ag specific CD8 cells.
    d. Development of neutralizing antibodies and ADCC.
    e. To analyze telomere length as a measurement of immunosenescence.
    f. To define Ab responses upon routine vaccination in HIV infected children. Final goal: design booster intervention in HIV infected children.
    g. Investigate whether the presence of protective HLA allele (HLA-B57, -B27, -B14, -Cw8, and -DRB1*10) interferes with HIV progression. Furthermore, we will define telomer length in association to HIV progression.
    h. To predict bNAb susceptibility for characterized bNAb. Ospedale Bambino Gèsu will store plasma samples for susceptibility to bnAbs until the final laboratory to do this test is identified.
    i. RNA sequencing and gene expression profiles on PBMC inhibitory check points evaluation on T cells.
    j. To identify metabolomics and untargeted proteomics by mass specrometry profile associated with viral control in the early phase of the infection.
5. To evaluate children with unplanned cessation of ART who persist with a low reservoir at age 2 and 4, if any exist.
6. To study potential new virological markers relevant for reservoir, as they arise from ongoing protocols of EPIICAL or other ground-breaking research elsewhere.
7. To study adherence issues and parental expectations from cohort membership and clinical trials, and to ensure acceptable benefit: risk balance in the trials.
8. To know the proportion of infants actually with HIV that had high-risk of transmission, according to HIV-exposed Infants Programmatic Update 2018, WHO, Geneva.

Substudies objectives

Substudy 1: Social/Behavioural Research Substudy (SBR)

a. To study caregivers' HIV experiences, ART attitudes, adherence barriers and facilitators and parental expectations from cohort membership and future clinical research.

b. To compare self-reported and biological measures (obtained in substudy of barriers to viral suppression) of adherence in mother and infant.

Substudy 2: Strategies to improve viral suppression (SIVS)

Primary Objective

- To determine exposure to ARV drugs in infants enrolled on EARTH through the quantification of plasma ARV concentrations.

Secondary objectives

- To determine exposure to ARV drugs in mothers of infants enrolled on EARTH through the quantification of plasma ARV concentrations

8. DESIGN

Design: Prospective cohort study.

Sites:
The participants will be enrolled at 6 rural and urban sites in 3 different countries (1 in Mali, 2 in Mozambique, 3 in South Africa).

The biological samples will be collected at the clinical sites, stored initially at the site laboratories, with some testing done in-country and some samples shipped and analyzed in specialized laboratories in Mozambique, South Africa, Italy and USA (the latter including Massachusetts, San Francisco and Miami).

***See Appendix 13:** LOCAL SPECIFITIES PER SITE AND COUNTRY about clinical procedures and methods.

Main outcome variable
Proportion of children with excellent HIV control (see endpoint definition).

Newly-diagnosed HIV-positive children will start ART as soon as possible, following local standard of care. Children on early ART will be followed up to 4 years after enrolment.

Clinical data and blood for viral load, immunology and serology will be collected in up to 12 visits. The HIV reservoir size and HIV-specific immune responses will be analyzed at visit 1 and 1, 2, 3 and 4 years after enrolment. We will identify HIV-positive children eligible for proof-of-concept trials intended towards HIV cure.

Substudies design:

Substudy 1: Social/Behavioural Research Substudy (SBR)
Mixed methods study comprising a set of quantitative measures and an in-depth, semi-structured interview.

Substudy 2: Strategies to improve viral suppression (SIVS)
Longitudinal study performed at 24, 48, 72 and 96 weeks after treatment initiation in the infant.
Cross-sectional sampling from their mothers at 48 weeks.
This study is intended to be developed currently only in 2 sites: Cape Town and CISM.

9. ENDPOINTS

Primary outcome variable

Proportion of children with excellent HIV control profile at 2 and 4 years of age. This excellent control profile includes a composite endpoint of all of the following:

1) Undetectable HIV RNA viral load in peripheral blood in the last year (blips, spikes and suboptimal viral control allowed, see below for definitions),

2) Reservoir size (Total DNA /million PBMC) below 25th percentile with ddPCR / IPDA.

3) Good immunological control (defined as a CD4 >30% and a good NK or CTL response) during the last year

If in the next years, any more sensitive and specific immunological assessments arise from specific humoral or cellular response assays or transcriptome, lack of antigenic stimulation might be substituted by a new test after proper amendments.

Secondary endpoints:

CLINICAL

- Mortality
- Viral load suppression
- WAZ, HAZ, WHZ (anthropometric measures)
- Adherence issues.
- HIV Drug Resistance Profiles: Resistance on non-supressed participants, will be performed, to try and identify causes of viral failure in children otherwise with acceptable adherence. Children will benefit of adjustment of ART if resistance is found. The tests will be done as per standard of care at National Health Systems laboratories.
- Progression to AIDS (WHO clinical stage 3 or 4, CD4 < 25%)
- High risk of vertical transmission, based on maternal history (viral load and ART)
- High risk of vertical transmission, based on maternal history (viral load and ART)
- ART resistance testing
- ARV levels

VIROLOGICAL

- Reservoir (total DNA/million PBMC) and intact proviral DNA assay (IPDA).
- Full length individual sequencing (FLIPS)/ Deep sequencing on PBMC
- Detailed reservoir profiling using HIV-1 envelope single-genome sequencing (SGS).
- bNAb susceptibility for characterized bNAbs.

IMMUNOLOGICAL

- Cytokine profile
- To identify cytotoxicity of NK and CD8.
- ICAM+ Ag specific CD8 T cells
- Neutralizing antibodies and ADCC
- Telomere length
- Ab responses upon routine vaccination

- Protective HLA allele (HLA-B57, -B27, -B14, -Cw8, and -DRB1*10)
- RNA sequencing and gene expression profiles on PBMC inhibitory check points evaluation on T cells.
- Metabolomic profile
- Proteomic profile

Definitions of blips, spikes and suboptimal viral control

- Blips: viral load 50 to 399c/ml after viral suppression <50 c/mL, with subsequent return to <50c/ml on repeat sampling in next visit (within 6 months and same treatment).
- Spikes: defined as single viral load measurements of 400 -1000 c/mL with subsequent return to <50 c/mL at next sampling (within 6 months and same treatment).
- Suboptimal viral control: ≥2 viral load measurements 50 to 1000 c/mL without any change in treatment.

Potential factors associated with control: Variables to be explored as potential factors associated with excellent control will be recorded (see Master File).

Substudies outcomes

Substudy 1: Social/Behavioural Research Substudy (SBR)

This is an exploratory study to generate hypotheses and thus does not have a primary and secondary outcome variable. Participants will take part in a brief questionnaire and an in-depth interview in a private setting. This data collection point will capture the caretaker's mental health using the WHO SRQ-20; internalized stigma using the AIDS-related stigma scale, social support using the ACTG social support measure; self-reported caregiver adherence (for HIV+ caregivers) using the Morisky simple medication adherence questionnaire; and self-reported pediatric adherence using the ICAMP measure with an additional purposively developed item set, and caregiver adherence (as relevant) using items modified from Knobel et al. (2002). Measures will be self-administered or administered by the interviewer. The in-depth interviews will then be conducted in English or a local language using a standardized, semi-structured interview guide. In-depth interviews will be audio-recorded.

Substudy 2: Strategies to improve viral suppression (SIVS)

Adherence assessments in infants:

- DBS of tenofovir diphosphate (which is a proxy to weeks of adherence) as a longer-term marker of adherence.
  Indicators of adherence such as dispensing records, pill counts and self- reported adherence.
- CD4 count and viral loads coupled to adherence measures.
- Plasma concentrations of lopinavir or nevirapine

Adherence assessments in mothers of infants:

- Simultaneous (with the infant) DBS of tenofovir diphosphate (weeks of adherence) as a longer-term marker of adherence.

Assays

- DBS of tenofovir diphosphate as a longer-term marker of adherence;
- Plasma nevirapine and/or lopinavir/ritonavir concentrations (above level of detection and above therapeutic minimum).

10. SELECTION AND WITHDRAWAL OF PARTICIPANTS

Inclusion Criteria

1. Perinatally infected infants who start ART at ≤90 days after diagnosis (HIV infection diagnosed and confirmed by molecular methods in 2 different samples, i.e., positive qualitative HIV DNA/RNA test).
2. Breastfed-infected children found to be infected ≤ 90 days of age and who started ART at < 90 days after diagnosis. This group will be younger than six months of age at ART initiation.
3. Caregivers (mother, if alive and available) able to provide informed consent.
4. Able to take ART.

Exclusion/Withdrawal Criteria

1. Second and successive RNA PCR negative
2. Malignancy
3. Current concomitant immunosuppressive therapy (including>15 days and >2 mg/kg/day of prednisone-equivalent).
4. Caregivers withdraw consent
5. Age >180 days.

Substudies eligibility

Substudy 1: Social/Behavioural Research Substudy (SBR)

Inclusion criteria:

1. self-reported primary caregiver of an infant enrolled in the EARTH study
2. aware of the infant's HIV+ status and give infant ARVs at least occasionally
3. Age 18 and above
4. ability to participate in the study in the local language(s) offered at each site

Only one caregiver will be enrolled in the SBR substudy for each infant enrolled in EARTH.

Exclusion/withdrawal criteria:

1. Determination of the interviewer or social/behavioral research team that the caregiver participant is unable to provide consent or to continue with the protocol, for reasons related to capacity
2. Caregiver under 18 years of age
3. Caregiver will not provide consent or withdraws from the substudy

Substudy 2: Strategies to improve viral suppression (SIVS)

Study Participants

All participants and their mothers enrolled in EARTH at TBH-CT and CISM Mozambique sites.

11. CLINICAL PROCEDURES AND METHODS

Planned Study Period: Up to 4 years from enrolment from the beginning (began in May 2018), and at least up to 4 years of follow-up for every participant.
Interventions: Up to 12 visits for all participants (enrolment visit + 11 visits), with 11 blood draws for virological and immunological measurements.

Recruitment

Eligible children who are meet inclusion criteria will be identified by clinical and/or research staff under the leadership of the local EARTH principal investigator.

Enrolling sites:

Mozambique:

- Fundaçao Ariel: maternity ward and High-Risk Pediatric Clinic at Boane Health Centre, Ndlavela HC, Matola Gare HC, Machava 2, Matola 2 HC and Matola Provincial Hospital, Beleluane HC, Campoane HC, Matola Rio HC, Tsalala HC, Liberdade HC
- Manhiça CISM sites: maternity ward and High Risk Pediatric Clinic at Manhiça District Hospital and HC and Xinavane Rural Hospital, Centro de Saúde de Maragra and Centro de Saúde de Palmeira (Malavela) Taninga, Ilha Josina, 3 de Fevereiro, Nwamatibjana, Maluana, Munguine, Chibucutso e Calanga

South Africa:

- Tygerberg Children's hospital (Cape Town): Family Center for Research with Ubuntu will be a research site in this international, multicenter study. FAMCRU, located in Cape Town, South Africa, conducts clinical trials in infectious diseases in adults and children with a focus on HIV and TB. FAMCRU forms part of the clinical trials unit of Stellenbosch University, whose medical school serves the tertiary level care hospital, Tygerberg. Recruitment will be done at Tygerberg Hospital in: the pediatric ward, FAMCRU (and its satellites) and the infectious diseases clinic.
- Chris Hani Baragwanath Hospital (Johannesburg) at the Perinatal HIV Research Unit.
- AHRI (Durban): maternity and pediatric wards and infectious diseases clinic.

*See APPENDIX: LOCAL SPECIFITIES PER SITE AND COUNTRY for Mozambique and Cape Town specificities.

Mali:

- Centre Hospitalier Universitaire Gabriel Touré

Centers associated to Centre Hospitalier Gabriel Touré:

a) Centre d'Ecoute, de Soins, d'Animation et de Conseils (CESAC de Bamako)
b) Unité de Soins, d'Animation et de Conseil, USAC Commune V, ARCAD-SIDA, Mali

Screening

Participants will be assessed against the above inclusion and exclusion criteria and the parent/guardian will be asked if they wish to participate if they are eligible for the study. An explanation of the study will be provided along with a copy of the participant information sheet. They will be given as much time as they need to consider their participation and will be informed that participation is voluntary and a decision not to participate will not affect

their clinical care. Written informed consent will be obtained from parent or legal guardian before they undergo any research procedures.

The caregiver will also be asked to give consent for access to their own and their child's past clinical notes and laboratory results for verification of ART history, viral load and CD4 measurements and concomitant medication and medical history.

The study will prospectively collect clinical information and infant's blood samples to determine HIV reservoirs and HIV-specific immune responses. Participants can be recruited from 0 to 6 months of age.

All children should have 12 visits (enrolment + 11 additional visits).

In special circumstances where consent cannot be obtained for logistical reasons prior to ART initiation, the participant may still be enrolled retrospectively. No study procedure will be carried out before informed consent.

1) ***See Appendix 13:** APPENDIX: LOCAL SPECIFITIES PER SITE AND COUNTRY about clinical procedures and methods.

Follow-up

Follow up in the first month after starting ART will be performed according to the National guidelines. Study visits are scheduled every three months (*see Figure 1*) across all recruitment sites.

The study includes 1 enrolment visit and up to 11 visits for clinical data and blood collection from the child to characterize the HIV reservoir size and replication as well as the HIV-specific immune responses. The baseline visit will occur at diagnosis. Reasonable efforts will be made to contact participants and re-schedule missed visits if the participant does attend the scheduled visit. The minimum visits required are enrolment, and twice a year for two years.

The study visits will be scheduled periodically after ART initiation to evaluate serology, viral load and CD4.

At the visit 1 and visit 5, 7, 9 and 11 (corresponding to baseline, 48 weeks~12 months, 96 weeks~2 years, at ~3 years and at ~4 years) blood for reservoir and immunological features will be collected and shipped.

If there is not enough blood for performing immunological/virological assays from baseline visit 1, and there is stored blood remaining from visit 0, 2 or 3, suitable for these assays, these samples can be used as baseline, as time of those visits are within 12 weeks after visit 1.

At the visit 4, 6, 8, 10 (corresponding to 24 weeks~6 months, 72 weeks~18 months, at ~2.5 years, ~3.5 years), blood will be obtained for standard of care and for in-site promoted sub-studies.

Action		Enrolment visit (V 0)	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11
			Weeks after visit 0										
			2*	6*	12*	24*	48*	72*	96*	120	144	168	192
Information and informed consent		X											
History and clinical assessment		X	X	X	X	X	X	X	X	X	X	X	X
ART initiation		X											
BLOOD EXTRACTIONS (CHILD)													
HIV-1 RNA Qualitative + confirmatory		X											
Standard of Care													
whole blood EDTA	In-site CD4	2 mL***				1 mL(^)	1 mL	1 mL(^^^)	1 mL	1 mL	1 mL	1 mL	1 mL
	In-site FBC												
	Viral Load					3mL***	2 mL***	2 mL(^^^)	2 mL***	2 mL***	2 mL***	2 mL***	2 mL***
whole blood (dry tube)	In-site Biochemistry	0.5 mL				0.5 mL(^)	0.5 mL	0.5 mL(^^^)	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL
RESEARCH SAMPLES													
Whole Blood EDTA	Viral Load	3 mL**,***		3mL***	3mL***								
	WP3 Cell Storage for total DNA in PBMC	**	2 mL***	**	**		3 mL		3 mL		5 mL		5 mL
	WP 4 Cell Storage Transcriptomic Phenotyping, HIV specific response, Serostatus		4 mL***				4 mL		6 mL		6 mL		6 mL
	In-site Storage					3 mL(^^)	3 mL	5 mL(^^)	4 mL		4 mL		4 mL
	Malaria slide/PK in DBS		1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop
Ideal Blood Volume		5.5 mL	6 mL	3mL	3mL	7.5mL	13.5 mL	8.5 mL	16.5 mL	4 mL	18.5 mL	4 mL	18.5 mL
Minimum Blood Volume		2.5 mL	4 mL	1 mL	1 mL	5 mL	7 mL	7 mL	7 mL	3.5 mL	7 mL	3.5 mL	7 mL
Social Sciences Assessments									x		x		x

Figure 1: Blood withdraw timeline and tests. In red, new visits included in amendment v2.
*: Visit 2 and 3 can be skipped if visit 1 is delayed.
**: In some instances, plasma and PBMC can be recovered from VL sample if enough blood.
***: Ideally, these volumes should be collected. For CD4+FBC, 1 mL may be acceptable. For Viral Load, 1.5 to 3 mL might be acceptable. For WP3 cell storage, 1 mL might be acceptable. For WP4 Cell Storage, 3 mL may be acceptable.
(^): Only CD4 and Viral Load is Standard of Care in Johannesburg, only these tests will be performed at this timepoint in this site.
(^^): In the sites where technique is available, immunophenotype in-site will be done in this visit.
(^^^): Where these tests are standard of care. Otherwise, Viral Load will be done as a Research Sample and FBC and Chemistry will not be performed.

Details of study visits

The study has 12 visits scheduled for all children enrolled: the enrolment visits and up to 11 study visits. The majority of participants will follow one of two profiles: participants who test positive at birth, and those who test positive around 4-6 weeks of age. The actions, material and storage that the physician or researcher needs to achieve in each visit are spelled out and detailed in Appendix 1 as check lists.

Windows of visits:

Visits windows are:

• _**Regular window for visit 1** should be 1 week before and 2 weeks after planned visit. It is possible to widen the window up to 4 weeks in exceptional cases given the relevance of visit 1.

Note: In these cases (wider window up to 4 weeks), we will have to skip visit 2 and go directly from visit 1 to visit 3, to regularize the schedule.

- _**Regular window for visit 2** should be 2 weeks before and 3 weeks after planned visit
- _**Regular window for visit 3** should be 3 weeks before and 6 weeks after planned visit
- _**Regular window for visit 4** should be 6 weeks before and 12 weeks after planned visit
- _**Regular window for visit 5 to 11** should be 12 weeks before and after planned visit

If some visit is scheduled too late and overlaps to the next timeframe, we should skip this visit and go to the next one.
Also, given the relevance of visit 4, if visit 3 is scheduled too late and overlaps with visit 4, it's better to skip visit 3 and go directly from visit 2 to visit 4, to regularize the schedule.
If needed, inside the window for any visit it is possible to perform 2 analytics/extractions corresponding to only one visit.
If needed and justified (for example in case it is not possible to draw the required blood volume for technical reasons), 1 visit might split extractions into 2 separate days, if mother/responsible adult agrees, and if both extractions are inside the window. For example, in visit 3, 3 weeks before and 6 weeks after planned visit. However, this should be avoided as much as possible, and all necessary blood should be taken in 1 extraction/1 day.
If this situation this happens, a comment should be added in the free field of the forms, to report the date of the second extraction.

A WHO review on blood volumes in pediatric research recommends limiting blood volume withdraw to between 1% and 5% of total blood volume on a single draw or over 24h and of up to 10% of total blood volume over 8 weeks.[24]

The blood volumes taken from children for clinical and research purposes should not exceed maximum allowable limits. Based on the WHO publication, a limit ranging from 1% to 5% of total blood volume over 24 hours and up to 10% of total blood volume over 4 weeks was considered. For healthy children, estimation will be 3% total blood volume in a 24 hour period (approx. 2.5 mL/kg); see Appendix XI for blood draw volumes per kg.
The amount of blood in all the visits will be in any case <5% of the total blood.

Visit Number	Week number	Year	Local VL	WP3 Virology	WP4 Immunology	In site storage	Total For research	Standard of care	Total
0	0	1	3ml	NC	NC	NC	3 mL	2.5 mL	5.5 mL
1	2	1	NC	2ml	4ml	NC	6 mL	NC	6 mL
2	6	1	3ml	NC	NC	NC	3 mL	NC	3 mL
3	12	1	3ml	NC	NC	NC	3 mL	NC	3 mL

4pb	24	1	NC	NC	NC	3ml	3 mL	4.5 mL	7.5 mL
5	48	1	NC	3ml	4ml	3ml	10 mL	3.5 mL	13.5 mL
6	72	2	NC	NC	NC	5ml	5 mL	3.5 mL	8.5 mL
7	96	2	NC	3ml	6ml	4ml	13 mL	3.5 mL	16.6 mL
8	120	3	NC	NC	NC	NC		3.5 mL	3.5 mL
9	144	3	NC	5ml	6ml	4ml	15 mL	3.5 mL	18.5 mL
10	168	4	NC	NC	NC	NC		3.5 mL	3.5 mL
11	192	4	NC	5ml	6ml	4ml	16 mL	3.5 mL	18.5 mL

Substudies procedures

Substudy 1: Social/Behavioural Research Substudy (SBR)

One to two data collection points within the EARTH study period

Data collection will occur via an investigator-administered brief questionnaire and an in-depth interview. The data collection is expected to take 60-90 minutes and may be split over two visits if needed.

Study participants will be recruited by site staff during the child's study visits for EARTH. Site staff will explain the purpose of the study and procedures and if the participant is interested, undergo a written informed consent process in the language of the participant's choice (English, or a s local language).

Mothers or other caregivers will be assessed against the substudy inclusion and exclusion criteria and, if eligible, will be invited to participate. An explanation of the study will be provided along with a copy of the participant information sheet. Potential participants will be informed that participation is voluntary and a decision not to participate will not affect their child's continued enrollment in EARTH or their own or their child's clinical care. Written informed consent will be obtained from participant.

The SBR substudy will include one to two study visits. In cases where the data cannot be obtained in one visit, such as if the participant become fatigued and requests a break, or if there is a disturbance or interruption (e.g., the child becomes too demanding of the caregiver for the data collection to continue), then the data collection may be spread across two visits. If after conducting several interviews we find that the data collection takes too long and becomes burdensome to the participants or sites, we will reduce the number of questions/measures used in the interviews.

Data collection will occur in person and in a private setting after substudy participants provide consent. Data collection will be conducted in the local language(s) and is expected to take 60-90 minutes. As much as possible, consistency will be maintained throughout the sub-study such that the same interviewer will interview a given participant at each data collection point. All interviewers will be trained in prior to the initiation of data collection.

In-depth interviews and measurement of factors influencing adherence

Participants will take part in a brief questionnaire and an in-depth interview in a private setting. This data collection point will capture the caretaker's mental health using the WHO SRQ-20; internalized stigma using the AIDS-related stigma scale, social support using the ACTG Social Support measure; self-reported adherence for HIV+ caregivers (as relevant) using the Morisky scale; and self-reported pediatric adherence using the ICAMP measure with an additional purposively developed item set, and caregiver adherence (as relevant) using items modified from Knobel et al. (2002). Measures will be self-administered or administered by the interviewer. The in-depth interviews will then be conducted in English or a local language using a standardized, semi-structured interview guide, with slight modifications as necessary at each site to make the questions culturally acceptable and understandable. Such adjustments are standard practice for semi-structured interviewing across multiple countries and cultures.

Recruitment will begin after the modification to the protocol is approved and will be conducted on a rolling basis—that is, at their child's next study visit to EARTH, the mother or caregiver will be invited to participate in the substudy, and if they consent, an appointment for the substudy will be scheduled. The timing of the substudy data collection will depend on the child's progress through the EARTH protocol. The preferred time for caregivers of children newly enrolled in EARTH to have their SBR substudy visit is after EARTH visit 3, but the study team will accommodate caregivers' scheduling needs as much as possible. Parents of children already enrolled in EARTH who agree to participate will have their SBR-substudy visits anytime between visit 3 and the termination of their child's EARTH enrollment.

Informed consent:

A specific information sheet for caregivers will be developed for this substudy, and a checkbox will be added to the general informed consent form.

Substudy 2: Strategies to improve viral suppression (SIVS)

Adherence assessments in infants:

- Indicators of adherence such as dispensing records, pill counts and self- reported adherence; included already in the database.
- CD4 count and viral loads coupled to adherence measures;
- Plasma concentrations of lopinavir or nevirapine

Adherence assessments in mothers of infants:

- Simultaneous (with the infant) DBS of tenofovir diphosphate (weeks of adherence) as a longer-term marker of adherence (sub-study).

Assays

- DBS of tenofovir diphosphate as a longer-term marker of adherence;
- Plasma nevirapine and/or lopinavir/ritonavir concentrations (above level of detection and above therapeutic minimum)

Informed consent:
A specific information will be added in the information sheet for caregivers, relating drugs levels in blood of the child, and the mother. A separate consent will be signed to provide the possibility to join the main study and not the sub-study.

The amount of extra blood for this substudy will be <0.5 mL, since it's a dried spot test and will be kept within the accepted limits stated above.

12. DRUG REGIMEN

ART choice and any further medical management will be according to standard of care at the treating site and not a part of this research protocol. However, a 3- drug regimen including 2 reverse transcriptase inhibitor backbone plus 1 NNRTI or protease inhibitor or will be required as standard ART. The drug regimen will be recorded for analysis purposes.
*See APPENDIX: LOCAL SPECIFITIES PER SITE AND COUNTRY for Mozambique specifics.

Substudies regimen

No comments relating this section.

13. POTENTIAL ADVERSE EVENTS REPORTING

Since all children will be managed according to the local standard of care in place, adverse events relating to HIV progression or ART toxicity or other aspects of the medical management of these children will be reported in compliance with the national rules and included in the case report forms (CRFs).
Adverse events related to the research will also be reported. These include but are not limited to loss of confidentiality, adverse events related to blood draws, stigmatization etc. For serious adverse events (SAEs), the investigators will report these into the next-visit CRF.

All adverse events will also be reported to the local regulatory boards following applicable rules.

Potential Risks and Protection against Risk
The major risks associated with participation include those associated with phlebotomy. The risks when drawing a blood sample may include some discomfort, bleeding or bruising at the site of entry of the needle, rarely the formation of a small blood clot or swelling of the vein, bleeding from the puncture site, and on rare occasion infection. Risks related to blood drawing are not anticipated to be serious and are of a mild and transient nature. All phlebotomists are trained and experienced in drawing blood on newborns, infants and toddlers. Every effort will be made to minimize any adverse experience by the study team.

Potential benefits of the proposed research to the subjects and others
In the consent form, it will be explained this benefit and that there is no other benefit to the subject. However, the information generated from this study may help us to better understand the HIV reservoirs and form the basis for strategies aimed to cure HIV.
The close monitoring of immunological and virological response to ART will allow prompt identification of infants who do not respond to ART and warrant early investigation of possible causes and eventually ART regimen switch.

*See APPENDIX: LOCAL SPECIFITIES PER SITE AND COUNTRY for Mozambique.

Substudies specificities adverse events

Substudy 1: Social/Behavioural Research Substudy (SBR)

Potential Risks and Protection against Risk

Substudy risks include distress or other adverse psychological reaction, identification of an urgent caregiver or child risk during data collection, and risk of loss of privacy and confidentiality.
Caregivers will be informed that participation in the substudy is voluntary and their decision will not impact their child's enrollment in the EARTH study. Further, they will be informed that they can refuse to answer any question or stop participation at any time. Interviewers will be trained to respond to emotional responses during data collection. Caregivers will be

referred to mental health services if investigators have concerns about the caregiver psychological wellbeing. We will only enroll adult participants in the SBR substudy.

The complete transcripts and recordings of the qualitative data will be maintained in secure files that are separated from the EARTH data. Only social/behavioral investigators engaged in the data collection and analysis will have access to the complete individual transcripts. Other EARTH investigators will not have access to the complete individual transcripts. They will only have access to aggregated reports with identifiers removed. In this way, SBR substudy participants' personal accounts will be maintained as private except in cases where the SBR investigators have urgent concerns about the mental or physical health of the participating adult or the infant enrolled in EARTH. Such concerns will be reported to the EARTH clinical team to facilitate appropriate referrals or responses. These exceptions to participant privacy are noted in the substudy consent form. Analysis of the SBR substudy data will be conducted at each EARTH site and at RTI International and University of North Carolina at Chapel Hill. Data analyzed at RTI and UNC will not include any identifiers.

The information sheet explains that there are no benefits to participation. The information will be used to better understand barriers and facilitators to adherence to ART and caregivers' thoughts about EARTH and other clinical research studies.

Substudy 2: Strategies to improve viral suppression (SIVS)

No further risks besides those stated in the main protocol

14. LABORATORY TESTING ASSAYS

Comments on samples

One of the aims of the EPIICAL consortium is to strengthen the link between local research centers and European/US centers. Efforts will be made for transfer of knowledge from European/US labs to local sites to implement or improve techniques if necessary, including the possibility of human resources mobility.

The biological samples will be collected at each clinical site. Some testing will be done in-country and others in centralized, specialized laboratories in Italy, South Africa (Cape Town, Durban), and USA (Miami, Massachusetts, San Francisco), as detailed below.

In particular, in Mozambique samples collected at CISM supported Health Facility will be sent to the laboratory at CISM, and samples collected at F. Ariel supported –Health Facility will be sent to the National Health Institute (INS) Lab.

In South Africa, samples collected at Tygerberg Hospital will be processed, in the Hospital and sent to the Division of Medical Virology, Faculty of Medicine, Tygerberg Campus. Samples collected at Chris Hani Baragwaneth Hospital will be sent to Clinical Laboratory Services in Johannesburg. Samples collected at AHRI will be sent and processed at AHRI Laboratory in Durban.

In Mali, samples collected, at the Centre Hospitalier Universitaire Gabriel Touré will be processed at the Bacteriology Laboratory, Centre Hospitalier Universitaire Gabriel Touré, Bamako, Mali, Faculté de Pharmacie, Université des Sciences, des Techniques et des Technologies de Bamako, Bamako, Mali.

Haematology, biochemistry, CD4 count, and viral load will always be done on site. Real-time performance of tests relevant for clinical care will be done always on-site, including viral load, and CD4 count.

<u>Samples workflow:</u>
At the visit 1 and visit 5, 7, 9 and 11 (corresponding to baseline, 48 weeks~12 months, 96 weeks~2 years, at ~3 years and at ~4 years) blood for reservoir and immunological features will be collected and shipped.
If there is not enough blood for performing immunological/virological assays from baseline visit 1, and there is stored blood remaining from visit 0, 2 or 3, suitable for these assays, these samples can be used as baseline, as time of those visits are within 12 weeks after visit 1.
These samples from baseline, v5, v7, v9 and v11 will be split in aliquots and shipped for:

i) AHRI (Durban, SA): Reservoir size (ddPCR/IPDA):
ii) Massachusetts, USA (Ragon Institute): Sequencing (FLIPS)
iii) Selected samples: from CISM and Stellenbosch to University Cape Town.
iv) Immunology tests: Samples for USA will be shipped to Rome first, and then from Rome to the USA. Final destines are:

a. Rome (Italy) Cytokine via proteomics, cytotoxicity of NK and CD8, transcriptome, transcriptional analysis on HIV-specific T and B cell subsets, specifically including intracellular cytokine production, cytotoxic and degranulation activity of NK and CD8 T cells, ICAM+ specific CD8 T cells, ADCC and Abs profile, HLA typing, telomere length, Ab responses to vaccination. Store samples for susceptibility to bnAbs.
b. Massachusetts, USA (The Children's Hospital Corporation d/b/a Boston Children's Hospital): Metabolomics and untargeted proteomics by mass spectrometry
c. Miami University (USA): RNA sequencing and gene expression profiles on PBMC inhibitory check points evaluation on T cells

At the visit 4, 6, 8, 10 (corresponding to 24 weeks~6 months, 72 weeks~18 months, at ~2.5 years, ~3.5 years), blood will be obtained for standard of care and for in-site promoted sub-studies, including:

- ART resistance in standardized in-country laboratories such as NHS (all sites).
- Detailed viral reservoir profiling on selected EARTH samples, using HIV-1 envelope single-genome sequencing (SGS) using Oxford Nanopore Technology (ONT): Stellenbosch University only.
- ART levels (as per substudy 2): Stellenbosch University, Manhiça.

These samples will also be stored during 10 years for future use if the informed consent form has been obtained, as proposed by EPIICAL researchers and approved by EPIICAL governance, including new, high complexity virological assays.
Any new use of the samples will only be done after approval by the competent IECs/IRBs.

Blood samples for virology:
Extraction of 2 mL of whole blood at visit 1, 3 mL at 1 year, 3 mL at 2 years, 5 mL at 3 years, and at 18.5 mL at 4 years after enrolment.
All PBMCs should be stored in Liquid Nitrogen (LN2), or in a minus 150 degrees freezer.

Blood samples for immunology:
Blood draws will be 4 ml of whole blood at visit 1, 6 ml at year one and 6 ml at year 2, 3 and 4 after enrolment).
All PBMCs should be stored in Liquid Nitrogen (LN2), or in a minus 150 degrees freezer.
The blood for Rome/Miami/Massachusetts will be equally separated in vials each containing 5 million PBMCs that will be stored in liquid Nitrogen and shipped using a dry shipper in a timely manner as detailed in SOPs.
Plasma samples will be stored at -80ºC, or -20ºC if this is not possible.

In-site
3 mL-samples for storage and research on site will be obtained and stored at 6 months and 18 months, and 5 mL at 72 weeks, 4 mL at 144 and 192 weeks.

Comments on Samples for Genetics Testing

Ongoing research strongly suggests that genetic factors have a very relevant influence in control of HIV. This is why we will ask the permission of the participants to store some material for a 10 year period for possible genetic testing. A future genetics assay could possibly revel the key for HIV excellent control. One of the assays recently suggested is HLA, as mentioned in page 18 and 29. A specific checkbox in the informed consent is now available for genetic testing.

Shipping of the Samples

Samples for USA will be shipped to Rome first, and then from Rome to USA

1st shipping When first 150 fulfilled visit 1 year of age.
2nd shipping (estimated, 24 months after the 1st shipping): in 2023

Substudies specificities about tests

Substudy 1: Social/Behavioural Research Substudy (SBR)
Not applicable.

Substudy 2: Strategies to improve viral suppression (SIVS)

- Pharmacokinetic measurements of ARVs:
- DBS of tenofovir diphosphate (weeks of adherence) as a longer-term marker of adherence (sub-study)
- Plasma nevirapine and/or lopinavir/ritonavir concentrations (above level of detection and above therapeutic minimum.

These samples will be shipped to the UCT pharmacology lab in Cape Town, South Africa.

15. STATISTICAL ANALYSIS AND ACCESS TO SOURCES OF INFORMATION

Sample Size

This is a study with a whose size is based on the ability to recruit participants fulfilling the inclusion criteria. We expect to recruit a minimum of 300 participants at all sites during the recruitment period of up to 3 years. Assuming a 30% loss to follow-up during the 2-year follow-up period, we will obtain a sample of 200 participants at the end of the study. Approximately 30% of participants from European Cohorts had a low reservoir (median age, 7 y). Thus, we expect a sample of 60 participants who fulfil the definition of excellent controllers and 140 who do not.

*See APPENDIX: LOCAL SPECIFITIES PER SITE AND COUNTRY for Mozambique and Cape Town.

Primary statistical methods

In summary tables qualitative variables will be presented as counts and frequency distribution. Quantitative variables will be presented as mean and standard deviation (± SD) when normally distributed and as medians and interquartile range (IQR: P25-P75) when non-normally distributed. Shapiro-Wilk test will be performed to test normality. Chi-squared and Fisher Tests will be performed when categorical variables. For normally distributed continuous variables, Student T test will be performed and U-Mann Whitney or Kruskal Wallis when non-parametric. All hypothesis testing will be carried out at the 5% significance level and p-values will be rounded to three decimal places. In summary tables, p-values less than 0.001 will be reported as <0.001.

To test the association between the primary outcome (presence / absence of excellent control) and factors of interest a multivariable logistic regression model will be used.
Variable selection will be performed using a stepwise Akaike Criterion method and interaction between predictors will be tested. A regression diagnostic will be performed to ensure that all the model assumptions are met as independence and collinearity. If it were the case that the model does not fit the assumptions and the number of predictors highly overcome the number of events for the primary outcome, alternative methods as penalized or ridge regression models will be used. Finally, in order to evaluate the predictive performance of the model, sensitivity, specificity, accuracy and the area under the receiver operating characteristics (ROC) curve (AUC).

Secondary statistical methods

To investigate factors associated with mortality, progression to AIDS, suppression, adherence and anthropometric measures, a multivariable logistic regression will be used. Variable selection and regression diagnostic will be assessed with the same methodology stated in the primary statistical methods. Alternatively, to investigate the association between survival and the different predictors time-to-event models will be used with right-censoring. Survival models as Cox proportional hazards, semiparametric models, or random forest survival models will be tested and compared.

To test the association between HIV DNA-1 reservoir and sociodemographic, clinical, virological, and immunological factors (secondary objectives 2, 4, and 6) a Generalized Linear Poisson model will be used. Regression diagnostic will be examined to test for all model assumptions including dispersion and zero-inflation. In case of overdispersion or zero-inflation, negative binomial distributions and hurdle models will be applied respectively.

European Cohort data models will be validated in our cohort assessing the model performance in terms of accuracy, sensitivity, and specificity. The AUC will be calculated and compare with our designed models.

To study the risk-benefit of including these vulnerable infants in clinical trials, adherence and parental expectations will be described by means of summary tables and summary plots to determine factors potentially associated with poor adherence.

Missing data

In order to avoid loss of information and statistical power in the association analysis, missing data will be imputed. To prevent to many assumptions, only variables with less than 20% of missing information will be considered for imputation. Imputation will be performed using random forest imputation implemented in missforest R package[2]. Out-of-bag (OOB) imputation error estimate will be assessed and error for imputing categorical (proportion of falsely classified) and continuous (normalized root mean squared error) will be reported. In case higher imputation error (>10%) were reached, alternative methods as multiple imputation chained equations will be performed implemented in MICE R package [3]. Finally, sensitivity analysis will then be undertaken to assess the robustness of the conclusions to assumptions regarding the missing data.

All analysis will be performed using R software. The statistically significant threshold will be set as $p < 0.05$. We will add to all the report the size effect of the analyses when statistically significance differences are reached and statistical power of the analyses when they are not.

Modelling

We will build predictive mathematical models with our data. Also, the participants' data will be checked into the mathematical models built using data from European participants to check whether or not these models have predictive value for control at two and four years of age, these models will be rebuilt and refined if necessary.

Data Analysis

The analysis will be performed at the Hospital 12 de Octubre, Madrid, with the assistance of Perinatal HIV Research Unit, Chris Hani Baragwaneth Hospital, University of the Witwatersrand, Soweto, South Africa.

Interim analysis will be performed after first 150 enrolled participants fulfil visit 1 and 5; and after all participants fulfil visit 1 and 5.

Data Management Plan and Data Safety and Monitoring Board

A Data Management Team (DMT) will be settled with one member of each site, two central data managers from Hospital 12 de Octubre, and a data manager member from PENTA. The DMT will create a Data Management Plan which will follow for data collection and handling. All study data will be collected confidentially by the clinical staff or data entry personnel at the research centers using designated source documents. Data will be written down in paper case report forms (CRFs) and then introduced in an electronic CRF (eCRFs) implemented in REDCap collection data system (https://www.project-redcap.org/). This electronic database will be securely allocated at PENTA (study sponsor). This centralized database, managed by the DMT, will be the central data repository for all sites participating in the study. Each site participating in the trial will be responsible for data entry and first cleaning.

User-specific usernames and passwords are required to log onto the database. User rights will be provided by PENTA to study staff, PIs, and co-investigators at the level appropriate for each individual's job description.

This database will be built and managed by the central data managers, with revision and approval from the protocol board. Only the protocol chairs, data managers and statisticians will have access to the whole pseudonymized data in REDCap.

In summary, the plan for data monitoring will be the following:

1. In-site researchers will include eligible patients and complete participant forms into REDCap eCRFs every time that a participant is enrolled.
2. Central data managers will produce monthly reports of enrolled participants and urgent issues
3. Quarterly, central data managers will performed standardized quality controls to monitor missing values, logical inconsistences, clinical inconsistences, and outliers. This report will be sent to the sites to correct or justify each of the queries.

Substudies specificities about data management and statistics

Substudy 1: Social/Behavioural Research Substudy (SBR)

Investigators will recruit the biological mother or another guardian/caregiver for each infant enrolled in EARTH. Thus, the anticipated sample size is between 100 participants and up to the size of the EARTH cohort.

Qualitative and quantitative data will be collected. Items or measures will be removed if they are not applicable to the caregiver being interviewed—for example, if the participant is a grandmother who is not HIV positive. Analysis of the SBR substudy data will be conducted at each EARTH site and at RTI International and University of North Carolina at Chapel Hill. All data (recordings, transcripts, summary reports, translations) will be coded

with the date of data collection and the EARTH identification number. Any identifiers will be removed at each site prior to the data being shared with the RTI/UNC research team.
Qualitative: An in-depth semi-structured interview will cover four domains: 1) the caregiver's personal history with HIV and treatment adherence, 2) their child's history with HIV and treatment adherence, 3) current EARTH study participation, and 4) future engagement in with research studies. The in-depth interview guides may be modestly adjusted as needed based on the emerging data provided by the participant and/or based on site-specific acceptability of specific questions. These data will be audio-recorded. Immediately following each interview, the interviewer will be asked to complete a debriefing form in English, or to be translated to English, that will provide information about the interview (i.e. duration, mood of interview, participant ID, etc.) as well as a summary report of the content, which will include staff's impressions of key issues related to the main outcomes of interest. All debriefing reports will initially be reviewed by local site staff and then sent to RTI/UNC via a secure file sharing platform (e.g., sftp). Audio recordings of the interviews will be transcribed and translated to English and will also be shared via a secure file sharing platform.
Standardized summary forms will be used to capture major themes and subthemes using a rapid analysis approach. Summary forms will be translated into English, if captured in another language. The forms will be used to develop a brief and structured analysis scheme, comprising scoring of affective and contextual content, that will be applied to each narrative. Transcripts of the recordings and translations of the full recordings to English will be performed as required for data analysis. Data will be structurally coded, and themes compared and summarized across participants and sites.
Quantitative: Participants will take part in a brief questionnaire to assess the caretaker's mental health, internalized stigma, social support, and child and caregiver adherence. Questionnaires will be completed either on paper-based forms or entered directly into an online database (REDCap). Quality checks will be conducted by EARTH sites and RTI/UNC. Data analysis will include descriptive statistics and simple modeling that includes key data from EARTH, for example the duration of the child's enrollment in EARTH, child age, and clinical data relevant to adherence. We will also explore the concordance between self-reported adherence data provided in this substudy with the biomarker data obtained in the viral suppression substudy.

Substudy 2: Strategies to improve viral suppression (SIVS)
Investigators will recruit the biological mother and infants enrolled in EARTH at two sites (Manhiça and Cape Town). The anticipated sample size is 80 mothers and 80 infants.

In previous literature, the proportion of participants with virological failure at 24 weeks was 21%, in patients with suboptimal levels, and 8% in those with optimal levels ($p<0.001$) (https://academic.oup.com/jac/article/64/1/109/754487).
For this substudy, we assume viral failure at 48 weeks 30% in those with suboptimal levels and 10% in those with optimal levels. For a level of confidence 5% and error beta 20%, sample size as is estimated in a minimum of 60 participants.

16. REGULATORY, ETHICAL AND LEGAL ISSUES

Declaration of Helsinki

The investigator and sponsor will ensure that this study is conducted in full conformity with the latest revision of the 1964 Declaration of Helsinki (2013), the 2017 Ethical Considerations for clinical trials on medicinal products conducted with minors from the European Commission, the International Ethical Guidelines for Biomedical Research Involving Human Subjects CIOMS-WHO (2016); and the Additional Protocol of the Oviedo Convention (2005).

Independent Ethics Committee/Institutional Review Board Approval

Initial Approval

Prior to the enrolment of participants, the competent Independent Ethics Committee/Institutional Review Board (IEC/IRB) must provide written approval of the conduct of the study at each site, the protocol and any amendments, the Information Sheet and Consent Form (and assent if the mother is minor), any other written information that will be provided to the mother/parents/legal guardians, any advertisements or additional documentation that will be used.

Approval of Amendments

Proposed amendments to the protocol and aforementioned documents must be submitted to the IEC/IRB for approval/notification as required by the applicable law. Amendments requiring IEC/IRB approval may be implemented only after written IEC/IRB's approval has been obtained.

Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving Sponsor or IEC/IRB approval. However, in this case, approval must be obtained as soon as possible after implementation.

End of Study Notification

The IEC/IRB will be informed about the end of the study, within the required timelines.

Informed Consent

Participants identified as HIV positive who fit the inclusion criteria and their parent(s)/legally designated representative(s) will be approached by a member of the research team. An explanation of the study will be then provided to parent(s)/legally designated representative(s) along with a copy of the information sheet and their level of interest will be gauged. The consent will be joint for: enrolment of the baby, for obtaining data and samples from the mother.

*See APPENDIX: LOCAL SPECIFITIES PER SITE AND COUNTRY for Mozambique.

Parent(s)/legally designated representative(s) will be given the Informed Consent leaflet. The trained study doctor will give time to read the leaflet and then go through the aims and procedures required to participate and answer any questions. All parent(s)/legally designated representative(s) will be informed that their child's care is not dependent on their willingness to participate in the study. If parent(s)/legally designated representative(s) agree with the participation of the child, the study doctor will go through the consent form with him/her, answer any questions and he/she will then sign the form.

Separate tick boxes will be required to be completed for baby enrolment, caregiver enrolment, storage of samples.
Caregivers will be given as much time as they need to consider the participation of their babies and will be informed that participation is voluntary and a decision not to participate will not affect their clinical care. Written informed consent will be obtained from parent(s)/legally designated representative(s) before subjects undergo any research procedures. Informed consent will be asked to the mother in order to collect data. The original signed consent forms if agreed will be retained with the source documents and a copy included in the participant's medical notes.

Parent(s)/legally designated representative(s) will be provided with a copy of the signed consent form and all consent form updates. The informed consent process must be traceable from the available documentation. If the parent(s)/legally designated representative(s) is/are unable to write, the consent may be given and recorded through appropriate alternative means in the presence of at least one impartial witness, who will sign and date the informed consent document, according to the applicable law.
Furthermore, detailed information to the parent(s)/legally designated representative(s) will have to be on the basis of fair compensation in the case of damage, on the rights and safeguards dictated by law for the child protection, on the right to refuse or to withdraw from the study at any time without reasoning the decision and on the way to ensure respect for privacy and confidentiality of the individual data.

The parent(s)/legally designated representative(s) is/are free to withdraw consent at any time and for any reason, whether expressed or not. No further data would be collected from the subject although data collected from the subject up until withdrawal would remain in the study records. The subject would continue standard care given at the specific site, without any penalty or loss of benefits to which the subject is otherwise entitled.
Mothers 16 to 18 years old will follow the same protocol as >18 years old. Mothers <16 years will have to sing an assent and will need an additional consent form from their parent(s)/legally designated representative(s), according to local laws.

Subject Confidentiality
Subject data protection and confidentiality will be guaranteed following the provisions stated in the Directive 95/46/EC of the European Parliament and of the Council as repealed by the European General Data Protection Regulation (EU) 2016/679 and in the applicable national legislations. The investigator must ensure that the subject's privacy is maintained. On the CRF or other documents submitted to the Sponsors, subjects will be identified by a subject identification number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator. Data will be access only by authorized people including principal investigators and their staff. Pseudonymized data will be accessed by data managers and statisticians.

The investigator shall permit direct access to subjects' records and source document for the purposes of monitoring, auditing, or inspection by the Sponsor, authorized representatives of the Sponsor, Regulatory Authorities and IECs/IRBs.

End of Study
The End of the Study is defined as the last visit of the last participant.

Study Documentation and Data Storage
The investigators must retain essential documents until notified by the Sponsor, for at least for five years after study completion. The participant identification codes shall be archived by the principal investigators and their staff. Pseudonymized data in the REDcap database will be stored for 25 years after the completion (or discontinuation) of the study. After the end of the storage period, data will be made anonymous. Subject files and other source data (including copies of protocols, CRFs, original reports of test results, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) must be kept for 25 years. Documents, without personal identifiers, should be stored in such a way that they can be accessed at a later date after the storage period. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the investigator. If the investigator wishes to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

Data Sharing and Material Transfer Agreements
Data sharing and sample transfer agreements will be signed by the receiving institutions, the9 providing sites and the sponsor. These documents will be stored by the sponsor.

Storage of specimens
A maximum volume of 18.5 mL per patient will be stored in-site if available and following the applicable local and International regulations.

Specimens will be stored in the sites for 10 years after the study completion and may be used by the sites, AHRI, Rome, Massachusetts or Miami Laboratories to evaluate objectives consistent with this protocol to advance our understanding of the characterization and predictors of favorable HIV reservoirs and HIV-specific immunity profiles. After the storage period samples will be destroyed. Should new techniques rise specifically for evaluation of reservoir and excellent control, an amendment to this protocol will be done in order to perform these techniques in the stored samples. Specific examples that are in development and dependent of further funding are CD32a+ lymphocytes or multiparametric visualization of infected cells.[25]

Informed consent will be asked for storage of samples. Usage of samples for purposes other than the ones stated in this protocol and the informed consent requires additional ethical approval. Any reasonable effort will be pursued to get consent from the subjects for the use of the samples for purposes different from the ones stated in this protocol.

Subject reimbursement

Subjects will not be paid to take part in the study however the parents/legal guardians could be compensated for expenses and loss of earnings directly related to participation in the trial, as per site regulation and practice.
*See APPENDIX: LOCAL SPECIFITIES PER SITE AND COUNTRY for Mozambique.

Substudies specificities about regulatory and ethics

Specific and separate consent for the substudies will be obtained from parent(s)/legally designated representative(s) to provide the possibility to be enrolled in the study without participating in the substudies. This will be clearly explained to the subject and the subject's parent(s)/legal guardian(s), both verbally and in the information sheet.

Substudy 1: Social/Behavioural Research Substudy (SBR)
A specific information sheet will be provided for this substudy.

Adult mothers, legal guardians or other caregivers are the participants in the SBR substudy. They will complete a separate consent process for their participation that follows a similar process and set of protections utilized for consent to EARTH: potential participants will receive an information sheet and be informed about the substudy by a member of the research team, in particular about the aims and procedures required to participate and answer any questions. All potential participants will be informed that their child's care or participation in EARTH is not dependent on their willingness to participate in the substudy. Caregivers will be given as much time as they need to consider participation and will be informed that participation is voluntary. Written informed consent will be obtained from the caregivers. The original signed consent forms if agreed will be retained with the EARTH source documents for the related child. Participants will be provided with a copy of the consent form and all consent form updates. If the participant is unable to write, the consent may be given and recorded through appropriate alternative means in the presence of at least one impartial witness, who will sign and date the informed consent document, according to the applicable law.

Participants are free to withdraw consent at any time and for any reason. No further data will be collected but data collected from the subject up until withdrawal will be maintained in the study records. If mental health concerns are identified or suspected in the participating caregivers they will be referred for specialty care.

We will not enroll minors in the SBR substudy.

Substudy 2: Strategies to improve viral suppression (SIVS)
Specific information for this substudy has been added to the general information sheet. A specific box has been added to allow blood drawn from the mother.

17. ADMINISTRATIVE MATTERS AND PUBLICATION POLICY

Source Data
Source Data will be participant medical records and laboratory reports.

Language
CRFs will be in French, English, Portuguese and local language if required. Generic names for concomitant medications should be recorded in the CRF wherever possible. Consent form will be in English or Portuguese and Changana (local language in Maputo Province, Mozambique), Afrikaans, Xhosa, Zulu and Sotho. All written material to be used by subjects must use vocabulary that is clearly understood and be in the language appropriate for the study site.

Study Management Group (SMG)
This group will oversee the day-to-day running of the study and the members will be primarily the clinical, laboratory and data management teams. Minutes of meetings will be taken and will form the basis of the progress report to the EPIICAL Steering Committee. The SMG will consist of the Principal Co-ordinating Investigator, a representative of the Sponsor, a representative from the regulatory and ethics work package 6, a representative of the Clinical platform work package 2, 3 and 4 and Site Principle Investigators. The SMG will report study data to the EPIICAL steering committee at the end of the study.

Study Steering Committee (SSC)
The Steering Committee for the EPIICAL project will be responsible for study supervision.

Monitoring
The study will be monitored by the Sponsor to assess the progress of the study, verify adherence to the protocol, and other national/international requirements and to review the completeness, accuracy and consistency of the data.

Quality Control and Quality Assurance
Quality Control will be performed according to Sponsor standard operating procedures. The study may be audited by a Quality Assurance representative of the Sponsor. All necessary data and documents will be made available for inspection.

Disclosure of data and publication
Information concerning the study, patent applications, processes, scientific data or other pertinent information is confidential and remains the property of the Sponsor. The investigators may use this information for the purposes of the study only.
Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor. Therefore, all information obtained as a result of the study will be regarded as CONFIDENTIAL, at least until appropriate analysis and review by the investigator(s) are completed.

The results may be published or presented by the investigator(s), but the Sponsor must be given the opportunity to review and comment on proposed publications or presentations

for at least 15 days before they are submitted externally. Preparation of a manuscript for rapid publication will be the responsibility of the SSC. High priority will be given to this and it is anticipated that a report will be completed within six months of the study. Any preliminary and final publications will require approval by the SSC and the Sponsor.

Publication Plan

EPIICAL has a publication policy with clear authorship policies, specified in a separate document. In summary, authorship rules will follow BMJ publication rules (http://www.bmj.com/about- bmj/resources-authors/articlesubmission/authorship-contributorship).

The main papers (e.g., results of clinical trials) should have a name authorship (e.g. EPIICAL XY STUDY) with a writing committee at the bottom of the page and a list of collaborators. This is needed because we might need to include a number of authors higher than what allowed by the journal.

For publications on the key work of each EPIICAL working group (WG), the following phrase should be included immediately after the authorship list: 'on behalf of the EPIICAL Consortium'.

Expected Consortium Abstracts/Publications using data from the EARTH study:

- Report of clinical outcomes at 6, 12, and 24 months of age of first 150 and 300 participants. Leading, EPIICAL WG2(Clinical).
- Specific publication on evolution of viral reservoir. Timing, first semester of 2021. Leading, EPIICAL WG3 (Virology).
- Specific publication on immunological response. Timing, first semester of 2021. Leading, WG4 (Immunology)

Substudies specificities publication policy

Substudy 1: Social/Behavioural Research Substudy (SBR)

- Barriers and facilitators to ART adherence and cohort retention
- Stigma, social support and unmet needs among caregivers of children in the EARTH cohort

Substudy 2: Strategies to improve viral suppression (SIVS)

Report on objectives reported at the end of the main study.

18. REFERENCES

1. Penazzato M, Revill P, Prendergast AJ, et al. Early infant diagnosis of HIV infection in low-income and middle-income countries: does one size fit all? *Lancet Infect Dis*. 2014;14(7):650-655. doi:10.1016/S1473-3099(13)70262-7
2. Penazzato M, Lule F, Essajee S. Paediatric HIV: the unfinished business. *Lancet HIV*. 2017;4(10):e425-e427. doi:10.1016/S2352-3018(17)30126-1
3. Violari A, Cotton MF, Gibb DM, et al. Early antiretroviral therapy and mortality among HIV-positive infants. *N Engl J Med*. 2008;359(21):2233-2244. doi:10.1056/NEJMoa0800971
4. Bamford A, Turkova A, Lyall H, et al. Paediatric European Network for Treatment of AIDS (PENTA) guidelines for treatment of paediatric HIV-1 infection 2015: optimizing health in preparation for adult life. *HIV Med*. 2018;19(1):e1-e42. doi:10.1111/hiv.12217
5. Nuttall JJC. Antiretroviral therapy during the neonatal period. *South Afr J HIV Med*. 2015;16(1). doi:10.4102/sajhivmed.v16i1.361
6. 2nd, ed. *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach*. Geneva: World Health Organization; 2016.
7. Persaud D, Palumbo PE, Ziemniak C, et al. Dynamics of the resting CD4(+) T-cell latent HIV reservoir in infants initiating HAART less than 6 months of age. *AIDS Lond Engl*. 2012;26(12):1483-1490. doi:10.1097/QAD.0b013e3283553638
8. Luzuriaga K, Tabak B, Garber M, et al. HIV type 1 (HIV-1) proviral reservoirs decay continuously under sustained virologic control in HIV-1-infected children who received early treatment. *J Infect Dis*. 2014;210(10):1529-1538. doi:10.1093/infdis/jiu297
9. Blankson JN, Persaud D, Siliciano RF. The challenge of viral reservoirs in HIV-1 infection. *Annu Rev Med*. 2002;53:557-593. doi:10.1146/annurev.med.53.082901.104024
10. Calmy A, Hirschel B, Cooper DA, Carr A. A new era of antiretroviral drug toxicity. *Antivir Ther*. 2009;14(2):165-179.
11. Masikini P, Mpondo BCT. HIV drug resistance mutations following poor adherence in HIV-positive patient: a case report. *Clin Case Rep*. 2015;3(6):353-356. doi:10.1002/ccr3.254
12. Castro H, Judd A, Gibb DM, et al. Risk of triple-class virological failure in children with HIV: a retrospective cohort study. *Lancet Lond Engl*. 2011;377(9777):1580-1587. doi:10.1016/S0140-6736(11)60208-0
13. Lowenthal ED, Bakeera-Kitaka S, Marukutira T, Chapman J, Goldrath K, Ferrand RA. Perinatally acquired HIV infection in adolescents from sub-Saharan Africa: a review of emerging challenges. *Lancet Infect Dis*. 2014;14(7):627-639. doi:10.1016/S1473-3099(13)70363-3
14. Mandalia S, Mandalia R, Lo G, et al. Rising Population Cost for Treating People Living with HIV in the UK, 1997-2013. Kaul R, ed. *PLoS ONE*. 2010;5(12):e15677. doi:10.1371/journal.pone.0015677
15. Bernays S, Jarrett P, Kranzer K, Ferrand RA. Children growing up with HIV infection: the responsibility of success. *Lancet Lond Engl*. 2014;383(9925):1355-1357. doi:10.1016/S0140-6736(13)62328-4
16. Sandgaard KS, Lewis J, Adams S, Klein N, Callard R. Antiretroviral therapy increases thymic output in children with HIV. *AIDS Lond Engl*. 2014;28(2):209-214.

doi:10.1097/QAD.0000000000000063
17. Giacomet V, Trabattoni D, Zanchetta N, et al. No cure of HIV infection in a child despite early treatment and apparent viral clearance. *Lancet Lond Engl*. 2014;384(9950):1320. doi:10.1016/S0140-6736(14)61405-7
18. 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*. September 2014. doi:10.1097/INF.0000000000000532
19. Persaud D, Luzuriaga K. Absence of HIV-1 after treatment cessation in an infant. *N Engl J Med*. 2014;370(7):678. doi:10.1056/NEJMc1315498
20. Ananworanich J, Puthanakit T, Suntarattiwong P, et al. Reduced markers of HIV persistence and restricted HIV-specific immune responses after early antiretroviral therapy in children. *AIDS Lond Engl*. 2014;28(7):1015-1020. doi:10.1097/QAD.0000000000000178
21. Persaud D, Patel K, Karalius B, et al. Influence of age at virologic control on peripheral blood human immunodeficiency virus reservoir size and serostatus in perinatally infected adolescents. *JAMA Pediatr*. 2014;168(12):1138-1146. doi:10.1001/jamapediatrics.2014.1560
22. Martinez-Bonet M, Gonzalez-Serna A, Clemente MI, et al. Relationship between CCR5((WT/Delta32)) heterozygosity and HIV-1 reservoir size in adolescents and young adults with perinatally acquired HIV-1 infection. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2017;23(5):318-324. doi:10.1016/j.cmi.2016.12.020
23. Palma P, Foster C, Rojo P, et al. The EPIICAL project: an emerging global collaboration to investigate immunotherapeutic strategies in HIV-positive children. *J Virus Erad*. 2015;1(3):134-139.
24. Howie SR. Blood sample volumes in child health research: review of safe limits. *Bull World Health Organ*. 2011;89(1):46-53. doi:10.2471/BLT.10.080010
25. Descours B, Petitjean G, Lopez-Zaragoza J-L, et al. CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. *Nature*. 2017;543(7646):564-567. doi:10.1038/nature21710