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An Integrated Strategy to Support Antiretroviral Therapy and Pre-exposure Prophylaxis Adherence for HIV Prevention in Pregnant and Breastfeeding Women

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An integrated strategy to support antiretroviral therapy and pre-exposure prophylaxis adherence for HIV prevention in pregnant and breastfeeding women: a pilot study

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PROTOCOL SUMMARY

Background: If the anticipated gains for the prevention of mother-to-child HIV transmission (PMTCT) programs are to be realized, adherence to antiretroviral regimens will be critical. To date, however, there are few evidence-based approaches that are scalable and sustainable in busy, programmatic settings where HIV burden is high. Based on our formative work, we developed a combination approach that includes patient-centered counseling and adherence supporter training. We seek to implement the intervention for both HIV-positive (for antiretroviral therapy, or ART) and HIV-negative (for pre-exposure prophylaxis, or PrEP) pregnant and breastfeeding women, thus providing a universal approach to support PMTCT programs holistically.

Objective: We seek to obtain preliminary data about the effectiveness and implementation of a multi-component adherence support strategy for HIV treatment and prevention among pregnant and breastfeeding women.

Design: We propose two parallel, pilot randomized trials among women seeking antenatal clinic (ANC) services in Lusaka, Zambia and Lilongwe, Malawi. In Trial 1, we will evaluate the proposed multi-component intervention in the context of ART support for HIV-positive women. In Trial 2, we will assess the same strategy applied to PrEP adherence among HIV-negative women at elevated risk for HIV acquisition.

Proposed intervention: We propose a multi-component strategy to support both ART and PrEP adherence in the context of antenatal care settings. The principal components include:

- Patient-centered counseling: Integrated next step counseling (iNSC) is a structured, patient-centered approach informed by motivational interviewing techniques. The goal of iNSC is to foster an environment for joint-problem solving in order that the participant may identify their individual needs to increase or sustain sexual health through biomedical and non-biomedical approaches. iNSC frames the counselling sessions as a non-judgmental discussion to explore the facilitators and challenges to sexual health and medication adherence. Through this process the participant is able to identify needs to optimize sexual health protection and medication adherence.

- Adherence supporter training: There is broad body of knowledge showing that peer or partner support can help to enhance adherence and retention in antenatal and HIV programs. We propose an adherence supporter intervention. Participants will be asked to identify a partner, family member, or friend to whom they are willing to disclose their HIV status (for HIV-positive women) or their intent to initiate PrEP (for HIV-negative women). Participants may also select a clinic-based peer to serve in this adherence supporter role. The emphasis of these trainings will be basic HIV knowledge, importance of antiretroviral adherence, potential drug side effects, and practical strategies for supporting adherence. Based on our formative research, we believe the addition of this adherence supporter intervention to iNSC will help to reinforce behaviors discussed during patient-centered counseling.

Study Arms: Participants randomly assigned to the *control arm* will receive supplemental counseling in safe obstetrics and newborn care, based on World Health Organization guidelines. Women randomly assigned to the *intervention arm* will receive a multi-component support strategy comprising iNSC and adherence supporter training.

Population: We will enroll pregnant women 18 years of age or older receiving antenatal care services at our designated study facilities. In Trial 1, we will enroll 100 HIV-positive women who started (or re-started, after a 6 months or longer treatment interruption) a first-line ART regimen within the past 30 days. In Trial 2, we will enroll 200 HIV-negative women willing to initiate and continue oral PrEP, in the form of tenofovir disoproxil fumarate and emtricitabine (TDF-FTC), and who report factors that place them at elevated risk for HIV acquisition.

Study Site: Chipata First Level Hospital (Lusaka, Zambia) and Bwaila District Hospital (Lilongwe, Malawi)

Duration and Follow up: The duration of recruitment is expected to be six months. The length of participant follow-up is six months from enrollment.

Study Outcomes: For Trial 1 (ART adherence support), the primary outcome is to be retained in care with HIV viral suppression at six months following study enrollment. Secondary outcomes include: adherence to ART as measured by self-report and pharmacy measures; acceptability, feasibility, and fidelity of adherence support components; and incidence of social harms and other adverse events associated with the study intervention. For Trial 2 (PrEP adherence support), the primary outcome is retained in care with adherence to PrEP as measured by plasma and intracellular tenofovir drug concentrations at six months following study enrollment. Secondary outcomes include: adherence to PrEP as measured by self-report and pharmacy measures; acceptability, feasibility, and fidelity of adherence support components; incidence of social harms and other adverse events associated with the study intervention; and incidence of maternal HIV infection.

Relevance: To achieve the goal of eliminating mother-to-child transmission of HIV in sub-Saharan Africa by 2030 and to meet the ambitious 95-95-95 target set forth by UNAIDS, new approaches are needed to support proven biomedical interventions during pregnancy and breastfeeding. Antiretroviral regimens for ART and PrEP have been shown to be highly efficacious in clinical trials; however, barriers to adherence in public health settings may mitigate their otherwise broad population impact. New, resource-appropriate, and scalable approaches are urgently needed to support the implementation of these evidence-based practices.

1.0 INTRODUCTION

Services to prevent mother-to-child transmission of HIV (PMTCT) have expanded rapidly and dramatically reduced pediatric HIV in sub-Saharan Africa. A rapid progression of scientific, programmatic, and policy advances have led to calls for the "virtual elimination" of pediatric HIV.[1] However, two sets of challenges remain.

First, despite policies supporting lifelong antiretroviral therapy (ART) for all HIV-infected pregnant and breastfeeding women (i.e., the Option B+ strategy), uptake is not universal[2] and a significant proportion drop out of care over time, nearly 20% in the first six months on treatment.[3-6] In addition, without adequate support, adherence to ART may be suboptimal, which may in turn lead to antiretroviral drug resistance, treatment failure, and subsequent horizontal or vertical transmission.[7] While interventions have been shown to improve adherence and retention during pregnancy and breastfeeding,[3] there is need for scalable models that can be integrated into existing health systems.

Second, existing antenatal services leave large gaps in primary HIV prevention. Women in sub-Saharan Africa face an unacceptably high risk of acquiring HIV during pregnancy and breastfeeding. In a meta-analysis of 19 studies, Drake and colleagues reported a pooled incidence rate of 4.7/100 person-years (95% confidence interval [CI]: 3.3–6.1) during pregnancy and 2.9/100 person-years (95%CI: 1.8–4.0) while breastfeeding.[8] Women who become acutely infected during pregnancy and breastfeeding have much higher rates of mother-to-child HIV transmission (MTCT).[9-12] Unfortunately, for the majority of pregnant women who test HIV-negative, services end with post-test counseling; few—if any—structured interventions are offered.

We propose a multi-component adherence support strategy to support HIV treatment and prevention in the context of pregnancy and breastfeeding. Our intervention combines both biomedical and behavioral components; it is also cross-cutting in nature, designed to support both HIV treatment (i.e., ART) and prevention (i.e., PrEP) in antenatal settings to reduce vertical and horizontal HIV transmission. We consider the HIV status of all pregnant and breastfeeding women and offer interventions tailored to those circumstances. It contrasts the typical PMTCT approach, which segments the antenatal population based on HIV status and provides targeted interventions to only select (i.e., HIV-positive) groups. If proven effective, this cross-cutting model could have a transformative effect on how HIV prevention, care, and treatment are integrated within antenatal care in African settings.

2.0 STATEMENT OF THE PROBLEM

To achieve the goal of eliminating mother-to-child transmission of HIV (EMTCT) in sub-Saharan Africa by 2030 and to meet the ambitious 95-95-95 target set forth by UNAIDS,[13] new approaches are needed to support proven biomedical interventions during pregnancy and breastfeeding. Antiretroviral regimens for ART and PrEP have been shown to be highly efficacious in clinical trials. However, barriers to adherence in public health settings may mitigate their otherwise broad population impact. New, resource-appropriate, and scalable approaches are urgently needed to support the implementation of these evidence-based practices.

3.0 RATIONALE

In this pilot study, we evaluate a cross-cutting approach to support antiretroviral adherence, one that can be applied to ART and PrEP. Our study intervention comprises two parts: patient-centered counseling and adherence supporter training. These components are informed by the current medical literature, as well as our own formative work. We focus on the antenatal and postnatal periods because they represent periods of elevated HIV risk—both for mother and infant—and windows of increased healthcare access. By evaluating the intervention's effectiveness, acceptability, and feasibility, we will obtain the necessary preliminary data to inform larger definitive studies for PMTCT.

4.0 LITERATURE REVIEW

4.1 Reduction of pediatric HIV in global settings

An estimated 1.6 million new HIV infections among children were prevented globally in the past two decades, due in large part to the availability of antiretroviral drugs to HIV-positive pregnant and breastfeeding women.[13] The majority of these prevented infections—approximately 1.3 million—have occurred since 2010.[14-16] These remarkable gains have been made through important scientific advancements, forward-thinking policy changes, and renewed investments from local governments and international donors.[17,18] Driven by global campaigns—including the *Global Plan Towards the Elimination of New HIV Infections among Children by 2015 and Keeping Their Mothers Alive*[19]—national programs in sub-Saharan Africa have reduced new pediatric HIV infections by 60% and reduced AIDS-related pediatric mortality under 5 years by 62%. The proportion of children living with HIV who received antiretroviral therapy (ART) increased from 15% to 51% globally in six years (2009-2015).[14-16] Despite this tremendous progress, however, challenges remain. The majority of pediatric HIV infections continue to be via MTCT and occur mainly in sub-Saharan Africa.[15,20] Although universal ART is routinely available during pregnancy and breastfeeding (i.e., Option B+),[21] uptake and adherence present ongoing challenges.[4,6,22] In addition, there is growing recognition that current programs, which emphasize HIV treatment for HIV-positive women alone, may overlook pregnant and breastfeeding women who acquire HIV during pregnancy, but are missed through existing PMTCT services.[23]

4.2. Evolution of PMTCT regimens and the continued need for ART adherence

Significant progress has been made globally to prevent mother-to-child HIV transmission in the past two decades.[18,24] In most African settings, recommended antiretroviral regimens have evolved from short-course regimens, including short-course zidovudine[25] and intrapartum nevirapine,[26,27] to lengthier combination regimens that covered the longer span of pregnancy and breastfeeding.[28] With the expansion of HIV services in the region—and driven by effective global campaigns—there has been a rapid move towards universal antiretroviral therapy (ART) for all HIV-positive pregnant and breastfeeding women. Initially introduced in Malawi, the so-called "Option B+" strategy was soon incorporated into World Health Organization HIV guidelines and adopted by countries in sub-Saharan Africa and worldwide.[29] Subsequent studies of this regimen have demonstrated the efficacy of its various components, including the PROMISE 1077BF/FF studies conducted in multiple African sites and in India.[30-32]

The evolution of PMTCT policy has led to significant reductions in pediatric HIV in sub-Saharan Africa. However, while coverage of services has improved across the region, there is growing concern about achievement and sustainment of virologic suppression during pregnancy and breastfeeding—the key determinant of successful PMTCT.[33,34] In one meta-analysis, the pooled proportion of antenatal mothers with adequate ART adherence (defined as >80%) was only 72%.[33] In Malawi, another study reported adequate adherence in only 73% of pregnant women, with a significant drop in the first three months after delivery.[35] As national programs seek to meet the new 95-95-95 targets set forth by UNAIDS, it is clear that new interventions are needed to support ART adherence during pregnancy and breastfeeding. There appears to be a growing armamentarium of evidence-based practices available to program managers and policymakers. Strategies include integrated HIV care within antenatal settings,[36-38] peer adherence supporters,[39] text messages and other mHealth interventions,[40] and cognitive behavioral interventions.[41]

4.3 PrEP in pregnancy and breastfeeding: new opportunities and challenges

In randomized trials, oral pre-exposure prophylaxis (PrEP), in the form of daily tenofovir disoproxil fumarate and emtricitabine (TDF-FTC), has been shown to be effective for prevention of HIV acquisition,[42-46] including in women.[47,48] This intervention may be particularly well-suited for pregnancy and breastfeeding. Not only are HIV incidence rates exceedingly high during pregnancy in many African settings,[8] features that characterize this period may lead to greater PrEP adherence: high levels of institutional healthcare,[49,50] altruistic motivations towards the unborn fetus,[51-53] a tradition of male partner engagement,[54] and a window of concentrated risk. In

order to be effective, however, adherence is critical. In studies where adherence was high, the risk for new HIV infections dropped by as much as 85%;[47] when adherence was low, results were equivocal.[55]

TDF-FTC has been used in the context of pregnancy for both HIV and hepatitis B therapy, and has a strong track record for maternal and infant safety.[57] Data from PrEP trials have shown that TDF-FTC in the first trimester did not result in adverse outcomes at birth or in infant growth.[58] A smaller, open-label study—where women were given the option of continuing PrEP once they became pregnant—demonstrated similarly encouraging outcomes.[59] Studies in Kenya and Uganda suggest that PrEP can be safely used during breastfeeding with minimal infant drug exposure.[60] In settings with high HIV incidence during pregnancy (i.e., greater than 3.0 infections per 100 person-years), PrEP has been shown to be highly cost-effective across a range of modeling assumptions.[61]

Early studies suggest that pregnant and breastfeeding women will initiate PrEP. In a pilot program in Kenya, a large proportion of HIV-negative women in serodiscordant relationships continued PrEP even after the diagnosis of pregnancy.[59] The decision to remain on the regimens was driven by the desire to prevent horizontal and vertical HIV transmission, the latter in the case of undiagnosed new HIV acquisition.[62] PrEP also appears acceptable in the general antenatal population. As part of the PrEP Implementation for Young Women and Adolescents (PrIYA) project, for example, 1008 pregnant women were approached across 10 health facilities in western Kenya. Overall, 347 (34%) agreed to PrEP counseling. Of these, 252 (73%) were found eligible for PrEP and agreed to initiate it the same day.[63] Few studies, however, have evaluated approaches to support PrEP adherence and there appear to be multi-level barriers to its continued use over time (Figure 1).[56] This represents an important gap in the current PrEP cascade in these settings.

Figure 1: Barriers to PrEP adherence identified in a systematic review of the literature [56]

4.4 Our formative work

During our formative phase, we completed a new systematic review and meta-analysis to estimate the risk of HIV acquisition during pregnancy and breastfeeding in sub-Saharan Africa. Overall, 41 studies met our inclusion criteria. These represented 35 independent cohorts that contributed over 100,000 PY of follow-up. The pooled HIV incidence during pregnancy and breastfeeding was 3.7/100 PY (95% CI: 3.0-4.5), a rate consistent with cohort studies of female sex workers, men who have sex with men, and HIV serodiscordant couples.[44,64-67]

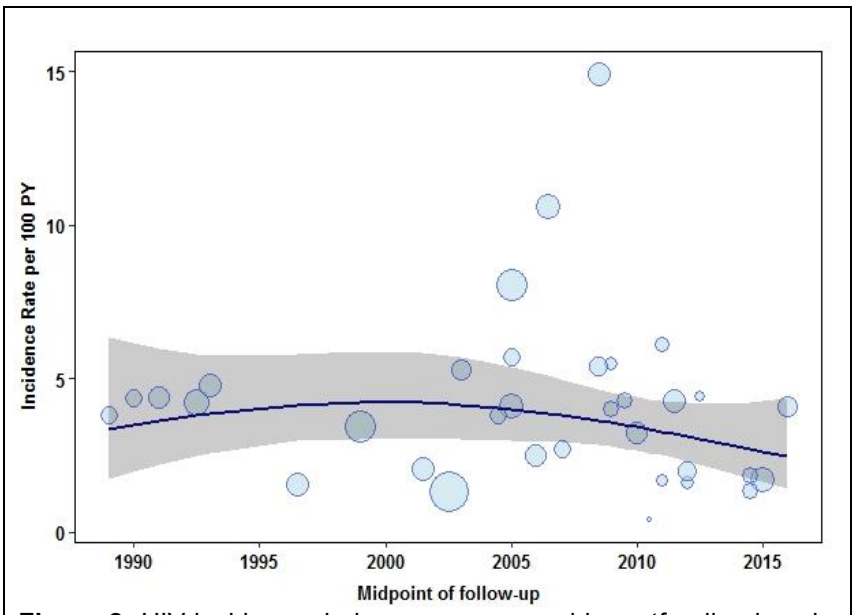

Figure 2: HIV incidence during pregnancy and breastfeeding in sub-Saharan Africa: a meta-analysis

We also found recent declines in estimated HIV incidence over time (Figure 2), coinciding with the expansion of combination prevention services in much of sub-Saharan Africa. While such trends are encouraging, the number of new HIV infections remains unacceptably high among pregnant and breastfeeding women.

We have developed a framework for HIV prevention during pregnancy and breastfeeding. In our framework (Figure 3), partner HIV status is used to stratify the population into six distinct groups (A-F). To optimally reduce horizontal and vertical HIV transmission during pregnancy, we argue that tailored interventions are needed. We hypothesized that three specific points along this dyad-based framework could have important downstream impact: (1) male partner HIV testing, (2) support for ART adherence, and (3) support for PrEP during pregnancy.

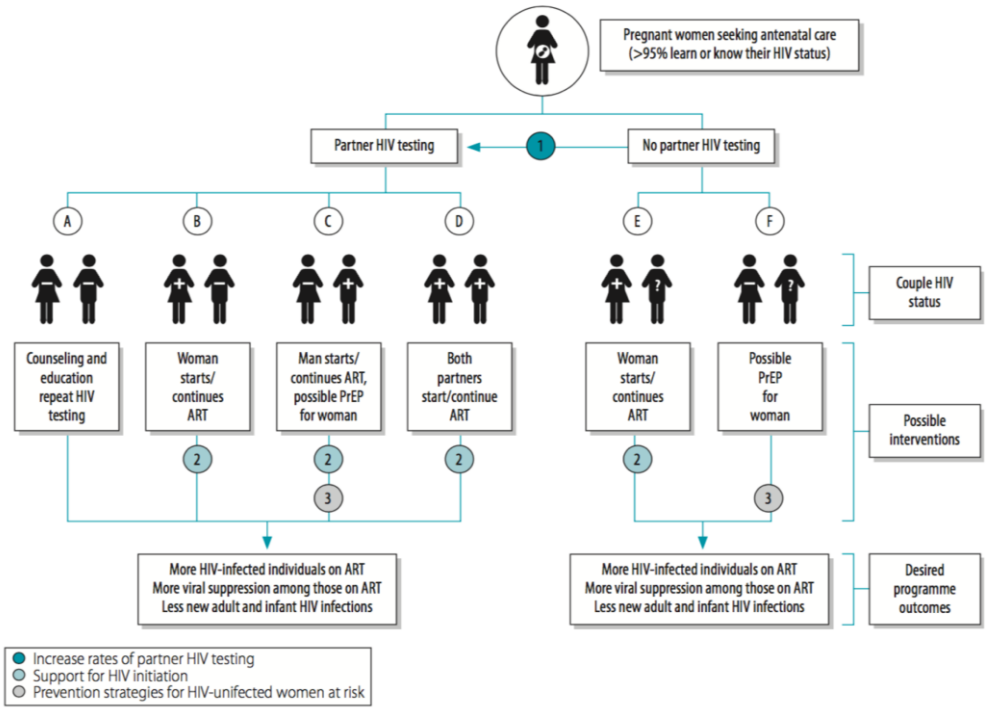

Figure 3: Proposed framework for HIV prevention during pregnancy and breastfeeding[68]

To test this hypothesis, we developed a mathematical model describing horizontal and vertical HIV transmission during pregnancy within patient-partner and patient-infant dyads, respectively.[69] The model was based on biological and behavioral inputs from the medical literature and from ANC program data from Malawi and Zambia. The downstream impact of three main HIV prevention strategies, alone and in combination, was assessed by varying: (1) male partner HIV testing from a base-case value of 15% to a target of 35%; (2) suppressive ART for HIV-positive ANC patients and partners from a base-case of 70% to a target of 90%; and (3) adherent PrEP use for HIV-uninfected female ANC patients from a base-case of 0% to a target of 20%. Using this model, the percentage of horizontal and vertical HIV infections that could be averted relative to the current (base-case) scenario (see Table 1) were estimated as follows:

- Increasing male partner testing to 35% coverage was predicted to reduce horizontal and vertical transmissions by 16.7% and 15.1%, respectively (scenario 2); corresponding reductions with 20% female PrEP use were 13.4% and 12.1% (scenario 4).
- Jointly increasing coverage of both male partner testing and female PrEP use by 20 percentage points was predicted to reduce horizontal and vertical transmissions by ~one-quarter (scenario 7); this reduction increased to ~one-third with a combination of these two interventions plus increasing suppressive ART (scenario 8).
- Across scenarios, a 20-percentage-point increase in suppressive ART for HIV-positive patients and partners had only a modest incremental impact (scenarios 3 vs. 1, 5 vs. 2, 6 vs. 4, 8 vs. 7).

The modeling suggests that combination HIV prevention in ANC settings, particularly approaches that increase male partner testing and female PrEP use, could substantially reduce HIV incidence among pregnant women, their partners, and their newborns in sub-Saharan Africa.

Table 1: Percentage of potential horizontal and vertical HIV infections averted

Scenario	% male partners tested for HIV	% HIV+ ANC patients & partners on suppressive ART	% HIV− female ANC patients on PrEP	% Horizontal transmissions* averted	% Vertical transmissions averted
1	Current (15%)	Current (70%)	Current (0%)	--- (Base case)	--- (Base case)
2	↑ to 35%	Current (70%)	Current (0%)	16.7%	15.1%
3	Current (15%)	↑ to 90%	Current (0%)	1.1%	1.8%
4	Current (15%)	Current (70%)	↑ to 20%	13.4%	12.1%
5	↑ to 35%	↑ to 90%	Current (0%)	21.5%	19.9%
6	Current (15%)	↑ to 90%	↑ to 20%	16.3%	13.9%
7	↑ to 35%	Current (70%)	↑ to 20%	27.8%	25.1%
8	↑ to 35%	↑ to 90%	↑ to 20%	32.1%	29.2%

***** both female-to-male and male-to-female

Based on this formative work, we propose an approach strategy to support HIV treatment and prevention in the context of pregnancy and breastfeeding. Our intervention is *integrated* because it combines both biomedical and behavioral components that could be operationalized in a routine health system. It is also cross-cutting in nature, designed to support both HIV treatment (ART) and prevention (PrEP) in antenatal settings to reduce vertical and horizontal HIV transmission. A strength of our approach is that we consider the HIV status of *all* pregnant and breastfeeding women and offer interventions tailored to those circumstances. It contrasts the typical PMTCT approach, which segments the antenatal population based on HIV status and provides targeted interventions to only select (i.e., HIV-positive) groups.

Table 2: Barriers to HIV prevention during pregnancy and breastfeeding, mapped to our proposed intervention components

Individual level barriers	
Lack of knowledge about PrEP	PCC, AS
Low perceived risk, feeling healthy	PCC, AS
Fear of stigma, incl. perceived infidelity	PCC, AS
Conflicting demands and responsibilities	PCC, AS
Traditional and spiritual beliefs	PCC
Substance use	PCC, AS
Mental health	PCC, AS
Relationship level barriers	
Powers dynamics – social, cultural	PCC, AS
Partner violence	PCC, AS
Fear of disclosure	PCC, AS
Poor social support	PCC, AS
Structural level barriers	
Poor treatment by healthcare providers	PCC
Transportation	PCC, AS
Discreet storage of medications	PCC, AS
Food requirements for medications	PCC
Clinic wait times	PCC, AS
Quality of counseling	PCC
Human resource constraints in clinics	AS
Lack of motivation for healthcare workers	AS

PCC=patient-centered counseling
AS=adherence support

Our intervention comprises two parts: (1) patient-centered counseling and (2) adherence supporter training. These two components were selected because of their adaptability and their ability to address a wide range of barriers at the individual, relationship, and structural level. We also emphasize patient-centered approaches based on recommendations from four PrEP clinical trials teams.[70] We show a list of common obstacles faced by pregnant and breastfeeding women, informed by our formative qualitative research and via expert opinion at our technical stakeholder meeting in September 2018 (Table 2). Each factor is mapped to our proposed intervention, showing how the component parts may address common issues faced by our population.

5.0 RESEARCH QUESTIONS

Does the addition of a multi-component adherence support strategy increase retention in care and HIV viral suppression (as a composite outcome), compared to the standard clinical care for ART adherence for HIV-positive pregnant and breastfeeding women in the antenatal setting?

Does the addition of a multi-component adherence support strategy increase retention in care and functional pharmacologically measured adherence to TDF-FTC for PrEP (as a composite outcome), compared to the standard clinical adherence education for HIV-negative pregnant and breastfeeding women in the antenatal setting?

6.0 OBJECTIVES AND OUTCOMES

The overall objective of this study is to obtain preliminary data about the effectiveness and implementation of a multi-component adherence support strategy for HIV treatment and prevention among pregnant and breastfeeding women.

6.1 Primary and secondary objectives

Our primary objective is:

- To obtain preliminary data about the effectiveness of a multi-component adherence support strategy to increase both ART and PrEP adherence during pregnancy and breastfeeding

Our secondary objectives are:

- To measure acceptability, feasibility, and fidelity of the separate components of the adherence support strategy in a "real world" setting
- To compare social harms between the intervention and control arms in the contexts of ART and PrEP
- To identify factors associated with successful ART and PrEP adherence among pregnant and breastfeeding women

Our exploratory objective is:

- To measure the incidence of maternal HIV infection among HIV-negative women on PrEP

6.2 Study Outcomes

6.2.1 Primary outcome for Trial 1 (ART adherence support)

For Trial 1, the primary outcome is retention in care with HIV viral suppression, defined as <40 copies/mL, at six months following study enrollment.

6.2.2 Secondary outcomes for Trial 1 (ART adherence support)

For Trial 1, secondary outcomes include:

- Retention in care with HIV viral suppression, defined as <1,000 copies/mL
- Adherence to ART as measured by self-report and pharmacy measures (e.g., pill count, medication possession ratio)
- Acceptability, feasibility, and fidelity of adherence support components (intervention arm only)
- Incidence of social harms and other adverse events (both arms)

6.2.3 Primary outcome for Trial 2 (PrEP adherence support)

For Trial 2, the primary outcome is retention in care with functional adherence to PrEP as measured categorically according to plasma and intracellular tenofovir drug concentrations at six months following study enrollment.

6.2.4 Secondary outcomes for Trial 2 (PrEP adherence support)

For Trial 2, secondary outcomes include:

- Adherence to PrEP as measured by self-report and pharmacy measures (e.g., pill count, medication possession ratio)
- Acceptability, feasibility, and fidelity of adherence support components (intervention arm only)
- Incidence of social harms and other adverse events (both arms)
- Incidence of maternal HIV infection

7.0 HYPOTHESES

The implementation of a multi-component adherence support strategy will increase retention in care with viral suppression among HIV-positive pregnant women on ART.

The implementation of a multi-component adherence support strategy will increase retention in care with functional PrEP adherence among HIV-negative pregnant women.

8.0 METHODOLOGY

8.1 Study design

We propose two parallel, pilot randomized trials to evaluate an integrated antiretroviral adherence support strategy for pregnant and breastfeeding women. In Trial 1, we evaluate the proposed multi-component intervention in the context of ART support for HIV-positive women. In Trial 2, we assess the efficacy of the same strategy applied to PrEP adherence among HIV-negative women at elevated risk for HIV acquisition. These pilot trials are designed to obtain preliminary data about the intervention's efficacy and its feasibility and implementation.

8.2 Study intervention

We propose a multi-component strategy to support both ART and PrEP adherence in the context of antenatal care settings. The principal components include:

8.2.1 Integrated Next Step Counseling

Integrated next step counseling (iNSC) is a structured, patient-centered approach informed by motivational interviewing techniques.[71,72] The goal of iNSC is to foster an environment for joint-problem solving in order that the participant may identify their individual needs to increase or sustain sexual health through biomedical and non-biomedical approaches. iNSC frames the counselling sessions as a non-judgmental discussion to explore the facilitators and challenges to sexual health and medication adherence. Through this process the participant is able to identify needs to optimize sexual health protection and medication adherence.

Study staff will be trained to provide iNSC to participants randomized to the intervention arm. iNSC uses eight distinct stages to guide the counselor and patient through a semi-structured discussion: introduce, review, explore, tailor, identify, strategize, plan, and document. Within each, the counselor guides the patient through motivational interviewing and other patient-centered strategies. iNSC will be provided at each scheduled study visit by trained study personnel. A standard case report form is used to document steps included in the discussion, as well as important content emerging from exploration of facilitators, challenges, needs and strategies.

8.2.2 Adherence supporter training

There is a broad body of knowledge showing that peer or partner support can help to enhance adherence and retention in antenatal and HIV programs.[40] We propose an adherence supporter intervention. Participants will be asked to identify a partner, family member, or friend to whom they are willing to disclose their HIV status (for HIV-positive women) or their intent to initiate PrEP (for HIV-negative women). Participants may also select a clinic-based peer to serve in this adherence supporter role. Study staff will provide training for this adherence supporter in the month following enrollment, using materials adapted from similar interventions. A second "refresher" session will be provided the following month as well. The emphasis of these trainings will be basic HIV knowledge, importance of antiretroviral adherence, potential drug side effects, and practical strategies for supporting adherence. Based on our formative research, we believe the addition of this adherence supporter intervention to iNSC will help to reinforce behaviors discussed during patient-centered counseling.

8.2.3 Standard of care (control arm)

Participants randomized to the control arm will receive a similar level of engagement—compared to those in the intervention arm—when visiting the health facility, based on the current standard of care. This will include supplemental information in safe obstetrics and newborn care, based on World Health Organization and local guidelines for safe motherhood and delivered via usual practice.

8.3 Eligibility criteria

Below, we separately list the eligibility criteria for our two parallel trials. Trial 1 focuses on ART adherence support for HIV-positive pregnant women. Trial 2 focuses on PrEP adherence support for at-risk HIV-negative pregnant women. Women who have previously enrolled in the study will not be permitted to enroll again. Following informed consent, participants will be enrolled into one of two parallel randomized trials based on their HIV status.

8.3.1 Eligibility criteria for enrollment in Trial 1 (HIV-positive women)

Trial 1 inclusion criteria

- Documented pregnancy by urine pregnancy test or physical exam
- Documented positive HIV status
- Initiated on first-line ART within the past 30 days, either for the first time or after treatment interruption of 6 months or longer (if previously started but stopped ART)
- Willingness to remain in the study site's catchment area over the course of study follow-up and to comply with visit schedule
- Ability and willingness to provide informed consent

Trial 1 exclusion criteria

- Risk for intimate partner violence or social harms as a result of participation, in the judgement of the study personnel
- Other conditions that, in the judgment of the study personnel, would make participation in the study inappropriate

8.3.2 Eligibility criteria for enrollment in Trial 2 (HIV-negative women)

Trial 2 inclusion criteria

- Documented pregnancy by urine pregnancy test or physical exam
- Documented negative HIV status within the past three months
- Identified factor(s) for elevated risk for HIV acquisition, such as known positive or unknown partner HIV status; report of secondary sexual partners over the past 12 months; diagnosis of STI over the past 12 months; use of post-exposure prophylaxis in the past 12 months; reported use of shared injection material or equipment; and/or unspecified concern about HIV acquisition during pregnancy and breastfeeding
- Willingness to initiate and continue PrEP over the course of study follow-up

- Willingness to remain in the study site's catchment area over the course of study follow-up and to comply with visit schedule
- Ability and willingness to provide informed consent

Trial 2 exclusion criteria

- Positive HIV test at time of screening
- Positive hepatitis B surface antigen test at time of screening
- Renal insufficiency, defined as creatinine clearance <90 mL/min, history of known renal parenchymal disease, or known single kidney at time of screening
- Risk for intimate partner violence or social harms as a result of participation, in the judgement of the study personnel
- Other conditions that, in the judgment of the study personnel, would make participation in the study inappropriate

8.4 Study site and study population

8.4.1 Study site in Zambia

Chipata First Level Hospital is a busy, health facility run by the Lusaka District Health Office serving a population of over 100,000. Chipata averages 400-450 new antenatal patients attending ANC clinic each month with an additional 900-1000 return ANC visits. Similar to other facilities in Lusaka the HIV prevalence in ANC clinic is 10-15%.

8.4.2 Study site in Malawi

Bwaila District Hospital is a high-volume district facility run by the Malawi Ministry of Health. Approximately 1,500 babies are delivered each month at the facility and approximately 500 women present each month for infant immunization visits. Bwaila Hospital has been providing PMTCT services since April 2002. Similar to many parts of Lilongwe, the antenatal HIV prevalence is estimated as high as 13%.

8.5 Recruitment and enrollment procedures

Staff will provide interested participants with additional information and referral to the study. Potential participants will be identified at any point in their pregnancy. All women attending the ANC clinic at enrolling study facilities who meet the eligibility criteria (section 8.3) will be invited to participate in the study. All participants will undergo an informed consent procedure to ensure they are well-informed about the study, its objectives, and its requirements (section 9.2)

8.5.1 Enrollment procedures for Trial 1

Following informed consent, HIV-positive pregnant women eligible for Trial 1 will be enrolled at the same visit. We will collect social, demographic, medical, and behavioral information from all participants. We will obtain from the locator information from the participant, including phone numbers, addresses, and directions. Participants will be randomly assigned to one of two study arms (control or intervention). Statistical software will be used to generate a list of random assignments with a 1:1 ratio, balanced for each site. The randomly generated numbers will be placed in opaque sealed envelopes and sequentially numbered with participant identification numbers.[73]

8.5.2 Enrollment procedures for Trial 2

Following informed consent, HIV-negative pregnant women eligible for Trial 2 will first undergo screening for HIV and hepatitis B surface antigen (HBsAg). Specimens will be drawn; participants will be asked to return in 1-2 weeks to confirm their eligibility to initiate PrEP. During that return visit, candidates who remain eligible (i.e., do

not meet any exclusion criteria) will be formally enrolled at this visit. Similar to Trial 1, we will then collect social, demographic, medical, and behavioral information from all participants. We will obtain locator information from the participant, including phone numbers, addresses, and directions. Participants will be randomly assigned to one of two study arms (control or intervention). Statistical software will be used to generate a list of random assignments with a 1:1 ratio, balanced for each site. The randomly generated numbers will be placed in opaque sealed envelopes and sequentially numbered with participant identification numbers.[73]

8.6 Study follow-up

8.6.1 Study procedures for Trial 1

In Trial 1, we plan study visits at enrollment, month 1, month 3, and month 6. The study procedures at each visit are listed in Appendix 1.

Participants will collect ART from the local PMTCT program. At each visit, we will obtain information about adherence, regardless of the randomization arm. Study visits and pharmacy visits will be scheduled to minimize the number of trips needed at the clinic.

At six weeks postpartum, we will collect information about delivery and neonatal outcomes, including HIV status of HIV-exposed infants. For most women, this will occur over the course of their 6-month follow-up; for those who have not yet delivered by the time they reach their primary study endpoint, we will schedule an additional follow-up visit accordingly.

For those in the intervention arm, we will also collect data about the iNSC and adherence supporter strategies

8.6.2 Study procedures for Trial 2

In Trial 2, we have included a screening phase to further assess eligibility. We plan study visits at enrollment, month 1, month 3, and month 6. The study procedures at each visit are listed in Appendix 1.

Because PrEP programs are only beginning to roll-out in Malawi and Zambia, we plan to dispense TDF-FTC on a monthly basis. At these pharmacy visits, we will obtain information about adherence, regardless of the randomization arm. Study visits and pharmacy visits will be scheduled to minimize the number of trips needed at the clinic.

At six weeks postpartum, we will collect information about delivery and neonatal outcomes, including HIV status of HIV-exposed infants. For most women, this will occur over the course of their 6-month follow-up; for those who have not yet delivered by the time they reach their primary study endpoint, we will schedule an additional follow-up visit accordingly.

For those in the intervention arm, we will also collect data about the iNSC and adherence supporter strategies.

For HIV-negative women who test positive for HIV over the course of the study, TDF-FTC will be stopped while the diagnosis is confirmed. We will collect a specimen for HIV RNA concentration (i.e., viral load) and storage for HIV resistance testing. If the HIV diagnosis is confirmed, the participant will complete exit study procedures (i.e., analogous to month 6 visit) and receive a guided referral to the HIV care and treatment clinic at the study clinic site.

8.7 Implementation assessment

8.7.1 Quantitative assessment of iNSC implementation

A core component of our adherence support intervention is iNSC, a patient-centered counseling approach that uses distinct stages to guide the counselor and patient through a semi-structured discussion. Within each, the counselor guides the patient through motivational interviewing and other patient-centered strategies. A standard case report form is used to document steps included in the discussion, as well as important content emerging from exploration of facilitators, challenges, needs and strategies. These instruments are adapted from other large network studies, including IMPAACT 2009 (pregnant and breastfeeding women in sub-Saharan Africa) and ATN 110/113 (men who have sex with men in the US).

While iNSC case report forms have been used to retrospectively assess fidelity, including in ATN 110/113,[72] this approach has important limitations, including improper, missing, and single reporter (i.e., counselor) documentation. In contrast, we propose to code recorded interviews to assess fidelity. This approach has been used to assess motivational interviewing interventions in numerous settings,[74,75] including for ART adherence in the US.[76] However, despite the similarities in the counseling techniques, such methodologies have not been applied to iNSC to date.

We will conduct detailed audits of iNSC sessions for all participants randomized to the intervention arm, in either Trial 1 or Trial 2 (n=150 total). As part of informed consent, we will seek permission to audio-record the discussions to be later reviewed by trained audit nurses. Two reviewers will independently rate the iNSC session according to key domains, providing both objective and subjective measures about the quality of the counseling. Through this process, our audit staff will also assess the appropriateness of documentation in the primary case report forms, including misinformation and missing data. It will also provide important feedback loops to improve counseling over time.

8.7.2 Quantitative assessment of adherence supporter implementation

A second component of our intervention is training for designated adherence supporters. At enrollment, we will ask what key attributes participants look for in an adherence supporter and then collect detailed information about the identified candidate. In most cases, this will be a close friend or family member, including the participant's primary male partner. We will document details about the adherence supporter training itself—the selected venue, preferences for format (e.g., one-on-one *vs.* group sessions), and completion of key curriculum modules. At each follow-up visit, we will administer a standard questionnaire to all participants (i.e., the index pregnant woman) regarding the adherence support provided by this designated individual. This will include ratings about the adherence supporter intervention along three primary domains: level of engagement of the adherence supporter, relationship dynamics (including trust and confidentiality), and satisfaction with the support provided.

8.7.3 Qualitative assessment of multi-component study intervention

We will conduct semi-structured interviews (SSIs) to explore the acceptability of the multi-component adherence support strategy through the experiences of participants and adherence supporters. We are interested in individual-level engagement with the intervention (including satisfaction with the different components), as well as barriers and facilitators to participation.

We will conduct serial SSIs to gain insight about the study intervention over time. We propose a sample size of up to 40 study participants between the two sites, all randomized to the intervention arm: 20 from Trial 1 and 20 from Trial 2 (Table 3). Three interviews are planned—within four weeks each of the enrollment, 3-month, and 6-month study visits. For individuals who are lost to follow-up or formally withdraw from the study, we will make efforts to trace them and conduct a final exit interview.

Table 3: Sample size for qualitative evaluation

	Participants in Zambia (total interviews)	Participants in Malawi (total interviews)
Trial 1 participants in intervention arm	10 (30)	10 (30)
Trial 2 participants in intervention arm	10 (30)	10 (30)
Total	**20 (60)**	**20 (60)**

We will use SSI guides, developed and piloted by our teams in the field, to provide a framework for the discussion. The SSIs will be conducted in rooms at or near the clinic that provide sufficient privacy to ensure confidentiality of information and are quiet enough to enable audio recording of the interviews. Each SSI is expected to last 30-60 minutes. Based on our past experiences in Malawi and Zambia, including from the formative phase of the parent study, the proposed sample size should allow us to reach thematic saturation.

8.8 Study drug

8.8.1 Study drug for Trial 1

In accordance to the eligibility criteria, all HIV-positive participants in Trial 1 will be on first-line ART according to local HIV guidelines. Dispensation of ART will be managed by the local PMTCT/ART program at the study facility. We will collect information about prescribed ART drugs, pharmacy dispensations, and adherence evaluations at short visits on a monthly basis, timed with routine clinic visits.

8.8.2 Study drug for Trial 2

To ensure a consistent medication supply over the 6-month follow-up period, we will provide TDF-FTC for PrEP to all study participants in Trial 2. Packaged product will be dispensed by an on-site study staff. Participants will begin daily administration of TDF-FTC from the day of randomization to 6 months after randomization. The drug will be dispensed on a monthly basis.

Tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) are antiretroviral agents, indicated and used in combination to reduce the risk of sexually acquired HIV in adults at high risk. Each dose contains 300 mg of TDF and 200 mg of FTC, to be dosed on a daily basis.

TDF is a fumaric acid salt of the bis-isopropoxycarbonyloxymethyl ester derivative of tenofovir. The chemical name is 9-[(R)-2 [[bis[[(isopropoxycarbonyl)oxy] methoxy]phosphinyl]methoxy]propyl]adenine fumarate (1:1). It has a molecular formula of C19H30N5O10P • C4H4O4 and a molecular weight of 635.52. TDF is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. TDF requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV-1 RT by competing with the natural substrate deoxyadenosine 5′-triphosphate and, after incorporation into DNA, by DNA chain termination. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase γ.

The chemical name of FTC is 5-fluoro-1-(2R,5S)-[2 (hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. FTC is the (-) enantiomer of a thio analog of cytidine, which differs from other cytidine analogs in that it has a fluorine in the 5-position. It has a molecular formula of C8H10FN3O3S and a molecular weight of 247.24. It has the following structural formula: FTC is a white to off-white crystalline powder with a solubility of approximately 112 mg/mL in water at 25 °C. The partition coefficient (log p) for emtricitabine is -0.43 and the pKa is 2.65. FTC, a synthetic nucleoside analog of cytidine, is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 reverse transcriptase (RT) by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA which results in chain termination. Emtricitabine 5′-triphosphate is a weak inhibitor of mammalian DNA polymerase α, β, ε and mitochondrial DNA polymerase γ.

8.9 Retention

Once participants are enrolled in the trial, the study team will make efforts to retain them in follow-up to minimize bias associated with loss to follow-up. The study team will closely monitor retention rates and address any issues prospectively. Strategies to minimize attrition include:

- Thorough explanation of the study visit schedule and procedures during informed consent.
- Collection of detailed locator information at enrollment.
- Use of appropriate and timely visit reminder mechanisms (including phone calls and text messages, if participants specifically agree).
- Follow-up after missed visits, including home or alternative, off-site visits where possible.
- Mobilization of trained outreach workers to complete in-person contact with participants at their homes and/or other locations.

If participants elect to discontinue their involvement in the study, we will document their stated reason(s). These will be reported in any reports about the study cohort.

8.10 Safety Monitoring

At each study visit, study staff will evaluate participants for social harms and adverse events (AEs). A social harm will be defined as a non-medical untoward consequence of study participation, including: difficulties in personal relationships, stigma, or discrimination from family or community. An AE will be defined as any untoward medical occurrence in a study participant including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the individual's participation in the research, whether or not considered related to participation in the research. In addition to events related to study procedures we can expect that this population of pregnant and postpartum women to experience adverse events unrelated to study procedures, including opportunistic infections, side effects from antiretroviral therapy or other medications, hospitalization, and death.

All adverse events and social harms will be documented, assessed for seriousness / severity, expectedness, and relatedness, and carefully monitored. The severity of events will be graded using the National Institute of Health's Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. We will also record information on all serious adverse events (SAEs) occurring in participants whether or not they are related to study participation or the study drug, including AEs that:

1. Result in hospital admission (unless hospitalization is preplanned, i.e. for delivery) or prolongation of existing hospitalization
2. Are immediately life-threatening, including drug reactions that necessitate discontinuation of study participation,
3. Cause significant, persistent, or permanent harm or disability, either physical or psychological,
4. Result in death to mother or infant, or
5. Are congenital anomalies or birth defects

8.11 Biological specimen collection and testing

All samples will be obtained from study participants by trained study staff according to approved standard operating procedures. All samples will be processed according to the assay manufacturers' specifications. Some specimens collected from patients in this protocol will be analyzed immediately per standard antenatal care guidelines. Others will be transported, processed, and temporarily housed at the University Teaching Hospital (Lusaka, Zambia) and the UNC Project Malawi (Lilongwe, Malawi). Use of stored specimens for testing that is not specifically designated in this protocol will require additional regulatory approval. All laboratory testing will be performed by trained staff using standard operating procedures and according to specific assay manufacturers' specifications.

8.12 Quality control and quality assurance procedures

Standard Operating Procedures (SOPs) following manufacturer's protocols and detailing technical procedures involved (e.g. sample collection, processing and storage, assay procedures and how to interpret test results) will

be developed and used by the study team. Tests will only be performed by certified laboratory personnel. Site coordinators will complete annual recertification. The certification process is an opportunity to ensure the highest specimen quality and standardize collection techniques.

8.13. Data Management and Storage

Data collected from each participant will include sociodemographic information, relevant HIV and obstetrical history, and results of HIV testing. Study data management (e.g., data transmission, query resolution, etc.) will follow site data management standard operating procedures. Study identification numbers will be used on all forms and communications related to the study. A separate confidential register will link study identification numbers and participant names. All data instruments and registers will be securely stored. Data will be entered into a custom-built database and, where possible, will be validated via double entry. Computers and tablets will be encrypted and password protected and their access restricted to authorized study personnel. Backups of the data will be made on a weekly basis. Data may be transmitted electronically to the study investigators through secure cloud-based servers. Study information will not be released without written permission of the participant, except when necessary for monitoring by the relevant ethical committees or their designees. Data will be disposed of after completion of the study per country guidelines. At that time, electronic records, including linkage codes and identifiers, will be deleted. Paper records will be shredded prior to disposal.

8.14 Sample Size

The two parallel randomized trials in this protocol are each designed as pilot studies, so that we may obtain the necessarily preliminary data about outcomes and implementation to inform future, larger studies. As such, the sample sizes are based on feasibility considerations. Despite this general approach, we provide estimated power and precision of each trial for large differences between the intervention and control arms.

8.14.1 Sample size for Trial 1

We will enroll **100 HIV-positive pregnant women in Trial 1**, randomized 1:1 between the intervention and control arms (n=50 each), with randomization balanced (stratified) by site. The primary endpoint for Trial 1 is the proportion of participants retained and in care with viral suppression at 6-month follow-up. This composite outcome is calculated based on the entire randomized sample, with the approximately 10% of women lost to follow-up included in the denominator.

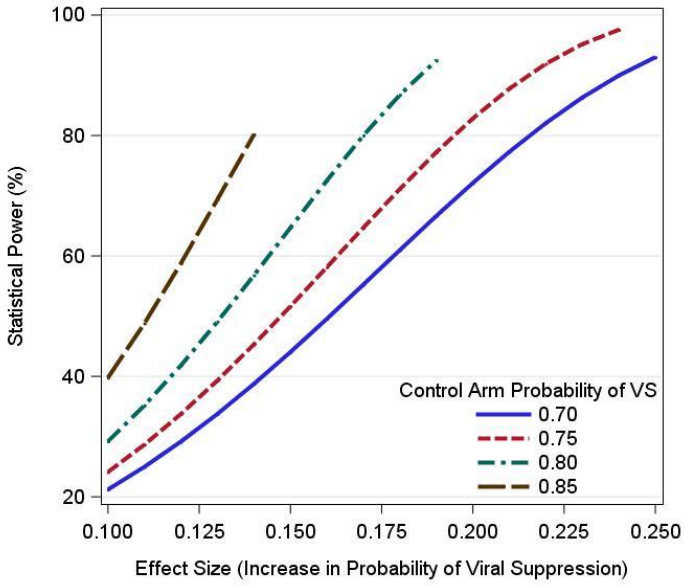

Figure 4: Statistical power in Trial 1 by effect size and control arm probability of retained in care with viral suppression (VS) at 6 months

Figure 4 presents anticipated statistical power for the Trial 1 primary analysis over a range of scenarios. Control arm probabilities of the primary outcome (retained in care with viral suppression <40 copies/mL) are shown ranging from 0.70 to 0.85.[ref] Effect sizes (risk differences) are shown ranging from 0.10 to 0.25. Assuming alpha=0.05 and the probability of being retained and in care with viral suppression in the control arm is 0.75, we anticipate approximately 82% power to detect an increase in viral suppression probability of 0.20 in the intervention arm (i.e., 75% vs. 95% retained with viral suppression in the control and intervention arms, respectively). For lower control arm probabilities of being retained in care with viral suppression (e.g., 0.70) there will be ≥80% statistical power to detect a risk difference of 0.22 or larger. Power calculations were based on a two-sided likelihood ratio chi-square test for two proportions.

The anticipated statistical precision for the Trial 1 and Trial 2 comparisons are presented in Table 4. For control and intervention probabilities of being retained and in care with viral suppression of 0.75 and 0.95, respectively, the anticipated 95% CI for the risk difference has length 0.263. This trial will provide preliminary data on the effectiveness of the intervention which can be leveraged in planning a larger, future trial.

Table 4: Anticipated statistical power and precision for Trial 1 and Trial 2

		Assumed true probability of outcome*			**Example results in expectation n tested / n evaluable (%)**			
Population	N enrolled per arm	Control	Intervention	Power†	Intervention	Control	Difference in probabilities (95% CI)	CI Width
Trial 1	50	75%	85%	24%	43/50 (86%)	38/50 (76%)	10 (-5.4, 25.4%)	30.8%
			90%	52%	45/50 (90%)	38/50 (76%)	14 (-0.6, 28.6%)	29.2%
			95%	*83%*	*48/50 (96%)*	*38/50 (76%)*	*20 (6.8, 33.2%)*	*26.3%*
Trial 2	100	30%	45%	59%	45/100 (45%)	30/100 (30%)	15 (1.7, 28.3%)	26.6%
			50%	*83%*	*50/100 (50%)*	*30/100 (30%)*	*20 (6.6, 33.4%)*	*26.7%*
			55%	95%	55/100 (55%)	30/100 (30%)	25 (11.7, 38.3%)	26.6%

* For Trial 1 (HIV-positive women), the outcome is retained and in care with viral suppression at 6 months. For Trial 2 (HIV-negative women), the outcome is retained and in care on PrEP at 6 months
† Using a likelihood ratio Chi-square test for two proportions, alpha=0.05

8.14.2 Sample size for Trial 2

We will enroll 200 HIV-negative pregnant women in Trial 2, randomized 1:1 between the intervention and control arms (n=100 each), balanced (stratified) by site. The primary endpoint for Trial 2 is the proportion of participants retained and in care with functional PrEP use at 6 months. This composite outcome is calculated based on the entire randomized sample, with the anticipated 10% of women lost to follow-up included in the denominator.

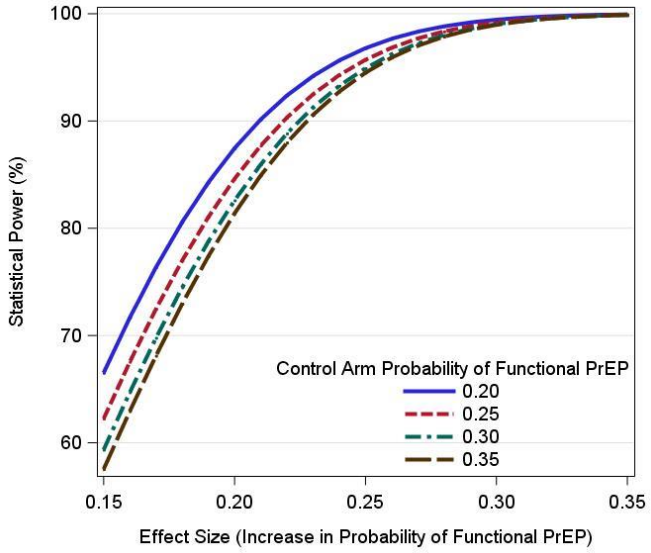

Figure 5: Statistical power in Trial 2 by effect size and control arm probability of retained in care with pharmacologically measured functional PrEP at 6 months

Figure 5 presents the anticipated statistical power for the Trial 2 primary analysis for a range of scenarios. The control arm probabilities of retained and in care with functional PrEP adherence at six months are shown ranging from 0.20 to 0.35; and effect sizes (risk differences) are shown ranging from 0.15 to 0.35. Trial 2 is powered to detect large increases in being retained in care with functional PrEP adherence between study arms. Assuming the probability of being retained in care with functional PrEP adherence in the control arm is 0.30, we anticipate approximately 82% power to detect an increase in the probability of functional PrEP adherence of 0.20 in the intervention arm (i.e., 30% vs. 50% in the control and intervention arms, respectively). For lower control arm probabilities of being retained in care with

functional PrEP adherence (e.g., 0.20) there will be ≥80% statistical power to detect a risk difference of 0.18 or larger. Power calculations were based on a two-sided likelihood ratio chi-square test for two proportions and alpha=0.05. The anticipated statistical precision for the Trial 2 comparisons are presented in Table 4. For control and intervention probabilities of being retained and in care with functional PrEP of 0.30 and 0.50, respectively, the anticipated 95% CI has length 0.267. Like Trial 1, the primary objective of Trial 2 is to obtain preliminary data regarding the efficacy of the intervention which can support future trials.

8.15 Data Analysis Plan

8.15.1 General Approach

The two randomized pilot trials will be conducted in parallel for HIV-positive (Trial 1) and HIV-negative (Trial 2) pregnant women. All primary and secondary endpoints will be evaluated separately for each trial. In order to evaluate effectiveness, analyses will be conducted using an intention-to-treat approach, with women analyzed according to the arm they were randomly assigned. Appropriate descriptive statistics will be used throughout, n (%) for categorical data and median (25th, 75th percentile), mean (SD) and min-max for continuous measures.

Given the pilot nature of these studies, emphasis will be put on estimation and precision of measured effects, rather than null hypothesis testing. An alpha=0.05 will be used throughout to compute 95% confidence intervals (CIs), with no adjustment for multiplicity. In the case of small cell counts or analyses of continuous data with n<30, 95% CI coverage properties of large-sample methods will be evaluated in simulation studies or existing exact statistical methods (e.g., exact CI for a risk difference) will be used. Given potential for precision loss with exact CI methods and the pilot nature of these studies, we will use large-sample methods (e.g., Wald CIs) when the nominal CI coverage level is tenable.

Missing (unevaluable) data are anticipated to be uncommon (≤10%). The primary analyses are based on a composite endpoint of being retained in care with either viral suppression (Trial 1) or functional PrEP use (Trial 2), and thus complete case analyses will be conducted (i.e., missing viral load data = excluded). Women who are not retained in care will be counted in the denominator for the primary analysis. Sensitivity analyses will exclude women who were not retained in care.

8.15.2 Primary endpoint analysis of Trial 1 (ART adherence)

We will assess ART adherence by evaluating the viral suppression status of each study participant 6 months after study enrollment. Participants not lost to follow-up will be classified as either virally suppressed (<40 copies/mL) or not virally suppressed (≥40 copies/mL). Women who are not retained for 6-month follow-up will be counted as failures and will contribute to the analysis denominator. The proportion of women who are retained and in care with viral suppression will be compared between randomization arms based on a linear-binomial model. To control for any site-specific effects, site (country) will be included as a covariate in the model. From the linear-binomial model, the site-adjusted risk difference of being retained and in care with viral suppression for the intervention arm versus the control arm will be calculated, along with a corresponding 95% CI. Results will also be described separately by country (Malawi and Zambia).

8.15.3 Primary endpoint analysis of Trial 2 (PrEP adherence)

We will assess PrEP adherence via drug concentrations of plasma tenofovir (TFV) and intracellular tenofovir diphosphate (TFVdp). Using published algorithms,[77] results from the plasma and upper layer of packed cells (ULPC) assays will be combined to develop a composite adherence score. The scale ranges from 0 to 5 and each category represents a distinct adherence pattern (Table 5). Our primary outcome for this analysis will be *functional* adherence, which we define as 4-5 or more doses per week (scores of 4 or 5). In sensitivity analysis, we will also consider a rigorous outcome of *optimal* adherence (score of 5, representing consistent daily dosing). Future published improvements upon this algorithm will be taken into consideration for sensitivity analyses prior to the final analysis.

Table 5: Adherence composite scores based on TFV and TVFdp concentrations, with doses estimated per interval [77]

Score	TFV in plasma	TVFdp in ULPC	Estimates doses per interval
0	None detectable	<10,000 fmol/mL	Low number or no doses in the interval
1	Detectable	<10,000 fmol/mL	A few doses in the entire interval
2	Any level	10,000 to 100,000 fmol/mL	1-2 doses per week
3	<10 ng/mL	>100,000 fmol/mL	Several doses early in the interval, followed by a stop in the 1-2 weeks leading up to sampling visit
4	>10 ng/mL	100,000 to 1,000,000 fmol/mL	4-5 doses per week
5	>10 ng/mL	>1,000,000 fmol/mL	Approximately daily dosing

In our primary analysis, we will compare the proportion of the sample retained in care with functional PrEP adherence at 6-month follow-up between the two randomization arms using a linear-binomial model. As with Trial 1, site (country) will be included as a covariate in the model. Women who are not retained for 6-month follow-up will be counted as failures and will contribute to the analysis denominator. From the linear-binomial model, the site-adjusted risk difference of being retained and in care with functional PrEP for the intervention arm versus the control arm will be calculated, along with a corresponding 95% CI. Results will also be described separately by country (Malawi and Zambia).

In addition to the primary analysis, assessing functional PrEP adherence as binary outcome, secondary analyses will compare the adherence composite scores between the study arms using a Wilcoxon rank-sum test. This analysis will be restricted to women with an evaluable adherence composite score.

8.15.4 Secondary endpoints analyses of Trial 1 and Trial 2

For both trials, incidence of social harms and other adverse events will be estimated by study arm, and individual events will be described using listings. Retention in the study will be described using frequency tables, and reasons for study drop-out will be tabulated. Participant deaths will be recorded along with the contributing cause(s) of death.

Adherence to ART and PrEP will be measured both by self-report and pharmacy measures. A medication possession ratio (MPR) will be estimated within study arm, and the difference in means between study arms will be compared using a 95% CI. An MPR of 80% or greater will be categorized as good adherence; this threshold will be used for additional analyses of a dichotomized endpoint (retained in care with MPR ≥ 80% at 6-month follow-up). Analyzed risk difference will be estimated using the same approach as in the primary endpoints described above. Data visualizations will display individual-level data points and box-plots of adherence measures (e.g., MPR, pill counts). Self-reported adherence responses will be described within study arm. In Trial 2, incidence of maternal HIV infection will be estimated with a corresponding exact Poisson 95% CI. Additionally, detailed statistical analysis plans will be finalized prior to analysis of each trial.

8.15.5 Implementation assessment: quantitative (intervention arm only)

For the iNSC and adherence supporter components, we will quantify implementation fidelity in separate 100-point scales that consider objective and subjective measures. We will describe their overall distribution and investigate how these differ when stratified by type of study visit, staff member providing iNSC, and calendar time (e.g., in biweekly increments). These assessments will help to characterize the quality of implementation over the course of study participation.

Because of the structure of the intervention, fidelity scoring for the adherence supporter will be summarized over the course of study follow-up for each study participant receiving the intervention. However, we will investigate associations with each of the component domains (i.e., engagement, relationship dynamics, and satisfaction) over follow-up time. We will also conduct stratified analyses based on key characteristics, such as the

relationship with the identified adherence supporter (e.g., male partner, other family member, close friend) and venue of training.

8.15.6 Implementation assessment: qualitative (intervention arm only)

The SSI audio recordings will be transcribed and translated for analysis. All identifiers will be redacted from the interview transcripts prior to analysis. Although we propose sample sizes for each group based on prior experience, the final number interviewed will be determined by theoretical saturation. Data will be analyzed using techniques that include coding, memoing, and matrices to summarize and interpret key patterns in the data. Comparative and thematic analyses will be used to provide an in-depth understanding of the experiences related to HIV testing. The interviews will be audiotaped and transcribed by the interviewers. Analysis of textual data will consist of 5 steps: 1) Reading for Content: Our analysis will begin with data reading until content becomes intimately familiar. As data are reviewed, emergent themes will be noted. Topics that the research has not adequately addressed and ones that emerge unexpectedly will be explored in continued fieldwork. 2) Coding: A list of codes will be created based on identified themes and assigned to specific sections of text so that the text can be easily searched. Code definitions will be documented in a code book. Qualitative interviewers will be trained to apply the codes using Nvivo to ensure inter-coder reliability, 10% of data will be double-coded. 3) Data reduction: Once transcripts have been coded, we will work within each code to identify principal sub-themes that reflect finer distinctions in the data. This entails taking an inventory of what is related to the given code, capturing the variation or richness of each theme and noting differences between individuals or among subgroup. 4) Data display: Matrices and tables that categorize and display data will be used to help facilitate comparisons. 5) Interpretation: Once text has been read and coded, and central ideas extracted, we will identify and explain the core meanings of the data. We will search for relationships among themes identified and develop diagrams in order to map out relationships in the data.

9.0 ETHICAL CONSIDERATIONS

9.1 Ethical approval

All study participants will be fully informed of the study procedures described above. Prior to study activation, for each participating site, ethical approval will be sought from the relevant institutional review boards / research ethics committees, national authorities, and other regulatory authorities.

9.2 Informed consent

All participants will be consented prior to participation and during the consent process they will be reminded that their participation is voluntary. Discussions with prospective participants and informed consent procedures will be conducted in private to protect patient confidentiality. We will obtain written informed consent from all participants. The study procedures, risks, and benefits will be discussed and we will answer all questions prior to obtaining consent. The consent forms will be translated into relevant local languages and back-translated into English to assure accurate translation. For illiterate participants, a literate impartial witness will be present during the entire consent process to ensure that all of the relevant information has been provided and the participant voluntarily gives consent. Eligible women who do not wish to participate in this study will continue to receive HIV and ANC care according to local clinical standards. We will obtain signed permission from the pregnant woman to collect locator information, including phone numbers, addresses, and directions. Permission for collection of locator information and contract tracing will be per usual practice at the clinic.

9.3 Data storage

The confidentiality of all study records will be safeguarded to the extent legally possible. To maintain participant confidentiality, all laboratory specimens, reports, study data and administrative forms will be identified by a coded number only. All databases will be secured with password-protected access systems, and computer entries will

be identified by coded number only. Forms, lists, logbooks, appointment books, and any other listings or data forms that link participant ID numbers to other identifying information will be stored in a separate, locked fireproof safe cabinet in a locked local office. For the data collected through audio recordings, all audio files will be deleted from the recorders after data are transferred into a computer. Study-related computers, tablets, audio tapes, field notes, and other study materials will be kept in a locked cabinet in a locked local office. All data analysis will be performed using datasets which have only study ID numbers as unique identifiers.

9.4 Confidentiality

Measures will be taken to ensure safety of data and confidentiality of all our study participants. All participants will be assigned a unique study ID number. The interview guides will not capture names of the participants but only their ID number. No study participant will be identified in any report or publication about this study. However, for quality control and safety purposes, data that we collect may be reviewed by the sponsor of this study (i.e. United States National Institute of Allergy and Infectious Diseases), the ethical and regulatory committees in Zambia, Malawi, and at the University of North Carolina at Chapel Hill. Clinical information with individual identifiers will not be released without the written permission of the participant. We expect these procedures to adequately protect participant confidentiality.

9.5 Potential risks of proposed research to study participants

Risks to participants in this study are not expected to differ significantly from the risks inherent in the local standard of care for pregnant women. Investigators will make efforts to minimize risks to participants. We do not expect any risks from the one-on-one interviews or counselling sessions. Participants may feel uncomfortable when being interviewed, but may decide to skip or refuse to answer any questions that they do not want to answer. It is expected that this study will expose subjects to minimal risks.

Side effects and serious adverse events with TDF-FTC administration are rare. In HIV-uninfected individuals in PrEP trials, adverse reactions that were reported by more than 2% of TDF-FTC users and more frequently than by placebo subjects were headache, abdominal pain and weight decrease[78]. Side effects of daily TDF-FTC use may include diarrhea, nausea, fatigue, headache, dizziness, depression, problems sleeping, abnormal dreams, and rash. These symptoms may be additive to normal changes associated with pregnancy; however, drug-typically decrease over time on medication. All antiretroviral regimens included in this study have been approved for use for ART and PrEP by the Malawian and Zambian Ministries of Health.

Physical risks also include the risk of discomfort, bruising or swelling from venipuncture. The risks that are associated with venipuncture are infrequent and minimized with the use of proper technique. Such risks include (1) bleeding, (2) bruising, or (3) rarely infection at the site of needle insertion. Individuals may also rarely become faint, in which case symptoms abate after several minutes in a recumbent position. Blood volumes for the study have been calculated to ensure safety. Collection of vaginal and rectal samples may also be associated with some discomfort and mild bleeding. Participants will be reassured in all cases that such feelings are transient.

Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of questions, particularly when discussing HIV infection or sexual behaviors. At each step in the study, we will protect participant privacy and confidentiality to reduce these risks (e.g., consenting participants in a private setting, not including names on case report forms, etc.). Although investigators will make every effort to protect participant privacy and confidentiality, it is possible that participant involvement in the study could become known to others, and that social harms may result (i.e., as participants could become known as HIV-positive). Participants will be given a phone number they can call at any time if they need assistance or feel they are at risk of harm.

9.6 Potential benefits of proposed research to study participants and others

The benefits to participants include: (1) increased adherence to ART, which will decrease HIV viral load and prevent transmission to both infant and partner(s); or (2) increased adherence to PrEP, which will prevent HIV infection; and (3) general improvement in sexual health through the development of individual strategies to overcome barriers to healthcare. Study findings may also inform local strategies to improve existing national HIV programs in Malawi and Zambia, promoting adherence to antiretroviral regimens for HIV-positive and HIV-negative pregnant women in the context of ANC.

9.7 Inclusion of children, sub-populations, and vulnerable populations

This study focuses on the outcomes of pregnant and breastfeeding women; as such, they must be included in our study population. Prisoners will be excluded as they receive care at separate facilities.

9.8 Reimbursement/compensation

There is no cost to participating in the study. Participants will be provided transport reimbursement for each study visit, according to local research standards.

9.9 Dissemination of findings

Study findings will be disseminated through appropriate local channels, including academic and public health research symposia. We will report findings to relevant local authorities in Malawi and Zambia. One or more publications will also be submitted to a peer-reviewed journal. Our study team plans to publish the study results whether positive or negative. The study participants' privacy and confidentiality will be strictly maintained in all results dissemination or publication activities.

10.0 TIME FRAME

In Table 6, we show our proposed timeline for the study activities.

Table 6: Study timeline

Objective	Month																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Site preparation	x																	
Data systems programming	x	x																
Site training	x	x																
Enrollment			x	x	x	x	x	x										
Follow-up				x	x	x	x	x	x	x	x	x	x	x				
Data entry and management			x	x	x	x	x	x	x	x	x	x	x	x	x			
Statistical analysis															x	x	x	
Publication and dissemination																	x	x

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12.0 APPENDICES

1. Schedule of evaluations – Trials 1 and 2

2. Informed consent forms
   - Trial 1 – adherence support for ART
   - Trial 2 – adherence support for PrEP
   - Qualitative interviews

APPENDIX 1:

Schedule of evaluations for Trial 1 – adherence support for ART

	Clinic visits				Pharmacy visit	Pregnancy outcome
	Enrollment	Month 1	Month 3	Month 6	Monthly	6 weeks postpartum
Laboratory studies						
HIV RNA (viral load)	6 mL		6 mL	6 mL		
Hemoglobin	2 mL					
Syphilis screening	2 mL*					
Urine dipstick	X		X	X		
Vaginal microbiome	X	X				
Storage for HIV resistance testing	6 mL**			6 mL **		
Medical exam						
Medical history	X					
Obstetric exam	X	X	X	X		
Questionnaires						
Demographic information	X					
Sexual history	X			X		
Partner information	X		X	X		
Social harms (including IPV)	X	X	X	X		
Stigma	X			X		
Self-efficacy	X			X		
Depression	X			X		
HIV risk behaviors	X	X	X	X		
Delivery information						X
Study drug						
ART dispensed at PMTCT clinic					X	
Pill counts					X	
Self-report adherence					X	
Intervention assessment *(intervention arm only)*						
Integrated next step counseling (iNSC)	X	X	X	X		
Adherence supporter	X					
Qualitative interviews (subset)	X***		X***	X***		

* will perform test if no results available in the medical record
** storage of leftover specimen for HIV resistance testing
*** schedule additional procedures within 30 days of visit for subset of participants enrolled in this component

Schedule of evaluations for Trial 2 – adherence support for PrEP

	Screening	Clinical visits				Pharmacy visit	Pregnancy outcome	HIV confirmatory testing
		Enrollment	Month 1	Month 3	Month 6	Monthly	6 weeks postpartum	(if rapid HIV test is positive)
Laboratory studies								
Rapid HIV antibody test	X	X		X	X			
Hepatitis B Antigen	4 mL							
ALT		2 mL						
Creatinine		2 mL		2 mL	2mL			
Syphilis screening		2mL *						
Urine dipstick		X	X	X	X			
Tenofovir concentration				4 mL	4 mL			
Vaginal microbiome		X	X					
HIV RNA (viral load)								4 mL
Storage for HIV resistance testing								6 mL
Medical exam								
Medical history	X							
Obstetric exam	X	X	X	X	X			
Questionnaires								
Demographic information		X						
Sexual history		X			X			
Partner information		X		X	X			
Social harms (including IPV)		X	X	X	X			
Stigma		X			X			
Self-efficacy		X			X			
Depression		X			X			
HIV risk behaviors		X	X	X	X			
Delivery information							X	
Drug dispensation and adherence assessment								
PrEP dispensation						X		
Pill counts						X		
Self-report adherence						X		
Intervention assessment *(intervention arm only)*								
Integrated next step counseling (iNSC)		X	X	X	X			
Adherence supporter		X						
Qualitative interviews		X***		X***	X***			

* will perform test if no results available in the medical record
** storage of leftover specimen for HIV resistance testing
*** schedule additional procedures within 30 days of visit for subset of participants enrolled in this component