**Evaluation of pre-exposure prophylaxis (PrEP) initiation, retention and adherence in pregnant and breastfeeding women**

PrEP-PP

Pre-exposure Prophylaxis in Pregnancy & Postpartum Period

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## 1. Background and rationale:

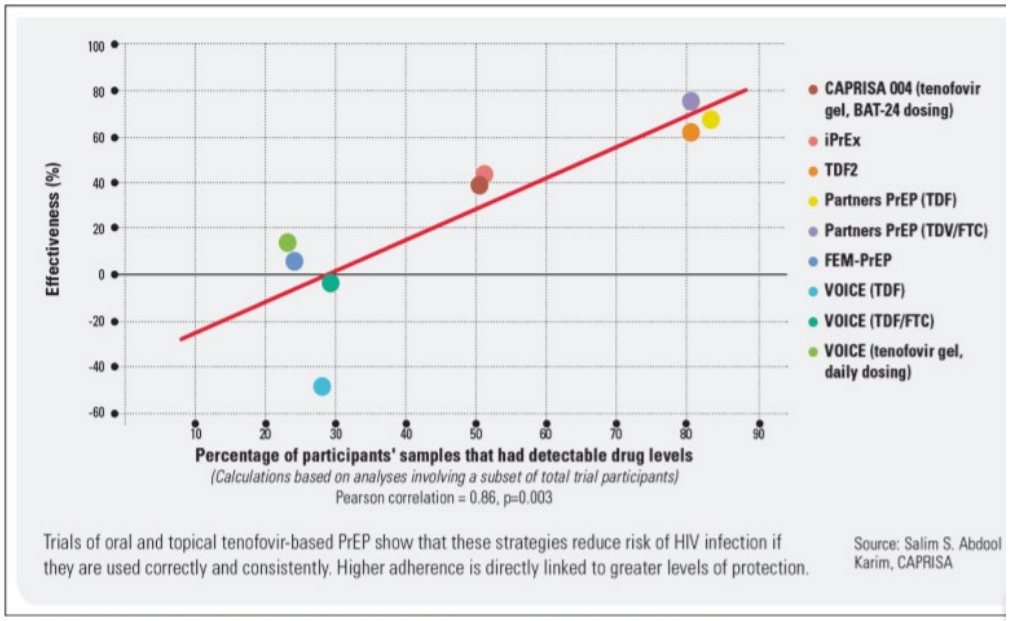
Pregnant and breastfeeding women in South Africa are at very high risk of HIV acquisition and vertical HIV transmission during pregnancy, labour and breastfeeding [1-4]. One-third to half of perinatal HIV transmission occurs in women who are seroconvert during the pregnancy and post-partum period [5-6]. Effective use of pre-exposure prophylaxis (PrEP) could contribute to eliminating maternal HIV acquisition, and hence mother to child transmission (MTCT) of HIV. However, PrEP efficacy requires high levels of adherence, and adherence requires high levels of acceptability, yet there are few data on acceptability and adherence in pregnant and breastfeeding women in high HIV prevalence communities.

**Risk of HIV acquisition in pregnant and breastfeeding women:** Despite increased access to HIV testing and counseling, condom promotions and antiretroviral therapy for male partners, maternal HIV incidence continues to be very high during and after pregnancy. In South Africa it is estimated that maternal HIV incidence was as high as 10.7 per 100-person years (PY) which is highest in urban health facilities at 12.4 per 100 PY [3]. High HIV incidence in pregnancy contributes to the majority of infant HIV infection [1, 4]. Mothers who seroconvert after their first antenatal visit account for approximately 34% of vertical transmission [3]. In a recent meta-analysis, vertical HIV transmission risk was significantly higher in women with incident versus chronic HIV infection in the postpartum period (odds ratio of MTCT in pregnancy [OR]=2.9, 95% confidence interval [CI]=2.2, 3.9) or in pregnancy and postpartum periods combined (OR=2.3, 95% CI=1.2, 4.4) [4]. Biological mechanisms of increased susceptibility of HIV acquisition during pregnancy and breastfeeding may be due to hormonal changes that alter genital mucosal surfaces or distribution of target cells at these surfaces [3]. However, behavioral factors also play a role including increased condomless sex during pregnancy, (multiple) sex partners of unknown serostatus and substance use during pregnancy and breastfeeding periods [3].

**Interventions to prevent maternal HIV acquisition:** We urgently need effective interventions to reduce HIV incidence in pregnant and breastfeeding women. PrEP is one of the only female-controlled methods that is effective for preventing HIV acquisition and safe in pregnancy. Recent meta-analyses demonstrated that use of tenofovir is not associated with increased adverse events during pregnancy or breastfeeding [6-11]. In new guidelines on PrEP, the World Health Organization (WHO) stated that "PrEP can also be considered as an additional prevention choice for HIV-negative pregnant women who are at substantial risk of HIV infection, as part of a comprehensive prevention of mother to child transmission (PMTCT) package" [12]. Currently, however, there is limited data on PrEP acceptability, initiation, and adherence in pregnant and breastfeeding women in high HIV incidence communities [2, 13-15].

PrEP for HIV involves the use of daily antiretroviral (ARV) medications to prevent HIV acquisition, similar to prophylaxis for malaria. Tenofovir (TDF) and tenofovir/emtricitabine (TDF/FTC) in a single tablet fixed-dose combination (FDC) are the ARV agents primarily used in oral PrEP studies to date, though studies of other formulations are under way. In December 2015, the TDF/FTC combination pill was approved for use as PrEP by the Medicine Control Council in South Africa, in combination with safer sexual practices [27]. Daily PrEP

may be used intermittently during periods of perceived HIV acquisition risk, rather than continually and lifelong, as is the case with ARV treatment. HIV testing, and estimation of creatinine clearance are recommended as baseline health measures. PrEP should not be given to those with abnormal renal function, nor should it be commenced in individuals with acute viral symptoms.




*Source*: AVAC Report 2013: Research & Reality: http://www.avac.org/sites/default/files/infographics/PrEP_by_Numbers_jan2016.jpeg

**FIGURE 1:** Effectiveness and adherence in trials of oral and topical tenofovir-based prevention.

To date there have been 10 randomized controlled trials of TDF-based PrEP reporting HIV outcomes. The studies have involved over 17,000 people and have demonstrated an overall reduction in HIV acquisition risk of 51% (women RR 0.57 [95% CI 0.34–0.94] and men RR 0.38 [95% CI 0.2–0.6]) [27-32]. Three studies in which there was high adherence to TDF (> 70% of drug detection) showed PrEP was most efficacious [27-30]. PrEP access is increasing in South Africa primarily in men who have sex with men (MSM) and sex workers. However, as of February 2018, only approximately 5000 people were on PrEP in South Africa, and there are two clinical trials ongoing in pregnant women in South Africa, including IMPAACT 2009, "Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants" which is an ongoing trial in Zimbabwe, Uganda, South Africa and Malawi (http://impaactnetwork.org/studies/IMPAACT2009.asp).

**PrEP safety in pregnant and breastfeeding women:** Studies of PrEP use in pregnancy and breastfeeding are more limited than those in nonpregnant adult populations. However, an increasing number of studies are addressing this question, and available evidence underscores its safety in this pregnancy and breastfeeding women and their infants (1–6). Importantly, a recent systematic review demonstrated that PrEP was not associated with increased pregnancy-related adverse events. No studies have found adverse effects among infants exposed to tenofovir disoproxil fumarate (TDF) as part of treatment for HIV-infected women during pregnancy [6-8] or breastfeeding [9-12]. Specifically, Mofenson et al's systematic review identified seven studies of TDF in HIV-uninfected women. They report, "No statistically significant differences were observed between TDF and comparison non-TDF regimens in pregnancy incidence, stillbirth/pregnancy loss, preterm delivery less than 37 weeks, low birth weight <2500/<1500 g, small for gestational age, birth defects, or infant (>14 days) or maternal mortality." Their study concluded that "given available safety data, there does not appear to be a safety-related rationale for prohibiting PrEP use during pregnancy/lactation or for discontinuing PrEP use in HIV-uninfected women receiving PrEP who become pregnant and are at continuing risk of HIV

acquisition." Based on the existing WHO guidelines and research, potential risks of PrEP adverse events outweigh the benefits of using PrEP when sexually active during pregnancy and breastfeeding periods.

**PrEP safety in young women:** There is very limited data on PrEP use specifically in pregnant adolescents less than 18 years of age (7–9). Very recently, one Phase II clinical trial has been completed in youth ages 15-17, looking at safety and feasibility in young men who have sex with men (10). This study demonstrated safety of TDF-FTC in 78 participants with only one adverse event possibly related to the study drug. There were no adverse effects on kidney function, liver function or bone density. A second study that includes adolescent girls has completed, but final results are not yet available (11). Finally, additional safety data is available from studies focused on the use of the TDF and FTC as antiretroviral therapy in HIV+ youth (7,12). One study looking at long term TDF use in 12- to 17-year-olds over a median of 96 weeks (longer than the proposed timeline of this study) showed no discontinuations due to the study drug and there was no significant decrease in bone mineral density, and kidney function was consistent with normal changes seen in adolescents. Thus, available data suggest that PrEP and its component drugs are safe in adolescent populations, though more studies are needed in both adolescents as a whole, and pregnant adolescents in particular.

**PrEP safety in infants:** Available data on infant outcomes after exposure to TDF-FTC suggest that there are no significant differences in health outcomes, including birth weight, preterm delivery, birth defects, or infant mortality, as summarized in a pair of recent systematic reviews (6,13). Long-term effects on infants have not been investigated in the context of PrEP, though this is becoming an area of active research, with studies such as IMPAACT 2009 focusing on both maternal and infant health outcomes after use of PrEP in the pregnancy and breastfeeding periods (14). However, one short-term study looked at drug levels in mothers and their breastfeeding infants in the context of PrEP treatment (5). They found that FTC was below 0.5% of the target therapeutic level given to infants to prevent postnatal HIV infection. TDF levels were even lower, <0.01% of target therapeutic levels and undetectable in 94% of infants.

Additional data on safety of TDF-FTC on HIV-uninfected infants is drawn from studies of HIV-infected mothers taking ART during pregnancy and breastfeeding while tracking health of infants who remain HIV-uninfected. One such study showed a mild decrease in infant serum phosphate levels between 6 and 12 months, but no decrease in serum creatinine levels with prolonged exposure (15).

No studies assessing long-term outcomes of infants exposed to PrEP are available. What data are available come from studies of health outcomes of HIV-uninfected infants in the context of ART use by HIV-infected mothers. One such study examined infant health after *in utero* TDF exposure with a median infant age of 25 months. This study found no differences in infant mortality, serum creatinine or phosphate levels, or immune cell function based on TDF exposure (16). In contrast, multiple studies demonstrate significant morbidity and mortality among infants exposed to HIV versus those who are not. One recent study looking at HIV-exposed, uninfected infants whose mothers were not on ART prior to labor and delivery, and who were subsequently formula-fed, found that up to 23% of infants had serious adverse events up to 6 months after delivery (17). Other studies have confirmed an increase in morbidity and mortality among HIV-exposed infants as compared to those not exposed, and are summarized in a recent meta-analysis that estimates HIV-exposure leads to a 70% increase in all-cause mortality, corresponding to a risk-ratio of 1.70 (18). However, this increased risk is attributed to HIV exposure, not TDF exposure (18).

Though the data on short- and long-term infant outcomes after exposure to PrEP are limited, comparing available data of TDF exposure versus exposure to HIV during pregnancy and breastfeeding clearly indicate that preventing maternal HIV infection confers significant benefit to the infant as well as the mother. The

benefit of PrEP treatment of pregnant and breastfeeding women must thus consider both the dramatically decreased risk of infant HIV infection (0.5% for chronic vs. 30% for acute maternal HIV infections (19)) as well as the increase in all-cause mortality among HIV-exposed infants (a 70% increase versus those who are not HIV exposed). These two metrics clearly demonstrate that the benefits of PrEP outweigh the minor risks by at least 5:1.

**Side effects of PrEP:** Side effects associated with FTC/TDF include diarrhea, nausea, fatigue, headache and rash, which are seen in about 10% of participants during the initiation of therapy [33]. While these early symptoms are generally well-tolerated and dissipate over a few weeks, such side effects may be less tolerable for pregnant or postpartum women. For HIV-infected pregnant and postpartum women, such risks – including the adverse bone and renal effects of TDF – are offset by the significant health benefits of taking the drug as part of ART. However, the risk of adverse drug effects in relation to the potential HIV prevention benefits for HIV-uninfected women at increased risk for infections requires further evaluation. Tenofovir is a highly-charged anion that does not readily permeate across epithelial barriers. The water soluble pro-drug administered by mouth, TDF, is well absorbed in the gut and is rapidly converted to the active form, tenofovir. While tenofovir is present in amniotic fluid and cord blood, a study of 50 HIV uninfected lactating women receiving 10 days of directly observed oral dosing with TDF/FTC PrEP found low infant drug levels and breastmilk penetration [11]. This suggests the risk of postnatal exposure to PrEP in breastfed infants is low**.**

**Renal damage:** TDF use has been associated with a small and asymptomatic decrease in glomerular filtration rate in HIV-infected individuals. Reports from completed PrEP clinical trials reveal no clinically significant elevations in serum creatinine but transient decrease in estimated glomerular filtration rates and a return to baseline levels within four weeks of PrEP discontinuation [34]. PrEP is not recommended for people with decreased renal function at baseline (GFR < 60ml/min).

**Bone demineralization:** Consistent with data on reduced bone mineral density (BMD) among HIV-infected adults on ARVs, PrEP use has been associated with an approximate 1% reduction in BMD among trial participants [35]. The effect on BMD was shown to be reversible in young African women adherent to PrEP in the VOICE trial with a follow-up dual-energy x-ray absorptiometry (DXA) scan 48 weeks after stopping study drug [35].

**Adverse pregnancy outcome:** A secondary analysis from the Partners PrEP study found no differences between women with incident pregnancies while taking PrEP in rates of preterm birth, congenital anomalies, or infant growth in the first year of life and women taking placebo; however, all women stopped the study medication at time of pregnancy detection, which occurred a median of 35 days (IQR: 29–45) after conception [36]. The rates of adverse birth outcomes did not differ according to study arm (78). The Partners Demonstration Project, an open-label delivery study in Kenya and Uganda, HIV-uninfected women in serodiscordant relationships were given the option of continuing PrEP even after pregnancy was diagnosed. Of the 34 who became pregnant, 30 elected to continue PrEP antenatally. When compared to 96 women who became pregnant in the Partners PrEP clinical trial, there were no differences in pregnancy loss, preterm delivery, or congenital anomalies, though sample sizes were small [36].

**HIV and pregnancy in young women:** Young South African women aged 15–24 years contribute nearly 30% of all new HIV infections in the region. In South Africa, this percentage translates to 113,000 new infections in young women per year, more than four-times the number contributed by their male peers (Figure 2). Further, adolescent girls have high pregnancy rates in South Africa. Approximately 3.7% of 16-year-old girls and 7% of 17-year-old girls were pregnant in 2013 (Figure 3).

Our study proposes to enroll pregnant adolescents 16-17 who provide their assent to participate in the study. The specific age range – enrollment of adolescent and young women 16-19 years – covers a critical period of risk of HIV acquisition. This age range falls squarely within the period of adolescence defined by the World Health Organization as 10-19 years of age. Because of the high HIV incidence and existing policy supporting PrEP among adolescent girls, it would be unethical to not provide adolescent girls and young women with PrEP

**Figure 2.** HIV incidence (%) by age and sex in South Africa

Our team is comprised of highly experienced adolescent HIV prevention researchers, including Dr. Linda-Gail Bekker and Dr. Landon Myer, who have a long history of research that seeks to address the key health issues facing South African adolescent girls while at the same time protecting this vulnerable population. The research sites have considerable experience in conducting clinical research in adolescent populations.

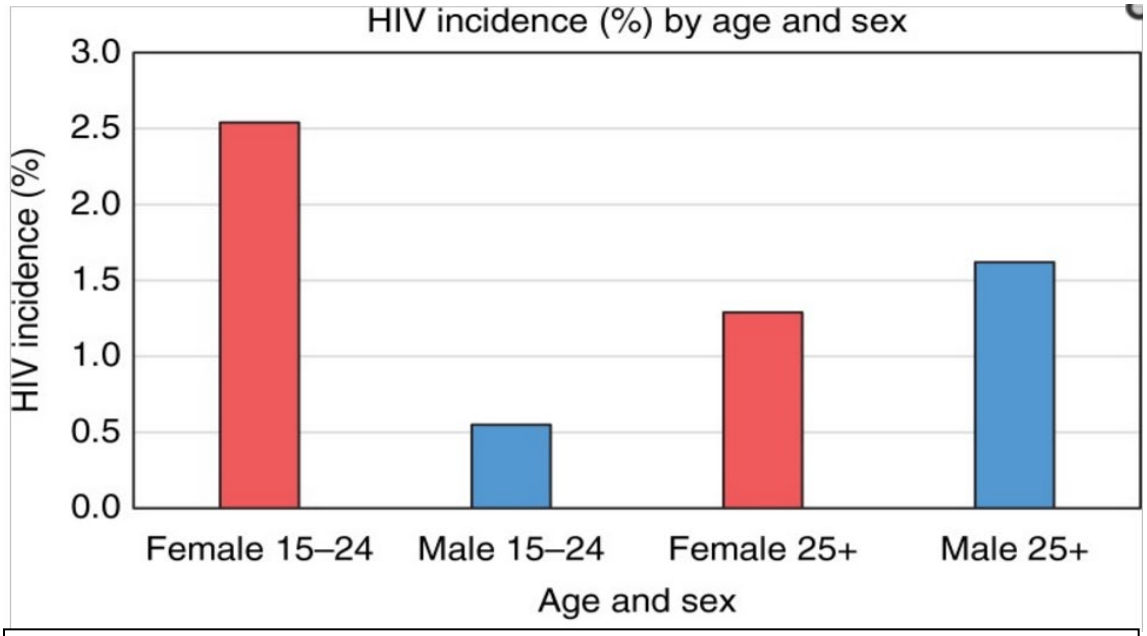
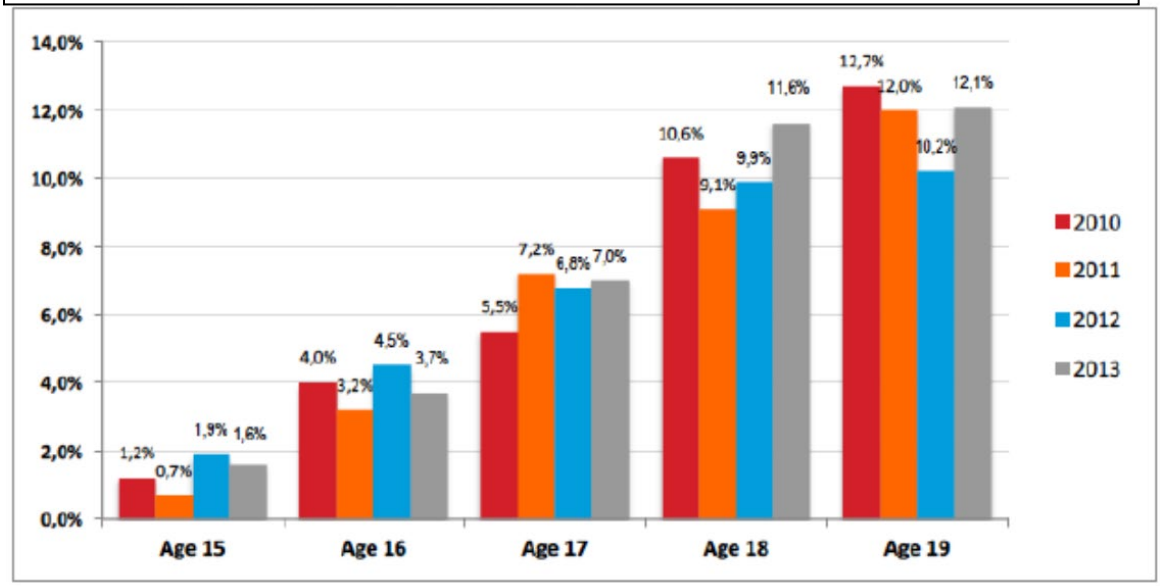



**Figure 3.** Adolescent pregnancy in South Africa, 2010-2013




Our study, PrEP-PP (PrEP in Pregnant and Postpartum women) will determine the distribution of women across the PrEP cascade (i.e. PrEP initiation, retention, and adherence) in a cohort of HIV- pregnant and breastfeeding

adolescent girls and women in two public health facilities in South Africa to inform policymakers about the efficacy of PrEP integration into antenatal (ANC) and postnatal care in high HIV incidence communities.

## 2. Study objectives:

The objectives of the study are to:

1. **Determine the distribution of women across the PrEP cascade:**
    - **(1) Evaluate the proportion of pregnant and breastfeeding women who initiate PrEP out of all women offered PrEP**
    - **(2) Evaluate the proportion of pregnant and breastfeeding women who are retained in the PrEP cohort**
    - **(3) Evaluate the proportion of pregnant and breastfeeding women who initiate PrEP who adhere to PrEP using objective dried blood spot measures and subjective measures of self-reported pill count and adherence**
    - **(4) Evaluate the proportion of pregnant and breastfeeding women on PrEP (and not on PrEP) who acquire HIV, who transmit HIV to their infant, and who report adverse events**
2. **Evaluate patient and provider-level factors associated with the PrEP cascade using quantitative and qualitative approaches (including in-depth interviews)**
3. **Apply an established mathematical model to simulate the impact of improvement in the PrEP cascade on HIV infections averted (maternal and perinatal)**
4. **Evaluate the effect of PrEP on vaginal microbiome in pregnant women and the consequent relationship to adverse pregnancy outcomes.**

## 3. Study design

To address these objectives, we will conduct an observational cohort study in 1200 pregnant women who will be recruited at the first antenatal care (ANC) visit from the Gugulethu and one additional facility in the Hanover Park subdistrict of the Metro Region (to be identified in conjunction with provincial and local health authorities) Midwife Obstetric Units in Cape Town (n=600 pregnant women per site). We will follow the enrolled women through 12-months post-delivery.

**Setting.** The study will take place in two urban townships in Cape Town (Gugulethu and Hanover Park) with high HIV incidence that span the different socioeconomic, cultural, and ethnic groups in South Africa.

- **Site 1: Gugulethu Midwife Obstetrics Unit (MOU)**
- **Site 2: Hanover Park**

We selected those communities because of the high HIV prevalence there in pregnant and breastfeeding women, and because of the high number of mothers visiting every month for ANC and labour/delivery. Further, our team has several years' experience implementing research studies in those MOUs which will facilitate the integration of the study there. In 2017, Gugulethu saw over 3300 new ANC consultations per month, and 72%

(n=2300) are HIV-negative. Hanover Park has saw over 4900 new ANC consultations/month of which 87% are HIV-negative (n=4200). Considering our experience and patient flow, we are confident that at least 60 women/month per facility can be enrolled. Further, Drs. Myer, Bekker and colleagues have conducted research in the Gugulethu and Hanover Park communities for more than a decade and have substantial experience partnering with local health services. Building on this platform, we will use existing infrastructure in each clinic, including capacitating existing clinical providers, pharmacists and counselors, to provide PrEP care to pregnant and breastfeeding mothers. We will hire study staff to assist in the coordination, data management, and overall quality assurance of the counseling, drug provision and participation follow-up.

4. **Study population**

Study counselors will enroll consecutive eligible, consenting pregnant adolescent girls (≥16 years) and women in ANC (n=600 women per site; N=1200 pregnant women) and follow them up for 12-months postpartum or until censorship for a mean of 18-months' follow-up.

Inclusion Criteria:

1. **≥16 years old**
2. **confirmed HIV-negative (confirmed with a 4th generation antigen HIV test),**
3. **Intend on giving birth in the MOU facility**
4. **confirmed to be pregnant**
5. **without psychiatric or medical contraindications to PrEP.**

Exclusion criteria:

Individuals not meeting the above criteria or meeting any of the following criteria will be excluded:

- **Concurrent enrolment in another HIV-1 vaccine or prevention trial**
- **Medical hospitalization in the past year, for reasons not associated with obstetric health (e.g. c-section, miscarriage, pre-eclampsia)**
- **Active TB or receipt of TB treatment within the past 30 days**
- **History of renal disease**
- **Current clinical diagnosis of hypertension**
- **Exhibiting psychotic symptoms (including hallucinations, suicidal or homicidal ideations, or violent behaviour)**
- **Currently or history of taking an anti-psychotic medication (such as for treatment of bipolar disorder, schizophrenia, or postpartum psychosis following a previous pregnancy)**
- **Positive Hepatitis B surface antigen (HBsAg) test on screening**
- **History of bone fracture not related to trauma**

- **Any other medical, psychiatric or social condition which in the opinion of the investigators would affect the ability to consent and/or participate in the study.**

Study censorship will include:

1. Seroconversion
2. Moves away
3. Transfers out of care
4. Lost to follow up (e.g. does not return for study or clinical visit for >8 weeks and study staff are unable to track the participant, or she does not want to continue in the study).

Eligible, consenting women will receive up to R250 in grocery vouchers, transportation and refreshments per visit for their time and effort in the study as well as transportation to the facility. All participants will also receive minor refreshments (e.g. sandwich and cool drink) on the day of their visit.

Procedures for the informed consent process are outlined below. Throughout, trained study staff will ensure that women are aware of their right to refuse and/or withdraw from the study at any time. In addition, study staff will emphasize that all study activities are entirely separate from routine ANC and postnatal care services received and that refusal or withdrawal from the study will have no impact on their ability to access any services provided at any public-sector health facility.

Inclusion of adolescent girls (16+ years old): Our study proposes to enroll pregnant adolescents 16 and 17 years old who will provide unassisted consent. The specific age range covers a critical period of risk of HIV acquisition. Further, *pregnant* adolescent girls are practicing condomless sex and are at elevated risk of HIV acquisition and mother to child transmission. Because of the high HIV incidence and existing policy supporting PrEP among adolescent girls, it would be unethical to not provide adolescent girls and young women with PrEP that might prevent them from acquiring HIV [18].

- All 16 and 17-year-old girls will provide unassisted consent if she chooses to participate.
- We will verify age on participant medical record and/or other official documents (eg, ID book)
- We will not have an upper age limit exclusion criterion for the study

Our definition of unassisted consent meets the following HREC conditions including:

- **The study is no more than minimal risk; and**
- **The nature of the research is acceptable to the Committee, parents or legal guardians or the community at large.**
    - **Our study was approved by the HREC and adolescent girl and young women PrEP is actively being rolled out in South Africa. In particular, we note that HREC has approved projects to use PrEP in adolescent girls ages 16-17 without parental consent in this area (see HREC REF: 567/2016).**
- **Justification for why adolescents are needed in the study:**
    - **Adolescent girls have high HIV incidence and pregnancy rates in South Africa. Girls 16-17 years old are at a critical period of risk of HIV acquisition. Because of the high**

**HIV incidence and existing policy supporting PrEP among adolescent girls, it would be unethical to not provide adolescent girls and young women with PrEP that might prevent them from acquiring HIV**

- **Justification for why adolescents should consent unassisted.**
    - **Our formative research has demonstrated that adolescent girls do not attend antenatal care with their parent/guardian and may find it difficult to get parental consent. Further, our study will include sensitive surveys on sexual behaviours which the adolescent may not want to share with their parents or guardians.**

Based on our work in this setting, we anticipate that young women ages 16-17 comprised <5% of all HIV-negative pregnant women at the Gugulethu MOU, and in turn we anticipate that this population will comprise <5% of our study cohort.

## 5. Study procedures

The study will be integrated into the MOUs. Women will be recruited directly from antenatal care. We will train existing counsellors, nurses and midwives in PrEP treatment (including risks and benefits) and the study. A trained recruiter will recruit directly from women at their first ANC visit, and if interested and eligible after the screening process, the women will go to the study space (Hanover Park) or study trailer (Green Clinic) in Gugulethu for the consent and study enrolment procedures. Consented, enrolled women will be invited to return every 3 months for study visits that correspond with their next ANC visit. All study staff will be trained, salaried staff working for UCT.

The purpose of the study visits will be to evaluate the study objectives. Below are the detailed steps for patient recruitment, enrolment, consent and study participation (Figure 5).

For enrolment/baseline visit

**Step 1:** Health care providers in Gugulethu and Hanover Park MOUs provide group counseling to women at their first ANC visit, beginning with a session orientation, so that the patients know what to expect from the ANC service. This includes information about HIV testing and counselling, ART treatment for PMTCT, and the importance of HIV prevention for those women who test HIV-negative. The counselor will also be trained to provide simple information about PrEP, including what PrEP is, its benefits and risks, and the importance of daily adherence. The counselor will discuss the side effects that may occur in the first 1-2 weeks (similar to new ART patients) and will answer any questions about PrEP in the group setting.

**Step 2:** Following the South African National guidelines, patients privately receive pre-test counseling for HIV, including conducting a risk assessment and assessing her level of concern about having HIV. A discussion follows about the client's most recent risk exposure or behavior before and during pregnancy. The patient again is counseled about how best to reduce the risk of acquiring HIV during pregnancy (and increased risk of MTCT), including previous risk reduction attempts, successful experiences in using condoms, knowledge of partner's serostatus, obstacles to risk reduction, and triggers and situations that increase the likelihood of high-risk behavior.

**Step 3**: Disclosure of HIV negative result and post-test counseling. The counselor shows the client the actual results on the test kit, and then explores the client's reactions to the test results**.** Additional counseling will be provided with information about PrEP if the woman is diagnosed HIV-negative.

**Step 4:** The counselor explains that the mother may be eligible to participate in a study of HIV prevention in pregnancy and breastfeeding periods, including PrEP for women who decide they want to take it. The counselor will review the benefits and risks of using PrEP and participating in the study and will conduct a screening questionnaire.

**Step 5: Screening and study consent process:** If the client agrees to participate in the study (*she does not have to agree to take PrEP*), she will be referred to the study recruiter on site where the study counselor will review the **screening consent form** to evaluate study eligibility (see study inclusion, exclusion criteria). Following affirmative answers on the screening consent form the counselor will conduct a rapid HIV test, to test for acute HIV infection using an antigen/antibody test, and a Hepatitis B surface antigen test. Women who test positive for HIV (antigen, antibody or both) and/or Hepatitis B are ineligible for the study and will be referred to the clinic nurse for treatment and care.

All participants who are eligible for the study and agree to participate in the study will receive the study consent form and listen to/watch the consent form on a previously recorded video in Xhosa to ensure that she understands the study design and consent form before signing, asking questions to ensure comprehension.

**Hepatitis B infection (all women at screening):** The blood draw will also be used to test for active Hepatitis B infection using Hepatitis B surface antigen (HBsAg). Women who test HBsAg+ will be excluded from the study and referred for care according to national protocol

- **In addition, we will draw 1ml of serum to be frozen for testing in the case of discrepant results between the Determine HBsAg+ and NHLS laboratory results.**

**Infant consent form:** The participant will review and sign the infant consent form when she returns for her first post-partum visit with her infant.

**Step 6:** Participants will self-collect two vaginal swabs for storage: (1) for laboratory STI testing and (2) for vaginal microbiome analysis. Results for STI testing will be provided in 2-3 weeks after analysis and participants will be called in with STI+ results for treatment and partner referrals

**Step 7:** All participants will complete the baseline survey, which may take approximately 30-45 minutes to complete.

**Step 8:** The participant will receive individual counseling about HIV prevention in pregnancy including PrEP. Risk reduction counseling will be provided for all women and will include:

- **Consistent and correct use of condoms**
- **Knowing your status and partner's status**
- **If partner is HIV infected, should be on ART and achieve viral suppression**
- **Use of drugs and alcohol increases risk of HIV**

**Step 9: For all participants who decide to take PrEP, the study nurse will draw blood to test for baseline creatinine tests**

- **Women with low creatinine can participate in the study but can only receive PrEP if their eGFR >60mL/min**
- **Test results will be received in 24-48 hours. If eGFR <60mL/min then the nurses will call participant to return and immediately stop using PrEP until they have a normal creatinine result (can return in following month)**

**Step 10:** Participants who opt to take PrEP will receive counselling on the risks and benefits of PrEP. Following the counseling they will be asked: "Do you want to start taking PrEP today to prevent HIV? You will need to take it daily for it to be effective." Her response will be recorded.

**If yes:**

1. **The nurse will provide the participant with a 1-month supply of TDF-FTC and invite her to return in 1 month for her next study visit (after then they will receive 3 months prescriptions to correspond with their quarterly PrEP-PP study visit).**
2. **Nurses will provide contact information to the participant and ask her to call to report any side effects or adverse events while on PrEP, and nurse will track and report serious adverse events to study PIs, Pediatrician, and Obstetrician to decide on how to treat women and infants who have adverse events.**

**If she says she does not want to start PrEP, woman will be in the deferred PrEP arm:**

1. **The nurse will schedule her to return in 3 months for the follow-up study visit**

**Step 10:** Participants will receive study staff contact information, instructions to call with any questions, and will be scheduled for their next visit in 1 month if on PrEP, or 3 months if not on PrEP, for the next study visit

**Step 11:** The study counselor will provide the mother with an appointment card and invitation for her partner to return to receive couples' HIV testing and counseling (if in a relationship). The session will end by encouraging the client to implement her/his risk-reduction plan (including PrEP if applicable and consistent condom use, couples testing) to prevent HIV acquisition.

**Step 12:** The participant will receive a snack and reimbursement for her time and transportation (up to R250 in a Shoprite voucher, refreshment and reimbursement for transportation) which is in line with our ongoing research at these sites. Further, we will combine the study visits with existing ANC or post-partum/well-baby visits to minimize the travel to the facilities outside of normal clinical checkups.

<u>2.For follow up return visits:</u>

Follow up visits will be at 1 month after baseline (to coincide with ANC visits) then after two months after birth then every 3 months thereafter (to coincide with post-partum, well-baby visits).

COVID update: During the COVID-19 lockdown, we will offer women the option of participating in interviews over the phone with participants who are willing and unable to attend the study visit. The interviewer will be in a private room and will ask the participant to be in a private, quiet space. The interviewer is an experienced isiXhosa -speaking research assistant and interviews will be conducted over the phone while the interviewer collects the data on the tablet using RedCap. Updated processes include:

- **Calling participants who miss study visits to assess if they wish to participate a phone interview (and schedule a time when they can be alone in a quiet place for 20-30 minutes)**

The interviewer will ascertain the participant ID and date of birth to ensure that they are speaking with the participant.

**Step 1:** In ANC: Healthcare providers provide counseling on HIV prevention and treatment at each ANC visit including HIV testing, as part of standard of care. In post-natal care and well-baby visits women may not receive regular HIV testing and messages and will receive this within step 2 (with study counselor). Following their ANC visit, enrolled women will be invited to attend the study site in each MOU to do the follow-up visit including the survey and prescription and adherence counseling (if on PrEP).

**Step 2:** Survey at each visit in a private study room

**Step 3:** In the study room, the study counselors will provide PrEP-specific counseling including adherence counseling for those on PrEP (and management of side effects), or counseling about the risks and benefits of PrEP for women not yet on PrEP. Women not on PrEP will be asked if they are interested in starting PrEP at this visit.

**Step 4:** *For participants on PrEP,* the study nurse performs a blood draw for creatinine clearance and a finger prick for DBS testing for adherence measures and (if applicable for this visit). See Table 1 for study calendar.

**Step 5 (for women on PrEP):** At each visit the study counselor will ask, and record responses, all participants on PrEP about any side effects associated with TDF/FTC, including diarrhea, nausea, fatigue, headache and rash (which are seen in about 10% of participants during the initiation of therapy). We will encourage participants to contact the study nurse if they experience side effects between visits.

**PrEP retention methods to improve adherence**: Each study visit will include counselling from peer counsellors who will discuss the importance of retention and adherence to PrEP for effectiveness. We will adapt messages from ongoing PrEP studies conducted by co-I Prof. Bekker and team. We will also provide participants with a brochure to take home that discusses retention, adherence and side effects of PrEP. Each visit will include group and individual counselling. Group counselling will be led by a mother who has successfully taken PrEP for 3+ months to guide a discussion about the importance of retention, reminders on how to take the medication daily and how to address side-effects (e.g. nausea, headaches, etc).

**Step 6:** PrEP provision (for 1 or 2 months) and appointment reminder

*Follow-up & retention:* Tracing information will be taken at the enrolment visit and updated at each study visit. Retention activities will focus on scheduled measurement visits. Following our established approach in this

setting, we will provide reminder calls to participants before their measurement visits, and initiate tracing, including home visits, for women who do not attend the measurement visit within one week of the scheduled visit.

*PrEP provision:* monthly for the first 3 months, and then 2-months intervals after that. Participants on PrEP will have regular monitoring with laboratory tests, including creatinine levels to check for changes in renal function and dried blood spots (DBS) for adherence measures. Those with HBsAg+ will be excluded from the study. We will test for HIV seroconversion at every study visit.

*In-depth interviews:* We will select 30 women at random among women on PrEP and 30 women will be randomly selected among those not on PrEP to participate in a 1-hour in depth interview (over the phone during the COVID-19 lockdown) at first post-partum visit and at study end. The interviewer will follow a semi-structured discussion to review decisions to take (or not take) PrEP, side effects, issues related to adherence, disclosure of PrEP to others, and relationship status with open-ended questions.

*In-depth interviews at end of study (n=45 women from PrEP-PP who used PrEP):* We seek to use qualitative methods to assess the acceptability of, as well as facilitators and barriers to, novel PrEP methods in development (e.g. long-acting PrEP methods such as injectable cabotegravir, the dapivirine vaginal ring, and differentiated PrEP delivery) among a subsample of n=20 postpartum women enrolled in PrEP-PP. In addition we will recruit n=28 women who used PrEP and reported alcohol use or IPV before or during pregnancy to understand risk factors and barriers to PrEP use in this population. The semi-structured interviews will take 20-30 minutes among women who used PrEP during pregnancy or postpartum.

Eligible, consenting women will receive up to R150 in grocery vouchers and refreshments for their effort in the study as well as transportation to the facility.

**Laboratory tests in women on PrEP:**

- **Renal function (in all women on PrEP): Blood draws for creatinine levels will be taken at baseline, 3-month follow-up and at the end of the study, as per SA HIV Clinician Guidelines [26].**
    - **At enrollment: women with eGFR<60ml/min will be excluded from the study**
    - **At 3-month follow up: Women will not be able to continue taking PrEP with an eGFR <60m/min but will be able to continue to participate in the study. If their creatinine results change at the following visit they will be eligible to start PrEP.**
- **DBS: Dried blood spots will be prepared from finger pricks every three months in women on PrEP to evaluate adherence.**
    - **DBS cards will be prepared and stored following standard protocols and stored at -80°C for analysis at the end of the study with the clinical pharmacoanalytic laboratory of Dr. Lubbe Wiesner at UCT. Using techniques developed by Dr. Peter Anderson at University of Colorado [16] adherence will be defined as red blood cells containing TDF-DP ≥40ng/dl which is indicative of daily dosing [16]. As supplementary measures of PrEP adherence, we will use self-reported items that we will adapt from previous studies (including visual analogue scales as well as 3-day, 30-day and last visit recall questionnaires) (22-25), and pharmacy refill records to compare objective versus subjective measures and report on the effectiveness of each measure in correlation to the true measures of TDF-DP. We will determine final dosing and measures following reporting from ongoing PK studies of pregnant women on PrEP (e.g. Impaact 2009).**
- **Two self-collected vaginal swabs will be taken at enrollment and at 1-month visit. These will be stored at -80oC at University of Cape Town until processing for STI testing (at 2-3 weeks) and end of study**

**for bacterial and viral metagenomic sequencing. Briefly this will include microbial DNA extraction and shotgun metagenomic sequencing as performed previously by the Jaspan Lab (Brown, BP Biorxiv 2019).**

**Laboratory tests in all women in the cohort:**

- **STI testing: Self-collected vaginal swab kits will be provided to all participants along with an explanation for their use. Swabs will be analyzed in the laboratory for chlamydia and gonorrhea. Results for STI testing will be provided in 2-3 weeks after analysis and participants will be called in with STI+ results for treatment and partner referrals**
- **HIV testing. As part of the standard of care, all pregnant and breastfeeding women will be tested for HIV using serial rapid HIV tests at each antenatal and postnatal care visit. In addition, PrEP-PP study staff will test for HIV using antigen and antibody HIV tests to rule out acute HIV infections.**
    - **Seroconversion. If a woman, infant or partner seroconverts at any point during the study (including antigen+, antibody+ or antibody/antigen+), they will stop PrEP immediately and be referred to the clinician on duty to initiate ART on the same day, following counseling about PMTCT, breastfeeding, disclosure, and ART adherence, per the SA National Protocols [17]. Seroconverted women will be censored from the remainder of the study and referred to the clinic to initiate ART.**
    - **If a woman seroconverts while on PrEP, there may be a risk of ART resistance, though research has demonstrated that the risk of ART resistance is very low [12, 13]. In the case of seroconversion, Drs Bekker and Myer will follow seroconverters with the study team to evaluate if the patient achieves viral suppression within 6 months of ART start, and if not, she will be referred for specialist attention for testing for ART resistance and clinical follow-up.**

**Adverse events:**

- **Maternal adverse events: We will collect data on maternal adverse events by asking mothers about intercurrent medical events, which will be reviewed by an obstetrician, including any hospitalizations or illnesses. We will also track and report changes in creatinine or other toxicities in women on PrEP.**
- **Infant adverse events: We will collect data on infant adverse events by asking mothers about infant health or intercurrent medical events, which will be reviewed by a pediatrician.**
    - **Adverse birth outcomes will include infants that were small for gestational age, pre-term births, miscarriage and stillbirths.**

Table 1: PrEP-PP cohort measures by participant study month

| | Pregnancy | | | | | | Post-partum | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Participant study month | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| Study enrollment and consent of consecutive sample of HIV-negative pregnant women (n=600 per site, N=1200) | X | | | | | | | | | | | | | | | | | |
| PrEP group, individual (or couples counseling where applicable) and information, education, counseling materials on PrEP in pregnancy | X | X | X | X | X | X | X | | X | | X | | X | | X | | | X |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| and breastfeeding in local languages (at each visit) | | | | | | | | | | | | | | | | | | |
| Routine ANC and postnatal care, well-baby visit follow-up (as available in standard of care), including rapid HIV testing, pregnancy, and birth outcomes | X | X | X | X | X | X | X | | X | | X | | X | | X | | | X |
| Survey to collect: behavioral acceptability, knowledge, and substance use data | X | X | X | X | X | X | X | | X | | X | | X | | X | | | X |
| Screening at enrolment for Hepatitis B and creatinine. If HBsAg+ = excluded from study; if creatinine eGFR<60, excluded from study | X | | | | | | | | | | | | | | | | | |
| Laboratory testing for STIs (chlamydia, gonorrhoea) & treatment if positive | X | | | | X | | X | | | | | | | | | | | |
| For women on PrEP: | | | | | | | | | | | | | | | | | | |
| Laboratory tests of creatinine (if eGFR<60ml/min cannot continue on PrEP) | X | | | X | | | | | | | | | | | | | | X |
| PrEP pharmacy refills and adherence supportive counseling monthly (for first 3 months on PrEP) then every 2 months for following months | X | X | X | | X | | X | | X | | X | | X | | X | | | X |
| Laboratory testing: renal function, hepatitis B; DBS taken for TDF-DP adherence measures (bi-monthly in participants on PrEP) | X | X | X | | X | | X | | X | | X | | X | | X | | | X |
| In-depth interviews for 30 participants per site (N=60) among 30 PrEP users and 30 non-PrEP users | | | | | | | X | | | | | | | | | | | X |
| Collect study data on: (1) PrEP counseling, (2) PrEP medication when requested, (3) regular appointments, (4) IEC materials, (5) adherence counselling and support by facility, through patient survey at every study visit | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Vaginal swab for microbiome | X | | X* | | | | | | | | | | | | | | | |

*between 24 and 28 weeks gestational age.

Training, quality assurance and supervision:

1. ***Existing DOH staff:*** **We will conduct an on-site half day training for existing counselors, nurses and midwives and Managers in the two study sites on the study protocol to ensure that they understand and support the integration of the study in their facility.**

a. **Inclusion of interviews for healthcare providers in health facility to understand their knowledge of and attitudes around PrEP: We seek to interview existing health care providers including nurses, counselors, midwives and managers to understand their knowledge about PrEP, HIV prevention in pregnancy and any attitudes about PrEP that may affect future integration of PrEP and PrEP uptake in the facility. We will interview approximately 35 health care providers from both facilities (Gugulethu and Hannover Park) and have one focus group in those providers in isiXhosa or Afrikaans. We will record, translate and transcribe their responses and analyze the results thematically in order to develop interventions around provider training and support.**

2. ***Study staff:*** **We will hire 2 counselors, 2 nurses, 2 coordinators who will receive a 3-day training program and they will be required to complete the entire training course. After completion of the training, the study PI will observe the counselor and nurse completing each step of the intervention with a mock patient. Upon satisfactory completion of observed sessions, the counselors and nurses will be certified to conduct the study intervention and will receive a completion certificate.**

3. ***Quality assurance*** **will occur on three levels. Day-to-day monitoring, supervision, and support of counselors will be carried out by the Study Coordinators (from UCT—1 per facility). The Study Coordinator will provide on the job training to ensure that counselors and nurses respect the study protocol. Adherence to the counseling protocol will be established by the Study Coordinator who will sit in on a random subsample of 20% of counseling sessions to ensure that counselors are adhering to study protocols.**

<u>Sources of data</u>

There are four sources of data/materials for this trial: (1) survey measures; (2) in-depth interview measures in 60 randomly selected women (n=30 who were on PrEP compared with 30 not on PrEP); (3) laboratory measures; and (4) information abstracted from routine care records, including related to PrEP. The collection of data materials will be conducted by a study measurement team comprised of one study coordinator, one study interviewer, and one fieldworker, per site. All procedures for data collection (regardless of source) will be outlined in a study manual of procedures, with individual activities guided by standard operating procedures (e.g., DBS, specimen transport, or questionnaire administration).

*Survey measures*

At study measurement visits, questionnaires will be administered to mothers participating in the study. Following standard procedures, the questionnaires will be translated into isiXhosa and Afrikaans and back translated to ensure appropriate phrasing. Questionnaires will include:

- Basic demographic information and obstetric history (enrollment only)
- Partner's HIV status
- Sexual behaviors in the past month and week including # of sex partners, type of sex, and frequency of sex and condom use
- Substance use information using the AUDIT-C and DAUDIT scales
- Risk perception scales
- Partner, community and social support for PrEP
- For PrEP users, questions related to PrEP adherence according to self-report (the 3-day and 1-month recall and a visual analog scale) and pharmacy-based pill counts will be conducted at each study measurement visit.

- Additional schedules will be used to collect data on symptoms in mothers that are potential side-effects of PrEP use (at all study visits)
- We will also use short questionnaires to collect data on all women's experiences in the study, and the acceptability of the PrEP initiation, including what kind of services were offered at each visit (e.g. counseling, peer mentorship, and IEC materials, medication)
- Self-reported symptoms related to STIs
- This will focus on the positive and negative aspects of their experiences of PrEP initiation in pregnancy and breastfeeding and any factors associated with PrEP initiation, retention, and adherence.

*Interview measures*

In addition, a subgroup of 60 randomly selected women participating in the study will be enrolled into a qualitative sub-study to complete one in-depth interview (telephonically during the COVID-19 lockdown) at the end of their study participation. We will randomly select 30 women per site to examine in more detail the acceptability of PrEP, barriers, and facilitators to PrEP adherence over time. We will randomly select 15 women per site who were on PrEP and 15 women not on PrEP. This type of 'process evaluation' information can play a critical role in interpreting the quantitative findings and understanding of how to generalize the intervention to other populations. We plan to conduct interviews after approximately 3 months of PrEP use, so at the first postpartum visit, and then at the end of the study. This sub-study will have a separate informed consent process, although these interviews will be timed to take place immediately after study visits scheduled for the trial.

**Updated in-depth interview data collection:**

During the COVID-19 lockdown, we will conduct in-depth interviews over the phone with participants who are willing to be on a call and conduct their interview telephonically. The interviewer will be in a private room and will ask the participant to be in a private, quiet space. The interviewer is an experienced isiXhosa -speaking research assistant and interviews will be digitally recorded over the phone (or in person, after the COVID-19 epidemic lockdown) for later transcription and translation. Updated processes include:

- **Calling participants to assess if they have time conduct an interview (and schedule a time when they can be alone in a quiet place for up to 1 hour)**
- **Recording interviews on the phone (with 2 devices)**
- **Confirm that it is the participant who they are speaking with by confirming their participant ID and date of birth prior to initiating interview**

The interviewer will use a simple question guide to help ensure that key topics are investigated. Interview recordings will collect participant study ID numbers but will not record participant names in any form. Key constructs to be investigated during these interviews include:

- Experiences of study participation, including experiences with study staff, counselors, and clinicians
- Experiences of PrEP initiation and any side effects
- Individual, partner, and family experiences that could be facilitators or barriers to PrEP

**Additional survey on long-acting PrEP attitudes and perspectives**

We will recruit a sample of n=300 pregnant and breastfeeding women during their study visits to complete a brief 10 minute survey about their perspectives about potential acceptability of long-acting PrEP options including vaginal rings, injectables or long-acting pills to understand the potential for further research on long-acting PrEP in this population.

## 6. Analytic Considerations

Sample size considerations

The projected sample size for the study is 1200 women enrolled from 1st ANC visit until 12-months postpartum (estimated mean of 18-months follow-up). This estimate is based on the following assumptions:

**(1) 60% of women will initiate PrEP during the study period (versus approximately 40% who will not);**

**(2) Approximately 75% of women on PrEP will be retained in care at 12-months (defined as not missing >1 study visit among women on PrEP)**

**(3) 70% of those retained in care (n=378) will adhere to PrEP during at-risk periods.**

A sample size of 1200 women yields 80% power to detect identify a relative risk of 2.0 or greater in key covariates including age (<25 vs. ≥25 years), pregnancy and relationship status associated with PrEP initiation versus non-initiation. A sample size of 378 women (e.g. 70% of women retained in the study on PrEP will be adherent as measured by TDF-DP measures) yields 80% power to detect relative risks of 2.0 or greater associated with adherence in the cohort. The analysis will be based on women who complete the 12-month postpartum cohort follow up. We will use relative risk ratios performed at a two-sided α level of 0.05.

Statistical analysis considerations

The primary analysis for Aim 1 will focus on measuring the distribution of women across the PrEP cascade including: initiation, retention and adherence. PrEP initiation will be based on a woman's uptake of PrEP and allow for stoppage and re-initiation; PrEP retention will be defined as not missing >1 visit among those on PrEP (with separate analyses of PrEP stoppage). For PrEP adherence for women on PrEP we will measure both perfect adherence (i.e. level of objective adherence throughout pregnancy and breastfeeding periods) as well as "prevention-effective" adherence (i.e. adherence during times of sexual activity) in participants through objective and subjective measures to evaluate agreement between the two measures, and the level of under- or over-reporting. Primary adherence analyses will use objective measures (TDF-DP from DBS) taken on all those who enrolled and initiated PrEP; subsidiary analyses will consider (i) other adherence measures and (ii) multiple imputation for missing adherence data.

- **Use of PrEP will be measured** through alternate definitions of the main outcomes (e.g. adherence by self-report versus adherence by pharmacy refill), compared using measures of agreement and will be analyzed as independent models to contrast estimated associations.
- **Measurement of prevention-effective execution:** Execution refers to PrEP adherence during a time when at-risk sex happens. It is calculated by: number of doses reported, or recorded/number of expected doses based on HIV risk and PrEP initiation/discontinuation
- **Prevention-effective continuation:** Prevention-effective continuation describes the duration of PrEP use during periods of HIV risk and references the absolute time of use during pregnancy and breastfeeding periods. We will adapt the methodology from previous PrEP studies in SA including FEM-PrEP to create a semi-ordinal composite adherence score from blood specimens from DBS every 2-months.

Table 2: Study measures for PrEP-PP

| Primary Outcomes | **Measurement** | **Source** | **Frequency** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| PrEP initiation | # of women who initiate PrEP over time / total # of women in active cohort = proportion of women who initiate PrEP | Electronic provider log (REDCap) | Daily entry, weekly analysis |
| PrEP retention | # of women on PrEP who return for study visits (do not miss more than 1 visit) / total # of women in active cohort who are prescribed PrEP= proportion of women on PrEP who are retained in PrEP study | Electronic provider log (REDCap) | Daily, analysis weekly |
| PrEP adherence-subjective | # of women taking PrEP, who self-report taking their medication daily (& pill count to confirm this) during periods of sexual risk over time / total woman-time on PrEP in active cohort = PrEP adherence rate (subjective) | Electronic provider log (REDCap) | Daily, analysis weekly |
| PrEP adherence-objective | # of women taking PrEP who have >80% levels at >40ng/mL TDF-DP (indicating dosing in past 24 hours) at 3rd trimester, delivery, and 6 weeks postpartum (and during sexual risk periods during that time) / total woman-time on PrEP in active cohort = PrEP adherence rate (objective) | Lab analysis of TDF-DP measures | Quarterly |
| Secondary Outcomes | | | |
| Predictors of attrition at key steps in the PrEP cascade | Individual, pill/study and disease specific predictors of attrition | Study survey | Study survey: Every study visit |
| HIV outcomes | HIV testing data: HIV-uninfected, HIV-positive for mother and child (if mother seroconverts) | Electronic provider log (REDCap) | Every visit |
| Health outcomes and adverse events | Any side-effects, creatinine, any negative maternal or neonatal outcomes (for mother and infant) | Electronic provider log (REDCap) | During routine lab tests and visits |

For **Aim 1,** each outcome will be described via cumulative incidences at fixed intervals (e.g., cumulative PrEP initiation by delivery) and incidence densities over time. The general statistical approaches to these outcomes are analogous; data will be described graphically, including product-limit graphs, with preliminary analyses based on standard approaches for rates and proportions. To address aims 1 and 2, the modelling approach will vary depending on the nature of the outcome. **PrEP initiation** will be modelled using a probit model (preferred to estimate risk factors for common outcomes) with individual-level covariates as fixed effects; when time-dependent or visit-specific covariates are included in the analysis of cumulative initiation (for **Aim 2)** we will shift to a mixed-effects setting with random effects around individual participants. **PrEP retention** will be examined using similar approaches; in addition, we will use time-to-event methods to estimate factors associated with time to loss (non-retention) among women having initiated PrEP. **PrEP adherence** will be modelled using mixed effects probit models as above and generalized estimating equations to examine the marginal effect of individual-level covariates on adherence during the study [85]. Throughout, robust estimates of standard errors will be used.

The same modelling approaches will be used to explore secondary implementation questions (**Aim 2**) such as the effect of individual, pill/study, and disease factors on PrEP initiation, retention and adherence outcomes (see Figure 1 for conceptual model). The main exposures include: **individual level factors**: risk perception, community/family support, sexual activity, risk, concerns about drug's side effects and harm on self

(pregnancy) and/or infant's health. **Pill regimen and study factors** include: PrEP side effects, time required to participate in study, patient-provider relationship measured from the participants' perspective**. Disease factors** include: stigma around HIV and PrEP, and partner's HIV status (see Table 5 for specific variables).

We will also describe the frequency of HIV outcomes, including maternal HIV acquisition and MTCT, and any laboratory or self-reported side effects (e.g. changes in creatinine or self-reported side-effects [GI symptoms, headache, and etc.]). Analyses will include pure-count and person-time approaches; for the latter, confidence intervals will be calculated following the method of Breslow and Day [86]. We will also describe infant outcomes over time (including birthweight, gestational age at delivery, pregnancy loss and any infant illness, hospitalization or death). While we expect the frequency of adverse HIV-related outcomes to be low, we will describe characteristics of women who did/did not acquire HIV, and consider further analyses using the approaches outlined above.

Aim 3: Mathematical modeling considerations

The objective of **Aim 3** of the PrEP-PP study is to evaluate the potential impact of PrEP on prevention of HIV acquisition and perinatal transmission in South Africa. We will use the results from the cohort as parameters in the model to predict the impact of PrEP initiation, retention, and adherence on a national scale. This model will help identify the potential impact to identify priorities for HIV prevention strategies. We hypothesize that providing access and counseling on PrEP to HIV-negative pregnant and breastfeeding women will bring us closer to the virtual elimination of HIV acquisition and MTCT of HIV. The model will consider two-time points of HIV prevention (during pregnancy and breastfeeding) and two forms of MTCT of HIV: perinatal (at/before birth) and postnatal (through breastfeeding). The model will simulate maternal HIV acquisition during pregnancy and breastfeeding, and the associated risks of vertical transmission, as previously described [18]. The model that will be used in this analysis is the Thembisa model, a combined demographic and HIV model developed for SA [37]. The model divides the population into groups that are defined in terms of demographic characteristics (age and sex), sexual behavior (marital status and level of HIV risk behavior), level of engagement in HIV and HIV stage. The model has previously been calibrated to age-specific HIV prevalence data from SA antenatal surveys, as well as HIV prevalence data from household surveys and recorded death data. The method has been used to model the impact of different PMTCT of HIV strategies and to show the significant contribution of maternal seroconversion during pregnancy and breastfeeding to overall MTCT of HIV [18]. The model has also been used to consider the potential impact of PrEP if it is offered to high risk groups such as youth and commercial sex workers [38, 39], but has not previously been used to model the impact of PrEP during pregnancy and during the postnatal period. The model makes assumptions about the annual uptake of PrEP in different risk groups, the efficacy of PrEP in preventing HIV, the rate at which PrEP is discontinued, and the extent of condom migration in individuals who use PrEP. Using the outcomes of the cohort study to parameterize and validate the mathematical model, the model will estimate the number of HIV infections averted per 100 person-years of PrEP use.

Qualitative data analysis considerations

Following our standard practice, study tools including in depth interview guides and survey materials will be translated into isiXhosa and Afrikaans, the predominant local languages, with independent back-translation for verification. We will digitally record the IDIs and then have the recordings transcribed verbatim. The recordings will be reviewed and verified for accuracy by the interviewers. Transcripts will then be translated and key quotations back-translated to verify meaning. A subset of 20% of transcripts will be double-coded to examine the inter-rater reliability of the coding process. After coding, Investigators will search by substantive codes and themes identified utilizing matrices to organize and allow for comparisons by group (e.g. age,

pregnancy stage, partner status, initiators and non-initiators). Patterns in the matrices will be used to identify themes. Tables of themes will be created using the verbatim quotations as evidence to support the themes that serve as the primary data analyses approach. Conclusions will be based on interpretation of these tables of themes with quotations. Data will be managed using NVivo Software (QSR International).

Data management

Data management will take place at the site following procedures established for other research protocols conducted in Gugulethu. Data collected on paper forms will be entered into a custom designed RedCap database, maintained in a firewall-protected UCT server with nightly backups. The study database will be password-protected following standard password safety procedures. The database will be designed and maintained by a senior data manager who will develop the data dictionary, direct queries, and data quality assurance / quality control activities, and will supervise the data entry clerk. Data quality assurance will be in the form of robust database structure and platform, and "front-end" data checks, including real-time database queries. Quality control will be through data checking scripts to identify out-of-range values, logic violations, and missing observations. Data editing will be based on reference to the form and/or source document in question; all data queries and responses will be logged, and edits will be implemented through separate program files. All study records will contain anonymous participant identification numbers, and no participant names or identifiers will be recorded. Patient routine folder numbers will be stored in a separate password protected database. Once all routine data are linked the folder number will be removed and only the study identification number will be used.

## 7. Ethical considerations

Ethical review

The study protocol, informed consent form, all data collection tools, and other requested documents will be reviewed and approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (UCT-HREC). After the initial review and approval, UCT-HREC will review progress of the study at least annually. Following the approval, we will submit the protocol and approval to University of California Los Angeles (UCLA) for secondary review and approval. UCLA IRB will also review progress of the study annually and we will report all adverse events or amendments to both Ethics Committees.

## Insurance

**Currently we propose that this protocol is covered by UCT's no-fault insurance policy as this is non-commercially sponsored interventional research.**

Ethics approach for including adolescent girls (16+ years old) in study

As per the Department of Health. Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa [21] we will collect parental or guardian consent to participate in the study as well as assent from the minor.

The risk is justified by the anticipated benefit to the subjects: the risks associated with PrEP-PP study procedures are minor and largely non-invasive (except for phlebotomy) and are justified by the anticipated benefit that PrEP would have on prevention of HIV acquisition in this vulnerable group. Thus, there is the potential for direct benefit associated with participation.

Informed consent

Our study will have two consent forms.

1. **The first would be for screening and would include the consenting for the screening survey (inclusion criteria questionnaire) and consenting for the HIV testing (using antigen/antibody testing to screen for acute HIV infection) and hepatitis B surface antigen testing. The study consent form will be for enrolled participants' consent.**
2. **The second is the main study ICF. The study informed consent for the study and questionnaire are modelled after that used in previous studies and will be delivered in participants' home language (isiXhosa or Afrikaans) by trained interviewers. This study ICF details the purpose of the study, study procedures, and the risks and benefits to mothers that participants may encounter at the additional study measurement visit.**

Here, study staff will emphasize to participants that:

- Participation is entirely voluntary, and their choice regarding participation will in no way influence the quality of routine medical care for mothers or their infants
- Women may exit the study at any time for any reason without compromising the quality of health care received.

English versions of the informed consent documents are provided in the Appendix to this document. Translated isiXhosa and Afrikaans versions (as well as a certification of their translation and back-translation) will be submitted to the HREC before the start of the study.

**Risks**

**The potential risks to participants in the study include:**

- **Risks associated with collection of self-reported behavioural and psychosocial information, related to psychosocial distress raised by questionnaire items involving social support, mental health, or disclosure of HIV status**
- **Risks due to loss of confidentiality due to study procedures—for instance, in the process of data collection**
- **Risk associated with asking participants to disclose their status, and potential for interpersonal violence by the partner resultant from the disclosure.**

The potential risks to participants who take PrEP include:

- **Risks associated with PrEP side effects (headaches, gastro-intestinal) for women on PrEP**
- **Risks associated with PrEP adverse events for women on PrEP**
- **Risks associated with collection of dried blood spots**

**All participants will be informed of these risks, and the strategies to minimize these, as part of the informed consent process. These strategies draw directly from prior experiences conducting research on HIV prevention and treatment in Gugulethu, Hanover Park and similar communities across Cape Town.**

## Benefits

*Direct benefit*

**The major potential direct benefit from participating in this study is that pregnant women will receive best possible HIV prevention including ongoing counseling, Hepatitis B screening, STI testing and treatment and PrEP if they choose to take it which can prevent HIV acquisition during pregnancy and breastfeeding periods. If women do not opt to take PrEP, or are not adherent to PrEP, she will still receive the ongoing counseling and STI testing. Their participation may help us answer our research questions and help us inform health programs for pregnant women in the future. The benefit of participating in the study and preventing HIV acquisition and onward infant transmission is likely to outweigh the risk of PrEP exposure or other risks.**

*Indirect benefit*

**By identifying the optimal strategy for delivering PrEP to pregnant women, this study has the potential to lead to improved HIV prevention interventions to protect against HIV acquisition and vertical transmission in HIV-uninfected women and their infants in Cape Town, the Western Cape Province, and across South Africa.**

## Compensation

Participants will be given up to R250 in grocery vouchers for their time and effort in every study measurement visit as well as additional money to cover the cost of transport for each study visit. This is in line with ongoing research in the two sites, as the study visits will correspond to regular ANC or postpartum/well-baby clinical visits wherever possible. All participants will also receive refreshment on the day of their visit.

## Confidentiality

**The following steps will be taken to minimize the risk of any loss of confidentiality throughout study design and conduct.**

- **All personnel involved in data collection and management will undergo specific training for the study in confidentiality and related patient protection issues.**
- **Following standard practice, all patient- and study-related information will be kept in locked cabinets at either the study office in Gugulethu, Hanover Park or at UCT.**
- **Anonymous participant identification numbers will be used on all study documents. Collection of participant names and other identifiers will be restricted to informed consent documents, patient tracing materials, and a study identification key, all of which will be kept in a locked cabinet in the study office at Gugulethu and at UCT separate from other study documentation and accessible only by the project coordinator and local PI. No CRF will include participant name, including CRF that may reflect HIV status of women or their children (including STI test results or treatment).**
- **All electronic records will be kept in password-protected files. All electronic communications of study data will be through password-protected, encrypted files. All data storage at the University of Cape Town will be within a firewall-protected server.**
- **The metagenomic sequencing and relevant metadata will need to be deposited into a database such as dbGaP after our publication. We will remove any identifying information from this data, including date of birth and leave age in days only.**

**While efforts will be made to minimize the loss of confidentiality, in the event that staff learn that the participant is a threat to themselves or to others or of possible abuse by partners, the proper authorities will be notified. This exception will be included in all study informed consent forms.**

**Internal monitoring**

**The purpose of monitoring is to verify the rights and well-being of human subjects are protected; that trial data is accurate, complete and verifiable with source data; that the trial is conducted in compliance with the protocol, GCP and the applicable regulatory requirements. Throughout the conduct of the study, internal study monitoring will be led by the study PIs. We are collaborating with the study Paediatrician, Dr. Lisa Frigati and Obstetrician, Dr. Greg Petro, who will monitor emergent clinical concerns as co-Investigators. They will monitor all adverse events and advise on treatment for all participants who have adverse events.**

**Throughout the duration of the study, study PIs, co-investigators and the study coordinator will participate in weekly conference calls to monitor the rate of participant enrolment and the integrity of protocol implementation (including the completion of informed consent and quality of study measures). In addition, participant retention and safety endpoints will be discussed as well. We will report all serious maternal and/or infant adverse events, including any laboratory or other measures that require women to stop PrEP, to the study's DSMB and Ethics Committee.**

Because of the vulnerable nature of the study population –pregnant women and adolescent girls –, and the innovative nature of providing PrEP to those women, we have chosen to invite an independent DSMB to review study progress. While independent DSMB review is not common in implementation research, we feel that external review of study progress will be critical to maximizing the benefits and minimizing the risks of the PrEP-PP evaluation. Briefly, the DSMB will monitor:

- **Study protocol and tools for data collection**
- **The rate of recruitment at different time points**
- **Adverse events, including social adverse events, identified during the study**
- **any missing or spurious data with the Investigator, which should be resolved in a timely manner.**

**DSMB members:**

- **Dr. Raphael Landovitz (UCLA) is an infectious disease and HIV clinician with both a clinical and research interest in HIV prevention, particularly the use of chemoprophylaxis as part of HIV prevention - PrEP, PEP, and microbicides - and their impact on risk behavior. He works in the ACTG, HPTN and ATN clinical trials networks.**
- **Dr. Barbara Moscicki (UCLA) is Professor of Pediatrics, Chief of Adolescent and Young Adult Medicine at UCLA who is a member of the Scientific Leadership Group in Pediatric HIV and AIDS Cohort Study with 20 years of experience conducting research on HIV infected adolescents.**
- **Dr. Grace Aldrovandi (UCLA) is Chief of Pediatric Infectious Diseases at the David Geffen School of Medicine at UCLA and PI of International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Laboratory Center and other R01 funded projects.**
- **Dr. James McIntyre (UCT) Ob-Gyn, is the CEO of the Anova Health Institute and Honorary Professor in the School of Public Health & Family Medicine at UCT, with significant experience in research on PMTCT and PrEP delivery in SA.**

- **Dr. Sinead Delany-Moretlwe (Wits) is a MD epidemiologist who is a PI on three PrEP demonstration projects for adolescent girls and young women across SA, with a particular focus on evaluating interventions that may address the social and structural barriers to PrEP use in young women.**

**Use of Information and Publications**

**Publication or presentation of the results of this study will be agreed upon in collaboration with the study investigators. Note that the funding agency has no input in the decision to present or publish study data or the nature of the data that are presented or published.**

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Evaluating the PrEP cascade in HIV-negative pregnant and breastfeeding women in South Africa (PrEP-PP): Statistical Analysis Plan


## 1. Review of statistical design

Our study, PrEP-PP (PrEP in Pregnant and Postpartum women) will determine the distribution of women across the PrEP cascade (i.e. PrEP initiation, continuation and persistance) in a cohort of 1200 HIV-negative pregnant and breastfeeding adolescent girls and women recruited and followed in two public health facilities in South Africa to inform policymakers about the effectiveness of PrEP integration into antenatal (ANC) and postnatal care in high HIV incidence communities.

### The objectives of the study are to:

**AIM 1:** Determine the distribution of women across the PrEP cascade (initiation, continuation and persistence on PrEP) and outcomes (maternal HIV incidence, infant HIV incidence, and adverse events) in a cohort of pregnant and breastfeeding women using quantitative and qualitative approaches

(1) Determine the distribution of women across the PrEP cascade and outcomes including HIV acquisition (maternal and infant) and adverse events
   1. PrEP initiation: defined PrEP start at any time point in the study
   2. PrEP continuation: defined as return to clinic and PrEP refill within 45 days post initiation
   3. PrEP persistence: defined as reporting taking PrEP in $\geq$5 out of last 7 days or $\geq$25 out of last 30 days (self-reported), or $\geq$600 fmol/punch from DBS TDF-DP (objective report)

(2) Evaluate key predictors of PrEP initiation, continuation and persistence on PrEP including age, substance use, relationship status and partner's serostatus

**AIM 2:** Evaluate patient and provider-level factors associated with the PrEP cascade using quantitative and qualitative approaches

(1) Evaluate individual-level factors (including risk perception, substance use, sexual activity, and partner/family/community encouragement) associated with PrEP initiation, continuation and persistence on PrEP

(2) Evaluate pill and study factors (including PrEP-related side effects, time to participate in study, participant-provider communication) associated with PrEP initiation, continuation, and persistence on PrEP

(3) Evaluate disease factors (including partner's HIV status, HIV-related stigma) associated with PrEP initiation, continuation, and persistence on PrEP

**AIM 3:** Apply an established mathematical model to simulate the impact of improvement in the PrEP cascade on HIV infections averted (maternal and perinatal)

## Sample size considerations

The projected sample size for the study is *1200 women enrolled from 1st ANC visit until 12-months postpartum (estimated mean of 18-months follow-up).* This estimate is based on the following assumptions:

(1) 60% of women will initiate PrEP during the study period (versus approximately 40% who will not);
(2) Approximately 75% of women on PrEP will be retained in care at 12-months (defined as not missing >1 study visit among women on PrEP)
(3) 70% of those retained in care (n=378) will adhere to PrEP during at-risk periods.

A sample size of 1200 women yields 80% power to detect identify a relative risk of 2.0 or greater in key covariates including age (<25 vs. >25 years), pregnancy and relationship status associated with PrEP initiation versus non-initiation. A sample size of 378 women (e.g. 70% of women retained in the study on PrEP will be adherent as measured by TDF-DP measures) yields 80% power to detect relative risks of 2.0 or greater associated with persistence in the cohort. The analysis will be based on women who complete the 12-month postpartum cohort follow up. We will use relative risk ratios performed at a two-sided α level of 0.05.

## Statistical analysis considerations for AIMS 1 & 2

The primary analysis for *Aim 1* will focus on measuring the distribution of women across the PrEP cascade including: initiation, continuation and persistence. ***PrEP initiation*** will be based on a woman's uptake of PrEP and allow for stoppage and re-initiation; ***PrEP continuation*** will be defined as not missing >1 visit among those on PrEP (with separate analyses of PrEP stoppage). For ***PrEP persistence*** for women on PrEP we will measure both *perfect persistence* (i.e. level of objective persistence throughout pregnancy and breastfeeding periods) as well as *"prevention-effective" persistence* (i.e. persistence during times of sexual activity) in participants through objective and subjective measures to evaluate agreement between the two measures, and the level of under- or over-reporting. Primary persistence analyses will use objective measures (TDF-DP from DBS) taken on all those who enrolled and initiated PrEP; subsidiary analyses will consider (i) other persistence measures and (ii) multiple imputation for missing persistence data.

- **Measurement of use of PrEP:** Conducted through alternate definitions of the main outcomes (e.g. persistence by self-report versus persistence by pharmacy refill), compared using measures of agreement and will be analyzed as independent models to contrast estimated associations.
- **Measurement of prevention-effective execution:** Execution refers to PrEP persistence during a time when at-risk sex happens. It is calculated by: number of doses reported, or recorded/number of expected doses based on HIV risk and PrEP initiation/discontinuation
- **Prevention-effective continuation:** Prevention-effective continuation describes the duration of PrEP use during periods of HIV risk and references the absolute time of use during pregnancy and breastfeeding periods. We will adapt the methodology from previous PrEP studies in SA including FEM-PrEP to create a semi-ordinal composite persistence score from blood specimens from DBS every 2-months.
  For *Aim 1*, each outcome will be described via cumulative incidences at fixed intervals (e.g., cumulative PrEP initiation by delivery) and incidence densities over time. The general statistical approaches to these outcomes are analogous; data will be described graphically, including product-limit graphs, with preliminary analyses based on standard approaches for rates and proportions.

For *Aim 1*, we will also describe the frequency of HIV outcomes, including maternal HIV acquisition and MTCT, and any laboratory or self-reported side effects (e.g. changes in creatinine or self-reported side-effects [GI symptoms, headache, and etc.]). Analyses will include pure-count and person-time approaches; for the latter, confidence intervals will be calculated following the method of Breslow and Day. We will also describe infant outcomes over time (including birthweight, gestational age at delivery, pregnancy loss and any infant illness, hospitalization or death). While we expect the frequency of adverse HIV-related outcomes to be low, we will

describe characteristics of women who did/did not acquire HIV, and consider further analyses using the approaches outlined above.

To address *Aims 1 and 2*, the modelling approach will vary depending on the nature of the outcome. PrEP initiation will be modelled using a probit model (preferred to estimate risk factors for common outcomes) with individual-level covariates as fixed effects; when time-dependent or visit-specific covariates are included in the analysis of cumulative initiation (for *Aim 2*) we will shift to a mixed-effects setting with random effects around individual participants. PrEP continuation will be examined using similar approaches; in addition, we will use time-to-event methods to estimate factors associated with time to loss (non-continuation) among women having initiated PrEP. PrEP persistence will be modelled using mixed effects probit models as above and generalized estimating equations to examine the marginal effect of individual-level covariates on persistence during the study. Throughout, robust estimates of standard errors will be used.

For each outcome, covariates in the multivariable models will be selected a priori using directed acyclic graphs; the main exposures of interest include: age (<25 vs. >25 years old), pregnancy status (by trimester and pregnant vs. postpartum), relationship status (married/cohabiting vs. not) and substance use (vs. none). We will explore associations within subgroups of participants using interaction terms in predicting outcomes involving these four covariates in particular. Throughout the analysis of outcomes in the PrEP cascade, losses of patients at early stages are highly informative about outcomes later in the cascade. To specifically address this, we will perform further bias analyses with inverse probability of censoring weighting in addition to complete case analyses. We will also use multiple imputation via chained equations for missing data [83] to examine the potential impact of missing data.

For *Aim 2*, the same modelling approaches will be used to explore secondary implementation questions such as the effect of individual, pill/study, and disease factors on PrEP initiation, continuation and persistence outcomes. The main exposures include: individual level factors: risk perception, community/family support, sexual activity, risk, concerns about drug's side effects and harm on self (pregnancy) and/or infant's health. Pill regimen and study factors include: PrEP side effects, time required to participate in study, patient-provider relationship measured from the participants' perspective. Disease factors include: stigma around HIV and PrEP, and partner's HIV status.

**For *Aim 2* qualitative data analysis, following our standard practice, study tools including in depth interview guides and survey materials will be translated into isiXhosa and Afrikaans, the predominant local languages, with independent back-translation for verification. We will digitally record the IDIs and then have the recordings transcribed verbatim. The recordings will be reviewed and verified for accuracy by the interviewers. Transcripts will then be translated and key quotations back-translated to verify meaning. A subset of 20% of transcripts will be double-coded to examine the inter-rater reliability of the coding process. After coding, Investigators will search by substantive codes and themes identified utilizing matrices to organize and allow for comparisons by group (e.g. age, pregnancy stage, partner status, initiators and non-initiators). Patterns in the matrices will be used to identify themes. Tables of themes will be created using the verbatim quotations as evidence to support the themes that serve as the primary data analyses approach. Conclusions will be based on interpretation of these tables of themes with quotations. Data will be managed using NVivo Software (QSR International).**

## Study measures

**Under Aim 1, we will measure the following primary outcomes in the PrEP cascade defined as PrEP imitation, continuation and persistence over time in the cohort.**

1. **PrEP initiation:** # of women who initiate PrEP over time / total # of women in active cohort = proportion of women who initiate PrEP
2. **PrEP continuation:** # of women on PrEP who return for study visits (do not miss more than 1 visit) / total # of women in active cohort who are prescribed PrEP= proportion of women on PrEP who continue in PrEP study

3. **PrEP persistence:** defined as reporting taking PrEP in ≥5 out of last 7 days or ≥25 out of last 30 days (self-reported), or ≥600 fmol/punch (~7 doses/week) from DBS TDF-DP at 3rd trimester, delivery and 6 weeks postpartum (objective report)

**In addition we will measure secondary outcomes defined as continuation and persistence during periods of sexual risk.** Different levels of PrEP continuation and persistence may be needed depending on sexual risk. Our study will measure both perfect persistence (e.g. level of objective persistence throughout pregnancy and breastfeeding periods) as well as "prevention-effective" persistence among participants. We will measure persistence through objective and subjective measures to evaluate the agreement between the two measures, and the level of under- or over-reporting.

a. **Use of PrEP will be measured via:** (i) quarterly testing of DBS to test for TDF-DP adapted from previous studies of PrEP objective measures; (ii) self-reported items that we have adapted from previous studies (including 7-day, 30-day and last visit recall questionnaires), (iii) pill counts and (iv) pharmacy refill records.
b. **Measurement of prevention-effective execution:** Execution refers to PrEP persistence during a time when at-risk sex happens. It is calculated by: number of doses reported or recorded/number of expected doses based on HIV risk and PrEP initiation/discontinuation.
c. **Prevention-effective continuation:** Prevention-effective continuation describes the duration of PrEP use during periods of HIV risk and references the absolute time of use during pregnancy and breastfeeding periods. We will adapt the methodology from previous PrEP studies including FEM-PrEP in SA to create a semi-ordinal composite persistence score measuring plasma TDF-DP from blood specimens collected from DBS every 3-months.
d. **In-depth interviews (IDIs):** We recognize the need for mixed methods in this study to better understand the reasons behind the biomarkers (i.e., laboratory test results of persistence) and survey responses, including why women use (or don't) use PrEP, and contextualize sexual and substance use behaviors. We will identify key risk factors to gain information about how risk factors affect the PrEP cascade to design interventions to address those factors.
    - At study month 7 and 12 we will purposefully select 60 study participants to better understand structural (or study-related) barriers to the PrEP cascade. We will select 30 participants who initiated PrEP compared to 30 participants who did not initiate PrEP (n=15) and reported poor adherence to PrEP (n=15) to compare motivations behind PrEP initiation.
    - We will interview key informants, including health care providers and managers in each facility (N=20, n=10 per facility).
    - We will develop semi-structured interview guidelines for the trained study interviewers to use. The Study Coordinator will sit in a random sample of 10% of interviews and counseling sessions to ensure that the interviewers follow the guidelines, and re-train as necessary. Based on our prior research, we expect to hit saturation after 30 interviews per site.
    - We will use factors hypothesized in our conceptual model to be salient and the findings from the CRFs to build the interview guide for the IDIs to help build upon our understanding of the PrEP cascade and factors associated with attrition in each step.
e. **HIV outcomes.** As part of the standard of care, all pregnant and breastfeeding women will be tested for ab and ag HIV using serial rapid HIV tests at each antenatal and postnatal care visit. In addition, PrEP-PP study staff will have a stock of rapid ab/ag HIV test kits to ensure that women get tested at each study visit.
    a. **Seroconversion.** If a woman, infant or partner seroconverts at any point during the study, they will be referred to the clinician on duty to initiate same-day ART, following counseling about PMTCT, breastfeeding, disclosure, and ART persistence, per the SA National Protocols].
f. **Health outcomes and adverse events.** We will collect side effects and serious adverse events in mother and infant including adverse pregnancy and birth outcomes and infant growth measures.

g. **Infant measures.** We will collect infant HIV outcomes and birth outcomes from the infant road to health card, and will collect infant growth outcomes at each quarterly visit.

| Table 1. Study measures for PrEP-PP for AIM 1 | | | |
|---|---|---|---|
| **Primary Outcomes** | **Measurement** | **Source** | **Frequency** |
| PrEP initiation | # of women who initiate PrEP over time / total # of women in active cohort = proportion of women who initiate PrEP | Electronic provider log (REDCap) | Quarterly |
| PrEP continuation | # of women on PrEP who return for study visits (do not miss more than 1 visit) / total # of women in active cohort who are prescribed PrEP= proportion of women on PrEP who continue in PrEP study | Electronic provider log (REDCap) | Quarterly |
| PrEP persistence (subjective) | # of women reporting taking PrEP in ≥5 out of last 7 days or ≥25 out of last 30 days (self-reported)/ total woman-time on PrEP in active cohort = PrEP persistence rate (subjective) | Electronic provider log (REDCap) | Quarterly |
| PrEP persistence (objective) | # of women taking PrEP who have ≥600 fmol/punch (~7 doses/week) from DBS TDF-DP at 3rd trimester, delivery, and 6 weeks postpartum (and during sexual risk periods during that time) / total woman-time on PrEP in active cohort = PrEP persistence rate (objective) | Lab analysis of TDF-DP measures | Yearly and end of study |
| **Secondary Outcomes** | | | |
| Prevention effective persistence | 4. # of women reporting adherence to PrEP (as per PrEP persistence subjective measure)/ total woman-time reporting sexual activity in past 30 days | Electronic provider log (REDCap) | Quarterly |
| | 5. # of women having TDF-DP in DBS (as per PrEP persistence objective measure)/ total woman-time reporting sexual activity in past 30 days | Lab analysis of TDF-DP measures | Yearly and end of study |

| | | | |
|---|---|---|---|
| Prevention effective continuation | # of women who don't miss any PrEP study visits/ total woman time reporting sexual activity in prior visit | Electronic provider log (REDCap) | Quarterly |
| Predictors of attrition at key steps in the PrEP cascade | Individual, pill/study and disease specific predictors of PrEP initiation, continuation and persistence | Study CRFs and in-depth interviews (month 7 and12) (mixed methods) | End of study |
| Healthcare provider attitudes and knowledge about PrEP in pregnancy | Provider knowledge about PrEP and barriers/facilitators to PrEP prescription in ante and postnatal care | In-depth interviews | Year 1 of study |
| HIV outcomes | HIV testing data: HIV-uninfected, HIV incidence in mother and child (if mother seroconverts) compared by PrEP use/non-use | Electronic provider log (REDCap) | End of study |
| Health outcomes and adverse events | Any side-effects, change in creatinine clearance, any negative maternal or neonatal outcomes (for mother and infant) compared by PrEP use/non-use | Electronic provider log (REDCap) | Weekly and quarterly |
| Infant growth outcomes | Measure infant weight, length, z-score and any adverse events in infants compared by PrEP use/non-use | Electronic provider log (REDCap) | Weekly and quarterly |

# 2. Analysis Plan

## 2.1 Preliminary evaluation of data:

***Purpose:***

To identify issues that will affect our approach to the primary analysis. Specifically:

(1) To summarize the amount of missing data for key variables,
(2) To summarize time between baseline and follow-up key measurements, and
(3) To examine the distributional characteristics of key variables in order to detect potential data entry/reduction errors.

***Methods:***

Relevant approaches may include:

(1) Inclusion of all participants in data evaluation regardless of intervention group,
(2) Tabulation of descriptive statistics for key variables (including missing observations),
    a. Key variables include: age, education, relationship status, partner's HIV status, IPV, depression, substance use, PrEP knowledge, STI status, persistence to PrEP at v2 and beyond, SAEs, birth outcomes
(3) Histograms, quantile plots of baseline levels and follow-up measures and changes in those measures.

## 2.2. Description of study process

***Purpose:***

To compare how the study unfolded with the pre-trial plan in order to determine if changes in the pre-trial analysis plans are warranted.

***Methods/Approach:***

(1) Study flow figure (see Continuation Flowcharts)
(2) Number screened per week and month (target= 18 per week, 72 per month) vs number enrolled
    a. The projected sample size for the study is 1200 women enrolled until 12-months postpartum (estimated mean of 18-months follow-up).
(3) Subjects initiating PrEP compared to those who do not
    a. Estimated that 60% of women (n=720) will initiate PrEP during the study period (versus approximately 40% who will not).
(4) Continuation and persistence by group
    a. Assumed that approximately 75% of women on PrEP (n=540) will continue on PrEP at 12-months (defined as not missing >1 study visit among women on PrEP);
    b. Assumed that 70% of those retained in care (n=378) will adhere to PrEP during at-risk periods.
(5) Final completion rates
    a. Compare final number of participants who enroll in study, who initiate PrEP, who are retained in care, and who adhere to PrEP to sample sizes needed to yield 80% power (i.e., n=1200, 720, 540, 378, respectively).
(6) Number of subjects in analysis data sets
    a. Consider number of participants that will potentially be excluded from analyses because of withdrawal from study, missing data on key covariates or outcomes, etc.

## 2.3. Description of participants

***Purpose:***

To determine distribution of baseline characteristic within study sample compared to target population (i.e., pregnant and breastfeeding women in Cape Town).

***Methods/Approach:***

Construct "Table 1" describing the baseline characteristics of each intervention group including:

(1) Demographic characteristics (e.g., age, socioeconomic status, ethnicity, gestational age at baseline, number of children, knowledge of PrEP, relationship status, risk factors)
(2) Baseline exposure characteristics (e.g., PrEP initiation)
(3) Baseline disease characteristics (i.e., STI results)
(4) Baseline values of primary and/or secondary outcome variables (note, this may be a separate table)

## 2.4. Primary analysis/results

***Purpose:***

To measure the distribution of women across the PrEP cascade including: initiation, continuation and persistence

***Methods/Approach:***

(1) *Primary outcome:* See **Table 1** for precise definitions of (1) PrEP initiation, (2) PrEP continuation, (3) PrEP persistence-subjective, and (4) PrEP persistence-objective
(2) *Primary analysis:* Describe each outcome listed above via cumulative incidences at fixed intervals (e.g., cumulative PrEP initiation by delivery) and incidence densities over time. The general statistical approaches to these outcomes are analogous; data will be described graphically, including product-limit graphs, with preliminary analyses based on standard approaches for rates and proportions.

a. **PrEP initiation** will be modelled using a probit model (preferred to estimate risk factors for common outcomes) with individual-level covariates as fixed effects.

b. **PrEP continuation** will be examined using similar approaches; in addition, we will use time-to-event methods to estimate factors associated with time to loss (non-continuation) among women having initiated PrEP.

c. **PrEP persistence** will be modelled using mixed effects probit models as above and generalized estimating equations to examine the marginal effect of individual-level covariates on persistence during the study.

    a. *Sub*-group analysis: we will analyze the primary outcomes by sub-groups including age (<25 and ≥25 years), STI diagnosis, relationship status, pregnant/postpartum

***Missing data:***

Primary persistence analyses will use objective measures (TDF-DP from DBS) taken on all those who enrolled and initiated PrEP; subsidiary analyses will consider (i) other persistence measures and (ii) multiple imputation for missing persistence data. Specify any sensitivity analyses. Specify how the impact of missing data on the primary results will be interpreted.

***Interpretation:***

The analysis will be based on women who complete the 12-month postpartum cohort follow up. We will use relative risk ratios performed at a two-sided α level of 0.05.

Summarize how the results will be interpreted. You can include what level of statistical significance will be used to determine the trial's main conclusion/recommendation. You can also describe how disagreement or coherence between primary and secondary results will be interpreted.

## 2.5. Secondary analyses

***Purpose:***

(1) To evaluate prevention-effective measures of PrEP continuation and persistence during periods of sexual activity/risk
(2) To evaluate patient and provider-level factors associated with the PrEP cascade using quantitative and qualitative approaches

Methods/Approach:

See **Table 1** for precise definitions of prevention effective PrEP continuation and persistence

(1) *Secondary analysis:* Describe each outcome listed above via cumulative incidences at fixed intervals (e.g., cumulative PrEP continuation after 1 year in study) and incidence densities over time. The general statistical approaches to these outcomes are analogous to the primary outcomes (above). Data will be described graphically, including product-limit graphs, with preliminary analyses based on standard approaches for rates and proportions.

(2) Other analysis approaches on the PrEP cascade:

    a. When time-dependent or visit-specific covariates are included in the analysis of cumulative PrEP initiation we will shift to a mixed-effects setting with random effects around individual participants.

Other outcomes (secondary outcomes):

a. **Predictors of attrition at key steps in the PrEP cascade:** Methods will involved mixed methods analysis. Utilizing the qualitative data from the IDIs at month 7 and 12 of the study from a random selection of 60 participants (30 randomly selected from those who initiated PrEP and 30 randomly selected from those who chose not to initiate) to complement the quantitative data to understand factors associated with PrEP persistence and non-persistence, including 1) individual factors, (including risk perception, sexual activity and risk, substance use, and partner/family/community discouragement), 2) the pill regimen (side effects and concerns about adverse pregnancy or infant outcomes) AND patient-provider relationship and 3) the disease-related factors (including reasons behind stigma associated with PrEP taking).
    i. **Individual factors:** Collected from CRFs in all participants (regardless of PrEP uptake) including: risk perception, sexual activity and risk, substance use, partner/family/community discouragement, sociodemographic variables, relationship characteristics and fear about adverse pregnancy and/or infant health outcomes. Collected from the sub-set of women participating in the IDIs.
    ii. **Pill/study factors:** Collected from CRFs in participants who decide to take PrEP, including: side effects on PrEP, and barriers to attending frequent study visits and DBS during those visits, and provider participant communication and support

(measured from participant perspective). Collected from the sub-set of women participating in the IDIs.

iii. **Disease-specific factors:** Collected from CRFs (in all participants, not just those who decide to take PrEP) on partner's HIV status (including knowledge of serostatus and uptake of couples testing and counseling), perceived risk of HIV exposure and acquisition, perceptions of PrEP efficacy, and stigma associated with PrEP and HIV. Collected from the sub-set of women participating in the IDIs**.**

b. **HIV outcomes:** Describe the frequency of HIV outcomes, including maternal and infant incident HIV. Analyses will include pure-count and person-time approaches. While we expect the frequency of adverse HIV-related outcomes to be low, we will describe characteristics of women who did/did not acquire HIV, and consider further analyses using the approaches outlined.

### 2.6. Adverse effects

***Purpose:***

To summarize and describe adverse intervention effects.

***Methods/Approach:***

Tabulation of adverse effects with each treatment group:

(1) Number of events by type of event

- Describe the frequency of any laboratory or self-reported side effects (e.g. changes in creatinine clearance, or self-reported side-effects [GI symptoms, headache, and etc]). Analyses will include pure-count and person-time approaches. Confidence intervals will be calculated following the method of Breslow and Day. We will also describe pregnancy (miscarriage, stillbirth) and infant outcomes over time (including birthweight, gestational age at delivery, pregnancy loss) by PrEP status (on/off PrEP and by persistence to PrEP)

(2) Number of subjects by highest grade of event

(3) Reasons for termination of treatment

### 2.7 Subgroup analyses

***Purpose:***

To determine if all subgroups experience similar treatment effects or if there are subgroups that behaved differently.

***Methods/Approach:***

(2) Pre-specify subgroups that will be analyzed. Consider subgroups defined by:

a. Different levels of key prior exposures: STI diagnosis, age, relationship status, pregnant/postpartum

## 3. Post-hoc (data driven) analyses

***Purpose:***

(1) To document which analyses were conducted after the results in section 2 were known.

(2) To document the rationale for these analyses.

(3) To pre-specify their interpretation in the context of the primary and secondary results and their impact on the overall trial conclusions.

***Methods/Approach:***

TBD

## 4. Table and Figure Templates

Outline tables and figures that will be produced for the analysis. This will include all analyses. Tables and figures for the paper will be constructed separately.

| **Table 1. HIV risk factors associated with PrEP uptake in HIV-uninfected pregnant women (N=XXX)** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Person time on PrEP (n=x)** | | **Person time not on PrEP (*n*=y)** | | **Crude HR (95% CI)** | **Adjusted HR (95% CI)** | |
| | ***n*** | **%** | ***N*** | **%** | | | |
| Age (median and IQR) | | | | | | | |
| Age <25 years | | | | | | | |
| Age >=25 years | | | | | | | |
| Primigravida | | | | | | | |
| Knew about PrEP | | | | | | | |
| Ready to start PrEP today (Agree + Strongly Agree) | | | | | | | |
| Intended to get pregnant | | | | | | | |
| Married or cohabiting | | | | | | | |
| More than 1 sex partner | | | | | | | |
| STI+ diagnosis (chlamydia, gonorrhoea, and/or trichomonas vaginalis) | | | | | | | |
| Reported depression or anxiety in pregnancy (EPDS <13 score) | | | | | | | |
| HIV+ partner | | | | | | | |
| Partner HIV status unknown | | | | | | | |
| Reported IPV in past year | | | | | | | |
| Reported alcohol/drug use in past year | | | | | | | |

| **Table 2. Continuation, persistence and outcomes in those on PrEP (n=XXX)** | | | | |
|---|---|---|---|---|
| | **On PrEP (*n*=XXX)** | | **Change from last quarter** | |
| | ***n*** | **%** | | |
| Returned for 1 month visit | | | | |
| Persistence (self reported taking PrEP 5+ days of last 7 days) | | | | |
| Persistence (self reported taking PrEP 25+ days of last 30 days) | | | | |
| Returned for 3 month visit | | | | |
| Persistence (self reported taking PrEP 5+ days of last 7 days) | | | | |
| Persistence (self reported taking PrEP 25+ days of last 30 days) | | | | |
| Returned for 6 month visit | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Persistence (self reported taking PrEP 5+ days of last 7 days) | | | | | |
| Persistence (self reported taking PrEP 25+ days of last 30 days) | | | | | |
| Returned for 9 month visit | | | | | |
| Persistence (self reported taking PrEP 5+ days of last 7 days) | | | | | |
| Persistence (self reported taking PrEP 25+ days of last 30 days) | | | | | |
| Returned for 12 month visit | | | | | |
| Persistence (self reported taking PrEP 5+ days of last 7 days) | | | | | |
| Persistence (self reported taking PrEP 25+ days of last 30 days) | | | | | |
| Returned for 15 month visit | | | | | |
| Persistence (self reported taking PrEP 5+ days of last 7 days) | | | | | |
| Persistence (self reported taking PrEP 25+ days of last 30 days) | | | | | |
| Returned for 18 month visit | | | | | |
| Persistence (self reported taking PrEP 5+ days of last 7 days) | | | | | |
| Persistence (self reported taking PrEP 25+ days of last 30 days) | | | | | |
| Continuation on PrEP at v3 (defined as not missing >1 Study visit in those on PrEP) | | | | | |
| Continuation on PrEP at v4 | | | | | |
| Continuation on PrEP at v5 | | | | | |
| Continuation on PrEP at v6 | | | | | |
| Continuation on PrEP at v7 | | | | | |

**Adverse events and serious adverse events (PrEP use defined as taking PrEP in last 30 days)**

| | **PrEP users (N)** | **%** | **PrEP non-users (N)** | **%** | **Comment** |
|---|---|---|---|---|---|
| **Adverse event reported (n=# of events and # of participants)** | | | | | |
| Type of events | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| **Serious adverse event reported (n= # of events)** | | % over # of participants in study | | | |
| Type of events | | | | | |
| | | | | | |
| **HIV testing** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| HIV ab+ | | | | | |
| HIV ag+ | | | | | |
| HIV ag/ab+ | | | | | |

**Birth outcomes**

| | PrEP user (n) | % | PrEP non-user (n) | % | Comment |
|---|---|---|---|---|---|
| **Number of active participants** | | | | | |
| **Number of women still pregnant** | | | | | |
| **Number of Births** | | | | | |
| Number of Live Births | | | | | |
| Number of Birth abnormalities | | | | | |
| Still births | | | | | |
| Miscarriage | | | | | |
| Other | | | | | |

**Monthly and quarterly report templates (can be by PrEP status for our DSMB and other analyses)**

**Table 1: Monthly enrolment information for participants**

| Description | N for this month | % for this month | Cum. total (for study) | % Cum. total | Comments |
|---|---|---|---|---|---|
| **Enrolment Status** | | | | | |
| **Total Screened** | | | | | |
| Ineligible | | | | | |
| Refusals | | | | | |
| **Enrolled participants** | | | | | |
| **Withdrawals (total N=X)** | | | | | |
| Study withdrawals | | | | | Monthly PIDs: |
| Censored from study | | | | | |
| Lost to follow up (90+ days without a visit and not contactable) | | | | | |
| **Total** | | | | | |
| **PrEP Status of Study participants (total N=X)** | | | | | |
| On PrEP (≥18 yrs) | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| On PrEP (16-17 yrs) | | | | | | |
| Not on PrEP (18≥yrs) | | | | | | |
| Not on PrEP (16-17 yrs) | | | | | | |
| **Total** | | | | | | |
| **Visit attendance** | | | | | | |
| Attended Refill visit (v1.1) | | | | | | |
| Attended v2 | | | | | | |
| Not on PrEP (v2- subset of above row) | | | | | | |
| **AE and SAE reporting** | | | | | | |
| AEs reported (≥18 yrs) | | | | | | |
| AEs resolved (≥18 yrs) | | | | | | |
| AEs reported (16-17 yrs) | | | | | | |
| AEs resolved (16-17 yrs) | | | | | | |
| **SAEs (denominator= total enrolments)** | | | | | | |
| **Birth outcomes** | | | | | | |
| Normal | | | | | | |
| Adverse (& reason) | | | | | | |
| **Total births** | | | | | | |
| **STI diagnosis** | | | | | | |
| CT | | | | | | |
| NG | | | | | | |
| TV | | | | | | |
| CT/NG | | | | | | |
| CT/TV | | | | | | |
| TV/NG | | | | | | |
| CT/NG/TV | | | | | | |
| **Total** | | | | | | |
| **Referrals** | | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| IPV | | | | | |
| EPDS (depression) | | | | | |
| AUDIT | | | | | |
| DUDIT | | | | | |
| **HIV results at Baseline** | | | | | |
| HIV negative | | | | | |
| Ag | | | | | |
| Ab | | | | | |
| Ag/Ab | | | | | |
| **Total Tested Participants** | | | | | |
| **HIV results at visit 2+** | | | | | |
| HIV negative | | | | | |
| Ag | | | | | |
| Ab | | | | | |
| Ag/Ab | | | | | |
| **Total tested** | | | | | |

| | |
|---|---|
| **Abnormal laboratory results (creatinine)** | |
| **Unanticipated problems** | |
| **Protocol Deviations** | |
| **Quality Management** | |

**Part 2: Baseline Characteristics**

**Table 2: Baseline Demographic Characteristics (can be by PrEP status in analysis)**

| **Variable** | **Total** | **Percentage** | **Comments** |
|---|---|---|---|
| **Age (Years)** | | | |

| | | | |
|---|---|---|---|
| 16-17 | | | |
| 18-24 | | | |
| 25 and older | | | |
| Mean (SD) | | | |
| Median (IQR) | | | |
| **Gestational age (Weeks)** | | | |
| Mean (SD) | | | |
| Median (IQR) | | | |
| **Education** | | | |
| None | | | |
| Primary (Grade 1-7) | | | |
| Secondary lower (8-10) | | | |
| Secondary upper (11-12) | | | |
| Tertiary (attended / completed) | | | |
| **Income** | | | |
| Yes | | | |
| No | | | |
| **Income Monthly (Rands)** | | | |
| 1 – 1 000 | | | |
| 1 001 – 5 000 | | | |
| >5 000 | | | |
| **Total** | | | |
| **Main Sexual Partner** | | | |
| No | | | |
| Yes | | | |
| **Relationship status (of those with main partner)** | | | |
| Married, living together | | | |
| Married, Not living together | | | |

| | | | |
|---|---|---|---|
| Not Married, living together | | | |
| Not Married, not living together | | | |
| **Total** | | | |
| **Do you have other sexual partners (concurrent sex partners)** | | | |
| No | | | |
| Yes | | | |
| **Has your current main partner(s) ever been tested for HIV?** | | | |
| Yes | | | |
| No | | | |
| I don't know | | | |
| N/A | | | |
| **Total** | | | |
| **Partner HIV status (of those previously tested)** | | | |
| HIV positive | | | |
| HIV negative | | | |
| **Total** | | | |
| **PrEP Knowledge** | | | |
| Never heard about it | | | |
| Heard about it before this study | | | |

**Part 3: PrEP persistence and side effects**

**PrEP persistence and side effects at the refill visit (v1.1) and V2+**

| | **V1.1** | **V2** | **V3** | **V4** | **V5** |
|---|---|---|---|---|---|
| | | | | | |
| **Taken PrEP in the last 30 days** | **N, %** | **N, %** | **N, %** | **N, %** | **N, %** |
| Yes | | | | | |
| No | | | | | |
| **Had symptoms or side effects while on PrEP** | | | | | |
| Yes | | | | | |
| No | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| **In the last 30 days, on how many days did you miss at least one dose of any of your PrEP** | | | | | |
| Never missed (0 days) | | | | | |
| 1 -5 days | | | | | |
| 6-10 days | | | | | |
| 11-20 days | | | | | |
| >20 days | | | | | |
| | | | | | |
| **In the last 7 days, on how many days did you miss at least one dose** | | | | | |
| Never missed (0 days) | | | | | |
| 1-3 | | | | | |
| 4-7 | | | | | |

**Part 4: Birth outcomes (by PrEP status for analysis)**

| | **Frequency** | **Percentage** | **Comment** |
|---|---|---|---|
| **Number of active participants** | | | |
| **Number of women still pregnant** | | | |
| **Number of Births, (n=X)** | | | |
| Number of Live Births | | | |
| Number of Birth abnormalities | | | |
| Still births | | | |
| Miscarriage | | | |
| Other | | | |

**Part 5: Participants Visit**

| | **Frequencies** | **Percentage** |
|---|---|---|
| **Refill visit** | | |
| Actual attended | | |
| Missed visits | | |

| | | |
|---|---|---|
| **Total** | | |
| **Visit 2** | | |
| Actual attended | | |
| Missed visits | | |
| **Total** | | |

**Part 6: Expected vs achieved participants for enrolment (weekly, monthly and quarterly)**

| | **Expected** | **Actual** | **% achieved** |
|---|---|---|---|
| Week 1 | | | |
| Week 2 | | | |
| Week 3 | | | |
| Week 4 | | | |
| **Total** | | | |

**TITLE OF RESEARCH:** Evaluation of pre-exposure prophylaxis (PrEP) initiation, retention and adherence in pregnant and breastfeeding women

**INTRODUCTION**

.

Good Morning/Afternoon. My name is _____. I work for the University of Cape Town. We would like to ask you to participate in a research study. The purpose of the study is to evaluate the use of pre-exposure propylaxis, also known as PrEP, which is an antiretroviral drug, to prevent HIV acquisition in pregnant and breastfeeding women. This study is being run by public health researchers from the University of Cape Town, South Africa in collaboration with researchers from the University of California, Los Angeles. We have selected (*select one:)* Gugulethu Midwife Obstetric Unit OR Hanover Park Midwife Obstetric Unit in Cape Town to recruit study participants.

Before you decide if you want to take part, I would like to tell you more about this study, what the risks and benefits are to you and your unborn baby, and what would be expected of you. This information is described in the consent form, which I will give to you now. Please read it carefully, or if you would rather me read it to you, I can do that as well. Please do not hesitate to ask me any questions. If you agree to participate, I will ask you to sign the form or make your thumb print mark confirming your willingness to participate. I will give you a copy of the signed consent form to keep.

**Why is this study being done?**

Adolescents and young women in South Africa are at high risk of HIV infection. During pregnancy and breastfeeding, the risk of HIV infection nearly doubles. There is also high risk of HIV transmission between mother and infant while breast-feeding. The purpose of the study is to evaluate if pregnant and breastfeeding women like you are interested in taking pre-exposure prophylaxis (called PrEP) to prevent HIV. If women do take it, we will evaluate how long they take it for and how they feel when they take it. Even though previous research has shown that antiretroviral therapy does not lead to increased risk of maternal or infant health problems, we will keek track of any side effects to the mother or infant during the study. At this time, PrEP is not used in South Africa for HIV prevention in pregnant and breastfeeding women.

By taking part in this study you will help us collect data that will help determine how to best provide PrEP for pregnant women, and reduce the number of pregnant women and babies infected with HIV.

**Why are you being asked to take part?**

We are asking you to take part in this study because you are pregnant and are HIV-negative, and you are coming to a community health center in [Gugulethu/Hanover Park] for your care. This study will help prevent you and your baby from getting HIV.

**What do we do to decide if you are eligible to take part?**

We will ask you questions to determine if you are eligible. For you to participate in this study you must be:

1) 16 years old or older,
2) attending your first antenatal care visit for this pregnancy,
3) be HIV-negative at your first antental care visit,
4) not have any psychological or health problems that could prohibit the use of PrEP
5) living in Gugulethu or Hanover Park and surrounding areas,
6) planning to give birth in Cape Town,
7) willing to participate in the study.

<u>You cannot participate in the study if you:</u>

- Are enrolled in another HIV-1 vaccine or prevention trial
- Were hospitalized in the past year for any reason
- Were on TB treatment within the past 30 days
- Have any history of infection of your liver
- Have or had any mental health issues that requires you to take medication
- Positive Hepatitis B surface antigen (HBsAg) test on screening
- Had any broken bones without an accident
- Have other medical, psychiatric or social condition which in the opinion of the investigators would affect the ability to consent and/or participate in the study

**How many people will take part in the study?**

We will recruit 1200 HIV-negative pregnant women.

**What other choices do you have?**

Taking part in this study is voluntary. If you choose not to participate, your care at this clinic will <u>NOT</u> be affected today or in the future. If after you join the study, you decide that you no longer want to be involved, you can speak with one of the nurses or study staff, and we will take you off our list and you will not be contacted about the study again.

If you decide to participate today, you will be given a copy of this form with a special study number on it that is your study identification number. Please keep this in case you want to withdraw from the study at any

time. Also, if any new findings are developed during the course of this study that may affect you, such as a new alternate treatment becoming available, we will let you know and ask if you want to remain in the study. Again, if you decide you do not want to be involved anymore, the care that the clinic will provide you and your baby will NOT be affected.

**How long will the study last?**

The study will begin today and last until 12 months after you give birth. There is one study visit every 3 months. The visits take place at the same time as your regularly scheduled clinic visits at [Gugulethu MOU/ Hanover Park MOU].

**What will happen if you decide to take part in the study?** If you agree to participate in this study, you will then have certain tests and procedures. These include:

- Recording your personal information, including for example your name, age, gender, ethnicity;
- Counseling about HIV prevention in pregnancy including condom use and HIV testing for yourself and your sex partner(s).
- We will draw about 2 tablespoons of you your blood today to test if you have a liver illness called Hepatitis B or kidney illness. If you test positive for Hepatitis Byou will not be eligible to participate in the study.

- As part of the study you will be offered PrEP, which is the oral pill containing TDF/FTC (300mg TDF plus 200 mg FTC). We will ask you if you are willing to take PrEP while you are pregnant and breastfeeding. If you are, you will receive counseling on the importance of taking the treatment every day to be most effective at preventing HIV.
    - If you take PrEP, blood tests will be done periodically to check the blood level of the PrEP medication to make sure you are taking the PREP medication properly. You will not be told the results of these tests.

- If you do not want to take PrEP, you will still be in the study. You will complete the other parts of the study as normal, but you will also be counselled about the risks and benefits of PrEP at each study visit to see if you have changed your mind and would like to start taking PrEP. We will offer you other resources, like condoms, to reduce your risk of getting HIV.

- *If you decide to take PrEP*, you will receive a prescription for PrEP, and drug supply for 1 month, then 2 months, and every 3 months after that. We will also give you condoms that you can also use to reduce your risk of getting HIV.
- We will also test your blood at month three and at the end of the study to see if your kidney health changes. If the kidney health changes you may have to stop taking PrEP until your kidney health improves.
- At every visit, you will receive an HIV test to confirm your status. We will invite your partner(s) to come in for testing as well (though this is not required).
    - If your HIV status changes, and you become HIV-infected, we will help you start treatment immediately and give you counseling and support to prevent mother to child transmission of HIV. We will follow you up for 6 months to make sure you become virally suppressed (to show that treatment is working) and refer you for additional specialized treatment if any problems are found.

- At three of the visits, we will collect samples using a vaginal swab to test you for three common sexually transmitted infections (STIs): gonorrhea, trichomonas vaginalis and chlamydia. Your results will be available in about 90 minutes.
    - A nurse can help collect the swab, or she can show you how to do it yourself.
    - These swabs will be collected at today's visit, in the third trimester, and then in the first week after you give birth
    - If you test positive for either infection, we will give you treatment the same day, and we will give you a letter from the clinic that you can give to your partner(s) so they can come get treatment if they want to.
- We will have access to your medical files to evaluate your or your baby's other health care, laboratory results or health, or hospitalizations during the study (antenatal, postpartum and infant medical files).
- You will receive the phone number of the study nurse to call or SMS if you have any questions or concerns, or any side effects you want to report. You can call any time even in between visits.
- In order to follow you up through post-birth, we need to collect several forms of contact information from you. This will include phone number(s), email addresses, and home addresses, for you, family members (including your partners or father of your child), friends, or others that may help us find you if we lose touch.
- Your participation in each visit may take up to 1-1.5 hours. This will include collecting samples from you, and the time it takes to complete the survey.

**What are the risks and discomforts of this study?**

*Short term side effects of PrEP (TDF/FTC)*

All medications can cause unwanted effects. We call these 'side effects' of the medicine. PrEP can have short-term side effects, including nausea, abdominal cramping, vomiting, dizziness, headache, and fatigue. These problems typically arise in the first week or two of PrEP, and often disappear in the next few weeks. Some of these side effects are similar to pregnancy side effects. We will ask you questions about side effects of pregnancy before and after taking PrEP to understand if the side effects are related to PrEP or pregnancy.

*Longer term side effects*

Pregnant and breastfeeding women in our study will take PrEP for less than 1.5 years on average. TDF taken as PrEP for 1 to 2 years poses only a small risk of negative effects that usually go away once you stop taking PrEP. One rare side effect is changes in kidney markers, which may mean the kidneys aren't working as well as they should. We will follow kindney health and stop PrEP treatment if we find any changes in kindey function. Kidney function changes usually go away after you stop taking PrEP. The other rare side effect of long term PrEP use is decreased bone density, which means they lose some of their mass and may fracture more easily, but will go away after you stop taking PrEP. This is seen more often in people with past fracture or bone loss risk factors. Our study will measure any fractures during the study.

*Infant side-effects*

A recent study of women taking PrEP in pregnancy showed low drug concentrations in the breast milk and the infants' blood, but it did not lead to any major side effects. There was less of the drug in the babys' blood than is normally given to babies with HIV-infected mothers to keep them from getting HIV. To make sure infant health is not affected, our study will record any health problems in the infant during the first 12-months of life.

*Psychological effects*

Telling your partner and/or family you are taking PrEP to prevent HIV may cause some stress for you and your partner(s). If you think your partner(s) will inflict emotional or physical abuse on you as a result of finding out that you are taking PrEP, please let a member of the study team know so we can discuss it with you.

If you are diagnosed with HIV, telling your partner and/or family may cause some stress for you and your partner(s). If you think your partner(s) will inflict emotional or physical abuse on you as a result of finding out that you are HIV infected please also discuss this with a member of the study team.

The study team will provide counselling to you and your partner(s) to ensure that you both get the care you need to be healthy. We can also refer you for counselling to address any potential problems in your relationship. If problems do arise, we will ask you to contact us at the number below so that we can assist in referring you for additional social services in your area.

**Are there any benefits to you for being in the study?**

If you decide to take PrEP during pregnancy and breastfeeding, your risk of getting HIV is very low. Studies have shown that 98% of women who take PrEP every day are protected against getting HIV. Your baby will also be protected from HIV if you take PrEP. If you do not decide to take PrEP, you will receive regular counseling on HIV prevention and testing at each visit to help you protect yourself and your infant from HIV. You will also get free testing for STIs during this study. STIs can be harmful to you and your baby, so getting them treated during pregnancy can benefit both you and your baby. If you test positive for an STI, we will provide you treatment right away, and give you a letter from the clinic to give to your partner(s) so they can come in for free treatment also.

**Will you receive any reward for taking part in this study?**

At the end of each visit, you will be given a R100 grocery voucher, R20 for transport, and food and drink while you are at the visit. There is no payment for participation.

**What happens if I get hurt taking part in this study?**

This research study is covered by an insurance policy taken out by the University of Cape Town if you suffer a bodily injury because you are taking part in the study

The insurer will pay for all reasonable medical costs required to treat your bodily injury, according to the SA Good Clinical Practice Guidelines 2006 which are based on the Association of the British Pharmaceutical Industry Guidelines. The insurer will pay without you having to prove that the research was responsible for your bodily injury. You may ask the study doctor for a copy of these guidelines.

The insurer will *not* pay for harm if, during the study, you:

- Use medicines or other substances that are not allowed
- Do not follow the study doctor or nurses's instructions

- Do not tell the study doctor or nurse that you have a bad side effect from the study medicine
- Do not take reasonable care of yourself and your study medicine

If you are harmed and the insurer pays for the necessary medical costs, usually you will be asked to accept that insurance payment as full settlement of the claim for medical costs. However, accepting this offer of insurance cover does not mean you give up your right to make a separate claim for other losses based on negligence, in a South African court.

It is important to follow the study nurse or doctor's instructions and to report straightaway if you have a side effect from the study medicine.

**What will happen when the study is over?**

When the study is over, 12 months after you give birth, you will no longer be able to get PrEP through the study to prevent HIV. If you are no longer breastfeeding your baby, this means your baby is no longer at risk of getting HIV from breastmilk if you get HIV. However, you may still be at risk of getting HIV from a partner who has HIV. At the end of the study, we will counsel you about other methods to reduce the risk of getting HIV, including condoms, and tell you how you can get free condoms from the clinic. Once PrEP is provided in the clinic, we will refer you to other sources of PrEP if you decide to continue taking it

**Will your test results be shared with you?**

We will share test results collected during your visits with you and the study nurse will explain what they mean. This includes HIV tests, STI tests, and kidney function tests, including a test for infection of the liver. It also includes HIV tests of your partner(s), which will be discussed with him/her directly. However, you will not receive tests that we will evaluate for adherence to PrEP. We will print out copies of any test results if you like, so you can take them with you.

**Will the results of the research be shared with you?**

Once all participants have finished the study, we will write a summary of the results and have it at the MOU. If you want a copy, we will let you know when it is ready and can provide it to you.

**Will any of your blood, tissue or other samples be stored and used for research in the future?**

No.

**Who will see the information which is collected about you during the study?**

Information that will be collected from your medical files and during your interviews with our study nurse will be kept confidential. No one but the researchers will be able to see it. We will not tell anyone about your participation, and we will make every effort to protect your privacy and confidentiality. Your name will not be linked to your information. Only the special number we give you will be able to identify you, and only the researchers will know what your number is. We will lock this information up with a lock and key.

**Can I leave the study?**.

It is your choice if you want to be in this research study. You can leave the study at any time. Please inform the study staff, or by calling the number below, if you would like to leave the study.

**Do you have any questions?**

If there is anything that is unclear or if you need further information, please ask us and we will provide it.

**FOR ADDITIONAL INFORMATION:**

The UCT's Faculty of Health Sciences Human Research Ethics Committee can be contacted on 021 406 6338 in case you have any ethical concerns or questions about your rights or welfare as a participant on this research study.

If you have any questions or have any problems while taking part in this research study, you should contact:

Professor Landon Myer
School of Public Health and Family Medicine
Faculty of Health Sciences, University of Cape Town
Tel: 021 406 6661
Email: Landon.Myer@uct.ac.za

If you have questions about this trial, you should first discuss them with your doctor or the Ethics Committee (contact details as provided above). After you have consulted your doctor or the Ethics Committee and if they have not provided you with answers to your satisfaction, you should write to the South African Medicines Control Council (MCC) at:

The Registrar

Medicines Control Council

Department of Health

Private Bag X828

PRETORIA

0001

**CONSENT FOR STUDY PARTICIPATION**

CONSENT STATEMENT:

I have read this form, or someone has read it to me. I have been offered a copy of this consent form. I was encouraged and given time to ask questions. I agree to participate in the PrEP study, including regular data collection. If I decide to take PrEP, I agree to provide lab specimens as explained in the consent form. I know that I may withdraw my consent at any time. My participation is voluntary. I understand that whether or not I take part will not affect my health care services received today, or at any time in the future.

Please indicate your consent with your signature.

Volunteer's name _____

_____

Signature of Volunteer Date (DD/MM/YYYY)

Staff member's name_____

_____

Signature of study staff Date (DD/MM/YYYY)

If the volunteer is unable to read or write the entire counselling process must be observed by an independent witness who can then confirm the procedure once the she has given consent.

Fingerprint of volunteer:

Witness:

I confirm that I am independent of the study and that I witnessed the entire informed consent counselling process in the home language of the volunteer

Name: _____

Signature: _____

Date (DD/MM/YYYY): _____

Thank you.

**CONSENT FOR STUDY PARTICIPATION (INFANT—to be signed at first post-partum study visit**)

CONSENT STATEMENT:

I have read this form, or someone has read it to me. I have been offered a copy of this consent form. I was encouraged and given time to ask questions. I permit my child to be in this study. I know that after choosing to be in this study, I may withdraw my child at any time. I am voluntarily allowing my child to participate in this study. I understand that whether or not my child takes part will not affect their health care services received today, or at any time in the future.

Please indicate your consent with your signature.

Child's name _____

Mother's name _____

_____

Signature of Mother Date (DD/MM/YYYY)

Staff member's name_____

_____

Signature of study staff Date (DD/MM/YYYY)

If the volunteer is unable to read or write the entire counselling process must be observed by an independent witness who can then confirm the procedure once the she has given consent.

Fingerprint of volunteer:

Witness:I confirm that I am independent of the study and that I witnessed the entire informed consent counselling process in the home language of the volunteer

Name: _____

Signature: _____

Date (DD/MM/YYYY): _____

Thank you.

**PATIENT CONSENT FORM (INFANT)- Participant Copy**

1. I have read the consent form, or it has been read to me.
2. I understand the contents of the consent form
3. I agree to permit my child's participation in the PrEP study
4. I understand that I may withdraw my consent at any time.
5. I understand that my child will receive the usual standard of care offered by this clinic.

Child's name _____

Patient name: _____

Signature: _____

Date (DD/MM/YYYY): _____

Name of the person obtaining consent: _____

Designation: _____

Signature: _____

Date (DD/MM/YYYY): _____