CONTEMPORARY CLINICAL TRIALS

Design and challenges of a large HIV prevention clinical study on mother-to-child transmission: ANRS 12397 PROMISE-EPI study in Zambia and Burkina Faso

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Abstract

Post-natal HIV infection through breastfeeding remains a challenge in many low and middle-income countries, particularly due to non-availability of alternative infant feeding options and the suboptimal Prevention of Mother to Child Transmission of HIV-1 (PMTCT) cascade implementation and monitoring. The PROMISE-EPI study aims to address the latter by identifying HIV infected mothers during an almost never-missed visit for their infant, the second extended program on immunization visit at 6-8 weeks of age (EPI-2). The study is divided into 3 components inclusive of an open-label randomized controlled trial aiming to assess the efficacy of a responsive preventive intervention compared to routine intervention based on the national PMTCT guidelines for HIV-1 uninfected exposed breastfeeding infants. The preventive intervention includes: a) Point of care testing for early infant HIV diagnosis and maternal viral load; b) infant, single-drug Pre-Exposure Prophylaxis (PrEP) (lamivudine) if mothers are virally unsuppressed.

The primary outcome is HIV-transmission rate from EPI-2 to 12 months. The study targets to screen 37 000 mother/infant pairs in Zambia and Burkina Faso to identify 2000 mother/infant pairs for the clinical trial.

The study design and challenges faced during study implementation are described, including the COVID-19 pandemic and the amended HIV guidelines in Zambia in 2020 (triple-drug PrEP in HIV exposed infants guided by quarterly maternal viral load). The changes in the Zambian guidelines raised several questions including the equipoise of PrEP options, the standard of care-triple-drug (control arm in Zambia) versus the study-single-drug (intervention arm).

Trial registration number (www.clinicaltrials.gov): NCT03869944

Keywords: HIV, mother-to-child transmission, randomized controlled trial, design, pre-exposure prophylaxis, Africa

Submission category: Study Design, Statistical Design, Study Protocols

1 Introduction

World Health Organization (WHO) recommendation for the prevention of mother-to-child transmission (MTCT) of HIV in 2013 (1), notably, includes a lifelong antiretroviral therapy for pregnant and breastfeeding women and short period of Pre-Exposure Prophylaxis (PrEP) to HIV exposed uninfected (HEU) infants (option B+).

While progress has been made in the last few years toward expanding prevention of mother-to-child transmission (PMTCT) programs and increased availability of antiretroviral therapy (ART), new HIV infections among children are still unacceptably high. In 2020, about 150 000 children were infected with HIV worldwide (2), a rate of infection 7.5 times higher than the target set by UNAIDS and partners as part of the Super-Fast-Track Framework to end AIDS (3).

Most cases of MTCT result from a) new HIV infection during late pregnancy or breastfeeding period (4) and b) non-attendance of antenatal care, or poor retention in care (5) including suboptimal adherence to maternal ART (6–8) especially when ART is initiated during late pregnancy or breastfeeding (9).

Improving maternal ART adherence is at the top of the research program agenda (10). However, even if adherence is improved through dedicated interventions, significant residual transmission will remain for several reasons. Many women do not have access to the program or do not comply with the PMTCT cascade (attendance of the antenatal consultation, HIV-1 screening, referral for care, initiation to ART). Furthermore, the 6 weeks' prophylaxis to exposed infants included in the B+ strategy do not cover the whole period of breastfeeding exposure (11).

The "prevention of mother-to-child transmission of HIV-1: program evaluation and innovative responsive intervention integrated in the expanded program of immunization" (PROMISE-EPI) study aims to provide a second chance to mothers who have dropped out at any stage of the PMTCT cascade to get back on track. These mothers are identified at a visit almost never missed for their infant: the second extended program on immunization visit (EPI-2) performed when the infant is six to eight weeks of age in sub-Saharan countries. During this visit, eligible mothers are invited to participate in the clinical trial part of this study with the aim to evaluate the efficacy of an innovative response intervention in order to protect their HEU infants against HIV-1 acquisition by breastfeeding.

The optimal PrEP intervention for HEU infants would result from a good risk-benefit balance between efficacy, safety and risk of resistance among newly infected infants. Nevirapine and zidovudine are the current WHO recommended drugs for HIV prophylaxis (12). Nevertheless, a high risk of resistance associated with nevirapine prophylaxis has been reported by several studies (13–15) and serious hematologic toxicity had been associated with zidovudine used as infant prophylaxis (16–18). The choice of lamivudine as study prophylaxis drug was motivated by its good efficacy / safety profile with no observed resistance demonstrated during the PROMISE-PEP study (19).

Herein, the study design, the challenges encountered and the lessons learnt during the implementation of the PROMISE-EPI study are reviewed.

2 Method

2.1 Local settings

In 2019, 81% and 86% of pregnant women living with HIV, received ART for PMTCT in Burkina Faso and Zambia respectively and the final HIV mother-to-child transmission rate including breastfeeding period was 15.1% in Burkina Faso and 10.7% in Zambia (20, 21).

Both countries have adopted the WHO recommendations for PMTCT including: timing of HIV tests; ART for pregnant and breastfeeding HIV infected mothers; early infant diagnosis for HIV and short period of PrEP for HEU infants. In particular, national recommendations differ in the two countries regarding infant sampling for early diagnosis. In Burkina Faso, a single blood sample is taken at the "42th day well baby visit" for PCR test whereas 2 samples are taken in Zambia, at birth and at EPI-2 visit.

2.2 Study objectives, associated design, eligibility criteria and endpoints

The study consists of 3 different components, each associated with distinct objectives.

Component 1 is proposed to all mothers attending the EPI-2 visit. Its description is presented in table 1.

Table 1: Description of Component 1

Objective	To monitor the 'real life' efficacy of the PMTCT cascade up to the second EPI visit
Design	Cross-sectional study
Inclusion/ Non-inclusion criteria	Inclusion criteria: -Mother aged 15 or older accompanying her infant to the EPI-2 visit -Infant between 5 and 16 weeks at the time of EPI-2
Procedures	Administration of a short questionnaire and HIV rapid test if not performed recently
Endpoints	-Proportion of mothers attending the 6-8 week EPI visit who: a) Attended PMTCT clinic at least once during their pregnancy, b) Have been tested for HIV-1 antenatally or during childbirth, c) Are HIV-1 infected -Proportion of HIV infected mothers : a) With suppressed viral load(<1000 HIV RNA copies/mL), b) Having initiated ART during pregnancy or following childbirth -Proportion of HIV exposed infant HIV tested with PCR at birth -Proportion of infants with a positive HIV-1 PCR who were initiated on ART at EPI-2

Component 2/3 is proposed to all HIV positive mothers from Component 1 (newly and previously diagnosed) and their infant pair, who meet the couple eligibility criteria. At this point infants are tested for HIV-1 using a POC HIV-1 DNA PCR test (GeneXpert HIV-1 Qualitative) to determine the component affiliation and subsequent procedures (table 2):

- If HIV positive, the participant becomes part of Component 2 and is referred to the National HIV treatment Program.
- If HIV negative, the participant becomes part of Component 3, and is randomized to one of the two study arms (control or intervention).

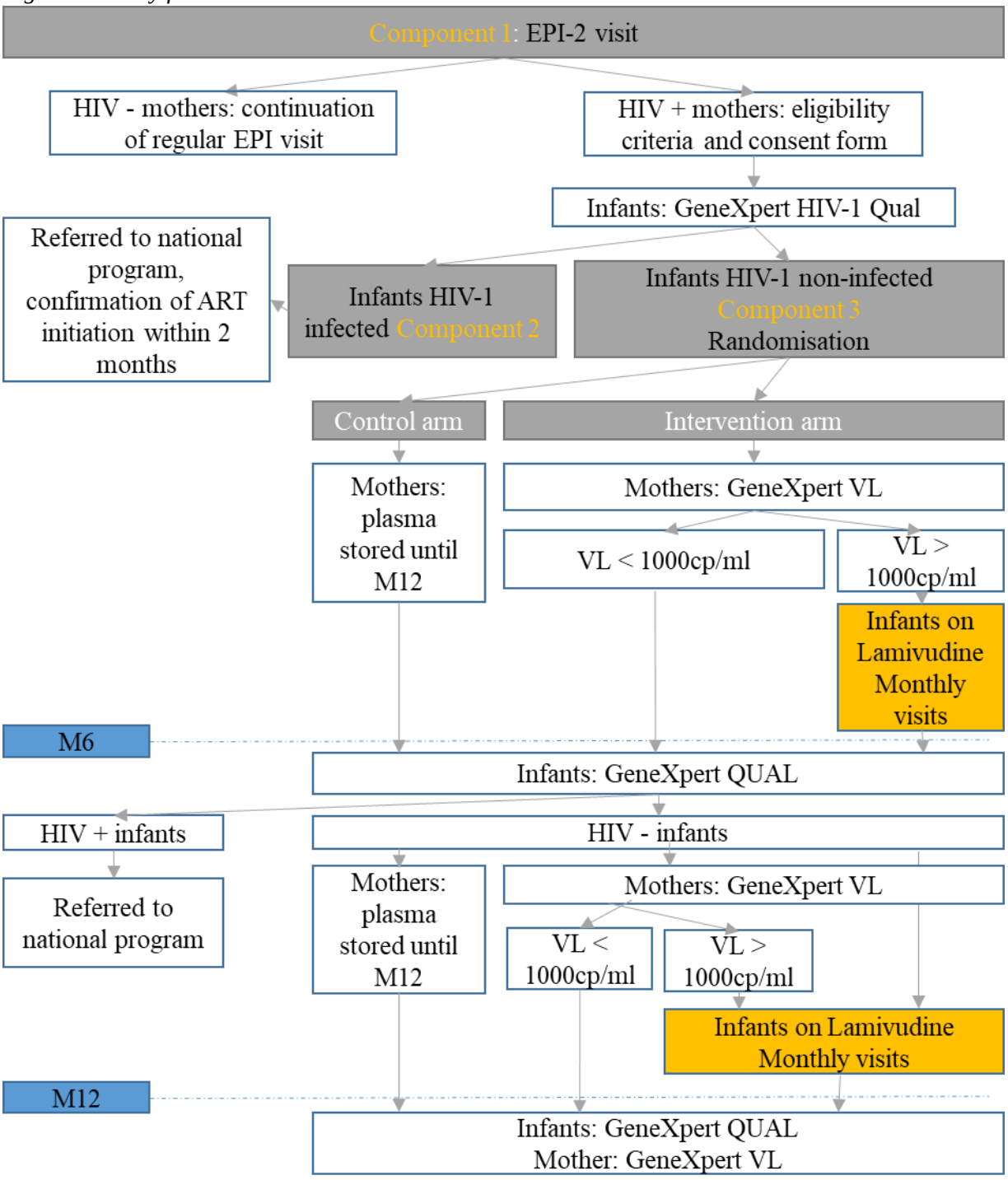
Table 2: Description of Component 2 and 3

	Component 2: HIV infected infants at EPI-2	***Component 3 : HIV exposed uninfected infants at EPI-2***
Objectives	To evaluate a reinforced access to early paediatric ART among HIV-1 infected infants not engaged in care at EPI visit	**Primary objective**: To evaluate the efficacy of an innovative response intervention including point of care (POC) testing (maternal viral load and infant HIV diagnosis with immediate results) and infant single-drug PrEP (lamivudine) for high risk infants of HIV-1 acquisition by breastfeeding **Secondary objectives:** - To evaluate the safety of the intervention - To evaluate the diagnostic performance of plasma HIV viral load compared to breastmilk HIV viral load to identify infants at-risk of transmission via breastmilk

<table>
<tr><td rowspan="3">Inclusion/ Non-inclusion criteria</td><td colspan="3">Inclusion criteria:
- HIV-1 mother (with or without HIV-2)
- Singleton infant
- Infant breastfeed at EPI-2 and the mother intends to continue breastfeeding at least until child is 6 months-old
Non-inclusion criteria
- Infant with:
-Clinical symptoms or biological abnormalities of DAIDS classification 3 or 4 for adverse events on the day of inclusion
-Severe congenital malformation
-Known allergy to the study drug or its components
-Already taking emtricitabine drug
- Mother:
-Living outside the study area or intending to move from the area within the next 12 months
-Participating in another clinical trial</td></tr>
<tr><td>Inclusion criteria:
- Infant with positive HIV-1 PCR POC test at EPI-2</td><td colspan="2">Inclusion criteria:
- Infant with negative HIV-1 PCR POC test at EPI-2</td></tr>
<tr><td colspan="3"></td></tr>
<tr><td>Design</td><td>Cross-sectional study</td><td colspan="2">Multi-centre and multi-country, parallel, controlled open-label trial</td></tr>
<tr><td rowspan="2">Procedures</td><td rowspan="2">Information on HIV diagnosis confirmation and ART initiation are sought in the hospital registers at least 2 months after the diagnosis</td><td>Control group
-EPI-2 and M6 visit: Mother’s plasma stored for viral load testing at M12.
(Outside the study, mothers can access the routine national program including HIV-1 plasma viral load testing).</td><td>Intervention group
- EPI-2 and M6 visit: Maternal viral load testing by POC HIV-1 PCR (GeneXpert HIV-1 viral load) and lamivudine initiation for infants of virally unsuppressed mothers
- Monthly visits for infants of virally unsuppressed mothers with lamivudine dispensation</td></tr>
<tr><td colspan="2">-M6 and M12: infant HIV-1 DNA PCR</td></tr>
<tr><td>Endpoints</td><td>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</td><td colspan="2">Primary endpoint:
- Proportion of HEU infants who are PCR positive at 12 months, using POC HIV-1 DNA PCR test (GeneXpert® HIV-1 Qualitative)
Secondary endpoints:
- Adverse events rates at 12 months of age, including death and Grade 3 or 4 events based on Division of AIDS (DAIDS) table for grading the severity of adult and paediatric adverse events
-Proportion of plasma HIV-1 viral load levels concordant with breastmilk HIV-1 viral load levels</td></tr>
</table>

At EPI-2 for Component 2 and 3 and at M6 and M12 for Component 3, the following procedures are performed in addition to the ones described in table 2: infant physical examination; questionnaires administration to the mother (socio-demographic data, medical history, attendance at counselling sessions, PMTCT questions on breastfeeding, ART) and ART and adherence counselling are provided. In the intervention group of Component 3, infants of virally unsuppressed mothers at EPI-2/ M6 visit receive PrEP (lamivudine) with monthly visits, until 12 months of age or until breastfeeding cessation (defined as 2 consecutive monthly visits where mother confirms the end of breastfeeding). In case of lamivudine cessation, the participant continues to be followed in the study with M6 and M12 visits. The intervention duration is 10 months (from 6-8 weeks to 12 months) (Figure 1).

Figure 1 : Study flowchart



2.3 Informed consent

An opt out consent was initially planned for Component 1 in both countries, but a signed consent was subsequently requested by the Zambian Ethic committees and Competent Authorities. A specific consent form is signed prior enrolment in Component 2/3.

Written informed consent is collected in the local language of the mother by investigators who underwent specific training. An independent third party assists mothers that are not able to read or write. Mothers between 15 and 18 years of age in Zambia and between 15 and 19 years of age in Burkina Faso

can be enrolled in the trial if they are accompanied by a referent adult of their choice who will represent their interests and those of the infant.

2.4 Location and personnel

The choice of sites in West Africa (Burkina Faso) and Southern Africa (Zambia) was aimed at ascertaining the generalizability of the proposed strategy in different cultural, epidemiological and health system contexts.

The study is being conducted by the Centre Muraz in Burkina Faso and the University Teaching Hospital in Zambia. Both institutions are experienced in MTCT prevention research programs through their participations in several clinical trials.

In Burkina Faso, the study is on-going in two districts (Do and Dafra) of Bobo-Dioulasso and two other districts (Baskuy and Boulmiougou) of Ouagadougou. Each district has one level/referral health centre (CMA/CMU) and various Centres for Health and Social Promotion (CSPS). A total of 31 CSPSs were selected for Component 1 based on their willingness to participate, space and staff capacity, and their accessibility/distance to the referral CMA/CMU. Eligible HIV positive mothers, willing to participate in component 2/3 are referred to the CMA/CMU for follow-up activities after obtaining informed consent.

In Zambia, 4 sites in the capital city, Lusaka, are involved (Chilenje, Bauleni, Matero Main and Chaisa) where the study activities are collocated with the Maternal Child Health (MCH) department. In contrast to Burkina Faso, site staff involved in the study in Zambia are solely dedicated to the study. This study organization adjustment resulted from the different prevalence of HIV observed in each country. In Burkina Faso, where prevalence is low, a high number of mothers must be screened at EPI-2 in order to achieve the objective of Component 3, and therefore multiplying the number of recruiting sites. In Zambia, each site deserves a dedicated team due to the high HIV prevalence. We do not expect differences in the conduct of the study in the two countries but it may be easier to get answers to queries in Zambia.

The PROMISE-EPI team received training on International Conference on Harmonisation Good Clinical Practice (22) including ethical principles that have their origin in the Declaration of Helsinki (23).

Community health workers are involved in the study at different levels depending on the country: In Burkina Faso they have a key role in the transfer of participants between the CSPS and the CMA/CMU and in both countries they are involved in providing support to mothers in order to avoid loss to follow-up.

2.5 Laboratory assays

In Component 1, the Determine™ HIV-1/2 rapid test is used for initial diagnosis of the woman and SD Bioline HIV-1/2 rapid test as confirmatory test. Due to the circulation of HIV-2 in Burkina-Faso, the mothers already known to be HIV-positive at the time of Component 1 perform a SD Bioline HIV-1/2 rapid test to avoid erroneously enrolment of mothers who are only HIV-2 infected.

The point of care HIV-1 PCR (GeneXpert HIV-1 Qualitative, Cepheid) is performed on the capillary blood collected from infants. Mother blood samples (5ml) are collected by trained study nurses at EPI-2, M6 and M12. Plasma is prepared for either HIV-1 viral load assay (GeneXpert HIV-1 viral load, Cepheid) if the mother belongs to the intervention arm or for storage and later HIV-1 viral load assay if the mother belongs to the control arm. Whenever blood is collected from mothers and infants dry blood spot (DBS), aliquots are saved for quality control assessment and future investigations.

In Zambia only, 10 ml of manually-expressed milk from each breast is collected from mothers at 6-8 weeks, 6 months and 12 months post-partum for storage of acellular and cellular fractions.

Laboratory and quality control procedures are monitored to ensure Good Laboratory Practice (24).

2.6 PrEP

The lamivudine oral suspension is administered according to the baby's weight bands: 7.5 mg (0.75 mL) twice daily if 2-4 kg, 25 mg (2.5 mL) twice daily if 4-8 kg and 50 mg (5 mL) twice daily if >8 kg. These dosages were calculated on the basis of previous pharmacokinetic study (25).
Study drug compliance is assessed by the investigator at each visit based on a discussion with the participant and the quantity of lamivudine in the returned bottles.

2.7 Data collection and data management

The data collected are recorded in an electronic Case Report Form (CRF), using REDCap (https://www.project-redcap.org/, Vanderbilt University, Nashville, USA), a secure web application. Included in the data is information on PMTCT experience, clinical evaluation (with duplicate measurements for weight), medical history, laboratory samples taken and tests performed, study drug intake, concomitant treatment, adverse events. All data recorded are strictly confidential and coded, using a unique study subject identification code.
Verification of the completeness and consistency of the data is performed through a) a regular on site monitoring visits by the Centre Muraz in Burkina-Faso and the University Teaching Hospitals in Zambia and b) a central monitoring by the Pathogenesis and Control of Chronic and Emerging Infections, UMR 1058– INSERM unit following the monitoring procedures.

2.8 Randomisation

The participants of component 3 are allocated to one of two arms using a centralized randomization scheme incorporated in the eCRF (REDCap). The randomization list was elaborated using a 1:1 ratio, stratification by site (district for Burkina Faso) and permuted blocks of size 4 or 6.

2.9 Statistical considerations

Study size

Sample size calculation was based on the primary outcome, i.e. the rate of infant HIV infection at 12 months. This rate was hypothesized to range between 3% and 6%. These rates are conservative as the 'official' PMTCT rate in Zambia was above 10% in 2019 (26). The responsive intervention is expected to lower this rate, to achieve around 2% transmission rate, based on the results of PROMISE-PEP study (1.5% (CI95%:0.8- 2.9) in the intention-to-treat population in the lamivudine arm) (19). A 50% reduction of the current PMTCT rate using the 'responsive' intervention would be deemed satisfactory enough to be worth implementing. The table 3 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 infants in Component 3 (1750 in Zambia and 250 in Burkina Faso) will allow covering the most reasonable hypotheses.

Table 3: Hypotheses of sample size accounting for the various hypotheses of transmission rates in the two arms

		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

Component 1 sample size of 37 000 participants (25 000 in Burkina Faso and 12 000 in Zambia) was based on HIV prevalence among women (1.1% and 14.9% in Burkina Faso and Zambia, respectively) (27).

Data analysis

Analysis methods will follow the CONSORT guidelines (28) and recommendations of the GHENT group related to the mother-to-child transmission studies (29,30) and breastfeeding patterns (31).

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.

Analyses for the primary outcome, the acquisition of HIV-1 (i.e. a positive POC HIV-1 DNA PCR) between EPI-2 visit and 12 months of age, will be undertaken on an intention-to-treat basis using chi-squared test ($\chi 2$ test) or Fisher's exact test depending on the number of observed events. 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. Data of HIV-uninfected withdrawals and deaths will be censored at the last outcome measurement. HEU withdrawals and deaths will be considered in these analyses following various assumptions, corresponding to sensitivity analyses (such as: all unknown status considered positive, all unknown status considered negative, weighting the probability of HIV infection according to baseline maternal characteristics).

Concordance between plasma HIV-1 viral load levels and breast milk HIV-1 viral load levels will be evaluated using Cohen's kappa statistic.

2.10 Authorizations and external boards

The study protocol has been submitted to and approved by the Ethic committee for Health Research (CERS) and competent authority (Agence Nationale de Régulation Pharmaceutique: ANRP) in Burkina Faso and by the Ethic committees (private Institutional Review Board: ERES converge and Ministry of Health, National Health Research Authority: NHRA) and competent authority (Zambia Medicines Regulatory Authority: ZAMRA) in Zambia.

The study is sponsored by France REcherche Nord and sud Sida-hiv Hépatites (ANRS).

A Scientific Advisory Board (SAB), including sponsor members, is established for the global supervision of the trial. A Data Safety Monitoring Board (DSMB) monitors the overall conduct of the study with the aim of protecting the safety and the interests of the study participants. An external independent ethical advisor is also involved to assess the aims, objectives and methodology of the study, the overview of the study operations and also provides guidance on ethical dilemmas.

2.11 Study schedule

The recruitment began in December 2019 in Zambia (19 months' inclusion period) and in December 2020 in Burkina Faso (10 months' inclusion period), with a 10 months' follow-up period.

2.12 Dissemination plan

A webpage was established to share information on the study (https://promise.w.uib.no/). Final results are expected end of 2022. Relevant results will be shared with participants, study staff and key relevant stakeholders, disseminated through peer-review international journals and presented at conferences and scientific meetings.

3 Challenges and adaptations

Modification of the national guidelines in Zambia

The control arm of the PROMISE-EPI trial, being the standard of care for PMTCT in the country, is subject to the distinct country policies and to amendment during the course of the trial.

When the study was implemented (2019), there was an important difference of standard of care for infants at-high risk of HIV transmission between the two countries. Zidovudine/lamivudine/nevirapine (AZT/3TC/NVP) was the PrEP recommended up to 12 weeks of age in Zambia (32), while in Burkina Faso, the applied national recommendation was 6 weeks of nevirapine for all HEU infants. In the Zambian guidelines, infants were considered at-high-risk of HIV acquisition if they were born to a woman with established HIV infection a) who was not on ART; b) who had received less than 12 weeks of ART at the time of delivery, c) whose viral load was greater than 1000 copies/ml in the four weeks before delivery. Because our intervention started at EPI-2, it was decided to postpone the initiation of the study drug to M3 for these high-risk Zambian infants. Therefore, the standard of care, and thus the control arm, were quite similar for both countries.

In January 2020, the Zambian government released new guidelines for treatment and prevention of HIV infection (33) that modified the standard of care (control arm) in the following ways: a) triple drug prophylaxis (AZT/3TC/NVP) prolonged until maternal viral suppression, and b) mothers viral load measurements scheduled every 3 months.

As a consequence, and for ethical reasons, all mothers in the study are now encouraged to perform the M9 viral load measurement as recommended by the 2020 guidelines (provided by the national program, using central lab facility). EPI-2, M6 and M12 viral load measurements are already performed within the study by a POC technique.

However, for the study, the main change brought by the 2020 Zambian guidelines was the infant PrEP: triple-drug (control arm) versus single-drug (intervention arm). The equipoise of both prophylaxis options needed to be re-evaluated in order to continue the study in Zambia. The Scientific Advisory Board helped us to define the relevant questions. We hypothesized that the PROMISE-EPI intervention arm is non inferior as compared to the standard of care in terms of efficacy (prevention of breastfeeding transmission) while being safer (fewer serious adverse events (SAEs)), allowing for a better adherence and not generating drug-resistant HIV mutants (either by transmission from mother to infant or by selection in infected infants). The arguments were the following:

- *The efficacy of a single drug (lamivudine) used as prophylaxis in the HIV exposed uninfected infants is not inferior to a triple-drug to prevent HIV acquisition.*

WHO recommendation for breastfed HIV-exposed uninfected infants is 6 weeks of infant single-drug prophylaxis in the majority of the cases (12). Triple-drug prophylaxis has a very low level of scientific evidence in this population, per current knowledge, and no clinical trial has assessed the efficacy and safety of a triple-drug prophylaxis (zidovudine + lamivudine + nevirapine) in breastfed HIV-exposed uninfected infants. No differences in efficacy between one- versus multiple-drug prophylaxis have been demonstrated with short (6 weeks) prophylactic regimens in the French Paediatric Study (34) nor with prophylactic regimens (nevirapine/zidovudine versus zidovudine) extended for 14 weeks in the PEPI trial (17). Nevertheless, in the NICHD/HPTN 040 study, intrapartum HIV infection rates were similar in the multidrug infant prophylaxis groups (zidovudine + nevirapine and zidovudine + lamivudine + nelfinavir) and reduced when compared to the control group (zidovudine alone) (35). It has to be highlighted that lamivudine taken as single PrEP had very low rates of HIV-1 postnatal transmission for up to 50 weeks of breastfeeding in the PROMISE-PEP trial (19).

A new secondary objective was added to the protocol to answer this hypothesis: To assess the non-inferiority of the efficacy of a single-drug versus triple-drug prophylactic regimen: a) to prevent HIV transmission at one year of age; b) to assess the HIV-1 free survival at one year of age.

Roll out of the 2020 guidelines in Zambia started in March 2020, four months after the beginning of the recruitment. A new sample size calculation showed that with an HIV transmission rate in comparison arm of 1% and a lower limit for the difference “intervention-comparison triple drug arm” of 2 %, 600

participants per arm will be needed (confidence level: 95% (one-sided); power 90%) to allow a conclusion of non-inferiority.

- *A single drug (lamivudine) prophylaxis is safer than a triple drug prophylaxis (zidovudine/lamivudine/nevirapine).*

Three studies have shown that the hematologic toxicity of zidovudine can lead to severe adverse events and even death in HIV-exposed uninfected infants (16–18). In addition to safety concern, adverse events can lead to prophylaxis cessation or suboptimal compliance to therapy which can in turn lead to HIV acquisition.

Furthermore, rare and long term side effects following antiretroviral drugs exposure are suspected (in particular with zidovudine) due to potential impact on the mitochondrial function (36,37). These adverse events are currently under investigations but they will be difficult to identify during clinical trials because of the long delay in clinical symptoms. It is reasonable to anticipate that the risk for rare and long-term side effects increases with the number of antiretroviral drugs exposure.

A new secondary objective was added to the protocol to answer this hypothesis: To evaluate the safety of a triple drug prophylaxis versus a single drug prophylaxis in infants up to one year of age. According to the zidovudine safety profile, full blood count will be performed in infants at EPI-2 visit, M6 and M12 in both arms to allow a better evaluation of the safety.

- *A single drug prophylaxis does not increase the risk of drug resistant HIV acquisition compared to a triple drug prophylaxis*

In a WHO report with data from 2014 to 2016, the prevalence of HIV drug resistance for individuals on ART in Zambia was 4.3% (95CI:1.9-9.5); all of them with Nucleoside Reverse Transcriptase Inhibitors (NRTI) drug resistant mutations (DRM) (38). However, the frequency of NRTI DRM is rather low in the infants and young children and overwhelmed by Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) DRM (39). Indeed, Poppe et al. showed that the frequency of NNRTI DRM can be as high as 40% in HIV infected infants at 5 months of age (40). The high risk of resistance associated with nevirapine prophylaxis was confirmed in several studies (13–15). As an inference, this observation suggest that wild type virus represents the founder virus in most of the cases of HIV acquisition postnatally. Other arguments supporting this assumption were found in cohorts of breastfed infants whose mothers are treated with ART. In these infants, the HIV drug resistance mutation subsequently identified likely emerged as a result of ingestion of sub-optimal levels of antiretroviral drugs in breastmilk. Indeed, the children had wild-type infection or drug resistance mutations profile that differed from that of the mother at the first time of PCR positivity in three studies (14,39,41). In these observations (14,15,41), most of the infants acquired a wild type virus and subsequently selected resistant mutants.

M184I/V variants, conferring resistance to lamivudine, which are among the most frequently encountered mutations in patients treated with lamivudine or emtricitabine-containing regimens, also displays reduced transmissibility as a result of impaired replication capacity (42).

Viral resistance genotyping was added to the protocol to follow this hypothesis. It will be performed for the infants with a positive HIV-1 PCR at M6 and M12.

- *Better adherence to therapy can be better achieved with a single drug (lamivudine in syrup) infant PrEP as compared to the triple drug regimen recommended by the 2020 Zambian guidelines*

The triple-drug prophylaxis regimen as recommended by the 2020 Zambian guidelines is composed of 2 different formulations with different schedule of administration. Zidovudine/lamivudine is a dispersible tablet to be taken twice daily and nevirapine is a syrup to be taken once daily. Many variables influence adherence to a single-drug regimen or to a more complex regimen such as formulation of drugs, number of tablet/syrup, schedule of administration, palatability (43). Suboptimal observance may increase the risk of HIV acquisition, including acquisition of a resistant virus.

The DSMB agreed with the revised rationale, adapted objectives and study design in response to the modification of the Zambian guidelines. The DSMB proposed to act as clinical event adjudication committee with quarterly review of the HIV transmission and serious adverse events in the two arms. The initial objectives remain applicable for participants included prior to the implementation of the 2020 guidelines in Zambia as well as for participants from Burkina Faso. The amended protocol was submitted to and approved by the ethic committees and competent authorities.

Impact of COVID-19:

Some challenges were experienced in Zambia due to the COVID-19 outbreak. Recruitment was suspended for one month due to the lockdown in April 2020 but the follow-up visits were performed as initially planned.

The main challenge faced was the difficulty in maintaining the supply of the study drug. In July 2020, the available stock of lamivudine expired. The study team was able to obtain a certificate of approval for extended use of the expired drug from the national medicines and regulatory authorities. The approval was granted following drug assay test results that confirmed non-degradation of the drug and safety of use for an extended period of 1 month. However, a few days after approval, a public uproar forced the Zambian team together with the Zambian authority (ZAMRA) to withdrawal the drug. In this emergency context, four options were considered by the Zambian PROMISE-EPI team: a) to withdraw lamivudine and give nothing to the HIV exposed infants of the intervention group; b) to give the triple drug offered in the national program (AZT/3TC/NVP) as for the control group; c) to give zidovudine/lamivudine; d) to give abacavir/lamivudine (abacavir/lamivudine). The first option was excluded because it was considered unethical and second and third options were ruled out due to the safety concern regarding zidovudine. Abacavir/lamivudine was considered as the best available alternative based on its good safety profile and drug availability. The main safety concern with abacavir is hypersensitivity, which is extremely rare in Zambia, given the very low prevalence of the HLA-B*57:01 allele in the African population compared with the European population (44). Abacavir/lamivudine is indeed recommended as first line treatment in Zambia, combined with lopinavir, for the HIV-infected children above two weeks of age (33). The abacavir/lamivudine dosage was calculated to correspond to the dosage of lamivudine as described in the protocol without having abacavir overdose.

All bodies of the trial were informed of this situation (Ethic Committees, Scientific Advisory Board, sponsor and DSMB). The DSMB recommendations were as follows: a) to closely monitor the adverse events; b) to switch back the infants to lamivudine, as soon as it becomes available, after having verified they were not HIV infected; c) to check resistance among the infants who seroconvert. A specific written informed consent form was provided to and signed by the participants. Monthly and M6 visits were maintained but inclusions were interrupted during the month-long absence of the study drug (lamivudine). Infants in the intervention group whose mothers were virally unsuppressed were on abacavir/lamivudine for about 1 month. None of them experienced serious adverse events while taking abacavir/lamivudine and all of them remained HIV negative at the time of lamivudine prophylaxis reintroduction. Eventually, a new drug supplier was identified to mitigate the risk of supply chain disruption.

5 Discussion

By focusing on identifying mothers at-risk of HIV transmission during an almost never-missed visit for their infant, the PROMISE-EPI implementation study addresses the suboptimal PMTCT program implementation and monitoring in low and middle-income countries, which is critical to the control of paediatric HIV. Furthermore, this study aims to demonstrate the usefulness of an infant single PrEP administered to high-risk infants, not only during the first 6 weeks of life, as internationally

recommended (1), but until the end of the recommended breastfeeding period, for the infants of virally unsuppressed mothers, thanks to point of care tests. According to the WHO guidelines, HIV positive mothers should exclusively breastfeed their infants for the first 6 months of life, introducing appropriate complementary foods thereafter, and continue breastfeeding for the first 12 months of life (12). The risk of late HIV postnatal transmission is not negligible. This has been shown in observational studies, including prospective cohorts, where mixed feeding has been associated with an increased risk of HIV-1 transmission as compared to exclusive breastfeeding (45–48). Potential mechanisms include greater gut damage with mixed breastfeeding than with exclusive breastfeeding (49).

The study design has some limitations. First, the primary objective evaluates the intervention at 12-month of age. It does not consider a possible prolonged period of breastfeeding and thus exposure to HIV beyond 1 year. Nevertheless, virally unsuppressed mothers at M12 are referred to national program for adherence counselling and ART optimization. Second, the rescue intervention proposed in PROMISE-EPI study is based on the EPI-2 visit and therefore does not identify breastfeeding mothers who seroconvert after 2 months' post-partum.

Unexpected difficulties were faced during the study implementation due to the COVID-19 outbreak and the change in HIV standard of care in Zambia. Setting up a study with 'standard of care' as the control arm is always challenging because of the possible modification of the recommendations during the study. In this case, modifications in HIV guidelines in Zambia in 2020, led the team to deeply review the equipoise of single- versus multiple-drug regimens for prophylaxis in HIV exposed uninfected infants. It has to be kept in mind that the targeted benefit/risk ratio of an antiretroviral drug regimen used as HIV prophylaxis in infants is much higher than the one expected for therapy in those infected. In the HIV context, the success of a prophylaxis combines good efficacy, safety and adherence. In proposing to administer triple-drug prophylaxis to HEU infants, the 2020 Zambian guidelines are driven by practicalities and the availability of drug formulations, but not on scientific evidence for a better tolerance and efficacy profile. Furthermore, the current trends are toward antiretroviral therapy simplification for HIV infected patients in order to make treatment more convenient avoiding toxicity and reducing costs (50). A similar approach seems relevant in the context of prophylaxis for infants.

The unexpected challenges raised by the modification of the Zambian guidelines and the intermittent non-availability of the study drug were promptly identified and ethical, cultural and scientifically relevant solutions were found. We took advantage of the PROMISE-EPI study to assess the relevance of these new HIV prevention recommendations, explore alternative options of drug supply and learn how to navigate the ethical dilemma with the regulatory authorities.

Funding

This study is part of the EDCTP2 programme supported by the European Union (RIA2016MC-1617-PROMISE-EPI).

Competing interests/conflict of interests

The authors declare that they have no competing interest and no conflict of interests.

Author's contribution

PVdP, CK and PF: coordinating investigators. PVdP, NN JPM, CK, NM and TT: study conception, planning and design. PVdP, NN, JPM, TT, BM, AM, SED, CK, CC, MM and PF: preparation of the final version of the protocol. AM, BM, MM, CC, LS and ST: study management. CK, MM, CC, MMW, PF, DK, LK and ST: field and laboratory work coordination in the two African study sites. NN and SED: data management. AM: drafting of the manuscript. PVdP and NN: editing the manuscript. All authors significantly contributed to the manuscript and approved the final version of the manuscript.

Acknowledgements

We thank the Data Safety Monitoring Board (Melissa Neuman [London School of Hygiene and Tropical Medicine, UK], Valériane Leroy [Toulouse III University, France], Albert Faye [APHP, France],

Thomas Bourlet [CHU St Etienne, France], Ameena Goga [SA-MRC, South Africa] and Connie Osborne [Kwame Nkrumah University, Zambia]) and the Scientific Advisory Board (Nigel Rollins [WHO, Switzerland], Roger L. Shapiro [School of Public Health, Harvard University, USA], Makoura Traore-Barro [CHUSS, Burkina Faso], Adama Dembele [CHUSS, Burkina Faso], Issiaka Sombie [OOAS, Burkina Faso], Kozwa Zyambo [Ministry of Health, Zambia]) for their commitment and their invaluable supports and advises for the ANRS 12397 trial.

We thank all the study site's staff and the women and their infants who agreed to participate in the trial.

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