PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HIV-1: PROGRAMME EVALUATION AND INNOVATIVE RESPONSIVE INTERVENTION INTEGRATED IN THE EXPANDED PROGRAMME OF IMMUNIZATION.

Protocol Number: ANRS 12397 PROMISE-EPI

VERSION 6.0_30Apr2021

ClinicalTrials.gov Identifier: **NCT03870438**

Table of Protocol versions	Approval/Opinion EC/IRB
Protocol Version 1.0_24Sep2018	Decision of IRB in Zambia on 19Oct2018 : To resubmit
Protocol Version 2.0_Amendement 1_11 DEC 2018	Approved by IRB in Zambia on 14Jan2019 Approved by NHRA in Zambia on 08 Feb 2019 Provisional Approval by ZAMRA on 01 Apr 2019
Protocol Version 3.0_16Jul2019	Decision of ZAMRA on 06Sep2019: to resubmit
Protocol Version 4.0_28Oct2019	Approved by NHRA in Zambia on 05 Nov 2019
Protocol Version 5.0_28Aug2020	Approved by IRB in Zambia on 06Nov2020 Approved by NHRA in Zambia on 22Nov2020 Approval by ZAMRA on 07Jan2021
Protocol Version 6.0_30Apr2021	Approved by IRB in Zambia on 20th May 2021 Approved by NHRA in Zambia on 19th July 2021

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Sponsor: **Inserm-ANRS**

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RESEARCH PROTOCOL INVOLVING HUMAN BEINGS
ON A MEDICINAL PRODUCT FOR HUMAN USE

ANRS 12397 Promise-EPI Study.
Prevention of mother-to-child transmission of HIV-1: programme evaluation and innovative responsive intervention integrated in the Expanded Programme of Immunization.

Version 6.0_30Apr2021

SIGNATURES PAGE

ClinicalTrials.gov registration number: **NCT03870438**
Ethics committee /IRB approvals
Institutional EC of the Muraz Centre: **Not yet submitted**
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NHRA of Zambia: **19th July 2021**

Sponsor

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In kind contribution:

- University Teaching Hospital (Zambia)
- Centre Muraz Research Center (Burkina Faso)
- University of Montpellier and INSERM (France)
- University of Bergen (Norway)

ABBREVIATIONS:

AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
ANCOVA	Analysis of Covariance
ANRP	Autorité Nationale de Réglementation Pharmaceutique
ANRS MIE	Agence nationale de recherches sur les Maladies Infectieuses Emergentes
AR	Adverse Reaction
ART	Antiretroviral therapy
ARV	Antiretroviral
BF	Breastfeeding
CAG	Community Advisory Groups
CFR	Code of Federal Regulations
CHEERS	Consolidated Health Economic Evaluation Reporting Standards
CMA	Centre de Santé avec Antenne Chirurgicale
CMP	Clinical Monitoring Plan
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CSPS	Centre de Santé et de Promotion Sociale
CTU	Clinical Trial Unit
DAIDS	Division of Acquired Immunodeficiency Syndrome
DALY	Disability-adjusted life years
DBS	Dry blood spot
DNA	Deoxyribonucleic Acid
DSUR	Development Safety Update Report
EA	Ethical Advisor
EC	Ethics Committee
eCRF	Electronic Case Report Forms
EDCTP	European & Developing Countries Clinical Trials Partnerships
EID	Early Infant Diagnosis
EPI	Expanded Programme of Immunization
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HAART	Highly active ART
HEI	HIV Exposed Infants
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation
IDSMB	Independent Data Safety Monitoring Board
INSERM	Institut national de la santé et de la recherche médicale
IRB	Institutional Review Board
ISO	International Organization for Standardization
ITT	Intention-To-Treat
MCH	Maternal Child Health

MedDRA	Medical Dictionary for Regulatory Activities
MMC	Methodology and Management Center
MOP	Manual of Procedures
MTCT	Mother-to-child HIV-1 transmission
NCT	National Clinical Trial
PCR	Polymerase chain reaction
PrEP	Pre-exposure prophylaxis
PI	Principal investigator
PMTCT	Prevention of MTCT
PHOs	Provincial Health Offices
POC	Point of Care
QA	Quality Assurance
QC	Quality Control
REDCap	Research Electronic Data Capture
RCT	Randomised Controlled Trial
RNA	Ribonucleic Acid
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAP	Statistical Analysis Plan
SmPC	Summary of Product Characteristics
SoA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master Files
TSC	Trial Steering Committee
UTHs	University Hospital Teachings
VL	Viral Load
WHO	World Health Organization
WP	Work Package
ZAMRA	Zambia Medicines Regulatory Authority

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PRINCIPAL INVESTIGATOR AGREEMENT SIGNATURE PAGE

Protocol Title:	Prevention of mother-to-child transmission of HIV-1: programme evaluation and innovative responsive intervention integrated in the expanded programme of immunization. PROMISE-EPI Study.
Protocol No.:	ANRS 12397 PROMISE-EPI
Version No.:	6.0
Date:	30Apr2021

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the Declaration of Helsinki, ICH-GCP guidelines and other applicable requirements. Approval of the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the EC before the changes are implemented to the study. In addition, all changes to the consent form will be EC-approved; a decision will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

Full Name	Signature	Date (dd-mmm-yyyy)

1 PROTOCOL AMMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
2.0	11Dec2018	The Non-inclusion criteria below were corrected: • Concomitant treatment with Emtricitabine; • Presence of known allergies to the study medication or its components	The below non-inclusion criteria had been incorrectly listed under the conditions to be met by the mother (instead of the baby): • Concomitant treatment with Emtricitabine; • Presence of known allergies to the study medication or its components
2.0	11Dec2018	The Non-inclusion criteria below were modified: • To describe the participation age • To detail the informed consent requirements	In order to offer more clarity
2.0	11Dec2018	The informed consent process section was developed to better describe the informed consent process specially for young women	In order to offer more clarity
2.0	11Dec2018	The framework for the possible continuation of the lamivudine prophylaxis after the Month 12 visit for children of mothers who continue breastfeeding was more detailed	In order to offer more clarity
2.0	11Dec2018	The % target for Component 2 has been corrected, from 90 to 80%. It now states: "To evaluate a reinforced (at least 80%) access to early paediatric ART among those HIV-1-infected infants not engaged in care at EPI visit."	The % target had been incorrectly entered as 90% instead of 80%. The correct target is 80%
2.0	11Dec2018	Clarification and justification about the age of participation in the research	In order to offer more clarity
2.0	11Dec2018	Homogenized the terminology by using only "participant", instead of "subject" and "participant"	In order to abide by the IRB recommendations and to offer more clarity
2.0	11Dec2018	Rewrote the Introduction, background section and site selection sections	In order to offer more clarity on the rationale of the study
2.0	11Dec2018	A separate section for the Randomization was created	In order to abide by the IRB recommendations and to offer more clarity
2.0	11Dec2018	Added more detail on patient information sheet and consent form translation	In order to abide by the IRB recommendations
3.0	16Jul2019	Discontinuation criteria for parts of the trial or entire trial were further developed	ZAMRA's recommendation

3.0	16Jul2019	ZAMRA has been added to the process of Safety Reporting	ZAMRA's recommendation
3.0	16Jul2019	Criteria for the termination of the trial are better developed	ZAMRA's recommendation
3.0	16Jul2019	Specified more clearly that direct access to source data/documents will be available for trial-related monitoring, audits, institutional review board/independent ethics committee review, and regulatory inspection(s).	ZAMRA's recommendation
3.0	16Jul2019	Specified more clearly the data sharing policy	ZAMRA's recommendation
3.0	16Jul2019	Updated the trial timetable section	To reflect more accurately the new timelines
3.0	16Jul2019	The consent process was modified to reflect that an impartial witness will always sign the consent form in Zambia and that mothers in Zambia will be able to consent for themselves and their child without the need of a representative or father	To reflect more accurately the correct guidelines
4.0	28Oct2019	The component 1 consent process was modified in Zambia: a signed consent was implemented instead of an opt out approach	ZAMRA's recommendation
4.0	28Oct2019	It was specified that a witness is needed in Zambia only for illiterate mother	To reflect more accurately the correct guidelines
4.0	28Oct2019	UTHs center was removed as recruitment site but retained as an administration site and Chipata first level health center was added as fifth recruitment site	To avoid bias in the recruitment as UTHs does not run an EPI unit.
4.0	28Oct2019	Estimated study schedule was updated	To reflect more accurate timelines
5.0	28Aug2020	The study primary objective was reformulated (responsive instead of rescue intervention)	The preliminary data of the study showed that "rescue" is maybe not the appropriate term to describe the intervention as the majority of the mothers are already engaged in care according to the national guidelines.
5.0	28Aug2020	The secondary objectives were reformulated and new secondary objectives were added based on the study subpopulations arising from the new Zambian guidelines	To take into account the modifications of the Zambian guidelines
5.0	28Aug2020	Cost effectiveness was withdrawn as a secondary objective	Cost effectiveness analysis will be detailed as a sub study in a later amendment
5.0	28Aug2020	Eligibility criteria were divided by component	To better reflect the several steps of the study

5.0	28Aug2020	A limit of age was added as inclusion criteria: the children coming for EPI-2 visit have to be between 5 and 16 week-old	For clarification
5.0	28Aug2020	In Zambia, according to the 2020 guidelines, HIV exposed uninfected infants receive a triple-drug prophylaxis (zidovudine, lamivudine and nevirapine) until the mothersare proven virally suppressed. In the intervention group, triple-drug prophylaxis will be switched to lamivudine PrEP in case of the mother is virally unsuppressed at EPI-2. In case of the mother is virally suppressed at EPI-2, the mother/child pair will be referred to the ART clinic for triple-drug prophylaxis discontinuation	Consequences of the HIV guidelines update in Zambia
5.0	28Aug2020	Full blood count was added for all participant of component 3 at EPI-2, M6 and M12 visit (venous blood collected and not capillary blood)	To better compare the safety of the participants in both groups
5.0	28Aug2020	Component 3: In Zambia, genotype will be performed for the children with a positive HIV-1 PCR at M6 and M12	To analyze if infections by mutant virus occur
5.0	28Aug2020	The rationale and hypotheses were updated	To take into account the modifications of the HIV guidelines in Zambia
5.0	28Aug2020	Component 3: The 2020 guidelines in Zambia recommend quarterly viral load testing for breastfeeding mothers. In Zambia, the mothers from our intervention arm will be encouraged to perform the M9 viral load as recommended by the national guidelines (provided by the national program). EPI-2, M6 and M12 viral load measurements are already performed within the study.	For the sake of equity
5.0	28Aug2020	In practice, randomisation performed before getting the GenXepert HIV-1 qualitative test for the child: following randomization GenXepert HIV-1 qualitative and viral load test (for intervention arm) will be performed in the same time. HIV positive child will be considered as mis-randomised as they will be part of the component 2 and not of the component 3.	To avoid for the participant to wait too long time GenXpert results if GenXpert VL was performed after GenXpert Qual
5.0	28Aug2020	GenXepert HIV-1 viral load test will be performed at week 6-8 for mothers from component 2 mis-randomised in the control group	To give viral load results to all participants from component 2 at week 6-8 visit as no more visit are planned for them
5.0	28Aug2020	Component 1: In Zambia, HIV rapid test proposed if not performed in the previous month	To detect HIV seroconversion after delivery
5.0	28Aug2020	Component 1: Addition of a point of care HIV-1 PCR proposed to the mothers with an indeterminate HIV rapid test result during the component 1	To detect the ongoing seroconversion
5.0	28Aug2020	In component 3: GenXpert HIV-1 qualitative test performed for child with 2 weeks of study drug interruption or more	To detect child seroconversion, to avoid monotherapy exposition to HIV infected infants and to allow quick and

			adequate antiretroviral treatment or study drug reintroduction
5.0	28Aug2020	Loss to follow up was more specified	Clarification
5.0	28Aug2020	Chipata site was withdrawn and Chaisa (and Mount Makulu as back-up site) site were added	To fulfill inclusion objectives as competitive study was ongoing in Chipata
5.0	28Aug2020	Safety data reported to National Competent Authority and Ethics Committee were updated	To follow local requirement
5.0	28Aug2020	Adverse events to be reported are not only those who occurred after the study drug initiation but all those from the signature of the consent form	To be able to compare the adverse event rate in the control (patient possibly on prophylactic tritherapy) and in the intervention group
5.0	28Aug2020	At M12 visit, lamivudine prophylaxis will be stopped except if mother is virally unsuppressed at M12 and the child is still breastfed.	For ethical considerations
5.0	28Aug2020	Study settings in Burkina Faso were updated	Update
5.0	28Aug2020	Sample size determination and statistical analyses were updated	To take into account the modifications of the HIV guidelines in Zambia
5.0	28Aug2020	The independent committee acts as clinical event adjudication committee with quarterly reports on both HIV transmission and SAEs in the two arms	At the request of the independent committee to take into account the modifications of the HIV guidelines in Zambia
5.0	28Aug2020	Four ancillary studies were added as appendices: 30 samples of blood from HIV non exposed children and 30 samples of breastmilk from HIV non infected mothers are needed to be used as control in the lab sub-studies. The information sheet and consent form were adapted accordingly.	To optimize the data and the samples collected within the study
5.0	28Aug2020	Updated the trial timetable section	To reflect more accurately the new timelines
5.0	28Aug2020	Paragraph “Analysis of The Primary Efficacy Endpoint” modified	rectification
6.0	30Apr2021	Modification of the ANRS MIE director	
6.0	30Apr2021	At M12, still at risk children are referred to national program for 3 drug prophylaxis initiation	For ethical consideration
6.0	30Apr2021	Addition of a new component in Zambia (Component 4) : observational phase with 2 visits (M18)	to know the final HIV status of the children and to assess stunting and mortality in HIV-exposed uninfected children at 18 months

6.0	30Apr2021	Updated the trial timetable section	To reflect more accurately the new timelines
6.0	30Apr2021	Capillary and not venous blood collected in infants	To avoid traumatic procedure
6.0	30Apr2021	Update on the Burkina Faso sites	
6.0	30Apr2021	Update of the Social sciences sub-study	

2 SYNOPSIS

Protocol V.6.0_30Apr2021.

Clinical Trials ID

Title of Study / Trial
Prevention of mother-to-child transmission of HIV-1: programme evaluation and innovative responsive intervention integrated in the expanded programme of immunization. PROMISE-EPI Study.

Short title – *Sponsor Number*
ANRS 12397 PROMISE-EPI

Sponsor: **Inserm-ANRS:** French National Institute for Health and Medical Research (INSERM) ANRS Infectious Emerging Diseases

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Participating countries:
Burkina Faso (Dr. Fao), Zambia (Dr. Kankasa), Norway (Prof. Tylleskär) and France (Prof. Van de Perre)

Objectives
For clarity, the study Component primarily linked to each objective has been indicated.

Primary Objective:
Component 3: To evaluate the efficacy of an innovative responsive intervention (including Point of care testing and infant single PrEP in high risk children) in order to protect HIV-1-exposed uninfected infants against HIV-1 acquisition by breastfeeding.

Secondary Objectives:
Component 1: To monitor the 'real life' efficacy of the PMTCT cascade up to the second EPI visit
Component 2: To evaluate a reinforced access to early paediatric ART among those HIV-1-infected breastfed infants not on ART treatment at EPI visit.
Component 3:

- <u>For participants of Component 3 in Zambia</u>
  - To evaluate the diagnostic performance of plasma HIV viral load compared to breast milk HIV viral load to identify infants at risk of transmission via breast-milk at 6-8 weeks, 6 months and 12 months.

- <u>For all the participants of the intervention arm and the comparison arm sub-population before the introduction of the 2020 Zambian guidelines (including Burkina Faso control arm)</u>
  - To assess the efficacy of a responsive intervention package to prevent HIV transmission at one year of age

- To evaluate the safety of a responsive intervention (including infant lamivudine PrEP in high risk children) in order to protect HIV-1-exposed uninfected infants against HIV-1 acquisition by breastfeeding
- To assess the efficacy of a responsive intervention package to improve HIV-1 free survival at one year of age

➢ For all the participants of the intervention arm and the comparison arm sub-population following the 2020 Zambian guidelines implementation

- To assess the non-inferiority of the efficacy of a single-drug versus triple-drug prophylactic regimen to prevent HIV transmission at one year of age
- To evaluate the safety of a triple-drug prophylaxis versus a single-drug prophylaxis in infants up to one year of age
- To assess the non-inferiority of a single-drug versus a triple-drug infant prophylactic regimen in terms of HIV-1 free survival at one year of age

Component 4 (in Zambia):
-To assess the risk of HIV acquisition during late breastfeeding (beyond 1 year).
-To assess stunting and mortality in HIV-exposed uninfected children at 18 months

Study Design: Phase III, Randomized Control Trial, parallel, open-label, multi-country and multi-centre trial.

Estimated Enrolment:

- For Component 1, the target population will be approximately 37,000 mother-infant pairs attending the EPI-2 in Bobo-Dioulasso, Burkina Faso and Lusaka, Zambia.
- For Component 2 – statistically, it is estimated that out of 37,000 mother/infant pairs participating in Component 1, there will be about 48 HIV-1 infected infants.
- For Component 3 - phase III trial (N = 2,000 mother-infant pairs) uninfected infants born to HIV-1-infected breastfeeding mothers followed up to 12 months post-partum.
- For Component 4 (in Zambia)– Observational phase: all reconsented participants included in Component 3

Endpoints

Primary Endpoint

Component 3: HIV-transmission rate from EPI-2 visit to 12 months of age in infants exposed to HIV-1 by breastfeeding.

Secondary Endpoints

Component 1 (PMTCT cascade):

- Proportion of women attending the 6-8 week EPI visit who:
  - Have attended ANC/PMTCT clinic at least once during their pregnancy
  - Have been tested for HIV-1 antenatally or during childbirth,
  - Are HIV-1 infected
- Proportion of women with a positive HIV test who had:
  - suppressed plasma viral load (<1000 HIV RNA copies/mL) (information collected in component 2 and 3)
  - Initiated ART during pregnancy or following childbirth,
- Proportion of children who were HIV tested with PCR at birth

- Proportion of babies with a positive HIV-1 PCR who were engaged on ART at 6-8 weeks

Component 2 Proportion of HIV-infected breastfed infants identified during the second EPI visit and who were not engaged in HIV care at this time, but who will be initiated on ART within 2 months after this visit.

Component 3:

- For all participants of Component 3 in Zambia:
  - Proportion of plasma HIV-1 viral load levels concordant with breast milk HIV-1 viral load levels
- For all the participants of the intervention arm and the comparison arm sub-population before the introduction of the 2020 Zambian guidelines (including Burkina Faso control arm)
  - HIV-transmission rate at 12 months of age
  - Adverse events rates at 12 months of age, including death and Grade 3 or 4 events on the paediatric DAIDS scale
  - HIV-free survival at 12 months of age, defined as the proportion of children alive and testing HIV negative at 12 months.
- For all the participants of the intervention arm and the comparison arm sub-population following the 2020 Zambian guidelines implementation
  - HIV-transmission rate at 12 months of age
  - Adverse events rates at 12 months of age, including death and Grade 3 or 4 events on the paediatric DAIDS scale
  - HIV-free survival rate at 12 months of age, defined as the proportion of children alive and testing HIV negative at 12 months

Component 4 (in Zambia):

- Proportion of children exposed to HIV through breastfeeding who acquired HIV between 12 and 18 months
- Proportion of HIV exposed uninfected children with stunted growth (weight and height) at 18 months.
- Mortality rate among HIV exposed uninfected children between 12 and 18 months

Exploratory Endpoint: None identified at this point.

Eligibility Criteria

INCLUSION CRITERIA FOR COMPONENT 1

A mother/infant pair will be included in the component 1 if the infant:

- **Has a mother who**:
  - Is the accompanying person to visit 2 of the EPI
  - Is 15 years of age or older
  - Has signed the consent form to participate in Zambia by herself and a witness (if illiterate)

COMMON INCLUSION CRITERIA FOR COMPONENT 2/3

A mother/infant pair will be included in the component 2/3 if the infant:

- Is a singleton
- Is breastfed at around 2 months (between 5 and 16 week-old) and the mother intends to continue breastfeeding until her child is 6 months old
- **Has a mother who**:
    - Is the accompanying person to visit 2 of the EPI
    - Is 15 years of age or older (in Zambia) and 20 years of age or older (in Burkina Faso)
      or
    - If between 15 and 19 years of age (inclusive) in Burkina Faso, and is accompanied by a referent adult of her choice representing her interests and the interests of the child (parent, family member or guardian, member of an association, etc.)
    - Has been confirmed to be infected with HIV-1 (with or without HIV-2)
    - Has signed the consent form to participate. For the mother in Zambia, the consent must be signed by herself and a witness (if illiterate); For the mother in Burkina Faso, the consent must be signed by herself and a witness (if illiterate) and/or a referent adult (if under 20 years of age in Burkina Faso).

For the child in Zambia, the consent must be signed by the mother. For the child in Burkina Faso, the consent must be signed by the mother and/or a referent adult (if under 20 years of age in Burkina Faso. In Burkina Faso, both parents need to sign the consent unless the mother exercises sole parental authority or if obtaining the father's consent is likely to endanger the mother and her child. In Zambia, the mother exercises sole parental authority.

- **SPECIFIC INCLUSION CRITERIA FOR COMPONENT 2**

A mother/infant pair will be included in the component 2 if the infant:

- Has a positive HIV-1 PCR POC test at visit 2 of the EPI

- **SPECIFIC INCLUSION CRITERIA FOR COMPONENT 3**

A mother/infant pair will be included in the Phase III (component 3) if the infant:

- Has a negative HIV-1 PCR POC test at visit 2 of the EPI

NON-INCLUSION CRITERIA FOR COMPONENT 2/3

A mother-child couple will not be included if the child:

- Has clinical symptoms or biological abnormalities of DAIDS classification 3 or 4 for adverse events on the day of inclusion
- Has a severe congenital malformation
- Has a known allergy to the study drug or its components
- Takes emtricitabine concomitantly
- **Has a mother who:**
    - Lives outside the study area or intending to move from the area within the next 12 months
    - Is participating in another clinical trial

Description of Study Intervention:

The phase III Randomised Controlled Trial (RCT) testing a responsive preventive intervention among HIV-1-uninfected children exposed to HIV-1 by their HIV-1-infected breastfeeding mothers against the national PMTCT guidelines. Following a brief questionnaire and counselling on ART adherence and breastfeeding, participant mothers aged 15 years or older who meet eligibility criteria will be randomized to the control or intervention arms at a 1:1 ratio.

Control arm

In the control arm, routine national guidelines including HIV-1 plasmatic viral load testing will be adhered to as part of the clinics' usual practice. In addition, in Zambia, children followed according to the 2020 guidelines are likely to receive triple-drug prophylaxis until the mothers are proven virally suppressed.
Within the study, the visits will take place at 6-8 weeks, 6 and 12 months post-partum to collect samples from the mother for the analysis of viral load results at 12 months. In addition, at 6-8 weeks, 6 and 12 months post-partum, POC tests will be done for the diagnosis of HIV-1 in their infants (by HIV-1 DNA PCR) and results will be shared within 2 hours. Children infected with HIV-1 will be referred to the National Programme for confirmed diagnosis and immediate ART.

Intervention arm

The intervention arm aims at reducing the risk of HIV transmission for infants exposed to large amounts of HIV-1 through breastfeeding, which correlates with the maternal plasma viral load. At 6-8 weeks post-partum, a venous blood sample will be taken from the mothers to assess their viral load using a GenXpert® HIV RNA POC test. Concomitantly, a capillary blood sample will be taken from the child for the detection of HIV-1 (by HIV-1 DNA PCR). Children infected with HIV-1 will be referred to the National Programme for confirmed HIV diagnosis and immediate ART.

For children that are not HIV-1 infected in the intervention arm, the results on the mother's viral load will guide the next steps:

- Mothers with an unsuppressed plasma viral load (≥ 1000 copies/mL) will receive reinforced counselling on ART adherence. In addition, their child will be initiated on PrEP, lamivudine syrup (7.5 mg twice daily if 2 to 4 kg; 25 mg twice a day if weight <8 kg; and 50 mg twice a day if weight >8 kg). Mothers will come every month to the study centre to collect drug supplies, PrEP adherence counselling, mother's ART adherence and reporting. In Zambia, if the child is on triple-drug prophylaxis at EPI-2, the triple-drug prophylaxis will be switched to single-drug prophylaxis (lamivudine) during this visit.
- Mothers with an suppressed viral load (<1000 copies/mL) will continue receiving ART adherence counselling. However, the children of these mothers will not be initiated on lamivudine PrEP on the study at 6-8 weeks of age. The Zambian children on triple-drug prophylaxis at W6-8 will be referred to the ART clinic for triple-drug prophylaxis discontinuation. Because ART compliance declines rapidly over time within the first year of initiation, additional monitoring on the viral load of the mother and the diagnosis of the child will take place at 6 months: If the maternal plasmatic viral load is ≥ 1000 copies/mL, the child will be initiated on PrEP, lamivudine syrup (7.5 mg twice daily if 2 to 4 kg; 25 mg twice a day if weight <8 kg; and 50 mg twice a day if weight >8 kg), until the baby is 12 months old or until the confirmed end of breastfeeding. Breastfeeding will be considered to be ceased if the mother confirms she is no longer breastfeeding for 2 consecutive monthly visits. Children infected with HIV-1 will be referred to the National Programme for confirmed diagnosis and immediate ART.

The intervention will last for 10 months.

An observational phase will follow the intervention in Zambia: The participants will have a visit at M18 with a quick questionnaire for the mother, the children's anthropometry: weight and height will be measured and will be subjected to a rapid HIV test.

Statistical methods

The primary outcome will be the acquisition of HIV-1 (i.e. without HIV confirmatory PCR) between week 6-8 and 12 Months of age for babies born to HIV-infected women. Analyses for the primary outcome will be undertaken on an intention-to-treat basis and reported upon as such.

- Cumulative HIV-1 incidence rate

For each arm, cumulative event probabilities between 6-8 weeks and 12 Months will be estimated with the Turnbull's extension of the Kaplan-Meier procedure to interval-censored data, and will be compared between arms with a log-rank test.

Sub-studies

Four ancillary studies are described in the appendices:

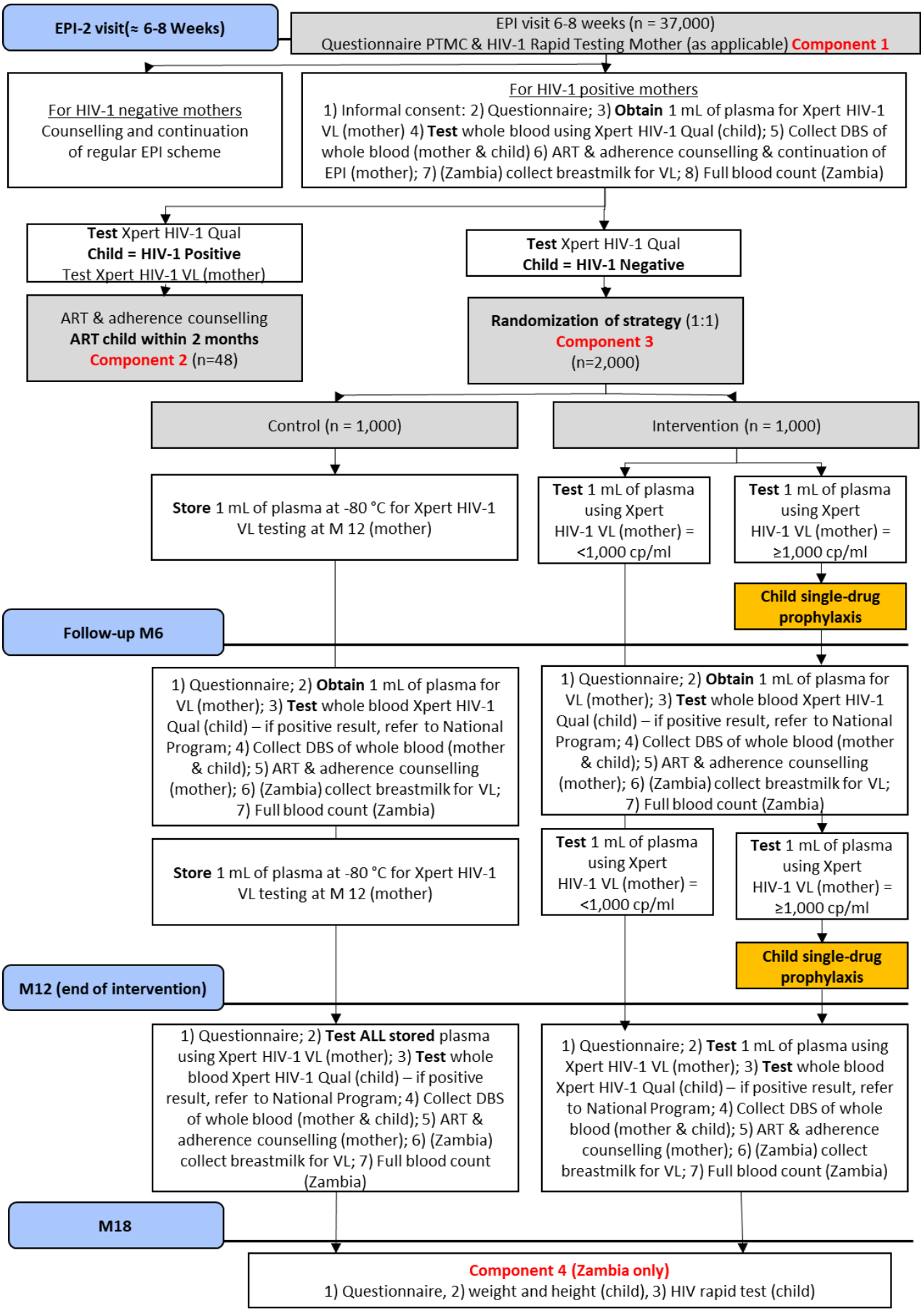
- Two lab sub-studies aim to analyze the breastmilk cell composition in order to better understand the HIV transmission via breast-milk: "HIV reservoir in Breastmilk" and "Breastmilk-induced microchimerism"
- One lab sub-study aims to analyze the mitochondrial toxicity following HIV and ARV exposure.
- One social sciences sub-study aims providers to better understand the factors influencing the strategy proposed in PROMISE-EPI study.

Estimated planning or Study / Trial timetable

Duration of inclusions: 19 months
Duration of follow up per participant: 16 months
Total expected study duration: 36 months
Expected end date of research: Fourth trimester 2022

Planned dates for:
FPFV: first patient first visit: Fourth trimester 2019
FPLV: first patient last visit: Fourth trimester 2021
LPFV: last patient first visit: Second trimester 2021
LPLV: last patient last visit: Fourth trimester 2022

Study / Trial design


EPI-2 visit(≈ 6-8 Weeks)
EPI visit 6-8 weeks (n = 37,000)
Questionnaire PTMC & HIV-1 Rapid Testing Mother (as applicable) Component 1
For HIV-1 negative mothers
Counselling and continuation of regular EPI scheme
For HIV-1 positive mothers
1) Informal consent: 2) Questionnaire; 3) Obtain 1 mL of plasma for Xpert HIV-1 VL (mother) 4) Test whole blood using Xpert HIV-1 Qual (child); 5) Collect DBS of whole blood (mother & child) 6) ART & adherence counselling & continuation of EPI (mother); 7) (Zambia) collect breastmilk for VL; 8) Full blood count (Zambia)
Test Xpert HIV-1 Qual
Child = HIV-1 Positive
Test Xpert HIV-1 VL (mother)
ART & adherence counselling
ART child within 2 months
Component 2 (n=48)
Test Xpert HIV-1 Qual
Child = HIV-1 Negative
Randomization of strategy (1:1)
Component 3
(n=2,000)
Control (n = 1,000)
Intervention (n = 1,000)
Store 1 mL of plasma at -80 °C for Xpert HIV-1 VL testing at M 12 (mother)
Test 1 mL of plasma using Xpert HIV-1 VL (mother) = <1,000 cp/ml
Test 1 mL of plasma using Xpert HIV-1 VL (mother) = ≥1,000 cp/ml
Child single-drug prophylaxis
Follow-up M6
1) Questionnaire; 2) Obtain 1 mL of plasma for VL (mother); 3) Test whole blood Xpert HIV-1 Qual (child) – if positive result, refer to National Program; 4) Collect DBS of whole blood (mother & child); 5) ART & adherence counselling (mother); 6) (Zambia) collect breastmilk for VL; 7) Full blood count (Zambia)
1) Questionnaire; 2) Obtain 1 mL of plasma for VL (mother); 3) Test whole blood Xpert HIV-1 Qual (child) – if positive result, refer to National Program; 4) Collect DBS of whole blood (mother & child); 5) ART & adherence counselling (mother); 6) (Zambia) collect breastmilk for VL; 7) Full blood count (Zambia)
Store 1 mL of plasma at -80 °C for Xpert HIV-1 VL testing at M 12 (mother)
Test 1 mL of plasma using Xpert HIV-1 VL (mother) = <1,000 cp/ml
Test 1 mL of plasma using Xpert HIV-1 VL (mother) = ≥1,000 cp/ml
Child single-drug prophylaxis
M12 (end of intervention)
1) Questionnaire; 2) Test ALL stored plasma using Xpert HIV-1 VL (mother); 3) Test whole blood Xpert HIV-1 Qual (child) – if positive result, refer to National Program; 4) Collect DBS of whole blood (mother & child); 5) ART & adherence counselling (mother); 6) (Zambia) collect breastmilk for VL; 7) Full blood count (Zambia)
1) Questionnaire; 2) Test 1 mL of plasma using Xpert HIV-1 VL (mother); 3) Test whole blood Xpert HIV-1 Qual (child) – if positive result, refer to National Program; 4) Collect DBS of whole blood (mother & child); 5) ART & adherence counselling (mother); 6) (Zambia) collect breastmilk for VL; 7) Full blood count (Zambia)
M18
Component 4 (Zambia only)
1) Questionnaire, 2) weight and height (child), 3) HIV rapid test (child)

Schedule of assessments for the mother

Procedures for Mother	**Screening Component 1** (≈6-8 weeks post-partum)	**ART Treatment HIV-1 positive child Component 2** (≈6-8 weeks post-partum)	**Phase III Component 3** (≈6-8 weeks post-partum)	**Visits M3, M4 & M5 (if child on single PrEP)**	**Month 6**	**Visits M7, M8, M9, M10 & M11 (if child on single PrEP)**	**Month 12 /End of intervention**	**Component 4 M18 (Zambia)**
Acceptable visit window (days)			**+ 7**	**± 7**	**± 14**	**± 7**	**± 14**	**± 14**
Visits								
Verbal Consent (Opt out) in Burkina Faso and opt in consent in Zambia and PMTCT Questions C1	X							
Eligibility of mother for C2/C3	X							
Informed Consent for C2/C3		X	X					
Socio-demographic data		X	X					
Medical History		X	X					
SAEs linked to blood drawing		X	X		X		X	
Counselling (ongoing)		X	X	X	X	X	X	
Laboratory Tests								
HIV-1 serology – rapid test**	X							
HIV-1 PCR and stored DBS***	X°	X	X		X		X	
Lactoserum (only in Zambia)****		X	X		X		X	

Schedule of assessments for the infant

Procedures for the Child	Screening Component 1 (EPI- 2)	ART Treatment HIV-1 positive child Component 2 (EPI- 2)	Phase III Component 3 (EPI- 2) †	Visits M3, M4 & M5 (if child on single PrEP)	Month 6	Visits M7, M8, M9, M10 & M11 (if child on single PrEP)	Month 12 /End of intervention	Component 4 M18 (Zambia)
Acceptable visit window (days)			**+ 7**	**± 7**	**± 14**	**± 7**	**± 14**	**± 14**
Child Visits								
Eligibility of infant for C2 or C3		X	X					
ART Treatment if child is confirmed HIV-1 positive (C2) according to national guidelines		X						
Randomization		X (mis-randomised)	X					
Intervention arm only: 1st dose of Lamivudine PrEP administrated to children of mothers with a VL ≥ 1000 copies/mL			X (if mother ≥ 1000 copies/mL at 6-8 weeks) †		X (if mother ≥ 1000 copies/ mL at 6 months)			
Clinical Assessments		X	X		X		X	X
Anthropometry		X	X	Weight only	X	Weight only	X	X
Resupply medication				X	X	X	X	

(single PrEP) based on weight								
Adherence Assessment				X	X	X	X	
Adverse Event Assessment		X	X	X	X	X	X	X
Concomitant Medication Collection			X	X	X	X	X	X
Breastfeeding/ Feeding Review *		X	X	X	X	X	X	X
Laboratory Tests								
HIV-1 DNA PCR and stored DBS*****		X	X		X°°		X°°	
Full blood count*****			X		X		X	
HIV rapid test								X

*Resupply medication (lamivudine PrEP) based on weight during breastfeeding.
*Definition of end of breastfeeding = 2 consecutive visits where mother confirms the end of breastfeeding
** HIV-1 rapid test (blood or saliva-based test following formative research recommendations) to be performed during EPI-2 (at 6-8 weeks post-partum) if previous HIV test was performed more than one month ago in Zambia and if the usual practice HIV-rapid testing results are not available at the time of the EPI-2 visit in Burkina Faso (within the last 3 months).
***Plasma will be obtained from mothers' 5ml of venous whole blood collected at 6-8 weeks, 6 and 12 months post-partum. In the control group, plasma will be stored at -80°C until Month 12. In the intervention group, point of care HIV-1 PCR (Xpert HIV-1 Viral Load, Cepheid) will be performed and results will be available in 2 hours) and in Zambia remaining plasma stored at -80°C. DBS collected for storage at 6-8 weeks, 6 and 12 months post-partum from whole blood for HIV-1 RNA quantification if requested and repository as quality control assessment.
****a minimum of 10 ml of manually-expressed milk from each breast to be collected only in Zambia from mothers in the control and intervention arms at 6-8 weeks, 6 months and 12 months of age for storage of acellular and cellular fractions. Breast milk RNA and DNA HIV-1 viral load will be quantified using a test to be determined.
*****capillary blood collected from children at 6-8 weeks, 6 and 12 months of age, point of care HIV-1 PCR (Xpert HIV-1 Qualitative, Cepheid; done on 100 µl of venous whole blood; result available in 2 hours). DBS collected for storage at 6-8 weeks, 6 and 12 months post-partum from 400 µl capillary whole blood for detection of HIV infection by HIV-1 DNA PCR as quality control assessment and full blood count performed with the remaining capillary blood sample
° For mothers with an indeterminate result by HIV rapid testing, a point of care HIV-1 PCR (Xpert HIV-1 Qualitative and Xpert HIV-1 viral load) will be performed.
°° viral resistance genotyping will be performed for the children with a positive HIV-1 PCR at M6 and M12
† In Zambia, according to the 2020 guidelines, HIV exposed uninfected infants receive a triple-drug prophylaxis (zidovudine, lamivudine and nevirapine) until the mothers are proven virally suppressed. In the intervention group, triple-drug prophylaxis will be switched to lamivudine PrEP in case of the mother is virally unsuppressed at EPI-2. In

case of the mother is virally suppressed at EPI-2, the mother/child pair will be referred to the ART clinic for triple-drug prophylaxis discontinuation.

3 INTRODUCTION/BACKGROUND

The WHO recommendation of lifelong antiretroviral therapy (ART) for all HIV-infected pregnant/lactating women together with a six-week nevirapine pre-exposure prophylaxis (PrEP) to their infants is implemented in most African countries since 2013, with the aim of eliminating paediatric HIV. Despite this PMTCT option B+ strategy, the actual impact of the strategy, on the MTCT rate in the general population is not known. By modelling, the UNAIDS estimated that, among 21 target African countries, the average residual HIV-1 transmission rate was about 14% at 12 months in 2015, incompatible with paediatric HIV elimination. Therefore, the goal of eliminating new HIV infections in children, can only be achieved if we succeed in engaging HIV-infected pregnant and postpartum women in successful long-term care in order to achieve the full individual and societal benefit of universal ART (Abrams, CROI 2016). In Zambia, the MTCT rates of HIV was 9% in 2014. (Zambia country report 2015). This study aims to further reduce the MTCT of HIV rates to less than 5% which has been a target for Zambia since 2016. (Zambia country report 2015)

3.1 STATEMENT OF THE PROBLEM

While progress has been made in the last few years toward expanding PMTCT programs and increased availability of highly active antiretroviral therapy (HAART), new HIV infections among children are still unacceptably high. In 2017, about 180 000 children were infected with HIV worldwide. (UNAIDS data 2018). One of the main obstacles is to identify infected mothers (pregnant and postpartum) and initiating them on treatment whilst also identifying the at risk children early-on and implement PrEP, with a very large coverage in the community.
Most cases of MTCT of HIV result from a combination of obstacles:

- Non-attendance at antenatal clinics (ANC) by some pregnant women, especially teenagers and young adults due to difficulties in terms of geographical, cultural or logistical access to ANC.
- Home delivery, mainly in rural areas.
- Difficulties in testing, treating and maintaining women who have initiated ART in HIV care in the care chain. In Malawi, one in five HIV-infected women diagnosed during pregnancy or breastfeeding never initiate ART (Tenthani, 2014).
- Difficulty in maintaining the PMTCT cascade of care. In Zambia out of the 100% women that tested for HIV and received their results , 91% received ART for MTCT of HIV and only, 36% and 37 % of their infants received ART prophylaxis and an HIV diagnosis within 2 months of birth respectively (Appendix). The PEARL study conducted in Cameroon, Cote d' Ivoire, South Africa and Zambia, showed that only 36.4% women completed the PMTCT cascade despite 98% women attending at least one antenatal clinic visit and 87% having an HIV test. (Dionne-Odom et al 2016
- In the PMTCT programme in Swaziland, postnatal retention in the care chain for HIV-infected women was only 37%, and 50% among women who started ART during pregnancy (Abrams, 2016).
- Women who start ART as part of PMTCT are five times more likely to be virologically uncontrolled than those who initiate ART for their own health (Tenthani, 2014).
- According to recent reports from various countries, up to 50% of mothers who initiated ART during pregnancy stopped taking their drugs before 12 months (UNAIDS 2015) after delivery. For their infants, the risk of acquiring HIV-1 is very high, and no alternative strategy is available to reduce this risk (Van de Perre, 2013). *Improving maternal ART adherence is at the top of the research program agenda (Rollins, 2014; Gourlay, 2013; Mwapasa, 2014; Oyeledun, 2014; Reimers, 2016; Rosenberg, 2014);*
- A very high risk of HIV-1 transmission through breast milk when mothers discontinue ARV treatment during breastfeeding, due to the viral rebound in breast milk (Manigart 2004);
- Even when mothers are successfully treated, residual HIV-1 transmission through breastfeeding is estimated at 0.2% per month of breastfeeding (corresponding to 2.4% at 12 months) (Rollins, 2012; Van de Perre, 2012). It is likely that this low residual transmission rate will be extremely difficult to reduce further by maternal ARVs alone, but it is consistent with the goal of eliminating MTCT (overall transmission rate <5%).

- In addition, mother-to-child transmission through breastfeeding from an ARV-treated mother carries a very high risk of selection of resistant viruses in HIV-1-infected infants due to the ingestion of suboptimal, non-suppressive antiretroviral doses by milk, subsequently compromising ARV response (Fogel, 2011; Lidstrom, 2010; Zeh, 2011).

Table 1: **PMTCT services global coverage in 2016**

Country	Percentage of pregnant women on ART	Estimated MTCT rates
Latin America and the Caribbean	72%	9%
Eastern Europe and Central Asia	46%	15%
South Asia	38%	27%
Middle East and North Africa	42%	16%
West and Central Africa	42%	18%
East and Southern Africa	88%	8%

Source: UNICEF analysis of UNAIDS 2017 data

PMTCT situation in Zambia - UNAIDS

In 2016, Zambia had 59 000 (52 000 - 69 000) new HIV infections and 21 000 (17 000 - 28 000) AIDS-related deaths. There were 1 200 000 (1 200 000 - 1 300 000) people living with HIV in 2016, among whom 65% (62% - 69%) were accessing antiretroviral therapy. Among pregnant women living with HIV, 83% (71% - 91%) were accessing treatment or prophylaxis to prevent transmission of HIV to their children. An estimated 8900 (7000 - 11 000) children were newly infected with HIV due to mother-to-child transmission. Among people living with HIV, approximately 58% (55% - 62%) had suppressed viral loads.

HIV transmission during late breastfeeding

WHO recommends HIV testing at the end of breastfeeding period to determine the child's final HIV status (WHO 2016). In Zambia, according to the Zamphia 2016 report, the majority of HEU children are breastfed more than 12 months. Zambia's 2020 consolidated guidelines for the treatment and prevention of HIV infection recommend serological testing at M18 and M24 for HIV exposed children. An observational follow up phase for PROMISE-EPI participants with M18 visits is therefore important to know the final HIV status of children.

Growth retardation in HIV-exposed uninfected children

Various studies have demonstrated growth retardation in HIV-exposed uninfected children compared with unexposed children (Omoni 2017; Le Roux 2019; Jumare 2019, Rosala-Hallas 2017). These studies have been conducted on populations differentially exposed to ARVs due to the constant evolution of HIV treatment and prevention. The assessment of stunting in children exposed to single PrEP (lamivudine: interventional arm) versus 3 drug prophylaxis (AZT/3TC/NVP: standard of care – control arm) appears to be of primary importance as an integral part of the benefit/risk analysis of both strategies.

3.2 RATIONALE OF THE STUDY

A 'responsive' intervention is therefore, needed to identify these at risk children in the community and link them to the health system to offer them PrEP. Infant PrEP is immediately protective and should leave enough time to address maternal ART adherence issues, without the stress of MTCT on the child. In addition, pharmacokinetic studies have demonstrated that infant PrEP could be safely combined with maternal ART because infants breastfed by mothers on ART have consistent ARV drug plasma levels largely below 5% of the therapeutic level (Shapiro, 2013).

In HIV-1-infected infants, early ART initiation confers an undisputable clinical benefit compared with deferred initiation (Kuhn, 2017). The extended follow-up of the CHER trial has demonstrated that early ART confers a persistent gain on reducing the size of HIV-1 reservoirs in children, confirming the utmost importance of diagnosing paediatric HIV-1 early and of initiating ART immediately (Payne, 2015). Today, the early ART initiation for HIV-infected children is hampered by i) the HIV test proposed only to mothers who come with their maternity card at

the EPI visit and who are notified as HIV-infected, ii) the long process of HIV molecular test, requiring central laboratory batch assays and a second visit four weeks later for the mother to collect the result.
In the vast majority of sub-Saharan African countries, all mothers attend the second immunization (EPI) visit when their child is 6 to 8 weeks old (the first EPI visit for BCG and OPV_0 vaccination, takes place at childbirth, which is too soon for this rescue strategy). This EPI-2 visit represents a unique opportunity to:

- Identify HIV-1-infected children early and refer them for early initiation of treatment
- Optimize the ART of HIV-1-infected mothers and also provide their children with a single antiretroviral drug that can protect them from HIV-1 depending on their mother's viral load.

Our study primarily aims at utilizing existing strategies to prevent postnatal HIV transmission through optimizing the use of lamivudine as pre-exposure prophylaxis (PrEP) for at risk children. For this purpose, we will use recently marketed diagnostic point of care diagnostic tests (POC) at the first level of care for maternal HIV viral load and early infant diagnosis (EID). This POC early infant diagnosis will also allow optimising treatment of HIV-infected infants by offering to all the benefits of an early ART initiation.

We will therefore address the call scope of "strategies for equitable and full-scale access to diagnostics, prevention and treatment intervention". During this study, in addition to counselling and the use reminders (for women to consent), peer support will be offered via the patient support groups and Community Advisory Groups (CAG). Our strategy is designed to be reproducible in all mother, neonatal & child health clinics (MNCH), hence the choice of sites in West and Southern Africa to ascertain it in different cultural, epidemiological and health system organisation contexts.

The study's rationale was revised following the 2020 guidelines implementation in Zambia. Indeed, the new Zambian guidelines recommend viral load tests every three months for HIV-infected breastfeeding mothers as well as triple-drug prophylaxis to HIV exposed uninfected children guided by maternal plasma viral load. The following arguments allow to concluding on an equipoise between the triple-drug (comparison arm) and a single-drug (lamivudine - intervention arm) prophylaxis in infants:

- No study demonstrated a superiority in term of efficacy for HIV transmission between a triple-drug and a single-drug prophylaxis in infants, and 6 weeks of infant single-drug prophylaxis is recommended by WHO in the majority of the cases for breastfed HIV exposed uninfected children (WHO, 2016). Furthermore, PROMISE PEP/ANRS 12174 trial demonstrated that one-drug prophylaxis among infants of HIV infected women who did not qualify for ART for their own health reduced the risk of postnatal transmission at 1 year of age to 0.5% per protocol or 1.4% in intention to treat (Nagot, 2016).
- When drugs are used as prophylaxis, safety is of utmost importance, as the drugs are administered to uninfected children. HIV-uninfected infants breastfed from mothers with unsuppressed viral load are at-risk of HIV acquisition (maximum risk estimated at 15-20% at 12 months). The benefit in terms of risk reduction of transmission should not be annihilated or counterbalanced by a higher risk of severe adverse events (including death) due to exposure to multiple drugs. Zidovudine is known to induce severe neutropaenia and severe anaemia leading to death in previous studies on HIV exposed uninfected children (Dryden 2011, Kumwenda 2008). Zidovudine may also impact mitochondrial function to a variable degree with possible late-onset neurological disorders (Zidovudine SmPC). In addition to safety concern, adverse events can lead to prophylaxis cessation or suboptimal observance that can in turn lead to HIV acquisition.
- Some observations suggest that wild type virus represents the founder virus in most of the cases of HIV acquisition postnatally. In these infants of mother on ART, the HIV drug resistance mutation subsequently identified likely emerged as a result of ingestion of non-suppressive levels of antiretroviral drugs in breastmilk (Zeh 2011). Therefore, even if NRTI drug resistant mutations are spread among individual on ART, it does not seem to be transmitted from mother to child in case of HIV transmission by breastfeeding.
- Many variables influence adherence to a single-drug regimen or to a more complex regimen. It includes formulation of drugs, number of tablet/syrup, schedule of administration, palatability, food interference with absorption, duration of treatment, adverse events (quality and frequency), The triple-drug prophylaxis regimen as recommended by the 2020 Zambian guidelines is composed of 2 different formulations with different schedule of administration. Suboptimal observance lead to a risk of HIV acquisition.

The study will additionally follow up the children at 18 months to evaluate the outcome of these interventions by evaluating the risk of HIV acquisition, the growth and mortality.

3.3 HYPOTHESIS OF THE STUDY

We hypothesise that the concept of a responsive intervention, based on the use of POC tests at EPI-2 visit:

- Will allow provision of single PrEP to HIV exposed high-risk infants (HEI) and can be incorporated into routine EPI services to achieve the goal of paediatric elimination of HIV transmission (HIV transmission rate of less than 5%)
- Will allow early identification of HIV infected children, reinforced (at least 80%) access to early paediatric ART and improve the prognosis of HIV infected infants (HII) by facilitating early ART initiation.

Such a responsive intervention would also provide a unique opportunity to monitor in real-time the performance of the PMTCT cascade. Adding only a very short questionnaire to all EPI-2 visit attendees would capture the early steps of this cascade, while POC molecular tests will provide crucial and quasi-exhaustive biological data, which are currently lacking. Overall, accurate and precise estimates of the cascade of care could be used by program managers to detect gaps and conduct appropriate corrective actions.

Furthermore, due the 2020 guidelines implementation, new hypotheses were defined based on the previous rationale:

- The efficacy of a single-drug (lamivudine) regimen used as a prophylaxis in HIV exposed uninfected children is not inferior to triple-drug regimen to prevent HIV acquisition.
- A single-drug (lamivudine) prophylaxis is safer than a triple-drug prophylaxis (zidovudine / lamivudine / nevirapine).

The following hypothesis will be assessed in projects associated to PROMISE-EPI study

- A single-drug prophylaxis does not increase the risk of HIV mutant acquisition compared to a triple-drug prophylaxis
    - This hypothesis will be assessed within the associated PhD program
- Optimal observance can be better achieved with a single-drug (lamivudine in syrup) compare to the triple-drug regimen (one syrup and one dispersible tablet) recommended by the 2020 Zambian guidelines (assessed
    - This hypothesis will be assessed within the social sciences sub-study

3.4 RISK/BENEFIT ASSESSMENT

2.4.1 Known Potential Risk

Potential risks for Participants

- Distress when learning their HIV status: Experienced staff will provide appropriate post-test counselling for these women. Their participation in the study will also reduce, even if they are in the 'control' arm, the likelihood that their child becomes infected.
- Breastfeeding Concerns: Participating mothers may worry about transmitting HIV-1 to their child during the breastfeeding period. Special dedicated lactation counsellors will discuss this possible worry with the mothers and give counselling on how to deal with the issue. Some women may feel that the questions posed are of private nature. They will be reassured that they do not need to answer the questions if they do not wish to do so.
- Stigma: Stigmatisation is an acknowledged risk that is discussed with the mother from Day 1 (EPI-2 Visit). Mothers will be counselled at every level regarding her experience and how to deal with this risk if she feels it is applicable to her. The ANC and the study clinics selected for the study are public health centres open

to the general population (i.e. not only dedicated to HIV care) which should reduce the risk of stigma. In addition, the study staff will receive training about the strict study procedures to preserve confidentiality.

- Invasive Procedures: HIV rapid blood tests performed by a finger prick are performed at 6-8 weeks post-partum per national guidelines to establish if the mother is HIV-1 positive or HIV-1 negative. During this study, HIV rapid tests using a finger prick may need to be performed if previous HIV test was performed more than one month ago in Zambia (due to the high prevalence of HIV in Zambia) and if the results of those mandated tests (per national guidelines) are not available at the time of the EPI-2 visit in Burkina Faso. For mothers with HIV rapid test indeterminate results, a venous blood collection will be needed for a point of care HIV-1 PCR. For HIV+ mothers providing an Informed Consent, a venous blood collection will be needed from those mothers in order to determine the viral load. In addition, their children will also provide a capillary blood sample in order to assess their HIV-1 status and check full blood count. Children with a confirmed HIV-1 positive status will be referred for immediate initiation of antiretroviral treatment according to national guidelines.
- Venepuncture procedure may cause discomfort to the mother and child. In order to minimise this discomfort, all procedures will be performed by skilled staff that have been trained in the various specimen collection techniques. Infection control procedures will be followed to minimise the risk of any infection during all procedures.
- For HIV+ mothers providing an Informed Consent in Zambia, collection of participant mother's breast milk would take place at 6-8 weeks, 6 months and 12 months post-partum. Breast milk will be tested for the purposes of testing viral load (secondary objective), HIV reservoir in breastmilk and breastmilk-induced microchimerism (sub-studies presented in appendices). A small quantity of manually expressed milk per breast (about 10 ml) will suffice per time point. In order to reduce discomfort, consenting mothers will be given sterile containers so that they can collect the samples themselves.
- Adverse reactions in children during daily lamivudine PrEP: The PROMISE-PEP study was very reassuring about the safety of lamivudine in very young children. We did not record any grade 3 or 4 drug-related events. However, the frequency of grade 3 and 4 events will be closely monitored. With the exception of gastrointestinal disorders in adults, no serious adverse events have been attributed to the use of lamivudine in the routine treatment of HIV-1 infection. Pancreatitis cases are rare. However, it is not clear whether these cases were due to antiretroviral treatment or the underlying HIV disease. Lamivudine therapy should be discontinued immediately if clinical signs, symptoms or laboratory abnormalities suggestive of pancreatitis appear. As this event is very rare, it is not currently recommended to routinely monitor pancreatitis biomarkers in children treated with lamivudine. Other adverse reactions have been reported with lamivudine; for a comprehensive list of all adverse reactions, please consult EPIVIR (lamivudine) SmPC. Mothers will be encouraged to come to the clinic whenever they wish in case their child has health problems. Our close collaboration with the heads of paediatric services in teaching hospitals will enable us to ensure rapid and optimal management of Adverse Events and Adverse Reactions, in accordance with national guidelines. The study drug may be stopped if necessary, depending on the doctor's decision.
- The 2020 guidelines in Zambia recommend quarterly viral load testing for HIV-infected breastfeeding mothers. For the sake of equity, in Zambia, the mothers from our intervention arm will be encouraged to perform the M9 viral load as recommended by the national guidelines (provided by the national program). The M9 viral load result will be asked by the investigator at next participant visit (questionnaire + medical record verification). EPI-2, M6 and M12 viral load measurements are already performed within the study and the results will be share with the national program.

Potential risks for research staff

Occupational exposure to HIV due to the additional blood sampling: All routine measures to control the risk of needles injuries and other occupational exposures will be checked before the study and amended if necessary. In addition, post-exposure prophylaxis using ART is readily available for the staff, through consultation with a HIV physician, and according to national guidelines.

The research staff assigned to this study is the research staff currently working in the site's laboratory as part of routine patient care activities. In the framework of this study, training will be reinforced and provided to the research staff. All routine measures to control the risk of needle injuries and other occupational exposures will be

checked before the study and amended if necessary. Namely: staff must assume that blood and other body fluids from all patients are potentially infectious. They should therefore, follow infection control precautions at all times: Routinely use barriers (such as gloves and/or goggles) when anticipating contact with blood or body fluids; Immediately wash hands and other skin surfaces after contact with blood or body fluids; Carefully handle and dispose of sharp instruments during and after use. Potentially contaminated surfaces will be cleaned routinely with registered disinfectants, following the manufacturers' instructions.
Although the most important strategy for reducing the risk of occupational HIV-1 transmission is to prevent occupational exposures, plans for post-exposure management of health care personnel will be in place. Post-exposure prophylaxis using ART is readily available for the staff, through consultation with a HIV-1 physician, and according to national guidelines.

2.4.2 Known Potential Benefits

All Component 3-participating mothers and babies will benefit during the course of trial from:

- Same day HIV DNA PCR results using POC HIV-1 tests
- Rapid referral to the national programme if the child is HIV positive
- Free medical care for acute common diseases at the research site for infants and mothers after randomization
- Participating mother/infant pairs will benefit from infant feeding counselling with the potential of optimising breastfeeding practices thus lowering the risk for MTCT, ART treatment and adherence reinforcement counselling.
- The mother's partner may also be offered free HIV counselling and testing and, if positive, referred to ART.
- HIV-1 prevention and family planning counselling.

2.4.3 Assessment of Potential Risk and Benefits

The risks of the intervention and control arm are acceptable due to the clinical equipoise explained in the rationale. For the control group, the most important point is for HIV-infected mothers to be compliant and adhere to the ART treatment and counselling provided by benefiting from the national prevention programme. Viral load results from the tests performed via the national recommendation can be made available to facilitate the mother's HIV infection management (i.e. adherence reinforcement). Furthermore, in Zambia, the infants are likely to receive triple-drug prophylaxis until their mothers are proven virally suppressed.

4 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary	
Component 3: To evaluate the efficacy of an innovative responsive intervention (including Point Of Care testing and infant single PrEP in high risk children) in order to protect HIV-1-exposed uninfected infants against HIV-1 acquisition by breastfeeding.	**Component 3:** HIV-transmission rate from EPI-2 visit to 12 months of age in infants exposed to HIV-1 by breastfeeding.
Secondary	
Component 1: To monitor the 'real life' efficacy of the PMTCT cascade up to the second EPI visit	**Component 1** (PMTCT cascade): 1. Proportion of women attending the 6-8 week EPI visit who: a. Have attended PMTCT clinic at least once during their pregnancy b. Have been tested for HIV-1 antenatally or during childbirth, c. Who are HIV-1 infected 2. Proportion of women with a positive HIV test who had: a. Suppressed plasma viral load (<1000 HIV RNA copies/mL), (information collected in component 2 and 3) b. Having initiated ART during pregnancy or following childbirth, 3. Proportion of children who were HIV tested with PCR at birth 4. Proportion of babies with a positive HIV-1 PCR who were engaged on ART at EPI-2
Component 2 To evaluate a reinforced access to early paediatric ART among those HIV-1-infected infants not engaged in care at EPI visit.	**Component 2** Proportion of HIV-infected breastfed infants identified during the second EPI visit and who were not engaged in HIV care at this time but who will be initiated on ART within 2 months after this visit,
Component 3: ➢ <u>For participants of Component 3 in Zambia</u> • To evaluate the diagnostic performance of plasma HIV viral load compared to breastmilk viral load to identify infants at risk of transmission at 6-8 weeks, 6 months and 12 months	**Component 3:** ➢ <u>For participants of Component 3 in Zambia</u> • Proportion of plasma HIV-1 viral load levels concordant with breast milk HIV-1 viral load levels
➢ <u>For all the participants of the intervention arm and the comparison arm sub-population before the introduction of the 2020 Zambian guidelines (including Burkina Faso control arm)</u> a) To assess the efficacy of a responsive intervention package to prevent HIV transmission at one year of age b) To evaluate the safety of a responsive intervention (including infant lamivudine PrEP in high risk children) in	➢ <u>For the intervention arm and the comparison arm sub-population before the introduction of the 2020 Zambian guidelines (including Burkina Faso control arm)</u> a) HIV-transmission rate at 12 months of age b) Adverse events rates at 12 months of age, including death and Grade 3 or 4 events on the paediatric DAIDS scale

OBJECTIVES	ENDPOINTS
order to protect HIV-1-exposed uninfected infants against HIV-1 acquisition by breastfeeding c) To assess the efficacy of a responsive intervention package to improve HIV-1 free survival at one year of age	c) HIV-free survival at 12 months of age, defined as the proportion of children alive and tested HIV negative at 12 months.
➢ For all the participants of the intervention arm and the comparison arm sub-population following the 2020 Zambian guidelines implementation a) To assess the non-inferiority of the efficacy of a single-drug versus triple-drug prophylactic regimen to prevent HIV transmission at one year of age b) To evaluate the safety of a triple-drug prophylaxis versus a single-drug prophylaxis in infants up to one year of age c) To assess the non-inferiority of a single-drug versus a triple-drug infant prophylactic regimen in terms of HIV-1 free survival at one year of age.	➢ For the intervention arm and the comparison arm sub-population following the 2020 Zambian guidelines implementation a) HIV-transmission rate at 12 months of age b) Adverse events rates at 12 months of age, including death and Grade 3 or 4 events on the paediatric DAIDS scale c) HIV-free survival rate at 12 months of age, defined as the proportion of children alive and tested HIV negative at 12 months
Component 4 (Zambia) a) To assess the risk of HIV acquisition during late breastfeeding (beyond 1 year). b) To assess stunting and mortality in HIV-exposed uninfected children at 18 months	**Component 4 (Zambia)** - Proportion of children exposed to HIV through breastfeeding who acquired HIV between 12 and 18 months - Proportion of HIV exposed uninfected children with stunted growth (weight and height) at 18 months. - Mortality rate among HIV exposed uninfected children between 12 and 18 months

5 LITERATURE REVIEW

Universal life-long antiretroviral therapy (ART) among pregnant and lactating women (Option B+) has been implemented since 2013 with the goal of MTCT elimination of HIV transmission (transmission rate <5%; WHO, 2016), but the real impact of this program on MTCT rate in the whole population is unknown. Available data shows that many women entering the PMTCT program during pregnancy will default (i.e. have a unsuppressed plasma HIV-1 viral load) before the child is no longer at risk of transmission (i.e. end of breastfeeding). Most transmission cases are attributable to breastfeeding exposure. Among all priority countries, only Botswana and South Africa have reached MTCT elimination (rate below 5%), mainly because their breastfeeding rate is very low. In 2014, Zambia reported a MTCT rate of 9%, from 24% in 2009. And has since 2016 targeted reducing this rate to below 5% by 2020. (Zambia country report 2015)

At the population level, one of the main difficulties faced is the lack of systematic evaluations to allow appropriate decision-making and corrective actions: while a large proportion of pregnant women enter the program, reliable data on the proportion of women with suppressed viral load and on the final step (HIV-1 testing of children at 12 months) is hardly available. The ultimate indicator, i.e. the MTCT rate in the population, is therefore universally not available. A study conducted in Kenyan, Malawian and South African households, showed that, among 11,000 HIV-1-infected pregnant or breastfeeding women, 27 to 73% had a plasma HIV-1 RNA > 1000cp/ml (Maman, 2015). These women with unsuppressed viral load were either undiagnosed for HIV-1, or had recent infections (occurring after antenatal screening), or had not initiated ART or were non-adherent to ART.

The HIV elimination target can only be achieved if we manage to engage most HIV-infected pregnant and post-partum women in successful long-term care to achieve the full individual and societal benefit of universal ART (Abrams, CROI 2016). However, despite many ongoing studies investigating ways to reinforce maternal ART

adherence, many children remain at risk of HIV infection either because their mothers have not attended the PMTCT program or because their treatment does not lead to a virological suppression (due to suboptimal adherence or HIV primary resistance). In Africa, the reasons for this high residual burden of child infections are multiple. The main reason is operational, with challenges in all phases of the care cascade (test, treat, and retain in care), including consistent testing of HIV exposed infants, initiating HIV infected infants (HII) timely on treatment, and retaining them in care (Van de Perre, 2017b). HIV-uninfected infants breastfeeding from mothers with unsuppressed viral load carry a high-risk of HIV infection, with incidence rates as high as 15-20%. The vertical transmission rate from mother-to-infant at six weeks was 5% but rose to 8.9% by the conclusion of breast feeding (Mofeson, 2017).

In contrast with other individuals exposed to such high HIV risk, such as MSM or sex workers, the universal and equitable access to PrEP is currently denied to these children (Van de Perre, 2017). The ANRS 12174 trial, showed that infant prophylaxis with either lamivudine (3TC) or boosted lopinavir (LPV/r) daily dose throughout breastfeeding for up to 12 months among infants of HIV infected women who did not qualify for ART for their own health was well tolerated and reduced the risk of postnatal transmission at 1 year of age to 0.5% (per protocol) or 1.4% (intention to treat) (Nagot, 2016). Adherence to infant PrEP in the trial was particularly high (over 90%) (Nagot, 2016). Consistently with other studies (Tenthani, 2014), unpublished data collected during the ANRS 12174 trial suggest that most pregnant or lactating mothers prefer to administer a prophylactic antiretroviral drug to their exposed infant than to adhere to their own ART.

While prevention of mother-to-child transmission (PMTCT) programmes have been extremely successful in recent years, it is more difficult to reduce new infections among adolescents (WHO defines adolescents as those aged 10-19 years, youth as those aged 15-24 years and the youth population as adolescents and youth aged 10-24 years). (UNAIDS, 2013).

Many factors put young people at high risk of contracting HIV. Adolescence and early adulthood are critical periods of development when significant physical and emotional changes occur. Adolescents and young people are increasingly autonomous and responsible for their personal health. The transition from childhood to adulthood is also an opportunity to explore and navigate peer relationships, gender norms, sexuality and economic responsibility (STOP AIDS, 2016). Youth access to ART is not well known because data are aggregated between children under 15 years of age and adults over 15 years of age. For those who have access to treatment, services for adolescents are rarely available and health care providers often have little experience in providing services to youth. They may not understand the needs of adolescents living with HIV and may have critical attitudes towards those who are sexually active. Failure to follow good practices and provide age-appropriate care in this area has resulted in low retention rates among adolescents compared to other age groups (MacPherson et al., 2015). Even once treatment has been accessed, some populations are more likely to be lost from care than others, e.g. younger women and those just initiating ART (Rollins *et al*., 2017).

In search for universal contact of mother/infant pairs within a health facility that could serve as an opportunity for a responsive PMTCT intervention, the second visit of the Expanded Program on Immunization (EPI) seems the most appropriate. Indeed, as in most African countries, early steps of EPI are particularly successful in attracting almost all mothers and infants, regardless of their previous attendance to ANC or their location of delivery/birth (health facilities or at home). Zambia is one example with a reported attendance rate of 80-90% of the second EPI visit (UNICEF).

Various studies have demonstrated growth retardation in HIV-exposed uninfected children compared with unexposed children (Omoni 2017; Le Roux 2019; Jumare 2019, Rosala-Hallas 2017). These studies have been conducted on populations differentially exposed to ARVs due to the constant evolution of HIV treatment and prevention. The assessment of stunting in children exposed to single PrEP (lamivudine: interventional arm) versus 3 drug prophylaxis (AZT/3TC/NVP: standard of care – control arm) appears to be of primary importance as an integral part of the benefit/risk analysis of both strategies.
In Zambia, according to the Zamphia 2016 report, the majority of HEU children are breastfed more than 12 months. Zambia's 2020 consolidated guidelines for the treatment and prevention of HIV infection recommend serological

testing at M18 and M24 for HIV exposed children, thus **HIV transmission during late breastfeeding is a possibility that can be assessed at this time points**

Early ART initiation in HIV-1-infected infants confers an undisputable clinical benefit compared with deferred initiation (Kuhn, 2017). The extended follow-up of the CHER trial has demonstrated that early ART confers a persistent gain on reducing the size of HIV-1 reservoirs in children, confirming the utmost importance of diagnosing paediatric HIV-1 early and of initiating ART immediately (Payne, 2015).

6 STUDY DESIGN

6.1 OVERALL DESIGN

- Component 1: To monitor the 'real life' efficacy of the PMTCT cascade up to EPI-2 visit
- Component 2 and 3
  - Incorporating the use of POC tests and provision of single PrEP to high-risk children into routine EPI-2 visits, may help achieve the goal of pediatric elimination and can improve the prognosis of HIV-infected children by facilitating early ART.
- Component 3
  - Phase III trial
  - Multicenter, Parallel, Open-Label, Randomized Controlled Trial
  - A description of methods used to minimize bias are listed in **Section 8**
  - Parallel, two arms-randomization at a 1:1 ratio: Control and Intervention.
- Component 4
  - Observational phase

5.1.1 Scientific Rationale for Study Design

This study primarily aims at improving existing strategies to prevent postnatal HIV transmission through optimizing the use of lamivudine as pre-exposure prophylaxis (PrEP) for high-risk children. For this purpose, recently marketed diagnostic POC tests will be used at the first level of care for maternal HIV viral load and infant diagnosis. This POC early infant diagnosis will allow optimising treatment of HIV-infected infants by offering to all the benefits of an early ART initiation. Therefore, the call scope of "strategies for equitable and full scale access to diagnostics, prevention and treatment intervention" will be addressed. In addition, this strategy is designed to be reproducible in all mother & child health clinics, hence the choice of sites in West Africa and South-East Africa to ascertain it in different cultural, epidemiological and health system organisation contexts.

According to recent reports from various countries, up to 50% of mothers who initiated ART during pregnancy stop taking their drugs before 12 months (UNAIDS 2015). For those infants, the risk of HIV-1 is very high, and no alternative strategy is available to reduce this risk (Van de Perre, 2013). However, in the vast majority of sub-Saharan African countries, all mothers attend the second immunization (EPI) visit when their child is 6 to 8 weeks old (the first EPI visit for BCG and OPV_0 vaccination, takes place at childbirth, which is too soon for this rescue strategy).
This EPI-2 visit represents a unique opportunity to:

- Identify HIV-1-infected children early and,
- Optimize the ART of HIV-1-infected mothers and also provide their children with an antiretroviral drug that can protect them from HIV-1, irrespective of their mother adherence to ART.

In search for universal contact of mother and infants with a health facility that could serve as an opportunity for a responsive PMTCT intervention, the second visit of the Expanded Programme on Immunization (EPI) seems the most appropriate. Indeed, as in most West African countries, early steps of EPI are particularly successful in attracting almost all mothers and infants, regardless of their previous attendance to ANC or their location of delivery/birth (health facilities or at home).

Additionally, Zambia's 2020 consolidated guidelines for the treatment and prevention of HIV infection recommends serological testing at M18 for HIV exposed children. This time points serves as an opportunity to reassess the risk HIV acquisition during late breastfeeding; to conduct growth monitoring in children that have been differentially exposed to ARVs, that could potentially cause stunting in HIV exposed children.

The risk of the intervention and control arm are acceptable due to the clinical equipoise explained in the rationale. For the control group, the most important point is for HIV-infected mothers to be compliant and adhere to the ART treatment and counselling provided by benefiting from the national prevention programme, and POC HIV-1 testing (using DNA PCR) will be performed for every child (result available in about 2 hours) at 6-8 weeks, 6 months and 12 months post-partum so that HIV-1-infected children are referred to the National Programme for confirmed diagnosis and immediate ART initiation. In Zambia, triple-drug prophylaxis will be given to children until the mothers are proven fully suppressed according to the 2020 guidelines.

5.1.2 Justification for Dose

The Intervention group will be administered Lamivudine (10mg/ml) as per the following weight bands:

7.5 mg (0.75 ml) twice daily if 2 to 4 kg
25 mg (2.5 ml) twice daily if 4 to 8 kg
50 mg (5 ml) twice daily if > 8 kg

The doses will be adapted to the weight at each visit. Lamivudine administered per the above weight bands was excellently tolerated in the PROMISE-PEP/ANRS 12174 trial (Nagot, 2016).

5.1.3 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3. The end of the study is defined as completion of the last visit or procedure shown in the SoA.

6.2 STUDY POPULATION

5.2.1 Inclusion Criteria

INCLUSION CRITERIA FOR COMPONENT 1

A mother/infant pair will be included in the component 1 if the infant:

- **Has a mother who**:
    - Is the accompanying person to visit 2 of the EPI
    - Is 15 years of age or older
    - Has signed the consent form to participate in Zambia by herself and a witness (if illiterate)

COMMON INCLUSION CRITERIA FOR COMPONENT 2/3

A mother/infant pair will be included in the component II/III if the infant:

- Is a singleton
- Is breastfed at around 2 months (between 5 and 16 week-old) and the mother intends to continue breastfeeding until her child is 6 months old
- **Has a mother who**:
    - Is the accompanying person to visit 2 of the EPI

- Is 15 years of age or older (in Zambia) and 20 years of age or older (in Burkina Faso)

or

- If between 15 and 19 years of age (inclusive) in Burkina Faso, and is accompanied by a referent adult of her choice representing her interests and the interests of the child (parent, family member or guardian, member of an association, etc.)
- Has been confirmed to be infected with HIV-1 (with or without HIV-2)
- Has signed the consent form to participate

For the mother in Zambia, the consent must be signed by herself and a witness (if illiterate); For the mother in Burkina Faso, the consent must be signed by herself and a witness (if illiterate) and/or a referent adult (if under 20 years of age in Burkina Faso).

For the child in Zambia, the consent must be signed by the mother. For the child in Burkina Faso, the consent must be signed by the mother and/or a referent adult (if under 20 years of age in Burkina Faso. In Burkina Faso, both parents need to sign the consent unless the mother exercises sole parental authority or if obtaining the father's consent is likely to endanger the mother and her child. In Zambia, the mother exercises sole parental authority.

- **SPECIFIC INCLUSION CRITERIA FOR COMPONENT 2**

A mother/infant pair will be included in the component 2 if the infant:

- Has a positive HIV-1 PCR POC test at visit 2 of the EPI

- **SPECIFIC INCLUSION CRITERIA FOR COMPONENT 3**

A mother/infant pair will be included in the Phase III (3) if the infant:

- Has a negative HIV-1 PCR POC test at visit 2 of the EPI

- **SPECIFIC INCLUSION CRITERIA FOR COMPONENT4**
  - Component 3 participants

5.2.2 Non-Inclusion Criteria for component 2 and 3

A mother-child couple will not be included if the child:

- Has clinical symptoms or biological abnormalities of DAIDS classification 3 or 4 for adverse events on the day of inclusion
- Has a severe congenital malformation
- Has a known allergy to the study drug or its components
- Takes emtricitabine concomitantly
- **Has a mother:**
  - living outside the study area or intending to move from the area within the next 12 months
  - participating in another clinical trial

5.2.3 Lifestyle Considerations

During this study, participants are asked to attend counselling sessions per the clinic's usual practice such as breastfeeding practices, ART adherence, family planning and best nutrition practices.

5.2.4 Screen Failures

Screen failures are defined as participants who consent to participate in the **Phase III** clinical trial, but are not subsequently randomly assigned to the study intervention or control in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, the questionnaire completed during Component 1 and any serious adverse event (SAE).

5.2.5 Strategies for Recruitment and Retention

Recruitment
For component 1, this study expects to screen approximately 37,000 mothers/infants attending EPI-2 (about 20,000 in Burkina Faso and about 17,000 in Zambia) over a period of 12 months. According to existing PMTCT programme data, screening 37,000 mothers/infants will allow diagnosing approximately 48 HIV-1-infected infants at EPI-2 (Component 2), as well as randomizing 2000 uninfected infants (about 1750 in Zambia and about 250 in Burkina Faso) (1,000 per arm) born to HIV-1-infected breastfeeding mothers aged 15 years or older who meet the eligibility criteria (Component 3 – Phase III).

In Zambia, in 2017, the 4 Health centres taking part in the study reported an HIV prevalence of 10%; cumulatively saw over 29 000 women attending ANC; over 20,000 mother attending EPI visits for their children and close to 3000 HIV exposed and HIV Infected children. These figures can be reached given the EPI-2 attendance rates in the selected sites. Based on 2017 data, it is estimated that the recruitment of HIV-infected mothers can be achieved in 12 months, accounting for a conservative 15% of women who will refuse participating in component 1 (evaluation of the PMTCT cascade) and who will therefore not be considered for the trial. To be safe, the present study allows for a 17-month screening period including 11 months recruitment period from June 2020 in order to have the 600 participants per arm needed for the non-inferiority analysis on the efficacy of a single-drug versus triple-drug prophylactic regimen. As a result, these figures can be reached given the EPI-2 attendance rates in the selected sites in Zambia.

In Burkina Faso, according to 2015 data from the existing programme, the screening of about 25,000 mother/infant pairs during the second visit of the EPI (EPI-2) at the Dô and Dafra health care clinics (CSPS) will allow the enrolment of 250 uninfected infants born to HIV positive mothers aged 15 years or older in a recruitment period of 12 months.

Two thousand participants will be engaged in the follow up process of the Phase III RCT. According to previous experience (Nagot, 2016) in the PROMISE-PEP study, which was implemented at the same sites by the same team, a dropout rate of less than 10% is expected during the first year of follow up (date of the primary outcome measurement).

Retention
The main retention strategy will consist on providing thorough and ongoing site training. If site staff is properly trained, they will be able to better advise and counsel patients on the importance of adhering to protocol visits, adherence to medication and best health practices.
Appointments will be scheduled in advance even if care providers may also phone participants. SMS reminders will be sent to participating mothers if they consented to receive SMS reminders during the informed consent process. In order to preserve confidentiality, the participant will receive a standard message: "Madam, the next appointment will take place in 2 days". A follow-up visit will take place when the child is 6 months old (4 months after the first visit) for participants in both arms, control and intervention. For participants on PrEP, monthly visits will be needed for PrEP resupply and accountability and to check on the participant's health. It is important that participants attend each visit. If the mother does not show up for her scheduled appointment, then the research staff will call her the next day, in she specifically agreed on the consent form, to arrange another appointment. Appointments need to be arranged before participants run out of medication. The End of intervention will take place at Month 12, after 10 months of follow up.

Treatment adherence will be further enhanced by a dedicated counselling during the monthly visits (drug resupply and adherence assessment) and the ongoing availability of the EPI network for counselling and support.

7 STUDY DRUG

7.1 STUDY DRUG(S) ADMINISTRATION

6.1.1 Study Drug Description

Lamivudine is available for HIV treatment as a generic paediatric suspension (10mg/ml) in Burkina Faso, through the national HIV control programme. In Zambia lamivudine is purchased from GMP-compliant Drug Companies.

6.1.2 Dosing and Administration of The Study Drug

The lamivudine oral suspension will be administered using commercial syringes adapted to the different dosages, and a mark will be affixed to these syringes by the care provider to indicate where to pull the plunger for each dose according to the baby's weight assessed at each visit.
Each dose should be given to the child at regular times, spacing each dose by about 12 hours as follows:

- 7.5 mg (0.75 ml) twice daily if 2 to 4 kg
- 25 mg (2.5 ml) twice daily if 4 to 8 kg
- 50 mg (5 ml) twice daily if > 8 kg

7.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Acquisition and Accountability

In Zambia, generic lamivudine will be purchased from GMP-compliant Drug Companies that supply ARVs to University Teaching Hospital. In Burkina Faso, lamivudine is available through the national HIV control program.
The bottles of lamivudine oral solution used will be returned to the site for monitoring of drug compliance and to protect the environment of inadequate disposal of medicines.

6.2.2 Formulation, Appearance, Packaging And Labelling

Lamivudine pediatric Oral Solution USP is a clear, colorless to pale yellow, strawberry-banana flavored liquid, contains 10 mg of lamivudine in each 1 ml in bottles with child-resistant closures. The lamivudine bottles will contain 100 ml or 240 ml depending on the child's weight. The lamivudine bottles bought locally for the purpose of the study will be re-labeled for the study at the UTH Pharmacy in Zambia and at the Centre Muraz in Burkina Faso before being shipped to the different sites. Procedures will be explained in the manual of operations.

6.2.3 Product Storage and Stability

Store at 20° to 25°C (68° to 77°F); excursions permitted to 15° to 30°C (59° to 86°F).

6.2.4 Preparation

This product does not require reconstitution.

7.3 STUDY DRUG COMPLIANCE

Consenting HIV-1 positive mothers with HIV-1 negative children will be randomized during the EPI-2 visit. Following Randomization to the Intervention arm, lamivudine syrup will be supplied by the care provider at the study clinic to "high-risk" children with mothers having plasma HIV mRNA load ≥ 1,000 cp/ml. Similarly, during the follow up visit at 6 months of age, lamivudine syrup will be supplied by the care provider at the Study clinic to children that were "low-risk" during the EPI-2 visit, but have been classified as "high-risk" following the viral load monitoring at Month 6.

Care providers in the Study clinics will have been trained in the protocol, the study medication and the study documentation during the formative phase. The first dose of the drug will be given by the mother, under supervision of the care provider. This will ensure that the mothers have understood properly how to give the drug to their babies and will have done it themselves.

The care provider will be accountable for the study drug, and will manage the drug supplies at each site.
Mothers with children on PrEP (lamivudine) will be instructed to perform Monthly visits for the resupply of the child's PrEP and in order to answer follow up questions on the mother's health, the child's health, to assess if the child is growing well and if the child is taking the treatment adequately. The care provider will counsel and review with the mother any possible adverse events, adherence to the study drug through questionnaires and bottle return and concomitant medication at every visit. Mothers will be encouraged to contact the caretaker if the baby experiences any unusual conditions at any time, either during planned study visits, unplanned (unscheduled) visits and/or by phone.

In addition, to fully assess the rates of adherence under different intervention conditions, aggregate data will be collected from all health centres about missed follow-up visit and End of Study intervention visits (at M6 and M12, respectively) and missed dispensations of ART and PrEP drug during Monthly visits.

In case of a study drug interruption of 2 weeks or more, a HIV-1 DNA PCR test Xpert® (HIV-1 Qualitative by Cepheid) will be performed before study drug reintroduction. Children tested positive will be immediately referred to the paediatric HIV unit for a confirmatory diagnosis (using a different sample and test) and early initiation of ART as applicable according to national program.

7.4 CONCOMITANT THERAPY

Concomitant medications initiated and changes in dose are to be reported in the electronic Case Report Form (eCRF). Concomitant medications include prescription medications (including Cotrimoxazole as per national guidelines), over-the-counter medications, supplements and traditional medications (such as traditional herbs).

Forbidden medications:
Lamivudine 10mg/ml Oral Solution should not be taken with other medications containing lamivudine (used to treat HIV infection or hepatitis B infection), emtricitabine, combination of tenofovir and abacavir or tenofovir and didanosine (medicines used to treat HIV infection), or large doses of cotrimoxazole or trimethoprim (an antibiotic), or cladribine (used to treat hairy cell leukaemia), or sorbitol (per current version of SmPC of EPIVIR (lamivudine).

ARV Treatment for HIV Positive Children
HIV-1 children with a confirmed positive status will be referred to the national programme and receive ART as per national guidelines.

8 STUDY DRUG DISCONTINUATION AND PARTICIPANT DISCONTINUATION/ WITHDRAWAL

8.1 DISCONTINUATION OF STUDY INTERVENTION

The administration of lamivudine should be discontinued immediately if clinical signs, symptoms or laboratory abnormalities suggestive of pancreatitis appear. The administration of lamivudine may also be stopped if necessary, depending on the doctor's decision. Lamivudine administration may also be discontinued after the confirmed end of breastfeeding. Breastfeeding will be considered to be ceased if the mother confirms she is no longer breastfeeding for 2 consecutive monthly visits.

However, discontinuation from the study treatment, lamivudine, does not mean discontinuation from the study. The remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrolment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

8.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants may withdraw from the study at any time without impact to their care. They may also be discontinued from the study at the discretion of the Investigator. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance as per the criteria of the IDSMB.
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
-
- If the participant meets a non-inclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- The death of the mother if the legal representative of the child decides to discontinue. If the legal representative of the child agrees to continue, the child will be followed up until Month 12 of age.
- New information concerning the study becomes available, especially information that is likely to affect the participant’s decision to continue in the study or that affect the risk benefit ratio or the participant’s best interest in the opinion of the participant or the Investigator.

The reason for participant discontinuation or withdrawal from the study will be recorded on the electronic Case Report Form (eCRF).

8.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if she continues to be unreachable at his M12 visit despite the effort made to contact her.
In order to reduce the risk of loss to follow up, care providers may phone participants and reminders could be sent via SMS if the participant has consented to them. Participants will only receive SMS messages if they explicitly agree when providing informed consent. In order to preserve confidentiality, the participant will receive a standard message: "Madam, the next appointment will take place in 2 days".

- Contact attempts should be documented in the participant’s medical record or study file.

- Should the participant continue to be unreachable at her M12 visit, he or she will be considered to have withdrawn from the study with a primary reason of loss to follow-up.

9 MEASURES TO MINIMIZE BIAS

This is an open-label study. It was decided to not design a double blinded trial since in order to implement and evaluate the responsive intervention under real life conditions. Although blinding with a placebo could be envisaged for the infant prophylaxis, it could not be possible for the viral load POC test. The potential consequences of this lack of blinding are minimized by an objective/biological measurement of the primary outcome, but raise a potential of contamination between arms (cf. below). Other potential biases anticipated in this study will also be addressed:

9.1 SELECTION BIAS

A selection bias could occur if:

- Not all women were screened during the second EPI visit for their child. The condition for a 'responsive' strategy is to cover all mothers attending the EPI visit. Therefore, a formative research phase will be implemented to discuss with the EPI staff how to accommodate this additional activity in their routine work. The aim is therefore to adopt an 'opt out' approach for HIV screening in Burkina Faso and a 'opt in' approach in Zambia. All HIV-infected mothers will then be seen by a health provider for study information, consent and enrolment. Any difficulty towards this 'completeness' goal detected during the study implementation will be discussed with the team and solutions implemented.

9.2 THE RATE OF LOST TO FOLLOW-UP IS HIGH

The two teams in each country are experienced in conducting PMTCT randomized controlled trials. Contact details and a buddy system may be put in place to ensure a similar achievement in this study.

9.3 INFORMATION BIAS

Information biases are unlikely. The POC HIV tests to be used (Cepheid) are well validated with excellent comparison against standard methods. All clinical study information shall be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification.
Quality assurance & quality control systems using key indicators that will be described in the Manual of Operations will focus on adherence to the protocol, protecting the rights and safety of study participants, obtaining complete follow-up information on all enrolled participants, and maintain high standards for data quality.

9.4 CONTAMINATION BIAS

The risk of contamination between arms concerns mainly mothers from the control arm willing to benefit from HIV viral load for them or for infant PrEP. This risk will be minimized by careful information of the study at enrolment. The research staff in charge will insist that; i) the important point is for her to be compliant with her ART regimen if she wants to reduce the risk for her child to be HIV-infected, ii) she benefits from the national prevention programme. Children will have POC HIV-1 testing (using DNA PCR) done at 6 months and 12 months study visits and results will be available in about 2 hours. Mothers in the control arm will have access to know their HIV-1 viral load via their ART clinic as per current practice.

9.5 CONFUSION BIAS

Confusion bias should not occur since the study is randomized. However, any baseline imbalance on the main maternal and infant characteristics between arms will be checked. If any occur, it will be adjusted for in the statistical analyses using appropriate models.

Randomization

At each site, the eligible infants will be allocated to one of two arms by a trained research nurse using a centralized randomization scheme incorporated in the eCRF solution (REDCap). The randomization arms will be open labelled. The randomization list will be elaborated by an independent statistician in Montpellier using a 1:1 ratio, stratification by site (site meaning district centre – such as CMA in Burkina) and permuted blocks of size 4 and 6.

In practice, randomization will be performed after the consent form for component 2/3 being signed and blood samples from mother and child collected for GenXpert (same blood samples are needed for both arms). This decision was taken in order to shorten the duration of participant visits as GenXpert QUAL and VL (for the intervention group) will be performed at the same time and not one after the other.

10 STUDY ASSESSMENTS AND PROCEDURES

10.1 STUDY SITES

PROMISE is a research consortium consisting of the following countries: Burkina Faso, Uganda, Zambia, South Africa, France, Sweden, Norway. The first study conducted was in 2005. Two major studies have been conducted so far where Zambia has been involved: The **PROMISE EBF** - **Prom**oting **I**nfant health and nutrition in Sub-Saharan Africa: **S**afety and **E**fficacy of **E**xclusive **B**reast-**F**eeding promotion in the era of HIV, from 2005-2008; the **PROMISE PEP** - **Prom**oting **I**nfant health and nutrition in Sub-Saharan Africa: **S**afety and **E**fficacy of infant **P**eri-**E**xposure **P**rophylaxis with lamivudine to prevent HIV-1 transmission by breastfeeding, from 2008 to 2011. The current study PROMISE EPI - **Prom**oting **I**nfant health and nutrition in Sub-Saharan Africa: **S**afety and **E**fficacy of Prevention of Mother-To-Child Transmission of Hiv-1: Programme Evaluation And Innovative Responsive Intervention Integrated In The **E**xpanded **P**rogramme Of **I**mmunization will run from 2019 to 2021.

In order to ensure the study findings can be generalized in most African settings, we chose the study sites in two distinct cultural, geographical, epidemiological and health system contexts: Zambia and Burkina Faso were selected based on their recruitment capacity, HIV-1 prevalence representative of the South-East (Zambian) and West African (Burkina Faso) environment, PMTCT management, study team experience with previous trials and option B + implementation by the National Program; this choice was facilitated by the long history of collaboration within the PROMISE consortium. The full list of study sites and team members will be detailed in the Manual of Operations.

Study Setting - Zambia

Zambia is divided into 10 administrative provinces and 105 districts. Health management is done through provincial health offices (PHOs) (10), DHOs (105), and statutory bodies. The country has eight third-level hospitals, 34 second-level hospitals, 99 first-level hospitals, 1,839 health centres, and 953 health posts. All third-level hospitals are Government owned. Of the second- level hospitals, 26 are Government-owned, and eight are owned by the Churches Health Associations of Zambia (CHAZ). (NHSP, 2017).

The proposed study will take place at 5 health facilities: Chilenje (First Level Hospital), Bauleni (Health centre), and Matero Health Centres (First Level Hospital), Chaisa, (and Mount Makulu as back-up site), which are all offering maternal child health (MCH) services. Among the MCH services include EPI services, HIV Counselling, Testing and Treatment facilities.

The University Hospital Teachings (UTHs). A government funded tertiary hospital, having a total bed capacity of 2,000. It primarily provides clinical care through its five hospitals namely: Lusaka Children's Hospital, the adult hospital, Maternal and New-born Hospital, The Eye Hospital and The Cancer Diseases Hospital. All these hospitals provide both outpatient and inpatient care facilities. It is also an academic medical centre/University Teaching Hospital hosting the University of Zambia – School of Medicine UNZA-SOM). The UTHs is the major tertiary referral institution for the whole country - including the 30 primary and secondary health centres within Lusaka which serve a population of about two million people, 50% of whom are children aged less than 15 years and 20% women of childbearing age. The UTHs will serve as central administration for the study as well as housing the laboratory and the pharmacy.

Study Setting – Burkina Faso

In Burkina Faso, the study is carried out in 2 districts of Ouagadougou: Baskuy and Boulmiougou and in 2 districts of Bobo-Dioulasso: Do and Dafra

Baskuy health district is one of five districts in the central region. Six Health and Social Promotion Centers (CSPS), all located in urban areas are involved for Component 1 and a Medical Center (CM): Goughin 6, carries out the Component 2 and 3 activities .

The Boulmiougou health district is one of the five districts in the central region. The district has forty Health and Social Promotion Center (CSPS): Eleven are located in urban areas and twenty-nine in rural areas. Eleven CSPS are involved for Component 1 and a Medical Center with surgical Antenna (CMA): Pissy, , carries out the Component 2 and 3 activities.

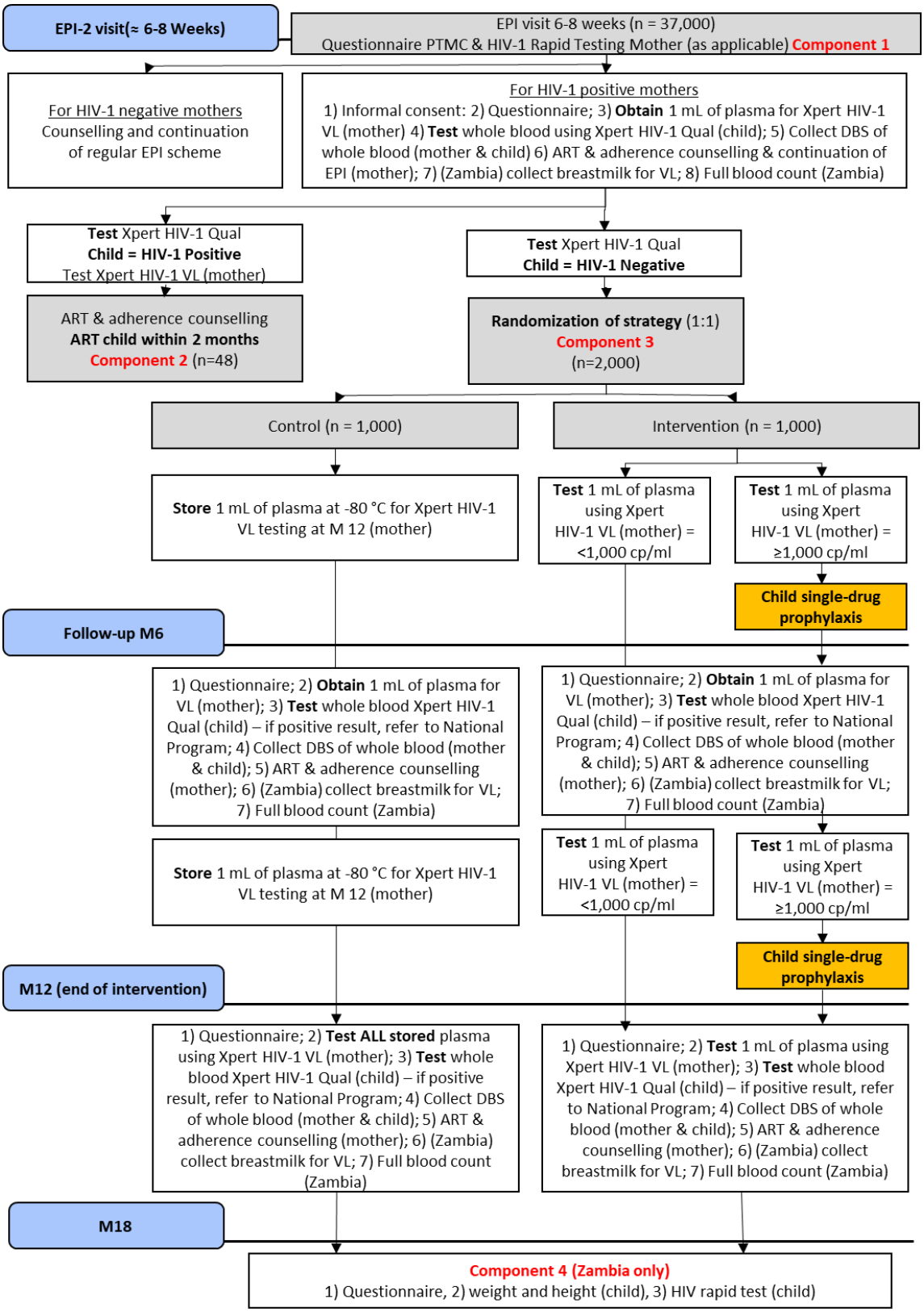
Bobo-Dioulasso, the second largest city in Burkina Faso, now has an estimated urban population of 661,455. The B+ strategy (ART for all pregnant and lactating women infected with HIV) was successfully launched in 2015 in the city, which includes two urban districts, Dô and Dafra.
In these two districts, the following data were collected for the year 2015 in prenatal consultation services (ANC), involved in PMTCT/HIV for option B + (Source: Regional Health Directorate, Bobo-Dioulasso):

The residual rate of HIV transmission in their baby remains high (7% at 2 months and an additional 8% transmission rate at 18 months, peaking at an overall transmission rate of 15%).
Accurate analysis of these data is difficult for several reasons: not all infants born to HIV-infected mothers could be followed, only some of them had access to HIV-1 (PCR DNA) testing at 2 months of age and some of the children tested for HIV-1 at 18 months of age were born in 2014, before Option B+ was launched. However, data collected during the first quarter of 2016 showed very similar transmission figures at 2 months (5/136, 4.4%) and 18 months (7/77, 9.1%), suggesting that the postnatal transmission rate reaches a high level at a time when an increasing number of HIV-1 infected mothers have received ART.

In search of universal contact of mothers and infants with a health facility that could serve as an opportunity for a "catch-up" PMTCT intervention, the second visit of the Expanded Programme on Immunization (EPI) was identified as an ideal entry point. Indeed, as in most West African countries, the first stages of the EPI are particularly effective in attracting almost all mothers and infants, regardless of their prior participation in NPC or their place of delivery (health facilities or home). In the Upper Basins region of Burkina Faso - of which Bobo-Dioulasso is the capital - nearly 100% of 8-week-old infants (around 2 months) accompanied by their mothers attend the second EPI visit (for polio vaccine, pneumococcus, rotavirus and combined diphtheria-tetanus-pertussis-hepatitis B- Haemophilus influenza). The attendance rate decreases slightly thereafter (visit 3 of the EPI at 12 weeks, visit 4 of the EPI at 16 weeks).

Through preliminary discussions between local researchers, the main managers of EPI centres and the Regional Health Directorate, the implementation of a strategy to "catch up" with PMTCT in EPI centres was appreciated by the various stakeholders, was considered very feasible and was well received.

10.2 STUDY FLOWCHART



- In Zambia, according to the 2020 guidelines, HIV exposed uninfected infants receive a triple-drug prophylaxis (zidovudine, lamivudine and nevirapine) until the mothersare proven virally suppressed. In the intervention group, triple-drug prophylaxis will be switched to lamivudine PrEP in case of the mother is virally unsuppressed at EPI-2. In case of the mother is virally suppressed at EPI-2, the mother/child pair will be referred to the ART clinic for triple-drug prophylaxis discontinuation.
- The 2020 guidelines in Zambia recommend quarterly viral load testing for breastfeeding mothers. For the sake of equity, in Zambia, the mothers from our intervention arm will be encouraged to perform the M9 viral load as recommended by the national guidelines (provided by the national program). EPI-2, M6 and M12 viral load measurements are already performed within the study.
- In Zambia, viral resistance genotyping will be performed for the children with a positive HIV-1 PCR at M6 and M12

10.3 SCHEDULE OF ASSESSMENTS

Schedule of assessments for the mother

Procedures for Mother	**Screening Component 1** (≈6-8 weeks post-partum)	**ART Treatment HIV-1 positive child Component 2** (≈6-8 weeks post-partum)	**Phase III Component 3** (≈6-8 weeks post-partum)	**Visits M3, M4 & M5 (if child on single-drug PrEP)**	**Month 6**	**Visits M7, M8, M9, M10 & M11 (if child on single-drug PrEP)**	**Month 12 /End of intervention**	**Component 4 M18 (Zambia)**
Acceptable visit window (days)			**+ 7**	**± 7**	**± 14**	**± 7**	**± 14**	**± 14**
Visits								
Verbal Consent (Opt out) in Burkina Faso and opt in consent in Zambia and PMTCT Questions C1	X							
Eligibility of mother for C2/C3	X							
Informed Consent for C2/C3		X	X					
Socio-demographic data		X	X					
Medical History		X	X					
SAEs linked to blood drawing		X	X		X		X	
Counselling (ongoing)		X	X	X	X	X	X	
Laboratory Tests								

HIV-1 serology – rapid test**	X							
HIV-1 PCR and stored DBS***	X°	X	X		X		X	
Lactoserum (only in Zambia)****		X	X		X		X	

Schedule of assessments for the infant

Procedures for the Child	**Screening Component 1 (EPI- 2)**	**ART Treatment HIV-1 positive child Component 2 (EPI- 2)**	**Phase III Component 3 (EPI- 2)**	**Visits M3†, M4 & M5 (if child on single-drug PrEP)**	**Month 6**	**Visits M7, M8, M9, M10 & M11 (if child on single-drug PrEP)**	**Month 12 /End of intervention**	**Component 4 M18 (Zambia)**
Acceptable visit window (days)			**+ 7**	**± 7**	**± 14**	**± 7**	**± 14**	**± 14**
Child Visits								
Eligibility of infant for C2 or C3		X	X					
ART Treatment if child is confirmed HIV-1 positive (C2) according to national guidelines		X						
Randomization		X (mis-randomised)	X					
Intervention arm only: Administer 1st dose of lamivudine PrEP to children of mothers with a VL ≥ 1000 copies/mL			X (if mother ≥ 1000 copies/mL at 6-8 weeks) †		X (if mother ≥ 1000 copies/mL at 6 months)			
Clinical Assessments		X	X		X		X	X
Anthropometry		X	X	Weight only	X	Weight only	X	X
Resupply medication (PrEP) based on weight				X	X	X	X	

Adherence Assessment				X	X	X	X	
Adverse Event Assessment		X	X	X	X	X	X	X
Concomitant Medication Collection			X	X	X	X	X	X
Breastfeeding/ Feeding Review *		X	X	X	X	X	X	X
Laboratory Tests								
HIV-1 DNA PCR and stored DBS*****		X	X		X		X	
Full blood count*****			X		X		X	
HIV rapid test								X

*Resupply medication (lamivudine PrEP) based on weight during breastfeeding.
*Definition of end of breastfeeding = 2 consecutive visits where mother confirms the end of breastfeeding
** HIV-1 rapid test (blood or saliva-based test following formative research recommendations) to be performed during EPI-2 (at 6-8 weeks post-partum) if previous HIV test was performed more than one month ago in Zambia and if the usual practice HIV-rapid testing results are not available at the time of the EPI-2 visit in Burkina Faso (within the last 3 months).
***Plasma will be obtained from mothers' 5ml of venous whole blood collected at 6-8 weeks, 6 and 12 months post-partum. In the control group, plasma will be stored at -80°C until Month 12. In the intervention group, point of care HIV-1 PCR (Xpert HIV-1 Viral Load, Cepheid) will be performed and results will be available in 2 hours) and in Zambia remaining plasma stored at -80°C. DBS collected for storage at 6-8 weeks, 6 and 12 months post-partum from whole blood for HIV-1 RNA quantification if requested and repository as quality control assessment.
****a minimum of 10 ml of manually-expressed milk from each breast to be collected only in Zambia from mothers in the control and intervention arms in component 3 at 6-8 weeks, 6 months and 12 months of age for storage of acellular and cellular fractions. Breast milk RNA and DNA HIV-1 viral load will be quantified using a test to be determined.
*****capillary blood collected from children at 6-8 weeks, 6 and 12 months of age, point of care HIV-1 PCR (Xpert HIV-1 Qualitative, Cepheid; done on 100 µl of venous whole blood; result available in 2 hours). DBS collected for storage at 6-8 weeks, 6 and 12 months post-partum from 400 µl capillary whole blood for detection of HIV infection by HIV-1 DNA PCR as quality control assessment and full blood count performed with the remaining capillary blood sample.
°For mothers with an indeterminate result by HIV rapid testing, a point of care HIV-1 PCR (Xpert HIV-1 Qualitative and Xpert HIV-1 viral load) will be performed.
°° viral resistance genotyping will be performed for the children with a positive HIV-1 PCR at M6 and M12† In Zambia, according to the 2020 guidelines, HIV exposed uninfected infants receive a triple-drug prophylaxis (zidovudine, lamivudine and nevirapine) until the mothersare proven virally suppressed. In the intervention group, triple-drug prophylaxis will be switched to lamivudine PrEP in case of the mother is virally unsuppressed at EPI-2. In case of the mother is virally suppressed at EPI-2, the mother/child pair will be referred to the ART clinic for triple-drug prophylaxis discontinuation.

10.4 DESCRIPTION OF STUDY VISITS

9.4.1 Information Component 1 (Day 0)

In Burkina Faso: Mothers attending the second visit of the Expanded Programme on Immunization (EPI-2) at the study sites are invited after information and expression of non-opposition to answer questions assessing their participation (or not) in the PMTCT Programme for their present child (Opt out approach). Mothers will then be

asked to perform a rapid HIV-1 test for HIV-negative women unless their last HIV test was done in the last 3 months in Burkina Faso.

In Zambia: Mothers attending the second visit of the Expanded Programme on Immunization (EPI-2) at the study sites in Lusaka are invited after information to sign an informed consent (opt in). Mothers will then be asked to perform a rapid HIV-1 test for HIV negative women unless a test had been performed in the previous month. .

For component 1, written informed consent (opt in) in Zambia and verbal consent (opt out) in Burkina Faso will be collected in the vernacular language of the mother, by investigators who would have undergone specific training.

For the mothers with an indeterminate result by HIV rapid testing a point of care HIV-1 PCR (Xpert HIV-1 Qualitative and Xpert HIV-1 viral load, Cepheid) will be performed to confirm their HIV status.

9.4.2 Informed Consent Process for component 2 and 3 (Day 0 To 7)

For HIV+ mothers who participated in Component 1: present the research: objectives, benefits and constraints for the mother and answer all questions. Check the eligibility criteria available (with the exception of the child's diagnosis and the mother's viral load). Before any specific trial procedure on Component 2 & 3 is performed, and after sufficient time for reflection, the investigator ensures that the person has understood the information contained in the information sheet.

Written informed consent for Component 2 & 3 will be collected in the vernacular language of the mother, by investigators (trained clinical officer and nurses) who would have undergone specific training.
Consent will include explicit authorization for the storage of blood samples (DBS) for overseas research.

In Zambia, the informed consent will be obtained with the assistance of an independent third party (the "trusted person" or "witness") for illiterate women. In Burkina Faso, the informed consent will be obtained with the assistance of an independent third party (the "trusted person" or "witness") for illiterate mothers or where translation is required. This witness will sign the consent form with the mother and the investigator. Participation in the study is free and voluntary. Any mother may withdraw her or her child from the study at any time and without giving any reason, and such withdrawal will not affect the level or standards of care offered to the mother or child. Further information on the informed consent process is provided in Section “Informed Consent Process”

9.4.3 Sample collection (Day 0 to 7)

Following the consent process, the signing of Component 2 & 3 consent by eligible mothers, blood samples from mother and child will be collected:

- For children, 2ml of capillary blood will be collected for the HIV-1 DNA PCR test Xpert® (HIV-1 Qualitative by Cepheid). Remaining whole blood sample will serve for full blood count and DBS for detection of HIV infection by HIV-1 DNA PCR as quality control assessment. Additional tests will be carried out on DBS: investigation on mitochondrial genotoxicity (sub-study described in appendices) and genotypes for ARV resistance in the instance of HIV-1 infected children.
- For mothers, 4 ml venous blood will be collected for point of care HIV-1 PCR (intervention group) or storage (control group) and for DBS for HIV-1 RNA quantification if requested and repository as quality control assessment.
- For mothers in Zambia only, breast milk will be collected for RNA and DNA quantification of HIV-1 viral load at 6-8 weeks post-partum in both randomization arms. Viral load will be measured. Breast milk RNA and DNA HIV-1 viral load will be quantified using a POC test at the end of the study. The remaining sample will be used for the sub-studies on microchimerism and on HIV reservoir (see appendices).

9.4.4 Randomization (Day 0 To 7)

The mother-child couples will be randomized at a 1:1 ratio to one of two study arms - Control and Intervention. The decision to randomize before knowing if the participant will be in component 2 or 3 was taken in order to shorten the duration of the participant visit as GenXpert QUAL and VL (for intervention group) will be performed at the same time and not one after the other.

9.4.5 Inclusion Screening for Components 2 &3 (Day 0 To 7)

For children, the process will be the same in the control arm and in the intervention arm:

- Children will be tested for HIV-1 using an HIV-1 DNA PCR test (Xpert® HIV-1 Qualitative by Cepheid) on 100 µl of capillary whole blood.
- The result of the HIV-1 diagnosis for the child will be available within 2-3 hours.
    - Children tested positive per the HIV-1 DNA PCR test (Xpert® HIV-1 Qual) belong to the component 2: they will be immediately referred to the paediatric HIV unit for a confirmatory diagnosis (using a different sample and test) and early initiation of ART as applicable according to national program. For these participants no more visit is planned within the study*.
    - Children tested positive per the HIV-1 DNA PCR test (Xpert® HIV-1 Qual) belong to the component 3 (phase III trial)

For mothers:

- In the control group: the plasma collected during the EPI-2 visit will be stored at -80°C until Month 12 (at Month 12 results will be shared) excepted for the mothers of children testing positive per the HIV-1 DNA PCR test for which the point of care HIV-1 PCR will be performed at this visit as no more visit and no intervention are planned for these participants
- In the intervention group: point of care HIV-1 PCR (Xpert HIV-1 Viral Load, Cepheid) will be performed and results will be available in 2 hours) (Ceffa, 2016):
    - Women with suppressed HIV-1 viral load (<1000 copies/ml by Xpert® HIV-1 Viral Load RNA) will be encouraged to maintain optimal ART intake, also a follow-up visit at 6 months and 12 months will be offered, as well as HIV-1 testing for their children, respectively by HIV-1 DNA PCR. The Zambian children on triple-drug prophylaxis at W6-8 will be referred to the ART clinic for triple-drug prophylaxis discontinuation.
    - For women with a unsuppressed plasma HIV-1 viral load whose baby is not infected with HIV-1: a care provider trained on the study will inform the mother of the correct administration of lamivudine in oral suspension (see Section 8.3.4). In Zambia, if the child is on triple-drug prophylaxis at EPI-2, the triple-drug prophylaxis will be switched to single-drug prophylaxis (lamivudine) during this visit. For the mother: she will be referred to adult HIV services (with viral load result) for immediate ART initiation or adherence enhancement.

The following data will be collected to ensure proper follow-up and will remain confidential. For the child: clinical assessment, anthropometry, date of birth, medical history, adverse events, concomitant medications, breastfeeding. For mother: socio-demographic data, year of birth, medical history, attendance at counselling sessions, PMTCT questions on breastfeeding, screening, ARV treatment, study procedure related AE.

*Children tested positive following Xpert HIV-1 Qual will be considered as mis-randomised as no randomisation should have been done for them because they belong to component 2. It was decided to randomize before GenXpert HIV-1 qualitative test been done in order to perform in the same time GenXpert HIV-1 qualitative for the child and GenXpert HIV-1 viral load for the mother of the intervention arm to shorten the duration of participant visits.

9.4.5 Treatment Administration at 6-8 Weeks (Day 0 To 7)

This visit corresponds to the first day of treatment for children in the intervention arm who are eligible for single PrEP because their mothers will have Viral Loads ≥ 1000 copies/ml.

During this visit at 6-8 weeks:

- The investigator will prescribe: lamivudine oral suspension for one month (until the next follow-up visit).
    - Dose: The first administration of the drug will be given by the mother under the supervision of a care provider trained on the study. A first supply of oral suspension lamivudine covering one month of PrEP will be provided.
- Unless consent is withdrawn, all participants will be followed, according to the schedule/schedule defined, until the end of the research, even if they interrupt their treatment.

9.4.6 Monthly Visit at M3, M4 And M5 (If Child is on single Prep)

For children prescribed lamivudine at M2, monthly visits will take place at M3, M4 and M5. During these visits:

- The following data will be collected to ensure proper follow-up and will remain confidential. For the mother: attendance at counselling sessions, PMTCT questions on breastfeeding, ARV treatment. For the child: adverse events, concomitant medications, breastfeeding review, adherence to single PrEP.
- Investigator will prescribe: oral lamivudine suspension for one month (until the next follow-up visit)
- PrEP accountability of lamivudine bottles
- Unless consent is withdrawn, all participants will be followed-up, according to the schedule/schedule defined, until the end of the research, even if they interrupt their treatment.

9.4.7 Follow up Visit at M6

A follow-up visit will be made at M6 for participants in both arms.

Children:

- Children will be tested for HIV-1 using an HIV-1 DNA PCR test (Xpert® HIV-1 Qualitative by Cepheid) on 100 ul of capillary whole blood
- The result of the HIV-1 diagnosis for the child will be available 2-3 hours later.
- Children testing positive per the HIV-1 DNA PCR test (Xpert® HIV-1 Qual) will be immediately referred to the paediatric HIV unit for a confirmatory HIV diagnosis (using a different sample and test) and early initiation of ART as applicable according to the national programme.
- For children, DBS will be collected using 400 µl of whole blood sample for detection of HIV infection by HIV-1 DNA PCR as quality control assessment. Additional tests will be carried out on DBS : investigation on mitochondrial genotoxicity (sub-study described in appendices) and genotypes for ARV resistance in the instance of HIV-1 infected children.
- Full blood count will be performed on the remaining blood sample.

Mothers:

- Plasma will be obtained from 5ml of mothers' venous whole blood collected at 6 months post-partum. In the control group, plasma will be stored at -80°C until the 12 Month visit. In the intervention group, point of care HIV-1 PCR (Xpert HIV-1 Viral Load, Cepheid) will be performed and results will be available in 2 hours) (Ceffa, 2016).
- In mothers from both arms, DBS collected from total blood by means of the same blood collection for HIV-1 RNA quantification if requested and repository as quality control assessment
- In Zambia only: Breast milk RNA and DNA quantification of HIV-1 viral load at 6 months post-partum from mothers in both randomization arms. Breast milk RNA and DNA HIV-1 viral load will be quantified using a POC test at the end of the study. The remaining sample will be used for the sub-studies on microchimerism and on HIV reservoir (see appendices).

The following data will be collected to ensure proper follow-up and will remain confidential. For the child: clinical assessment, anthropometry, adverse events, concomitant medications, breastfeeding. For mother: socio-demographic data, attendance at counselling sessions, PMTCT questions on breastfeeding and routine follow up regarding HIV, ARV treatment, study procedure related AE.

For children placed on lamivudine PrEP at M6:

- The investigator will prescribe: oral lamivudine suspension for one month (until the next follow-up visit)
- Dose: The first administration of the drug will be given by the mother under the supervision of a care provider trained on the study. A first supply of oral suspension lamivudine covering one month of PrEP will be provided.

For children already on lamivudine PrEP since M2:

- The investigator will prescribe: oral suspension of lamivudine for one month (until the next follow-up visit)
    - PrEP accountability of lamivudine bottles
- Unless consent is withdrawn, all participants included must be followed, according to the defined schedule/schedule, until the end of the research, even if they interrupt their treatment.

9.4.8 Monthly Visits at M7, M8, M9, M10, M11 (If Child is on lamivudine Prep)

For children who have been prescribed lamivudine at M2 or M6, monthly visits will take place at M7, M8, M9, M10 and M11. During these visits:

- The following data will be collected to ensure proper follow-up and will remain confidential. For the mother: attendance at counselling sessions, PMTCT, questions on breastfeeding, ARV treatment. For the child: adverse events, concomitant medications, adherence to PrEP.
- The investigator will prescribe: oral suspension of lamivudine for one month (until the next follow-up visit).
- PrEP accountability of lamivudine bottles
- Unless consent is withdrawn, all participants will be followed, according to the defined schedule/schedule, until the end of the research, even if they interrupt their treatment.

9.4.9 Visit at M12 or End of intervention

A visit at M12 will take place for participants in both arms.

Children:

- Children will be tested for HIV-1 using an HIV-1 DNA PCR test (Xpert® HIV-1 Qualitative by Cepheid) on 100 ul of capillary whole blood.
- The result of the HIV-1 diagnosis for the child will be available at the end of the visit.
- Children testing positive per the HIV-1 DNA PCR test (Xpert® HIV-1 Qual) will be immediately referred to the paediatric HIV unit for a confirmatory HIV diagnosis (using a different sample and test) and early initiation of ART as applicable according to the national programme.
- For children, DBS collected using 400 µl of whole blood sample for detection of HIV infection by HIV-1 DNA PCR as quality control assessment. Additional tests will be carried out on DBS: investigation on mitochondrial genotoxicity (sub-study described in appendices) and genotypes for ARV resistance in the instance of HIV-1 infected children.
- Full blood count will be performed on the remaining blood sample.

Mothers:

- Plasma will be obtained from 5ml of mothers' venous whole blood collected at 12 months post-partum. In both group, the plasma collected at M12 will be tested using point of care HIV-1 PCR (Xpert HIV-1 Viral Load, Cepheid) and results will be available in 2 hours).
- In mothers from both arms, DBS collected from total blood by means of the same blood collection for HIV-1 RNA quantification if requested and repository as quality control assessment.
- In Zambia only: Breast milk RNA and DNA quantification of HIV-1 viral load at 12 months post-partum from mothers in both randomization arms. Breast milk RNA and DNA HIV-1 viral load will be quantified using a POC test at the end of the study. The remaining sample will be used for the sub-studies on microchimerism and on HIV reservoir (see appendices).

The following data will be collected to ensure proper follow-up and will remain confidential. For the child: clinical assessment, anthropometry, adverse events, concomitant medications, breastfeeding. For mothers: socio-demographic data, attendance at counselling sessions, PMTCT questions on breastfeeding and routine follow up regarding HIV, ARV treatment, study procedure related AE.

For children already on lamivudine PrEP since M2 or M6:

The investigator will explain that the treatment by lamivudine for the child is ended. Children still at risk of HIV acquisition by breastfeeding (children still breastfed whose maternal viral load is unsuppressed at M12) will be referred to the national program for 3 drug prophylaxis initiation.

9.4.10 Visit at M18 in Zambia (Component 4: observational phase)

A follow-up visit will be carry out at M18 for all reconsented participants of Component 3 in Zambia.
Children will be tested for HIV using a serology test (HIV rapid test), as per national recommendations.

The following data will be collected:
- For children: clinical assessment, anthropometry, adverse events, concomitant medications, breastfeeding.
- For mothers: attendance at counselling sessions, PMTCT questions on breastfeeding and routine follow up regarding HIV, ARV treatment

9.4.11 Anticipated Therapeutic Management for Participants at The End of The intervention or Early Discontinuation

Women will be counselled from the beginning of the study on breastfeeding, the importance of ARV adherence and HIV transmission. It is anticipated that most of the mothers in the study will have controlled viral loads at 12 months. However, it is possible that some mothers may fail virologically despite advice on adherence and support. For the main analysis, the cut-off date will be the 12-month visit.

11 LABORATORY ASSESSMENTS

Component 1:

At Component 1 visit during EPI-2, at 6-8 weeks post-partum, the following laboratory procedures will take place:
Point of care HIV-1 rapid test (blood- or saliva-based test following formative research recommendations) to be performed at 6-8 weeks post-partum if previous HIV test was performed more than one month ago in Zambia and if f the national guidelines-mandated-HIV screening results are not available at the time of the EPI-2 visit in Burkina Faso (during the last 3 months).

If the first HIV-1 rapid test is positive, a second test using a different HIV-1 rapid test kit will be performed for confirmation (for example, DETERMINE HIV1/2 as first line and SD BIOLINE HIV-1/2 as confirmatory test).

For the mothers with an indeterminate result by HIV rapid testing a point of care HIV-1 PCR (Xpert HIV-1 Qualitative and Xpert HIV-1 viral load, Cepheid) will be performed to confirm their HIV status.

Component 2 & 3:

At 6-8 weeks, 6 months and 12 months post-partum, the following tests will be done:

- **In mothers:**
  - Plasma will be obtained from 5ml of mothers' venous whole blood collected. In the control group, plasma will be stored at -80°C until Month 12. In the intervention group, point of care HIV-1 PCR (Xpert HIV-1 Viral Load, Cepheid) will be performed and results will be available in 2 hours) (Ceffa, 2016).
  - In mothers from both arms, DBS collected from about 400 µl of total blood by means of the same blood collection for HIV-1 RNA quantification if requested and repository as quality control assessment.
  - In Zambia only: Breast milk RNA and DNA quantification of HIV-1 viral load from mothers in both arms. About 10mL will be collected from each breast. Breast milk RNA and DNA HIV-1 viral load will be quantified using a POC test performed at the end of the study. The remaining sample will be used for the sub-studies on microchimerism and on HIV reservoir (see appendices).

- **In infants born to HIV-1-infected mothers:**
  - In infants of both arms: capillary whole blood collected, point of care HIV-1 PCR (Xpert HIV-1 Qualitative, Cepheid; done on 100 µl of capillary whole blood; result available in two hours) (Ceffa, 2016).
  - In infants from both arms, DBS collected using 400 µl of whole blood sample for detection of HIV infection by HIV-1 DNA PCR as quality control assessment. Additional tests will be carried out on DBS: investigation on mitochondrial genotoxicity (sub-study described in appendices) and genotypes for ARV resistance in the instance of HIV-1 infected children.
  - Full blood count will be performed on the remaining blood sample.

Component 4: Children will be tested for HIV using a serology test (HIV rapid test), as per national recommendations.

At any point during the study, children testing positive per HIV-1 DNA PCR test (Xpert® HIV-1 Qual) will be immediately referred to the paediatric HIV unit for diagnosis confirmation (using a different sample and test) and early initiation of ART as applicable. In Zambia, infant confirmatory HIV diagnoses and viral loads will be done at the Reference Central Early Infants' Diagnosis laboratory (EID) attached to the Paediatric Centre of Excellence (PCOE at UTH-Children's Hospital). The turn-around time (TAT) for the EID and VL test is about 4 days, and the laboratory has -80^{0}C deep freezers for storage of specimen).

Samples Transfer to France

- DBS and milk samples will be transported to France for specific laboratory assessments not available in trial sites reference laboratories, either for exams planned in the protocol or for further ancillary/sub-studies.
- A Material Transfer Agreement will be submitted to regulatory authorities for approval.

12 SAFETY AND SAFETY REPORTING

12.1 DEFINITIONS

11.1.1 Definition of Adverse Events (AE)

An adverse event is any untoward or unfavourable medical occurrence in a human participant participating in the research, whether or not considered related to the participant's participation in the research

11.1.2 Definition of Adverse Reaction (AR)

An adverse reaction is any untoward and unintended response to an investigational medicinal product related to any dose administered.

11.1.3 Serious Adverse Event/Reaction (SAE/SAR)

A serious adverse event/reaction refers to any untoward medical occurrence or reaction that at any dose:

- Results in death;
- Is life-threatening (means that the participant was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- Requires hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect;
- Is a grade 3 or 4 biological or clinical event;
- Is an "important medical event" (medical events, based upon appropriate medical judgment, which may jeopardize the participant or may require medical or surgical intervention to prevent one of the above characteristics/consequences). Examples: allergic bronchospasm requiring intensive treatment at an emergency room or at home, blood dyscrasias, convulsions that do not result in inpatient hospitalization.

11.1.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)

An unexpected adverse reaction is an adverse reaction, the nature, the outcome or severity of which is not consistent with the applicable Reference Safety Information (RSI): Summary of the Product Characteristics (SmPC) of EPIVIR (lamivudine) 10mg/ml, oral solution.

11.1.5 New Fact

A new fact is defined as any safety data that could modify significantly the evaluation of the benefit/risk ratio of the investigational medicinal product or the clinical trial, likely to affect the safety of participants or that could modify the investigational medicinal product administration, the trial documentation or the conduct of the trial, or to suspend or interrupt or modify the protocol or similar trials. Examples: a serious adverse event which could be associated with the trial procedures and which could modify the conduct of the trial, a significant hazard to the participant population such as lack of efficacy of an investigational medicinal product used for the treatment of a life-threatening disease, recommendations of the IDSMB, if any, where relevant for the safety of participants.

12.2 RESPONSIBILITIES OF THE INVESTIGATOR

11.2.1 AE Notification

All biological and clinical adverse events reported for the infant, have to be reported in the proper electronic CRF AE form from the signature of the consent form. If a clinical or biological event is present at inclusion, only its aggravation will be notified.

11.2.1 SAE Notification to the Sponsor

What Needs to be Notified

For the infant, the investigator has to notify to the sponsor all Serious Adverse Events (SAE), except those that the protocol identifies as not requiring immediate reporting. For the mother, only the SAE related to the study procedure have to be reported.

SAE Not Requiring Immediate Reporting To The Sponsor

Hospitalizations or prolongation of existing hospitalizations that are not considered as serious:

- Outpatient care: the patient has been formally admitted to a hospital for medical reasons with no seriousness criteria (described above) and does not require overnight hospitalization;
- Elective or previously scheduled surgery or medical treatment;
- Hospitalization for social or administrative reasons;
- Pre-specified study hospitalization for observation.

Situation that are not considered as adverse event but as medical history:

- Pre-existing diseases or present conditions or detected at baseline and which do not worsen.

The investigator should collect all relevant documentation related to reported SAE (e.g. hospitalization report, laboratories results…) and send it to the sponsor, without omitting to make it anonymous (avoid any possible identification) and note the identification number of the participant in the trial.

The investigator must follow the participant until clinical recovery is complete and laboratory results have returned to normal or baseline, or until the event has stabilized. Follow-up should continue after completion of the trial if the SAE is possibly related to the study drug. All SAE must be reported since the participant signed the informed consent to the clinical trial and during the follow-up scheduled by the trial. SAE occurring to a participant after the treatment of that participant has ended should be reported to the sponsor if the investigator becomes aware of them. The investigator does not need to actively monitor participants for adverse events once the trial has ended, unless provided otherwise in the protocol.

Reporting Time Frames

SAE requiring reporting and all relevant documentation related to these SAE have to be sent by the investigator to the sponsor, immediately and no later than 24h after being made aware of it. The investigator should ensure that all relevant information is forwarded to the sponsor within 7 days after the initial notification.

How to Report

The initial report should be notified as a detailed, written report, using "SAE initial notification form" (in CRF SAE section). The initial report must include the minimum information following: the identifiable coded participant, the identifiable reporter, one AE, the study drug and a causality assessment. The initial report shall be followed by complementary detailed, written reports using "SAE complementary notification form" (in CRF SAE section).

Automated notification to sponsor via eCRF:

- **Notification via l'eCRF:**

All SAE/SAR should be recorded in corresponding form of the eCRF.

When the eCRF SAE notification form is completed, dated and signed by the investigator, an automatic email is sent immediately to the ANRS Clinical Research Safety Office and to the Clinical Project Manager of the CTU at *pharmacovigilance@anrs.fr*.

- **Back up circuit when eCRF is unavailable:**

The investigator must report all SAE/SAR using the CRF SAE printed form, dated and signed, to the ANRS by fax: 01 53 94 60 02 or email: pharmacovigilance@anrs.fr.

- **Relevant documentation related to SAE** *(e.g. hospitalization report, laboratories results…):*

The investigator sends all relevant anonymised documentation related to the SAE, to the ANRS by fax: 01 53 94 60 02 or email: pharmacovigilance@anrs.fr.

In Zambia, we commit to sending to ZAMRA the SAE using ZAMRA template.

11.2.3 AE Evaluation

The Adverse Event

The investigator should assess, if possible, the diagnosis of all adverse events. Diagnosis, or if not available, syndrome should be reported whenever possible. Date of "event onset", an adverse event should be earlier (or the same day) than date of seriousness. When medical or surgical procedures (e.g.: surgery, endoscopy, tooth extraction, transfusion) occurred; the condition that leads to the procedure should be notified.

The Severity

The severity (i.e. intensity) of all AE should be graded using the appropriate table below and reported by the investigator in the corresponding form of the CRF. *Example of table: "DAIDS table for grading the severity of adult and paediatric adverse events", (see appendix).
Or general table:

Grade 1	Mild	Mild or transient discomfort, without limitation of normal daily activities; no medical intervention or corrective treatment required.
Grade 2	Moderate	Mild to moderate limitation of normal daily activities; minimal medical intervention or corrective treatment required.
Grade 3	Severe	Marked limitation of normal daily activities; medical intervention and corrective treatment required possible hospitalization.
Grade 4	Life-threatening	Severe limitation of normal daily activities; medical intervention and corrective treatment required, almost always in a hospital setting.

The Seriousness

The judgment as to whether the event is serious is usually made by the reporting investigator (see section SAE definition). Deaths must be reported for participants as the outcome of an adverse event and not as an adverse event itself if the cause is known. If the cause is unknown, the death should be reported as "unknown cause of death".

The Causality

The investigator must assess the causality of all AE in relation to the study drug, concomitant medication and the research. All SAE for which the investigator or the sponsor considers that a causal relationship is a reasonable possibility are considered as suspected SAR.

The Expectedness

Assessment on expectedness is usually done by the sponsor.
Expectedness is assessed in the light of the applicable Reference Safety Information (SmPC of EPIVIR).

The Adverse Event Outcome

The adverse event outcome at the time of reporting should be provided on the initial SAE notification form.
Any change in the initial outcome (e.g. resolved, back to previous status, worsening…) should be reported using complementary reporting form(s). As long as the adverse event is not resolved, any new worsening will be reported using complementary forms of the corresponding initial SAE.

Potential Risks of The Research and Management Guidelines In Case Of Adverse Event

Refer to the Section "Risk/Benefit Assessment"

12.3 RESPONSIBILITIES OF THE SPONSOR

11.3.1 Recording and Assessment Of SAE

The sponsor shall keep detailed records of all SAE which are reported to him by investigators. The sponsor is responsible for the assessment of the causality of the SAE in relation to the study drug, concomitant medication (in case of drug-drug interaction) and the research. In the absence of information on causality from the reporting investigator, the sponsor should consult the reporting investigator and encourage him to express an opinion on this aspect. The causality assessment given by the investigator should not be downgraded by the sponsor. If the sponsor disagrees with the investigator's causality assessment, the opinion of both the investigator and the sponsor should be provided in the report to the National Competent Authority.

All SAE for which the investigator or the sponsor considers that a causal relationship is a reasonable possibility are considered as suspected SAR. The expectedness of the SAR shall be determined by the sponsor. The sponsor assesses if the SAE is expected or not using the applicable Reference Safety Information. If information on expectedness has been made available by the reporting investigator, this should be taken into consideration by the sponsor.

11.3.2 Reporting of Safety Data to The National Competent Authority and The Ethics Committee

SUSAR Reporting (Suspected Unexpected Serious Adverse Reaction)

All Suspected Unexpected Adverse Reactions (SUSAR) have to be reported, within the legal timeframe, by the sponsor to the Ethic Committees in Burkina Faso and Zambia (in so far as these ethic committees want to receive the SUSAR) and to all the members of the DMSB. The timelines for expedited initial reporting (day 0) starts as soon as the information containing the minimum reporting criteria has been received by the sponsor. For fatal and life-threatening SUSAR, the sponsor should report at least the minimum information without delay after being made aware of the case. SUSAR which are not fatal and not life-threatening are to be reported within 15 calendar days to the Ethics Committees and Competent Authorities in Burkina Faso. In Zambia all SAE which are associated to death have to be reported in 24 hours and all other SAE have to be reported in 48 hours to the Ethics Committees and Competent Authorities using a specific template. If significant new information on an already reported case is received by the sponsor, this information should be reported as a follow-up report within 8 days after being made aware of the relevant complementary information.

New Fact Reporting

When a new event is likely to affect the safety of participants, the sponsor and the investigator take appropriate urgent safety measures to protect participants against any immediate hazard. The sponsor informs without delay the Burkina Faso and Zambia Ethic Committees that may be relevant in terms of participant safety, or safety issues which might alter the current benefit-risk assessment of the trial. The safety office shall transmit a written report, within 15 days to the Burkina Faso and Zambia Ethic Committees and Competent Authorities (ZAMRA in Zambia and ANRP in Burkina Faso). If the new fact requires substantial amendments, the sponsor should notify any substantial protocol modification, to the Burkina Faso and Zambia Ethic, within 15 days of the safety measures implementation.

Annual Safety Reporting

Once a year throughout the clinical trial, the sponsor should submit to the Burkina Faso and Zambia Ethic Committees and Competent Authorities (ZAMRA in Zambia and ANRP in Burkina Faso), an annual safety report. The annual safety report is prepared in collaboration between the Inserm-ANRS Pharmaco-vigilance unit and the CTU and includes:

- A line-listing of all suspected serious adverse reactions which have occurred over this period (expected and unexpected SAR);
- A cumulative summary tabulation of the all expected and unexpected SAR by System Organ Class (SOC) name;
- A cumulative summary tabulation of all SAE by SOC name;
- A line-listing of deaths;
- IDSMB opinion and Steering committee report (if applicable);
- A concise, critical analysis of the participants' safety.

The annual safety report may be submitted to the coordinating investigator for approval. The RSI in effect at the start of the reporting period serves as RSI during the reporting period. The annual safety report should clearly indicate the version number and date of the SmPC used for this purpose. If there are significant changes to the RSI during the reporting period, they should be listed in the report. Despite the change to the RSI, the RSI in effect at the start of the reporting period serves as RSI during the reporting period. The annual safety report should be submitted no later than 60 calendar days from the date of the sponsor's first authorization to conduct the clinical trial in any country.

13 STATISTICAL CONSIDERATIONS

13.1 STATISTICAL HYPOTHESES

We hypothesize that the concept of responsive intervention, based on the use of POC tests at EPI-2 visit and provision of PrEP to high-risk children can be incorporated into routine EPI services to achieve the goal of paediatric elimination and improve the prognosis of HIV-infected children by facilitating early ART. Such a responsive intervention would also provide a unique opportunity to monitor in real-time the performance of the PMTCT cascade. Adding only a very short questionnaire to all EPI-2 visit attendees would capture the early steps of this cascade, while POC molecular tests will provide crucial and quasi-comprehensive biological data which are currently lacking. Overall, accurate and precise estimates of the cascade of care could be used by programme managers to detect gaps and conduct appropriate corrective actions.

Furthermore, due the 2020 guidelines implementation, new hypotheses have been defined based on the previous rationale:

- The efficacy of a single-drug (lamivudine) regimen used as a prophylaxis in HIV exposed uninfected children is not inferior to triple-drug regimen to prevent HIV acquisition.
- A single-drug (lamivudine) prophylaxis is safer than a triple-drug prophylaxis (zidovudine / lamivudine / nevirapine).

13.2 ENDPOINTS

12.2.1 Primary Endpoint

Component 3: HIV-transmission rate from EPI-2 to 12 months of age in infants exposed to HIV-1 by breastfeeding.

12.2.2 Secondary Endpoints

Component 1 (PMTCT cascade):
Proportion of women attending the 6-8 week EPI visit who:

- Have attended PMTCT clinic at least once during their pregnancy
- Have been tested for HIV-1 antenatally or during childbirth,
- Are HIV-1 infected

Proportion of women with a positive HIV test who had:

- Suppressed plasma viral load (<1000 HIV RNA copies/ml) (information collected in component 2 and 3)
- Having initiated ART during pregnancy or following childbirth,

Proportion of children who were HIV tested with PCR at birth Proportion of babies with a positive HIV-1 PCR who were engaged on ART at 6-8 weeks

Component 2: Proportion of HIV-infected breastfed infants identified during the second EPI visit and who were not engaged in HIV care at this time but who will be initiated on ART within 2 months after this visit.

Component 3:

- For all participants of Component 3 in Zambia:
  - Proportion of plasma HIV-1 viral load levels concordant with breast milk HIV-1 viral load levels
- For all the participants of the intervention arm and the comparison arm sub-population before the introduction of the 2020 Zambian guidelines (including Burkina Faso control arm)
  - HIV-transmission rate at 12 months of age
  - Adverse events rates at 12 months of age, including death and Grade 3 or 4 events on the paediatric DAIDS scale

- HIV-free survival at 12 months of age, defined as the proportion of children alive and testing HIV negative at 12 months.

➢ For all the participants of the intervention arm and the comparison arm sub-population following the 2020 Zambian guidelines implementation

- HIV-transmission rate at 12 months of age
- Adverse events rates at 12 months of age, including death and Grade 3 or 4 events on the paediatric DAIDS scale
- HIV-free survival rate at 12 months of age, defined as the proportion of children alive and testing HIV negative at 12 months

Component 4:

- Proportion of children exposed to HIV through breastfeeding who acquired HIV between 12 and 18 months
- Proportion of HIV exposed uninfected children with stunted growth (weight and height) at 18 months.
- Mortality rate among HIV exposed uninfected children between 12 and 18 months

13.3 SAMPLE SIZE DETERMINATION

Sample size calculations are based on the primary outcome, i.e. the rate of infant HIV infection at 12 months. This rate is hypothesized to be about 5%, ranging between 3% and 6%. These rates are conservative; the 'official' PMTCT rate in Zambia was well above 10% in 2015. The responsive intervention is expected to lower this rate, to achieve around 2%. A 50% reduction of the current PMTCT rate using the 'responsive' intervention would be deemed satisfactory enough to be worth implementing. The table below shows various hypotheses of sample size accounting for the various hypotheses of transmission rates in the two arms, with 80% power, 5% significance level and 15% lost-to-follow-up rate. The enrolment of 2000 children will allow covering the most reasonable hypotheses (see table below):

In 2017, 4 out of the 5 Health centres taking part in the study reported an HIV prevalence of 10%; cumulatively saw over 29 000 women attending ANC; over 20,000 mother attending EPI visits for their children and close to 3000 HIV exposed and HIV Infected children

		Transmission rate in control group			
		3%	**4%**	**5%**	**6%**
Transmission rate in intervention group	**1%**	1992	1127	766	575
	1.5%	3827	1725	1058	741
	2%	9246	2852	1500	978
	2.5%	39537	3048	2254	1327

A sample size calculation was performed following the 2020 guidelines implementation in Zambia in order to be able to add a non-inferiority analysis on the efficacy of a single-drug versus triple-drug prophylactic regimen to prevent HIV transmission at one year of age: with a HIV transmission rate in comparison arm of 1% and a upper limit for the difference “intervention arm - comparison arm” of 2 %, 600 participants per arm will be needed (confidence level: 95% (one-sided); power 90%).

13.4 POPULATIONS FOR ANALYSES

- Intention-to-Treat (ITT) Analysis Dataset will include all participants randomized to the Phase III trial.
- Safety Analysis Dataset: defines the subset of participants for whom safety analyses will be conducted and will include all participants included in component 3.
- Per-Protocol Analysis Dataset: defines a subset of the participants in the full analysis (ITT) set who complied with the protocol sufficiently to ensure that these data would be likely to represent the effects of study intervention according to the underlying scientific model as defined in the Statistics Analysis Plan (SAP) and IDSMB. The per-protocol study population will be validated by the scientific committee and described in the plan of analysis. A priori, participants with an estimated drug compliance greater than 80% and who attended at least the 6 months follow up visit will be included in the Per-Protocol analyses.

13.5 STATISTICAL ANALYSES

12.5.1 General Approach

All tests will be two-sided. Descriptive results, efficacy and safety estimates and their corresponding 95% CIs will be presented. The statistical significance is set at $p < 0.05$. Potential confounders may be considered for further adjustment if they are deemed imbalanced at baseline. Missing data will be considered, and appropriate imputations (detailed in the Statistics Analysis Plan (SAP) will be done when necessary.
SAS and R statistical software will be used to analyse the data.

12.5.2 Baseline Descriptive Statistics

The baseline characteristics of the two arms will be presented, and assessed for differences without any statistical tests but pre-defined thresholds described in the SAP. Categorical variables will be summarized as percentages and continuous variables as means with standard deviation for Gaussian variables, otherwise as medians with interquartile ranges. Global descriptive analysis will be carried out and reported before the assessment of the intervention efficacy. In particular, the breastfeeding duration, drug adherence, deaths and lost-to-follow up.

12.5.3 Analysis of The Primary Efficacy Endpoint

Analyses for the primary outcome will be undertaken on an intention-to-treat basis and reported upon as such. All the randomized and eligible infants will be analysed in their initial group of treatment. HIV-uninfected withdrawals and deaths will be censored at the last outcome measurement. If an infant is lost to follow-up or dies after a single positive DNA-PCR, s/he will be considered HIV-1 infected. The primary outcome will be the acquisition of HIV-1 (i.e. without HIV confirmatory PCR) between week 6-8 and 12 Months of age for babies born to HIV-infected women.

HIV transmission rate will be compared using chi-squared test ($\chi 2$ test) or Fisher's exact test depending on the number of observed events.

All possible efforts will be undertaken to obtain the HIV status of the children at 12 months. A sensitivity analysis will be implemented, accounting for various hypotheses of HIV-1 infection among children lost-to-follow up or dead at 12 months, without a known HIV status. Among these hypotheses, weighing the probability of HIV infection according to baseline maternal characteristics will be envisaged.
HIV-1 infections occurring between 6-8 weeks and 12 Months will also be compared between the two arms using proportional hazard modelling with hazard ratio (Cox Model) and its 95% CI adjusted for any potential confounders identified at baseline.

12.5.4 Analysis of The Secondary Endpoint(S)

For component-1 (PMTCT cascade): Proportion of EPI-2 women not having attended PMTCT clinic at least once during their pregnancy, proportion having been tested for HIV-1 antenatally or during childbirth, proportion with HIV-1 infection and proportion of them with suppressed HIV viral load (<1000 copies/ml), proportion having initiated ART during pregnancy or following childbirth, proportion of babies with a positive HIV-1 PCR, proportion of children who were HIV tested with PCR at birth, proportion of HIV-1-infected babies engaged in HIV care at 6-8 weeks (i.e. initiated on ART).

For component 2: Proportion of HIV-infected infants identified during the second EPI visit and who were not engaged in HIV care at this time but who will be initiated on ART within 2 months after this visit.

For component 3:

- For all participants of Component 3 in Zambia:

For HIV+ mothers providing an Informed Consent in Zambia, collection of participant mother's breast milk will take place at 6-8 weeks, 6 months and 12 months post-partum.
Diagnostic performance of plasma HIV viral load to identify infants at risk of transmission compared to breast milk HIV viral load will be evaluated using sensitivity, specificity, positive predictive value, and negative predictive value. Concordance between plasma HIV-1 viral load levels and breast milk HIV-1 viral load levels will be evaluated using Cohen's kappa statistic.

- For all the participants of the intervention arm and the comparison arm sub-population following the 2020 Zambian guidelines implementation

HIV-transmission rate and HIV-free survival rate at 12 months of age will be compared to assess the non-inferiority of a single-drug versus a triple-drug infant prophylactic regimen. The difference between the two arms (intervention arm – comparison arm) will be calculated with its 95% confidence interval and the upper limit of the interval will be compared to 2% (non-inferiority margin). The non-inferiorioty will be demonstrated if the upper limit of the interval is below non-inferiority margin.

Safety analysis are described section 12.5.5

For component 4: This part of the study is an observational phase with descriptive analyses. The point estimate (proportion) with its 95% confidence interval (95% CI) will be calculated for the endpoints:
-Proportion of children exposed to HIV through breastfeeding who acquired HIV between 12 and 18 months
-Proportion of HIV exposed uninfected children with stunted growth (weight and height) at 18 months.
-Mortality rate among HIV exposed uninfected children between 12 and 18 months

This section will be developed in the Statistical Analysis Plan (SAP).

Given that this responsive strategy is based on a large coverage of young children attending EPI-2, it will be important to conduct cost effectiveness/cost savings analyses. The cost and cost-effectiveness analyses will be detailed in a sub-study. It will be conducted from the governments' perspective because ultimately all intervention costs and ART costs will be borne by the governments, who also will be the beneficiary of any cost savings that accrue over time. The CHEERS (Consolidated Health Economic Evaluation Reporting Standards) checklist will be used to document the economic analysis and to report the results (Husereau, 2013). Cost-effectiveness analyses will quantify the disability-adjusted life years (DALYs) saved and cost savings.

12.5.5 Safety Analyses

The safety of the intervention will be evaluated among HIV-1-uninfected children as:

- Death rate, calculated as the number of deaths divided by the number of randomized children, with 95% confidence intervals

- The proportion of children who had at least one DAIDS paediatric scale event with grade 3 or 4, with its 95%CI, at 6 and 12 months.
- The total number of DAIDS paediatric scale events with grade 3 or 4
- A descriptive analysis of all these events

12.5.6 Planned Interim Analyses

Not-applicable.

12.5.7 Sub-Group Analyses

All sub-group analyses will be considered as exploratory. The determinants of the HIV-1 infection will be identified including the breast milk viral load. This section will be developed in the SAP.

12.5.8 Tabulation of Individual Participant Data

If applicable, this section will be developed in the SAP.

12.5.9 Exploratory Endpoint

No Exploratory Endpoints identified at this point.

14 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

14.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

13.1.1 Informed Consent Process

In obtaining and documenting informed consent, the investigator shall comply with the applicable regulatory requirement(s), and shall adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Prior to the beginning of the trial, the investigator should have the IRB/IEC's written approval/favorable opinion of the written informed consent form and any other written information to be provided to subjects.
Neither the investigator, nor the trial staff, should coerce or unduly influence a subject to participate or to continue to participate in a trial. None of the oral and written information concerning the trial, including the written informed consent form, should contain any language that causes the subject or the subject's legally acceptable representative to waive or to appear to waive any legal rights, or that releases or appears to release the investigator, the institution, the sponsor, or their agents from liability for negligence.

The investigator, or a person designated by the investigator, should pay special attention to under aged emancipated participants as explained below. Some of the mothers recruited for this research will be teenage girls or young adults (15 to 18 (Zambia), (15 to 20 years of age - Burkina Faso). These adolescents and young adults will be included in the study because they are likely to benefit directly from it, the research cannot be conducted only with mothers aged 20 years or older (young mothers are at higher risk of uncontrolled infections) and the research involves only minimal risks. Because of the stigma surrounding HIV, obtaining the consent of the child's parents, legal guardian or father may expose a younger mother to a higher risk of interrogation or even intimidation and physical or verbal abuse by her parents, legal guardian or father.

The enrollment criteria for mothers aged 15 to 18/20 will be the same as for mothers aged 18 or older - all requirements of the informed consent process will apply, such as voluntary participation, provision of all relevant information and absence of coercion, undue influence and inappropriate incentives. The potential benefits to the research participant will not be exaggerated and the risks will be minimized. In the case of mothers aged 15 to 18/20, additional precautions will be taken to ensure that they are informed of the research to the extent consistent with their understanding.

The language used in the oral and written information about the trial, including the written informed consent form, shall be as non-technical as practical and understandable to the subject or the subject's legally acceptable representative and the impartial witness, where applicable. In Zambia, an impartial witness (not part of the study team) will always be required when obtaining consent form from illiterate mothers. Before the informed consent may be obtained, the investigator, or a person designated by the investigator, shall provide the subject or the subject's legally acceptable representative ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial shall be answered to the satisfaction of the participant or the subject's legally acceptable representative.

Prior to a subject's participation in the trial, the written informed consent form shall be signed and personally dated by the subject or by the subject's legally acceptable representative, and by the person who conducted the informed consent discussion. In order to be able to perform an HIV diagnosis on the infants of HIV+ mothers, mothers will be providing informed consent on behalf of their children. Mothers will receive the information to enable them to decide on behalf and in the best interests of their infants. Attention will be made to minimize the risk and discomfort to all participants. Participation in the study is free and voluntary. Any mother can withdraw herself, her child or both from the study at any time and without giving any reason for the decision to do so, and this withdrawal will have no consequences whatsoever for the level or standard of care given to the mother or her child. The consent shall include explicit permission to store blood samples for future research uses after the study.

The informed consent process will be implemented with an independent third person (the 'witness') for illiterate mothers. In Burkina Faso, the informed consent process will be implemented with an independent third person (the 'witness') for illiterate mothers or when translation is required. This witness will sign the consent form together with the mother and the investigator. The information sheets and the informed consent forms will be translated into local languages (Bemba & Nyanja) once the template version in English has been approved. The translated versions (and back-translations) will be provided to the IRB for stamping before study initialisation.

Participants will only receive SMS messages reminding them of the next visit if they express their agreement during informed consent. In order to preserve confidentiality, the participant agreeing to receive SMS messages will receive a standard message: "Madam, the next appointment will take place in 2 days". Likewise, the Informed Consent will include explicit approval on the conduct to be followed in case of Incidental Findings (below). Study staff must ensure that, whenever an illiterate participant has consented, a witness sits in during the informed consent process; the witness may also meet and discuss candidate requirements with the individual at the end of the visit. A witness must be someone other than the person obtaining informed consent and must be impartial, not part of the study team. The witness must be present during the entire consent process and verify that, to the best of his/her knowledge, the prospective participant has understood the informed consent form and agrees to participate in the research project .

Summary of the informed consent process:

1. In case the mother is illiterate, the witness writes the participant's name in capital letters on the "Participant's Name" line and records the current date. The witness must write his initials after each registration made for the participant (both copies).
2. The participant must make her mark or thumbprint on the "fingerprint (if the mother is illiterate)" line (both copies).
3. In Burkina Faso and in Zambia, if the mother is illiterate, the witness, after the participant has made her mark, writes her own name in capital letters, signs and dates in the control section (both copies).

4. In Burkina Faso, if the mother is between 15 and 20 years of age, a representative of the patient association checks that the patient has received the information in accordance with her level of understanding and signs and dates the consent.
5. The study staff who obtains consent must write their own name, sign and date on the appropriate lines on both copies. Provide one of the original signed and dated informed consent forms to the participant and file the other in her study booklet. If the participant does not want to take her copy home, document it in the clinical file and file it in her file. Give the participant the contact information of the person to contact in case of emergency.
6. Document in the participant's clinical file the name of the investigator who obtained the consent, the date on which informed consent was obtained and the participant's identification in the study.
7. Once the informed consent process is complete and all required signatures have been obtained, conduct a preliminary review of the study.

13.1.2 Amendments, Consent/Assent and Other Informational Documents Provided to Participants

Prior to the beginning of the trial, the investigator should have the IRB/IEC's written approval/favourable opinion of the written informed consent form and any other written information to be provided to participants. Protocol amendments require approval by the Scientific Committee prior to submitting the amendment to the EC/IRB. Any amendment to the protocol will require review and approval by the EC/IRB before the changes are implemented to the study. Likewise, all changes to the consent form will be EC/IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

13.1.3 Consent Procedures and Documentation

Component 1:
As part of the routine national programmes, women attending EPI-2 will be informed (or reminded) about the principle of HIV screening. After orally consenting to the screening procedure by the women in Burkina Faso and informed consent signed by the women and a witness in case of illiterate mothers in Zambia, the women will be consecutively invited to answer a simple and brief standardized questionnaire. An HIV-1 rapid test (blood-based test provided by the national programme) will be performed during EPI-2 (at 6-8 weeks post-partum) if previous HIV test was performed more than one month ago in Zambia and if the usual practice HIV-rapid testing results are not available at the time of the EPI-2 visit in Burkina Faso (within the last 3 months). Results on the HIV screening performed at EPI-2 will be available during the EPI-2 visit (usually within one hour). HIV-uninfected mothers will be invited to continue the EPI-2 visit.

Component 2:
HIV-infected mothers will also be invited to continue the EPI-2 visit, but they will also be offered a written Informed Consent in order to assess their viral load and diagnose their children. Written informed consent will be obtained prior to any activities related to Component 2 or Component 3. Informed Consent will be obtained in vernacular language from the mother of participating mother-infant pairs, by research staff after specific training. The consent will include explicit permission to store blood samples for future research uses after the study. Participants will only receive SMS messages reminding them of the next visit if they express their agreement during informed consent. In order to preserve confidentiality, the participant agreeing to receive SMS messages will receive a standard message: "Madam, the next appointment will take place in 2 days". The informed consent process will be implemented with an independent third person (the 'witness') in Zambia for illiterate women. In Burkina Faso, Informed consent will be obtained with the assistance of an independent third party (the "trusted person" or "witness") for illiterate mothers or when translation is required. This witness will sign the consent form together with the mother and the investigator. In Burkina Faso, the mothers between 15 and 19 years of age (inclusive) will be accompanied by a referent adult of her choice representing her interests and the interests of the child (parent, family member or

guardian, member of an association, etc.). This referent adult will also sign the consent form. Participation in the study is free and voluntary. Any mother can withdraw herself, her child or both from the study at any time and without giving any reason for the decision to do so, and this withdrawal will have no consequences whatsoever for the level or standard of care given to the mother or her child. In order to be able to perform an HIV diagnosis on the children of HIV+ mothers, mothers will be providing informed consent on behalf of their children. Mothers will receive the information to enable them to decide on behalf and in the best interests of their children. Attention will be made to minimize the risk and discomfort to all participants.

The study site will keep one original signed Informed Consent, a second original or a copy of the original will be given to the participating mother or witness as it contains important information. The source-notes at the study site should document who signed the ICF, when the ICF was signed and dated.

Incidental Findings (the policy will be added to the Patient Information Sheet and Informed Consent Form): Unexpected medical incidental findings may be identified during the course of the study. If this occurs, an assigned study caretaker will contact a study doctor to determine if the finding is significant and whether further investigation is needed. If the finding is significant and further investigation is needed, the study doctor will inform the participant and, with the participant's consent, a referral will be made to an appropriate expert for further follow up.
Unexpected non-medical incidental findings including but not limited to physical, sexual, emotional abuse or neglect will be flagged by the assigned study caretaker to the study doctor. The study doctor will be responsible for the patient's support and for the reporting of those cases to the appropriate authorities.

13.1.4 Study Discontinuation/Termination and Closure

This study is not expected to be temporarily suspended or prematurely terminated. However, if the study is prematurely terminated or suspended by the scientific committee decision, the Coordinating Investigators and the sponsor will promptly inform study sites, the IRBs, ECs, national authorities must be informed for the termination or suspension. Study sites will contact participants, as applicable, and participant's care will continue.

It will be the duty of the Independent Committee (equivalent to independent DSMB) to provide a recommendation to stop or alter the design mostly based on evidence about safety and toxicity concerns. If significant safety concerns emerge, the Independent Committee will have full access to relevant efficacy and safety data to assess the relative benefit-to-risk profiles of the study regimens when developing their recommendations. Study may resume once concerns are addressed, and satisfy the sponsor, IRBs, ECs and Regulatory Authorities as applicable.

13.1.5 Legal and Regulatory Considerations

The relevant ethics boards and regulatory authorities in each country will approve the final version of the protocol, the information sheet, the consent form and any other relevant trial documents before study commencement. They will be kept updated on the study progress until termination, and then be informed of the study conclusions.
The study will be conducted in compliance with each country's laws and regulations and, as well as with the international rules.
This includes:
– Guidelines for Good Clinical Practice for biomedical research on drugs for human use and ICH Good Clinical Practice E6 (R2) 09 November 2016 and European Directive no. 2005/28/EC establishing the principles and detailed guidelines concerning the application of good clinical practice with regard to investigational drugs for human use, as well as the requirements for granting authorization to manufacture or import these drugs. (accessible at: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4_2016_1109.pdf).
– Good Clinical Laboratory Practice (GCLP. World Health Organization on behalf of the Special Programme for Research and Training in Tropical Diseases, 2009. Accessible at: http://www.who.int/tdr/publications/documents/gclp-web.pdf).

– In the country of the sponsor: The Public Health Code, as modified by Public Health Law no. 2004-806 of August 9, 2004 and its subsequent texts, and the Law n° 2012-300 of March 05th 2012 on research involving the human person (Jardé Law) and its subsequent texts.

The trial will be carried out in compliance with relevant national and international regulations, including the CIOMS International Ethical Guidelines for Biomedical Research Involving Human Subjects, the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects), the Nagoya protocol, and the ANRS Ethics charter for research in developing countries.

Insurance
The sponsor of the trial will subscribe to a civil liability insurance policy covering any harm directly resulting from the participation in the trial. The sponsor Inserm-ANRS subscribes to a civil liability insurance contract with Beah: European Hospital Insurance Office in accordance with the provisions of Article L1121-10 of the French Public Health Code. A copy of the certificate of insurance is attached as Annex A3. In addition, any amendment involving a modification of the terms of the insurance contract will be subject to an update of the contract and the certificate and attached to the protocol

13.1.6 Data Protection and Confidentiality

All the collected patient data will be strictly confidential and coded. Only persons mandated by the sponsor and involved in the study management and health authorities are able to access to medical files of patients in order to check the accuracy of the collected data. In order to respect patient confidentiality, each subject will be allocated a code.
For component 1: the code will be anonymous. An entry number into the study will be assigned to each participant automatically by the REDCap software. This number will be sequential and incremented according to the arrival of the participant. This number is unique to component 1 and will not be reused in component 2 & 3.
Example: | : | _0 | 0_ | _0 | _0 | 0_ | _0 | 1_ | _0 | 0 |
Site number Patient number
For component 2 & 3:
All information collected on the persons participating in the study will be kept strictly confidential and coded. Only persons mandated by the sponsor, involved in the study and representatives of health authorities may have access to patients' medical records to verify the quality of the data collected. In order to respect the confidentiality of participants, each participant will be assigned an identification code when signing the Component 2 & 3 consent.
Format of the code used: | _ | _ | _ | _ | _ | - | _ | _ | _ | _ | (Code Site Sequential Number and Letter). Only the code of the subject will be reported in the Case Report Form (CRF). The trial data will be recorded and be managed electronically at INSERM UMR 1058, Montpellier, France and the ANRS pharmaco-vigilance unit. Inserm-ANRS declares to the French National Commission of Informatics and Liberty (CNIL) that data management is compliant with the requirements of the modified Law n° 78-17 of January 6 1978 – Law 2018-493 - of June 20 2018 relative to information technology, files and civil liberties. The investigator should make available the individual documents and data strictly necessary for monitoring, quality control (including monitoring) and audit of the trial to persons having access to these documents and data (persons mandated by the sponsor for quality control and audit; representatives of the judicial authority). Any person having access to the data, including the investigator, shall be subject to professional secrecy.

13.1.7 Data Handling and Record Keeping

Data collection is the responsibility of the clinical trial site staff, under the supervision of the site investigator. It is the investigator's responsibility to ensure the accuracy, completeness, legibility and veracity of the reported data. The data recorded in the eCRF derived from the source documents must be consistent with the data recorded on the source documents.
The data will be entered into the **R**esearch **E**lectronic **D**ata **Cap**ture System, REDCap software. REDCap is an EDC system developed by Vanderbilt University that is compliant with 21 CFR Part 11, a data capture system provided by

INSERM UMR 1058 Data Coordination Centre, Montpellier, France. REDCap is validated to meet data security, data quality and data monitoring requirements in accordance with ICH GCP guidelines. In addition, REDCap has an easy-to-use design and study specific adaptability and meets HIPAA compliant data collection standards. The data system includes password protection and internal quality controls, such as automatic range checks, to identify data that appears inconsistent, incomplete or inaccurate. The data manager will implement integrated testing and verification controls to ensure data completeness, consistency and reliability. All audits will be approved by the investigators, the study team and the statistician prior to programming. Each operation performed in REDCap is tracked by an audit trail and can be easily found at the project level.

13.1.8 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is following the currently approved protocol/amendment(s), with International Conference on Harmonization Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by GCP-trained monitors with extensive therapeutic knowledge and external to the clinical work performed at each site.
- Monitoring will be done on-site by study monitors. However, centralized monitoring will also be done centralized by the Project Coordinating group (e.g., Data Manager and Medical Monitor).
- Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP).
- The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports. At minima, the monitor will regularly visit each trial site during the whole trial progress, including set up, implementation, and end of the trial. During these visits, the monitor will be in charge of the following, according to the CMP:
    - Verify that the trial is being carried out in accordance with the protocol, SOP and Good Clinical Practice;
    - Establish and maintain up-to-date the investigator's Trial Master Files (TMF);
    - Verify that the informed consent process was adhered to for each patient;
    - Monitor compliance with the trial protocol, SOPs and Good Clinical Practices, including eligibility criteria and reporting of SAEs;
    - Perform Source Data Verification as per the CMP;
    - Perform SDV on the key trial data identified in the CMP. At minima:
        - For component 1 in Zambia: a 100% monitoring at each site on the informed consent on the first week of inclusion. Afterwards a 10-20% sample checked during each monitoring visit. In case of major deviation found in a consent from for component 1 process, the ten next informed consents after the monitoring visit where the deviation was identified will be checked.
        - 100% of regulatory data: informed consent and serious adverse events for component 2/3/4; 100% of key data and libraries (to be defined in the monitoring plan).
    - Verify that privacy and confidentiality are respected;
    - Monitor that SAEs notifications forms have been properly declared and transmitted to the Inserm-ANRS pharmaco-vigilance department and relevant study team, and that the other event notification forms have been filled out in due time;
    - Monitor the management of medicinal products;
    - Monitor the management of blood samples and bio-banks;
    - Monitor that quality controls and quality management for laboratory assessments are implemented;
    - Follow up and contribute to corrective actions/preventive actions implementation and resolution.

Are part of the monitoring visits:

- *A site initiation visit will be conducted in each site before starting inclusion*
- *A closing visit should take place in each of the centres that includes at least one participant.*

Co-monitoring visits will be conducted by the Project Manager to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP.

13.1.9 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. A Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. That is, direct access to source data and source documents will be made available for trial-related monitoring, audits, institutional review board/independent ethics committee review, and regulatory inspection(s).

13.1.10 Future Use of Stored Biological specimens And Data

Stored biological specimens
With the participant's approval and as approved by local IRBs and ECs, Dried Blood Samples (DBS) samples labelled with a unique participant study number will be shipped to INSERM UMR 1058 Bio sample Repository, Montpellier, France for long term storage (15 years after the end of the study) and future analysis (including investigation in mitochondrial genotoxicity as described in appendices). DBS cards will be stored at -20°C. Breast-milk samples will be stored in Zambia but they will be shipped to France as sub-studies analyses require technology not available in Zambia (HIV reservoir and microchimerism). Breast-milk samples will be stored up to 15 years after the end of the study. Investigators will ensure that biological specimens are not disposed of or removed from the trial sites without prior notification and approval from the sponsor or his representative.

Data collected for this study will be analysed and stored at the Data Coordinating Center at INSERM UMR 1058, Montpellier, France for 15 years after the end of the study. After the study is completed, the de-identified, archived data will be transmitted to and stored at the Data Coordinating Center at INSERM UMR 1058, Montpellier, France.

Any utilization of the study's biological samples and/or data, for analyses for other studies not listed in the protocol should be approved by the study coordinating investigators, the Scientific Advisory Board (SAB) and the sponsor. These studies should receive approval from the national ethics and regulatory authorities of each country where there will be conducted. After dissolution of the SAB, the use of data will be subject under the responsibility of ANRS and the coordinating investigators.

13.1.11 Audit and Inspection

The sponsor may request an independent auditor to audit the trial at any time.
National health authorities may request an independent inspector to inspect the trial at any time.
All audits will be approved by the sponsor, the coordinating investigators, the study team and the statistician prior to programming.

13.1.12 Study Records Retention

This is a trial of approved investigational products. Essential study documents will be kept secured for a minimum of fifteen (15) years after trial completion, under the responsibility of each investigator and the sponsor. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained. Investigators will ensure that trial records are not disposed of or removed from the trial sites without prior notification and approval from the sponsor or his representative. Each Investigator will keep a hard copy of the Trial Master File (TMF) containing all the study documents (protocol, ICF/PIS, CRFs, insurance, IRB approvals, site task delegation logs, CVs of team members, training logs, etc.) The list of essential study documents will be detailed in the Study Manual and reviewed with the study monitors throughout the study.

13.1.13 Protocol Deviations

A protocol deviation is any non-compliance with the clinical trial protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), or study requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.
These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

The investigator/institution should conduct the trial in compliance with the protocol agreed to by the sponsor and which was given approval/favourable opinion by the Independent Review Board or Ethics Committee and Regulatory Authorities as applicable. The investigator should not implement any deviation from, or changes of the protocol except where necessary to eliminate an immediate hazard(s) to trial participants, or when the change(s) involves only logistical or administrative aspects of the trial (e.g., change in monitor(s), change of telephone number(s).

The principal investigator, or person designated by the principal investigator, should document and notify the Study Team any deviation from the approved protocol via the "Protocol Deviation Form" in the eCRF using REDCap. The study team must inform the sponsor immediately in case of major deviation and regulatory the scientific committee of all protocol deviations.

Protocol Deviations will be classified as MAJOR or MINOR –:

- **MAJOR Deviations** to protocol are conditions, practices or processes that may affect, or affect, the rights, safety or well-being of participants or the quality and integrity of data.
  Major deviations from the protocol will be immediately notified by the investigator (or delegated person) within 48 hours to the study team and the Ethics Committee.
  They will also be reported by the investigator (or delegated person) in the source file and to the study team via the "Protocol Deviation Form" in the eCRF using REDCap.
- **MINOR Deviations** to protocol are fully permissible in the management of circumstances not provided for in the protocol. These are deviations that do not increase the minimal risk and should not be reported to the Ethics Committee. They will be reported by the investigator (or delegated person) in the source file and to the study team via the "Protocol Deviation Form" in the eCRF using REDCap.

Further details about the handling and definition of protocol deviations will be included in the Project Plan and Statistics Analysis Plan (SAP).

15 KEY ROLES AND STUDY GOVERNANCE

15.1 SCIENTIFIC COMMITTEE

14.1.1 Composition

It is composed of the following persons: Investigator Coordinators, Trial Steering Committee members: the methodologist, statistician, lab coordinators, project manager, patient associations, experts in epidemiology, virology, gynecology, pediatric and family health, the project managers from each CTU, a representative of the sponsor, and a representative of ANRS pharmaco-vigilance department. The Ethical Advisor will be invited to the Scientific Committee meetings as a non-voting member. EDCTP will be invited to attend.

14.1.2 Meeting Frequency

The Scientific Committee meets before the clinical sites open and at least two (2) times per year until the research is completed. An extraordinary meeting may, at any time, be convened by decision of the President of the Scientific Committee at the request of the sponsor or of one or more members.

14.1.3 Role

The Trial Scientific Committee is the group that provides overall supervision of the trial.

- It regularly ensures that the research runs smoothly and that the protocol is followed, particularly with regard to the safety of those who take part in the research,
- It provides information to all investigators and other participants in the research,
- It ensures the scientific follow-up of the research: maintaining the relevance of the research questions and the validity of the methods used to answer them,
- It ensures the application of the rules of access to research data and communication and publication of its results,
- It maintains an ongoing relationship with the sponsor, the Independent Committee and investigators.
- It validates the publication plan.
- It shall decide on any relevant amendments to the protocol necessary for the continuation of the research, including:
    - Measures to facilitate recruitment in research,
    - Protocol amendments prior to submission to Ethics Committees,
    - Decisions to open or close trial sites,

After each meeting, a report containing the meeting minutes, signed by the Trial Scientific Committee chair will be sent to the members of the scientific committee according to the ANRS SOP. Depending on the needs and stage of the project, the Independent Committee and Ethics Advisor will be invited on an ad hoc basis. The Programme Officer from EDTCP is also welcome to attend as an observer.

15.2 INDEPENDENT COMMITTEE (EQUIVALENT TO INDEPENDENT DSMB)

14.1.4 Composition

It is composed of the following persons: an epidemiologist, a paediatrician, a virologist, a biostatistician, a health and reproductive family expert.

14.1.5 Meeting Frequency

The IDMC will meet before the beginning of the inclusion phase, and twice a year until the end of the trial. The sponsor, the trial scientific committee or the IDMC may request to increase the frequency of these meetings. The first meeting can take place jointly with the Scientific Committee

14.1.6 Role

The IDMC is a consultative board for the Trial Scientific Committee and the sponsor. It monitors the main safety and efficacy outcome measures and the overall conduct of the trial, with the aim of protecting the safety and the interests of the trial participants. Its members will provide general advice on the progress of the trial, including the rate of inclusions, the quality of follow-up, the overall rate of drug-related adverse events, changes in biological markers, the overall incidence of primary outcomes, and the number of subjects needed. It will be responsible for examining data with respect to clinical outcomes and virologic failures and for informing the Trial Scientific Committee about any decisions it needs to take to pursue or discontinue the trial, such as:

- Premature discontinuation of the trial (because the rate of adverse events is high, the trial is no longer feasible, or the available data are sufficient);
- Substantial changes to the protocol that becomes necessary during the inclusion or follow-up phases, or to account for new scientific information.

The IDMC will have access during its implementation to overall safety and efficacy data, as well as to any information justifying any change affecting the course of the trial. It may request an intermediate statistical analysis. During the trial, the IDMC may be asked to deliberate on questions relative to the scientific and ethical integrity of the trial, after the agreement of the Trial Scientific Committee, or initiated by the coordinating investigators, the coordinating CTU or other participants in the trial. The IDMC will provide a formal written report, containing the IDMC's opinion, to the Trial Scientific Committee and the sponsor after each IDMC meeting.

Due to the use of a different PrEP regimen in the intervention arm from that recommended in the new Zambian national guidelines, the IDMC will act as clinical event adjudication committee. Quarterly reports on both HIV transmission and SAEs in the two arms will be analyzed by the IDMC from July 2020.

15.3 TRIAL STEERING COMMITTEE

14.1.7 Composition

The PROMISE-EPI study is led by the Coordinating Investigator, Prof. Philippe Van de Perre, and divided into 6 Work Packages. The leaders of each Work Package are listed below. The Trial Steering Committee is composed of the Sponsor, the leaders of each Work Package and the team members appointed by those leaders.

Investigator Coordinators	
Pr. Philippe Van de Perre, Dr Paulin Fao, Dr. Chipepo Kankasa	
UMR 1058 " Pathogenesis and Control of Chronic Infections" INSERM - Université Montpellier – EFS	
Inserm ADR Languedoc-Roussillon 60, rue de Navacelles 34394 Montpellier Cedex 5	
Phone: +33(0)4 34 35 91 11	
p-van_de_perre@chu-montpellier.fr	
Study Work Packages	
Description of Work Package	**Leader of Work Package**

WP[1] Project coordination and management (including management of the Trial Steering Committee, Scientific Committee and Independent Committee)	Pr. Philippe Van de Perre
WP[2] Clinical trial, treatment, care and support in Burkina Faso	Dr. Paulin Fao
WP[3] Clinical trial, treatment, care and support in Zambia	Dr. Chipepo Kankasa
WP[4] Biological investigations	Dr. Jean-Pierre Molès
WP[5] Data management & statistical analysis	Prof. Nicolas Nagot
WP[6] Communication, dissemination, capacity building	Prof. Thorkild Tylleskär

14.1.8 Meeting Frequency

Regular meetings of the project team allow the progress of the research to be monitored. The participants of the Trial Steering Committee (TSC) will communicate frequently and will have regular monthly group calls or as deemed appropriate depending on the needs of the study.

14.1.9 Role

The TSC is the study coordination body, for all scientific and administrative aspects. The TSC will be responsible for the conduct of the study. The TSC will send technical reports to the sponsor, ethical committees and regulatory bodies, and financial reports to the sponsor. TSC Meetings will receive and provide feedback to EDCTP, the Scientific Committee, the Independent Committee, and the Community Advisory Group by circulating the agenda and the minutes from those meetings by e-mail and through the website. In addition, the Scientific Committee and Independent Committee will also hold periodic meetings (described below). The action items from those meetings will be tracked and implemented with support from the Project Management team (WP1).

15.4 THE COMMUNITY ADVISORY GROUPS (CAG)

The Community Advisory Groups (CAG) in Burkina Faso and in Zambia will advise the Scientific Committee and Trial Steering Committee on the community perspectives. This group will be made up of volunteer members from the community identified through non-governmental organisations working in the field of HIV-1 and civic organizations. The purpose of this group is to provide advice and insights to the investigators regarding community attitudes, perceptions and behaviours that might influence the implementation or outcome of the trial. In particular, the CAG will have an important role in offering peer support to the population that is more likely to be lost from care than others, such as younger women and those just initiating ART. The role of the CAG is purely advisory. It is not a decision making body, and as such does not hold any veto powers. However, the advice that is given by the CAG is usually well-informed and useful to the trial. The CAG will also act as a communication structure between the community which includes participants and the investigators to give feedback on participant concerns and to communicate key messages about the study. CAG members will meet on a regular basis and will be paid an honorarium for their time spent.

15.5 EXTERNAL INDEPENDENT ETHICS ADVISOR (EA).

External Independent Ethics Advisor (EA). The EA should: Assess the aims, objectives and methodology of the study; Overview the operations throughout the study; Provide clear guidance on ethical dilemmas; Encourage disclosure of questions and issues; Encourage transparency; The EA must report directly to EDCTP in order to ensure its independence. The reports to EDCTP would include: A reference to the relevant deliverable/task/issue at hand; The actions and decisions agreed on; Next steps

16 PUBLICATION AND DATA AND SAMPLE SHARING POLICY

This study will be conducted in accordance with the following publication and data and sample sharing policies and regulations: All oral or written presentations or publications of the trial results must be approved beforehand by the Coordinating Investigators and the Trial Scientific Committee. Reporting of the main results will follow the CONSORT statement (http://www.consort-statement.org/). Oral or written presentations or publications will mention "this research is part of the EDCTP2 Programme supported by the European Union (grant NUMBER RIA2016MC-1617 — PROMISE-EPI", the trial reference number and acronym (ANRS 12397 PROMISE-EPI), the name of all investigators who enrolled or followed patients in the trial and the composition of the Trial Scientific Committee members. Oral or written presentations or publications will mention « this research is Sponsored by Institut national de la santé et de la recherche médicale-ANRS . The Trial Scientific Committee alone will be competent to decide upon the mention of any other person in publications relative to the trial, if it thinks this is justified. The Scientific Committee will validate the publication plan.

All research data and all collected samples will be under the responsibility of the MMC and coordinating investigators. In case of request by other research teams to use results obtained during the research and/or to use stored samples, these teams will write a proposal and send it to the principal investigators, the scientific committee and the Ethics committees for approval. After the dissolution of the Scientific Committee, data and samples will be under the responsibility of ANRS and the coordinating investigators. In case of request by other research teams to use results obtained during the research and/or to use stored samples, these teams will write a proposal and send it to ANRS, which will take a decision in agreement with coordinating investigators and Ethic Committees. The protocol details can be accessed via Clinical Trials.gov (identifier: NCT03870438). In addition, this will be developed in the Dissemination and Exploitation Plan to be posted in the project's website (work in progress - https://promise.w.uib.no/projects-studies-trials/)

In the case of ancillary studies, the results of these studies may only be published with the agreement of the Coordinating Investigator and the Trial Scientific Committee, and only after publication of the results from the main study to be cited (ANRS 12397 PROMISE-EPI). Abstracts or publication of the results of ancillary studies carried out on the basis of biological data or samples from ANRS 12397 PROMISE-EPI should IMPERATIVELY be submitted to Inserm-ANRS, the trial sponsor, prior to submission.

14.2 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the Sponsor has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

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18 APPENDICES

1. Substudy “HIV reservoir in Breastmilk
2. Substudy “Breastmilk-induced microchimerism”
3. Substudy “Mitochondrial genotoxicity and ARV exposure”
4. Substudy on social sciences
5. Revised information sheet and Consent form for Component 1
6. Information sheet and Consent form for Component 1-Sub-studies
7. Revised information sheet and Consent form for Components 2 and 3
8. Revised information sheet and Consent form for Components 4
9. Updated CRFs to accommodate component 4

APPENDIX 1: **"HIV reservoir in Breastmilk" sub-study**

Edouard Tuaillon (MONTPELLIER, FRANCE)

Study design / Rationale	Descriptive analysis The number of cells harboring the HIV provirus is low in blood, estimated around 3% of the CD4+ cells, the other cell types are rather unsystematic[1]. Other cell populations susceptible to harbor HIV provirus could be investigated like (i) unconventional T cells and (ii) progenitor/stem cells. Recently, new classes of immune cells have been described in blood that are positioned at the edge of the acquired and innate immunity. Among them, Innate Lymphoid Cells, Mucosal Associated Immune T cells, semi-invariant T cells and memory T stem cells are detected in breastmilk[2]. Some of them expressed CD4 at their surface, therefore being permissive to HIV infection[2]. CCR5 and CXCR4, the main co-receptors used by HIV to enter CD4+ T cells, are also expressed at the surface of CD34+ stem cells. Of note, CXCR4 was more widely expressed on multipotent CD34+ cells than CCR5, and its sole expression renders these cells permissive to HIV-1 infection when the sole expression of CCR5 could not[3].
Main question	To identify alternative HIV reservoirs in human breast milk
Endpoints	Primary endpoint: % of HIV provirus positive cells subsets and its dynamic through 1-year lactation Secondary outcomes: 1. Impact of HIV-infection on the % of different innate immune cell populations 2. Dynamic of the % of different innate immune cell populations during the course of one year of breastfeeding.
Subjects / population	Healthy and HIV-infected breastfeeding mother
Sample size & Calculation method	• Nature of the Sample: Breastmilk cell pellets and blood cell pellets • No data is available in the literature. Groups (HIV- and HIV+) of 25 samples will be first tested. • Method: cytometry analysis / cell sorting / molecular biology
Conduct	• Where: Montpellier, France • How: collaborative study • Who: supervision by E. Tuaillon

Reference	1- Josefsson et al. Majority of CD4+T cells from peripheral blood of HIV-1–infected individuals contain only one HIVDNA molecule. **PNAS** 2011 108(27): 11199–11204 2- Bedin AS, et al. MAIT cells, TCR γδ+ cells and ILCs cells in human breast milk and blood from HIV infected and uninfected women. **Pediatr Allergy Immunol.** 2019 30(4):479-487. 3- Carter et al. HIV-1 utilizes the CXCR4 chemokine receptor to infect multipotent hematopoietic stem and progenitor cells. **Cell Host Microbe.** 2011;9(3):223-234.

APPENDIX 2: **"Breastmilk-induced microchimerism" Substudy**

JEAN-PIERRE MOLES (MONTPELLIER, FRANCE)

Study design / Rationale	Descriptive analysis Breastmilk cell composition presents a high amount of stem cells, 100 times more than the frequency observed in blood[1,2]. We recently demonstrated the co-existence of two milk stem cell populations depending on the expression of specific cell surface markers, the canonical one expressing CD45dim/CD34+ but also the one expressing CD45+/CD133+. Their distributions varied from colostrum to transitional milk[2]. The determinants for such distribution are unknown but current hypothesis suggests that these cells participate in the child development and child regeneration. A small proportion of cells can migrate during pregnancy through the placenta from the mother to the foetus and from the foetus to the mother and reside for an extended period of time in the new host. Such process is named microchimerism[3]. We observed recently that breastfeeding is also a period when mother cells could migrate to the neonates at the intestinal mucosae level[4]. We hypothesized that part of the behavior of breastmilk stem cells is to undergo a microchimeric process that could participate in the child development. To be capable of a long-term residency, these cells need to be tolerated by the immune system of the child. The main factor controlling immunotolerance during pregnancy, an example of macrochimerism, is the expression of HLA-G at the surface of the syncytiotrophoblast[5].
Main question	Would HLA-G, known as the main factor controlling immunotolerance during pregnancy, be expressed at the surface of breastmilk stem cells?
Objectives	To determine if HLA-G is expressed at the surface of breastmilk stem cells : a) To determine the dynamic in the expression of HLA-G during the course of one year of breastfeeding. b) To compare the stem cells subsets in breastmilk of HIV + and of HIV – mothers
Endpoints	a) % of CD45dim/CD34+ and CD45+/CD133+ stem cell subsets expressing HLA-G b) % of stem cell expressing HLA-G in HIV positive mothers at EPI-2, M6 and M12 and comparison with HIV negative mothers
Subjects /	Healthy and HIV-infected breastfeeding mothers

population	
Sample size Calculation / method	• Nature of the Sample: cell pellets from breastmilk. • No data is available in the literature. Groups (HIV- and HIV+) of 25 samples will be first tested. • Method: cytometry analysis
Conduct	• Where: Montpellier, France • How: collaborative study • Who: supervision by JP Molès
Reference	1- Fan, Y. et al. (2010). Unravelling the Mystery of Stem/Progenitor Cells in Human Breast Milk. **PLoS ONE**, 5(12), e14421. 2- Valverde-Villegas et al. Large Stem/Progenitor-Like Cell Subsets can Also be Identified in the CD45- and CD45+/High Populations in Early Human Milk. **J Hum Lact.** 2019 doi: 10.1177/0890334419885315. 3- Kinder et al. Immunological implications of pregnancy-induced microchimerism. Nat Rev Immunol. 2017 17(8):483-494. 4- Molès JP, et al. Breastfeeding-related maternal microchimerism. **Nat Rev Immunol.** 2017 17(11):729-1. 5- Ferreira LMR et al. HLA-G: At the Interface of Maternal-Fetal Tolerance. Trends Immunol. 2017 Apr;38(4):272-286.

APPENDIX 3:" **Mitochondrial genotoxicity and ARV exposure" Substudy**

JEAN-PIERRE MOLES (MONTPELLIER, FRANCE)

Study design / Rationale	A longitudinal descriptive analysis Many unfavourable health outcomes have been reported in HIV-Exposed Uninfected (HEU) children such as metabolic disorders, increased infectious disease morbidity and higher mortality, impaired growth, neurodevelopmental delay, altered immunity, and mitochondrial toxicity in comparison to never HIV-exposed children[1]. In fact, HEU children cumulate exposure to both maternal HIV and antiretroviral drugs throughout pregnancy, postnatal prophylaxis and breastfeeding. Reduction of the mitochondrial copy number (MCN) is a well-known biological adverse effect of nucleoside reverse transcriptase inhibitors[2]. In a previous safety studie on PROMISE-PEP children, we reported that lopinavir/ritonavir or lamivudine prophylaxis regimen reduced the number of mitochondrial DNA copy number (mtDNA) at 1 year. Furthermore, we also reported that HEU children at birth have a detectable level of mtDNA deletion[3]. While this observation may be a cause for concern, we were able to show that the MCN was normalized in the same children at 7 years (unpublished results).
Objective	To investigate the mitochondrial genotoxicity in HIV Exposed Uninfected children compared to HIV unexposed children
Endpoints	mtDNA copy number and % of deleted mitochondrial DNA in HIV Exposed Uninfected children compared to HIV unexposed children
Subjects / population	Inclusion criteria: infant included in PROMISE-EPI study. Non-inclusion criteria: child who acquired HIV during the trial. Groups: HIV exposed uninfected children receiving lamivudine vs HIV exposed uninfected children receiving triple therapy vs HIV exposed uninfected children not receiving prophylaxis vs HIV non exposed children
Sample size & Calculation method	• Nature of the Sample: DBS at W6, and W50 • Number: 30 per group • Method: quantitative PCR and deep sequencing. The sequencing method remains to date, the only one that can quantify exactly the proportion of deleted mtDNA and that can determine the border of the deletion. As an inference, it will provide the genetic information related to mtDNA of each analysed individuals. This genetic information can be sensitive because it can also identify mutations responsible for genetic diseases. However, the genetic information will cover the 13 mitochondrial genes out of the 30,000 genes encoded by the genomic DNA

Conduct	• Where: Montpellier, France and UTH, Zambia • How: collaborative study • Who: supervision by Jean-Pierre Molès
Reference	1- Afran, et al. "HIV-exposed uninfected children: a growing population with a vulnerable immune system?" Clin Exp Immunol. vol. 176, no. 1, pp. 11–22, 2014. 2- C.A. Koczor and W. Lewis: "Nucleoside reverse transcriptase inhibitor toxicity and mitochondrial DNA." Expert Opin Drug Metab Toxicol. vol. 6, no. 12, pp. 1493–1504, 2010. 3- Monnin et al. Mitochondrial DNA defects in blood of infants receiving lopinavir/ritonavir or lamivudine prophylaxis to prevent breastfeeding transmission of HIV-1. BMRI submitted

APPENDIX 4: **Social sciences Substudy**

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