Immediate or Deferred Pre-exposure Prophylaxis for HIV Prevention: Safe Options for Pregnant and Lactating Women

An Open-Label Randomised Control Study

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TABLE OF CONTENTS

GLOSSARY	
Acronyms	
AE	Adverse Event
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AP	Antepartum
ART	Antiretroviral therapy
ARV	Antiretroviral
AST	Aspartate aminotransferase
AUC	Area under the curve
BF	Breastfeeding
BHITS	Breastfeeding and HIV International Transmission Study
BMD/C	Bone mineral density/content
CBV	Combivir
CDC	US Centers for Disease Control and Prevention
CI	Confidence Interval
Cr/Cr CL	Creatinine/Creatinine Clearance
CRF	Case Report Form
DBS	Dried blood spot
DXA	Dual Energy X-Ray Absorptiometry
EAE	Expedited Adverse Event
EBF	Exclusive Breast Feeding
EC	Ethics Committee
ELISA	Enzyme-Linked ImmunoSorbent Assay
FDA	US Food and Drug Administration
FDC	Fixed dose combination
FF	Formula feeding
FTC	Emtricitabine
GCLP	Good clinical lab practice
HBV	Hepatitis B Virus
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
IP	Intrapartum
IRB	Institutional Review Board
LAR	Legally Authorized Representative
L/D	Labor and delivery
LFT	Liver Function Test
MOP	Manual of Procedures
MTCT	Mother-to-Child Transmission
NAT	Nucleic Acid Test
PK	Pharmacokinetic
PMTCT	Prevention of Mother-to-Child Transmission
PP	Postpartum
POC	Point of Care

GLOSSARY	
PoR	Pharmacist of Record
PrEP	Pre-exposure prophylaxis
SAE	Serious Adverse Event
SID	Study Identification Number
SIP	Site Implementation Plan
SOC	Standard of care
TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
TRV	Truvada (fixed dose combination Emtricitabine-Tenofovir disoproxil fumarate)
ULN	Upper limit of normal
HCT	HIV Counselling and Testing
WBC	White blood count
WHO	World Health Organization
WITS	Women and Infants Transmission Study
WY	Women years

SYNOPSIS

Background:

There is increasing evidence that women remain vulnerable to HIV during pregnancy and even more so postdelivery. In a meta-analysis, using data from 19 international cohorts, Drake et al concluded that the pooled HIV incidence rate during pregnancy/postpartum was 3.8/100 PWY (95% CI 3.0-4.6): 4.7/100 PWY during pregnancy and 2.9/100 PWY postpartum. In earlier South African studies (2005-2009) we reported HIV seroconversion rates ranging from 1.3% to 3.0% in pregnancy [4-6]. In a more recent population based evaluation (2011-2012), Dinh et al reaffirmed the high seroconversion rate among pregnant women attending public health facilities in South Africa; 3.3% (95% CI: 2.8%-3.8%) women seroconverted in pregnancy [7]. In a recent (Jan-Aug 2015) evaluation of antenatal HCT and PMTCT program indicators in KwaZulu-Natal among 78 906 pregnant HIV uninfected women who retested during pregnancy, 1.8% (95%CI 1.0-2.9) seroconverted before delivery [KZN DOH, 2016]. In a similar evaluation of postnatal HCT indicators for Apr-Dec 2015 during which 26 455 women who were HIV negative during pregnancy were retested postnatally we reported a 5% seroconversion rate in the postnatal period alone. Certain districts had distinctly higher postnatal seroconversion rates: Ethekwini (6.2%), Umgungundlovu (7.1%), Umzinyathi (9.1%), and Uthukela (7.1%) [KZN DOH, 2016]. The higher HIV incidence in women postpartum is hypothetically due to a longer exposure/observational period (12 – 18 months) compared to exposure during the antepartum period (maximum 6-8 months). Other plausible reasons for the higher HIV incidence postpartum include possible behavioural changes and changes in relationship dynamics over a 12-18 month period.

Rationale:

Gilead Sciences developed Truvada, a product containing FTC 200 mg and TDF 300 mg in a fixed-dose combination (FDC) tablet formulation that was approved by the US FDA on August 2, 2004 and widely used in combination treatment regimens and as prophylaxis to prevent mother-to-child transmission of HIV in Sub Saharan Africa. PrEP using either daily oral tenofovir disoproxil fumarate (TDF) of co-formulated TDF/emtricitabine (TDF/FTC) has been shown to be effective for HIV prevention in men who have sex with men and in discordant couples. TDF-containing PrEP is currently recommended by the World Health Organization (WHO) for people at substantial risk of HIV infection (incidence $\geq$3%) and in December 2015. Pregnant women were excluded from participating in PrEP trials because of the lack of safety data in pregnancy as cautioned in the Drug Package Insert ("Because studies in humans cannot rule the possibility of harm, Truvada should be used in pregnancy ONLY if clearly needed"). Available human and animal data suggest that TRUVADA does not increase the risk of major birth defects overall compared to the background rate and there are limited safety data for TDF/FTC when used in HIV uninfected pregnant women. In the past five years, PrEP trials have explored the use of Tenofovir alone or in combination with Emtricitabine in a once daily regimen, resulting in varying efficacy outcomes that have been largely associated with adherence. Much of the safety data have been generated in studies involving ART in HIV infected pregnant women. The majority of these studies indicate no added adverse effect of TDF on pregnancy outcomes. The few studies of TDF use in HIV uninfected women are treatment studies to prevent mother-to-child transmission of Hepatitis B and only 2 studies of TDF as PrEP use among women who subsequently fell pregnant post enrolment. However, exposure to TDF during pregnancy in these PrEP studies is limited since TDF was halted once pregnancy was diagnosed. Only a handful of studies have demonstrated the excretion of TDF into breastmilk and have concluded that these are relatively low doses of exposure to the infant. When women were given TDF 300mg/FTC 200mg as part of the triple drug regimen in Option B+ program in Malawi the Tenofovir concentrations in breastmilk were very low (breast milk/maternal plasma ratio=0.08) [59]. Additional studies are needed to explore the effect of long term TDF exposure during breastfeeding on child growth and child health. Considering HIV uninfected women are

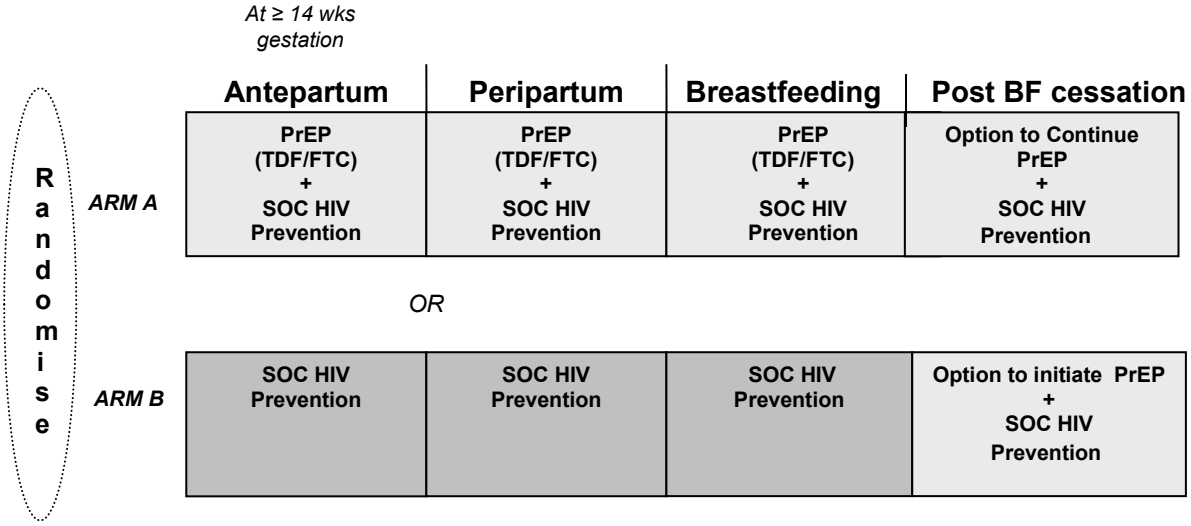
encouraged to breastfeed for at least a year, studies exploring the long term effect of breastmilk exposure to TDF when used as PrEP in the mother, are needed.

Goal:
To explore the safety of Truvada when used as PrEP in combination with current recommendations for prevention of sexually transmitted infections in young women at substantial risk of HIV acquisition during pregnancy and lactation.

Study Population:
Pregnant women, at their first antenatal visit, HIV uninfected, without evidence of maternal or fetal complications and at substantially high risk for HIV infection.

Sample Size:
To compare the frequency and seriousness of adverse events in women receiving tenofovir/emtricitabine in pregnancy as opposed to women not receiving tenofovir/emtricitabine in pregnancy, we have based our sample size on the main clinically relevant pregnancy outcome. Although all pregnancy outcomes will be compared between the intervention (immediate PrEP) and control (deferred PrEP) groups, we based our sample size on the expected preterm delivery proportion among HIV uninfected women (South Africa 18.5%, Botswana 19%). A sample size of 421 in the Immediate PrEP group and 421 in the Deferred PrEP group (i.e. 842 in total) achieves 80% power to detect a non-inferiority margin difference between the group proportions of 0.075 or 7.5%. The reference group proportion (deferred PrEP) of pre-term delivery pregnancy outcome as a main clinically-relevant adverse event is 0.185 or 18.5%.

Study Design: An Open Label randomized control study. Pregnant women considered at high risk for HIV infection will be randomized to commencing PrEP in pregnancy with continued use throughout breastfeeding or deferred PrEP until breastfeeding cessation. Women in either group will receive the standard of care for prevention of HIV and other sexually transmitted infections.



SOC – Standard of Care: Risk reduction counselling, STI screening and treatment, inviting the sexual partner to receive HCT and referral for treatment and support if he tests HIV positive and condom promotion.

Study Duration:
Mother: From the first antenatal visit in pregnancy until 18 months post-delivery.
Infant: From birth until 18 months of age.
Approximately 12 months for planned accrual and 24 months for complete follow-up. The study period is expected to span 3 years.

Study Products:
Truvada (emtricitabine 200mg/tenofovir disoproxil fumarate 300mg) is in a fixed-dose combination (FDC) tablet formulation and is proven safe for the prevention and treatment of HIV in non-pregnant and non-lactating women.

Study Arms:
Arm A: (Intervention)
Standard HIV Prevention strategy **PLUS** a once daily dose of Truvada (FTC 200mg/TDF 300mg tablet) initiated in pregnancy and continuing until cessation of breastfeeding or 18 months postdelivery whichever is earliest and thereafter the option to continue PrEP post breastfeeding cessation.

Arm B: (Control)
Standard HIV Prevention strategy throughout pregnancy until 18 months postdelivery **PLUS** the offer to initiate PrEP post breastfeeding cessation.

Primary Objective:
- To compare the frequency and seriousness of specific adverse events related to TDF/FTC in women and their children in Arm A (exposure to TDF/FTC) and Arm B (no exposure to TDF/FTC) during pregnancy until breastfeeding cessation.

Secondary Objectives:
- To estimate HIV incidence in women in ARM A with ARM B at delivery and at 18 months post-delivery.
- To determine the interdependency of adherence to a once daily oral TDF/FTC regimen and standard HIV prevention methods during pregnancy and postdelivery.
- To measure antiretroviral drug (TDF/FTC) level in women and their infants in association with adherence, safety and level of protection (plasma and breastmilk)
- To detect drug resistance among women who acquire HIV infection and correlate the treatment outcomes (after initiating immediate ARV treatment) with drug resistance
- To determine mother-to-child transmission of HIV among women who acquire incident infections during pregnancy and breastfeeding and the frequency of drug resistance in their infected babies

Main Outcome Measure:
- Serum Creatinine Clearance, adverse pregnancy outcomes (e.g., stillbirth, preterm delivery at < 37 weeks gestation, low birth weight <2500 grams, and congenital anomalies) and Bone Mineral Density among women
  Bone Mineral Density and growth parameters in neonates and infants until 18 months of age.

Other Outcome Measures:
- Confirmed presence of HIV infection detected by HIV NAT at scheduled 3 monthly visits until 18 months.
- Adherence –self reported use of study product, pill count during pregnancy and postdelivery

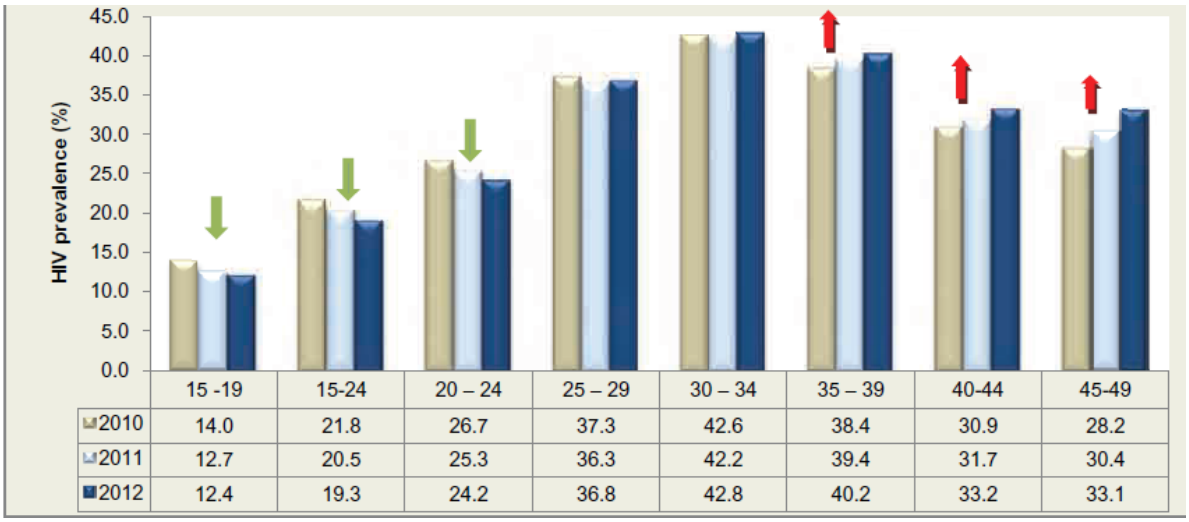
- Condom Usage, unprotected sex acts, adherence to PrEP and perceived partners HIV status
- Antiretroviral drug level in plasma as a marker of adherence.
- Drug resistance – drug resistance mutations as detected by genotype analysis
- Mother-to-child transmission-reactive NAT testing performed in children born to mothers who acquire HIV infection during pregnancy and breastfeeding.

1. BACKGROUND

1.1. HIV Prevalence in Pregnant Women in South Africa

South Africa has the largest burden of HIV disease globally [1]. Although South Africa (SA) recorded the greatest decline in new HIV infections in 2013 globally, 98 000 fewer than in 2010, SA still contributes the highest proportion of new infections (23%) in sub-Saharan Africa. The rapid scale-up of ART provision and Universal Test and Treat programmes are expected to eliminate the HIV epidemic in South Africa, but mathematical models have seemingly overestimated the timing of the effect [2], and the effect of this new strategy on HIV incidence in the country will be more likely realised only after 10 years of implementation [2]. South Africa with a largely heterosexual HIV epidemic has through its expansive PMTCT programme initiated more women than males on ART. The effect of this can be seen in the antenatal seroprevalence trend. The HIV prevalence among ante-natal women aged 15 – 49 years in the 2012 South African antenatal survey is estimated at 29.5% (95% CI 28.8-30.2%) and remains unchanged from 29.5% in 2011 (95% CI 28.7-30.2%) [3]. The prevalence in the older age groups is reflective of established infections, with the increase in prevalence in the older age groups (>35) due to greater antiretroviral treatment access and increased survival and increased parity among women with established HIV infections [4].

The antenatal HIV prevalence in the 15-24 year age group is a suggested proxy measure of HIV incidence because these adolescent girls and young women (AGYW) are more likely to have experienced recent sexual onset. The prevalence in this age group was 19.3% in 2012, not markedly lower than in previous years (20.1% in 2011 and 21.8% in 2010) (Figure 1). These young pregnant women are likely to present with recently acquired infections (either preconceptual or very early in pregnancy) when they first seek antenatal care [4].



	15 -19	15-24	20 – 24	25 – 29	30 – 34	35 – 39	40-44	45-49
2010	14.0	21.8	26.7	37.3	42.6	38.4	30.9	28.2
2011	12.7	20.5	25.3	36.3	42.2	39.4	31.7	30.4
2012	12.4	19.3	24.2	36.8	42.8	40.2	33.2	33.1

Figure 1. Antenatal HIV Prevalence by Age Group in South Africa 2010-2012.

1.2. HIV Incidence in Pregnant and Lactating Women in South Africa

Women remain vulnerable to HIV during pregnancy and even more so postdelivery. In earlier South African studies (2005-2009) we reported HIV seroconversion rates ranging from 1.3% to 3.0% in pregnancy and postnatally [5-7]. The national incidence of HIV in pregnancy alone in 2005 was estimated as 10.7/100 PWY [95% CI 8.2–13.1][5].

In a population-based evaluation (2011-2012), Dinh et al reaffirmed the high seroconversion rate among pregnant women attending public health facilities in South Africa; 3.3% (95% CI: 2.8%-3.8%) women seroconverted in pregnancy [7].

In a recent (Jan-Aug 2015) evaluation of antenatal HCT and PMTCT program indicators in KwaZulu-Natal among 78 906 pregnant HIV uninfected women who retested during pregnancy, 1.8% (95%CI 1.0-2.9) seroconverted before delivery [KZN DOH, 2016]. In a similar evaluation of postnatal HCT indicators for Apr-Dec 2015 during which 26 455 women who were HIV negative during pregnancy were retested postnatally we reported a 5% seroconversion rate in the postnatal period alone. Certain districts had distinctly higher postnatal seroconversion rates: Ethekwini (6.2%), Umgungundlovu (7.1%), Umzinyathi (9.1%), and Uthukela (7.1%) [KZN DOH, 2016].

In the past year (2015), most districts reported antenatal seroconversion rates of 2% or more but more significantly seven districts reported high postnatal seroconversion rates ranging from 3.9 to 9.1% (Figure 2 below). Postnatal seroconversion rates were 2-6 times higher than antenatal seroconversion rates.

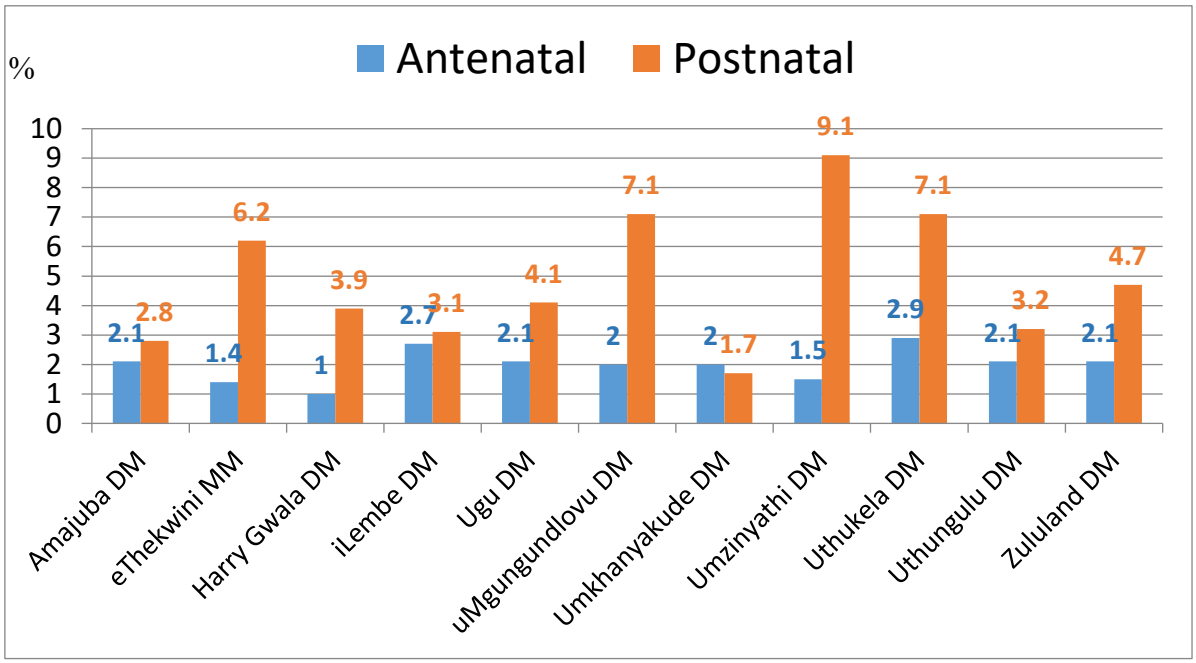


Figure 2. Antenatal and Postnatal Seroconversion Rates by District in KwaZulu Natal January-December 2015.

1.3. International Studies of HIV Incidence in Pregnancy and Postpartum

In a meta-analysis, using data from 19 international cohorts, Drake et al concluded that the pooled HIV incidence rate during pregnancy/postpartum was 3.8/100 PWY (95% CI 3.0-4.6): 4.7/100 PWY during pregnancy and 2.9/100 PWY postpartum (p=0.18) [9] (Fig 3). When compared to earlier studies, current programmatic data reflects a shift in the new HIV infection epidemic (Figures 2 and 3). The higher HIV incidence in women postpartum is hypothetically due to a longer exposure/observational period (12 – 18 months) compared to exposure during the antepartum period (maximum 6-8 months). Other plausible reasons for the higher HIV incidence postpartum include possible behavioural changes and changes in relationship dynamics over a 12-18 month period. These risk factors are yet to be confirmed in prospective studies.

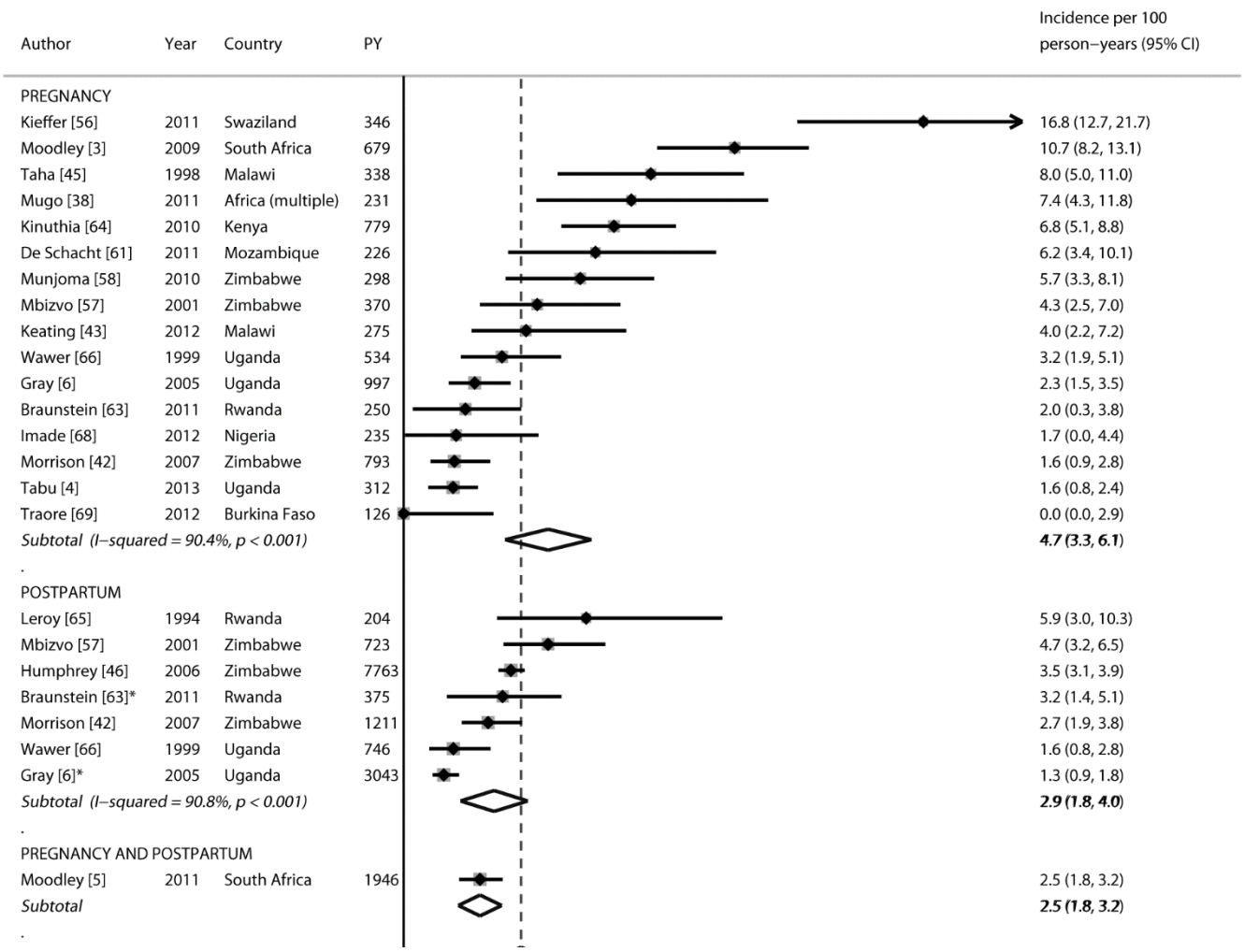


Figure 3. A forest-plot of HIV incidence in pregnancy and postpartum as per a meta-analysis of international studies by Drake et al, 2014 [9].

HIV incidence was significantly higher in African than non-African countries (3.6% versus 0.3%, respectively; p<0.001) and more importantly the meta-analysis revealed that risk of HIV in non-pregnant/non-postpartum women was not significantly different from the pregnant (HR 1.3, 95% CI 0.5-2.1) or postpartum women (HR 1.1, 95% CI 0.6-1.6). These findings support our rationale for pregnant and postpartum women to be regarded at similarly high risk as their non-pregnant counterparts. However, the impact of newly acquired maternal HIV infections on the unborn/lactating infant has far more outreaching consequences and for this reason these young pregnant women or lactating women need to be prioritized for access to PrEP.

1.4. Behavioural Risk for HIV Acquisition in Pregnancy and Post-delivery

In the KwaZulu-Natal HIV Incidence study involving 2835 pregnant women, the vast majority (63%) who tested HIV negative at their first antenatal visit continued to engage in unprotected sex during pregnancy and post-delivery, with abstinence during pregnancy reported among 17% of the women. A behavioural intervention study in a South African urban community reported similar high risk sexual behavior among postpartum women [10]. At 14 weeks, 42% of women reported resumption of sexual activity since delivery, and this increased to 87% at 9 months. An estimated 17% of the sexually-active women tested positive for C*hlamydia* or *N.gonorrhoea* or *T.vaginalis* infections within 14 weeks of giving birth. Furthermore, inconsistent condom use was reported for 45% of the sexually active women at the 14 week post-delivery visit,

increasing to 59% at the 9 months visit post-delivery. Inconsistent condom use was significantly more common among HIV uninfected women at 14 weeks and at 9 months. In Malawi, where HIV incidence in pregnancy is reportedly 1%, sexual activity was most frequent in early pregnancy and in the non-pregnant state [11]. Although women continued to be sexually active during the course of pregnancy and postpartum, the number of sex acts decreased in the late pregnancy and early postpartum period (62%). Women also noticed a change in their partner's behaviour in their late pregnancy and postpartum periods. Their partners spent more nights away from home or returned late at night. In south-west Nigeria and in other African countries, traditional norms impose abstinence during pregnancy and in the postpartum period [12]. The question is whether such a practice alters partner behaviour, i.e. do men abstain as well or do they engage in extramarital sex. In a community-based study in Nigeria, although men are known to generally have extramarital relationships (42.1%), the proportion of men with extramarital relationships is increased (43.7%) during the pregnancy and postpartum period and the chances of extramarital relationships in the abstinence period postpartum was much higher than in pregnancy (42.1% vs 48% p <0.001) [13].

1.5. HIV Acquisition in Pregnancy and Post-delivery and Effect on Mother-to-Child Transmission of HIV

An estimated 40 000 children are newly infected per annum in South Africa and the current mother-to-child transmission (MTCT) rate is 4% at 4-8 weeks despite the 88% ARV prophylaxis coverage [14]. Despite the encouraging reports of lower incidence of paediatric HIV infections as a result of PMTCT programmes, a study by Johnson et al modelled a 34% projected increase in MTCT from recently infected mothers in the absence of any intervention [15]. The study further cautions that MTCT from women who seroconvert during lactation will become the dominant mode of MTCT. In the KZN seroconversion cohort study, the group of HIV-exposed children was 2.3 times at higher risk of infection (MTCT 20.5% [8/9] vs 9.0% (83/925]) [5].

1.6. HIV Prevention in Women

For all the reasons previously argued in the search for female controlled methods to prevent HIV acquisition, women either during pregnancy or after delivery are also faced with similar challenges. However, the need for a safe and effective method of HIV prevention is more compelling and complicated because one would want to consider the unborn or breastfeeding child who could be exposed to antiretrovirals. Although there are no clinical trials that enrolled pregnant women in successful biomedical HIV intervention studies, there are reports of women falling pregnant subsequently [16,17]. In such cases, the study protocol may have dictated that study drugs be stopped and participants monitored for adverse events until end of pregnancy and end of lactation before recommencing study drugs.

There are currently several proven interventions to prevent HIV acquisition in women. Figure 4 below provides an overview of all known prevention methods for men and women. One approach is to reducing infectiousness of the HIV infected sexual male partner through early antiretroviral treatment [18]. In a large African population-based prospective cohort study, it was found that individuals in a rural community with high ART coverage were 38% less likely to acquire HIV than individuals in a rural community with a lower ART coverage [19]. Another successful strategy is to provide pre-exposure antiretroviral prophylaxis to the HIV uninfected female partner [20].

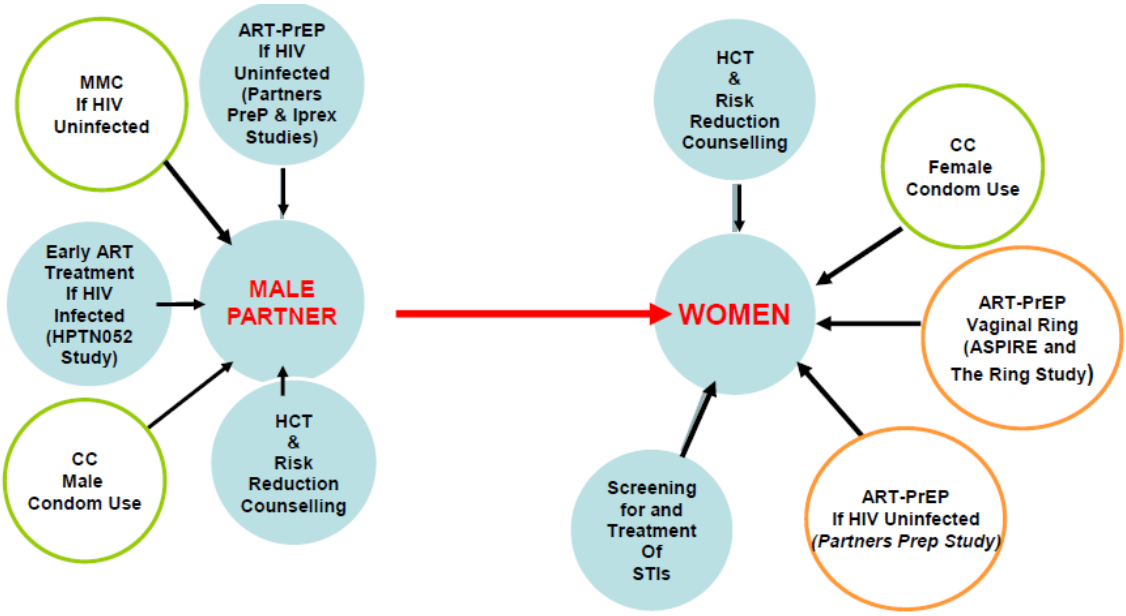


Figure 4: Evidence Based HIV Prevention Approaches for Men and Women

1.7. Antiretroviral PreExposure Prophylaxis to Prevent Mother-to-Child Transmission of HIV

A short course antiretroviral regimen given to HIV exposed infants during breastfeeding and that which prevented perinatal HIV transmission served as evidence of biological plausibility for using a simple antiretroviral regimen to prevent HIV acquisition in adults [21]. Progressive modifications in antiretroviral regimens and choice of drugs and wider access to populations at risk have resulted in a significant reduction in mother-to-child transmission rates worldwide [22]. In KwaZulu-Natal alone, we have seen transmission rates by 2 months of age drop from 23% to 2.4% within a decade (Fig 5), a decline in transmission rate once believed to be only possible in high-income countries [14,23].

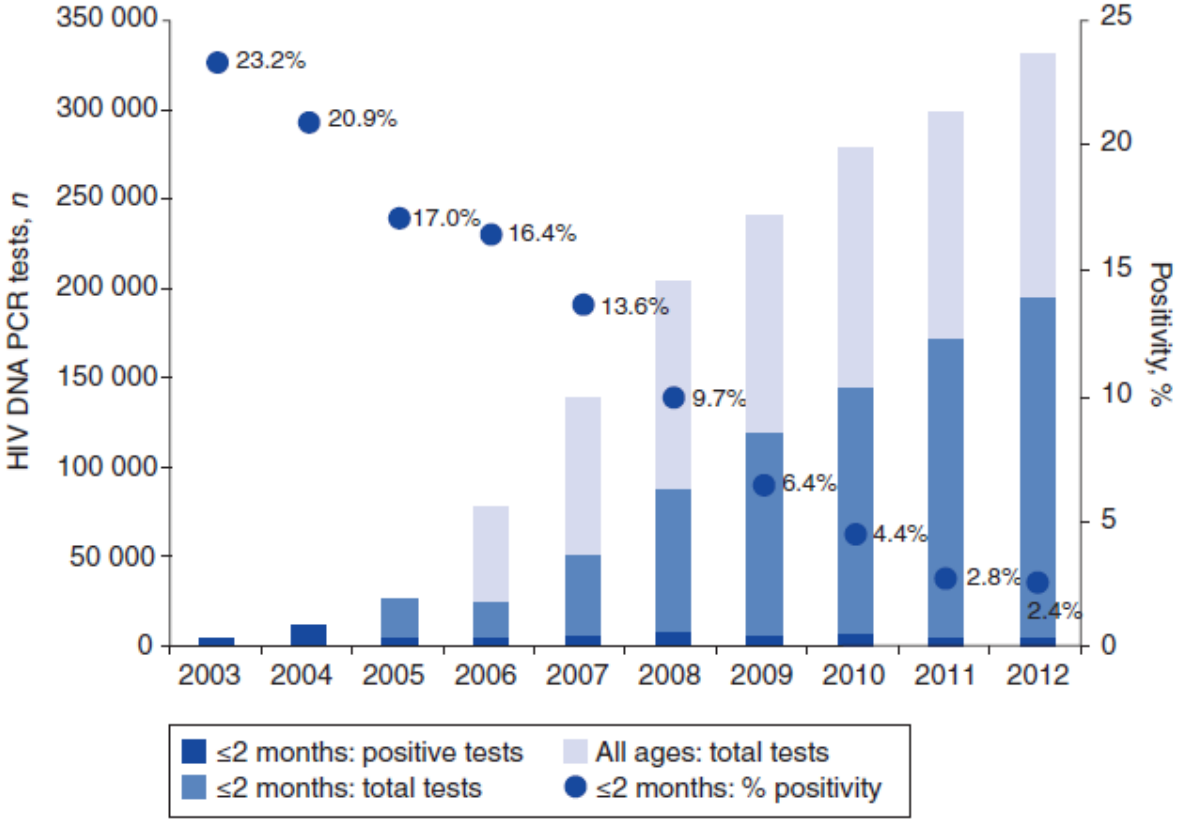


Figure 5. Perinatal HIV transmission Rates in KwaZulu Natal (2003-2012)

However, we have yet to eliminate all risks of mother-to-child transmission both during pregnancy and breastfeeding [22, 24]. Current PMTCT guidelines supporting a fixed dose combination of Tenofovir/Emtricitabine/Efavirenz as treatment of choice for all HIV positive pregnant women aim to maintain undetectable viral levels throughout pregnancy, breastfeeding and beyond (Option B+) [25,26]. The positive impact of this new strategy on the incidence of perinatal infections is yet to be reported but the positive impact on pregnancy outcomes among HIV positive women is already being realized [27]. In this multivariable analysis of HIV positive women delivering at a large regional hospital in KwaZulu-Natal, having received any antiretroviral regimen as opposed to not receiving any ARV was significantly associated with reduced odds of a stillbirth (OR 0.08, 0.21 and 0.18 respectively), preterm birth (OR 0.52, 0.68 and 0.56 respectively) and low birth weight (0.37, 0.61 and 0.52 respectively) [27].

1.8. Antiretroviral PreExposure Prophylaxis to Prevent HIV Acquisition in Women

In the past five years, scientists have made groundbreaking progress in identifying a safe and efficacious antiretroviral regimen to prevent HIV acquisition in men and women. These PrEP trials have explored the use of Tenofovir alone or in combination with Emtricitabine in a once daily regimen, resulting in varying efficacy outcomes that have been largely associated with adherence (Fig 6). Importantly, no major safety concerns have been identified. Three studies in particular, Partners Prep Trial in Uganda and Kenya, the TDF-2 Trial in Botswana and the Fem Prep Trial in South Africa, Kenya and Tanzania have contributed to an intended policy change both at a local and international level [20, 29, 30].

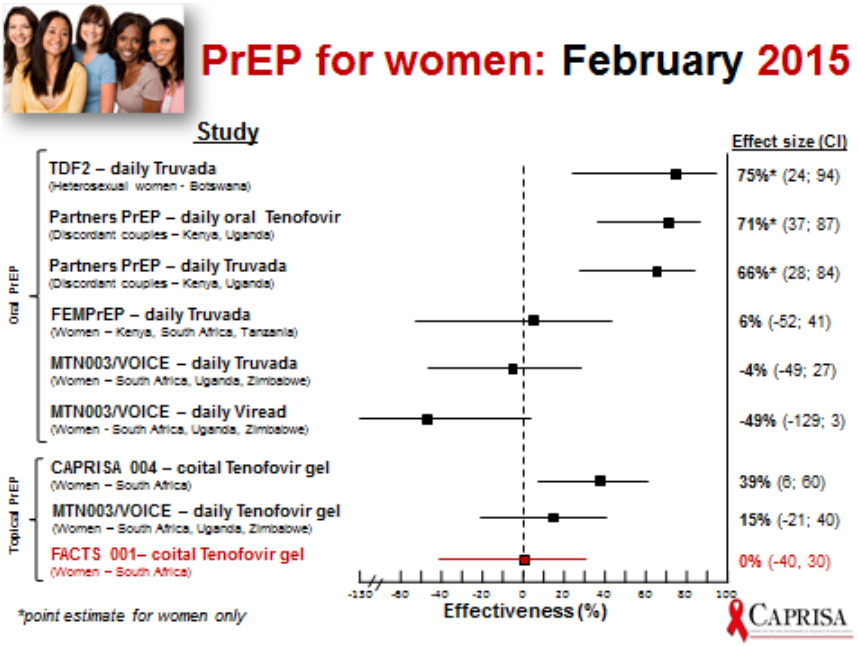


Figure 6: Antiretroviral Prevention Strategies for Women

1.8.1. Partners Prep Trial (Truvada or Tenofovir in Uganda, Kenya) [20]

A phase 3 randomised double-blind placebo controlled trial of daily oral Truvada (TDF/FTC) or Tenofovir (TDF) to prevent HIV transmission between 4,758 HIV discordant heterosexual partners in Uganda and Kenya. Study drug was discontinued among women who became pregnant during follow-up. Prevention efficacy among women was 71% for TDF and 66% for TDF/FTC. Adherence to study medication among pregnant women, notably for a shorter period since women were taken off study drug, was more than 90% by pill count and more than 80% by plasma drug level. Rates of adverse events did not differ between study groups, however gastrointestinal disorders and fatigue were more commonly reported in the groups receiving antiretroviral as compared to the placebo group. Resistance testing revealed TDF or FTC resistant viruses in 3 of 14 infected individuals (21.4%) who were infected at enrollment, but no resistant viruses were detected in persons who were subsequently infected.

1.8.2. TDF-2 Trial (Truvada in Botswana) [28]

A phase 2 randomised double blind placebo controlled study to determine the safety and efficacy of daily oral Truvada (TDF/FTC) in preventing HIV acquisition among 1219 men and women was conducted in Botswana. TDF/FTC afforded 62% protection and efficacy did not differ by gender. Follow-up was particularly low in this cohort with 33% of participants not completing study visits. Adherence to study medication was more than 80%, and nausea, vomiting and dizziness was reportedly more common among participants receiving TDF/FTC in the first month. Laboratory abnormalities and serious adverse events were similar in both groups. One of the 3 (33%) participants known to be infected at enrolment had TDF/FTC resistant viruses, while none of the 33 participants infected subsequent to enrolment demonstrated resistant viruses. Women who fell pregnant subsequent to enrolment were taken off study drugs and fetal loss was comparable in TDF/FTC and placebo groups as expected since exposure to TDF/FTC in pregnancy was limited.

1.8.3. FEM-PREP Trial (Truvada in South Africa, Kenya, Tanzania) [29]

A phase 3 randomized double blind placebo-controlled study conducted in South Africa, Kenya and Tanzania revealed no statistically significant difference in HIV acquisition between women receiving TDF/FTC (4.7 infections per 100py) and women receiving placebo (5.0 infections per 100py). Adherence measured by level of study drug in the plasma was less than 50% among women receiving TDF/FTC. Nausea and vomiting in the first month were more commonly reportedly among women receiving TDF/FTC. Laboratory investigations also revealed slight elevation in ALT/AST levels in this study drug group. TDF/FTC resistant viruses were detected in 5 of 68 (7%) women who acquired infections after enrolment; 1 woman in the placebo group and 4 in the TDF/FTC group. Study drug was discontinued in women who fell pregnant subsequent to enrolment.

1.8.4. The Ring Study (Dapivirine vaginal ring in South Africa and Uganda) [30]

A phase 3 placebo controlled study has shown that a long acting HIV prevention method (monthly vaginal ring containing Dapivirine, a non-nucleoside reverse transcriptase inhibitor) for women can safely reduce HIV infection in women by 31%. Higher efficacy (37%) however was only observed among women older than 21.

1.8.5 ASPIRE *(Dapivirine vaginal ring in South Africa and Malawi) [31]*

The second long acting vaginal ring study reportedly reduced HIV infections by 31%, but showed higher efficacy (61%) among women older than 25. The older women supposedly were more adherent to ring use.

1.9. Intervention

TRUVADA (tenofovir disoproxil fumarate/emtricitabine) is the antiretroviral drug to be used in this trial to prevent HIV acquisition. TRUVADA tablets are fixed dose combination tablets containing emtricitabine and tenofovir disoproxil fumarate. EMTRIVA is the brand name for emtricitabine, a synthetic nucleoside analog of cytidine. Tenofovir disoproxil fumarate (tenofovir DF) is converted in vivo to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5′-monophosphate. Both emtricitabine and tenofovir exhibit inhibitory activity against HIV-1 reverse transcriptase.

Emtricitabine*:* The chemical name of emtricitabine is 5-fluoro-1-(2*R*,5*S*)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. Emtricitabine is the (-) enantiomer of a thio analog of cytidine, which differs from other cytidine analogs in that it has a fluorine in the 5-position. It has a molecular formula of C8H10FN3O3S and a molecular weight of 247.24. Emtricitabine is a white to off-white crystalline powder with a solubility of approximately 112 mg/mL in water at 25 □C. The partition coefficient (log p) for emtricitabine is -0.43 and the pKa is 2.65.

Tenofovir Disoproxil Fumarate (TDF)*:* TDF is a fumaric acid salt of the bis-isopropoxycarbonyloxymethyl ester derivative of tenofovir. The chemical name of tenofovir disoproxil fumarate is 9-[(*R*)-2 [bis][(isopropoxycarbonyl)oxy]-methoxy]phosphinyl]methoxy]propyl]adenine fumarate (1:1). It has a molecular formula of C19H30N5O10P □ C4H4O4 and a molecular weight of 635.52. TDF is a white to off-white crystalline powder with a solubility of 13.4 mg/mL in water at 25°C.

The partition coefficient (log p) for tenofovir disoproxil is 1.25 and the pKa is 3.75. All dosages are expressed in terms of TDF except where otherwise noted.

Formulation: TRUVADA tablets are for oral administration. Each film-coated tablet contains 200 mg of emtricitabine and 300 mg of tenofovir disoproxil fumarate, (which is equivalent to 245 mg of tenofovir disoproxil), as active ingredients. The tablets also include the following inactive ingredients: croscarmellose sodium, lactose monohydrate, magnesium stearate, microcrystalline cellulose, and pregelatinized starch (gluten free). The tablets are coated with Opadry II Blue Y-30-10701, which contains FD&C Blue #2 aluminium lake, hydroxypropyl methylcellulose 2910, lactose monohydrate, titanium dioxide, and triacetin.

Pharmacokinetics:
Emtricitabine: Following oral administration of EMTRIVA, emtricitabine is rapidly absorbed with peak plasma concentrations occurring at 1–2 hours post-dose. Less than 4% of emtricitabine binds to human plasma proteins in vitro and the binding is independent of concentration over the range of 0.02–200 µg/mL. Following administration of radiolabelled emtricitabine, approximately 86% is recovered in the urine and 13% is recovered as metabolites. The metabolites of emtricitabine include 3′-sulfoxide diastereomers and their glucuronic acid conjugate. Emtricitabine is eliminated by a combination of glomerular filtration and active tubular secretion. Following a single oral dose of EMTRIVA, the plasma emtricitabine half-life is approximately 10 hours.

Tenofovir Disoproxil Fumarate: Following oral administration of VIREAD, maximum tenofovir serum concentrations are achieved in 1.0 □ 0.4 hour. Less than 0.7% of tenofovir binds to human plasma proteins in vitro and the binding is independent of concentration over the range of 0.01–25 □g/mL. Approximately 70–80% of the intravenous dose of tenofovir is recovered as unchanged drug in the urine. Tenofovir is eliminated by a combination of glomerular filtration and active tubular secretion. Following a single oral dose of VIREAD, the terminal elimination half-life of tenofovir is approximately 17 hours.

Overall, Study GS-US-104-172 demonstrated bioequivalence between the FTC-TDF combination tablet and the FTC capsule and TDF tablet formulations when administered separately. Administration of the FTC-TDF combination tablet with either a high-fat meal or light meal increased tenofovir exposure by approximately 30% compared with fasted-state administration. Clinical experience with TDF indicates that the effect of food on tenofovir exposure is not of clinical relevance. FTC and TDF, either administered as a combination tablet (containing FTC 200 mg/ TDF 300 mg) or co-administered as FTC 200 mg capsule and TDF 300 mg tablet were well tolerated.

1.10 Adverse Reactions from Clinical Trial Experience in HIV-1 Uninfected Adult Subjects

No new adverse reactions to TRUVADA (TDF/FTC) were identified from two randomized placebo-controlled clinical trials (iPrEx, Partners PrEP) in which 2830 HIV-1 uninfected adults received TRUVADA once daily for pre-exposure prophylaxis [20,32]. Subjects were followed for a median of 71 weeks and 87 weeks, respectively. These trials enrolled HIV-negative individuals ranging in age from 18 to 67 years. The iPrEx trial enrolled only males or transgender females of Hispanic/Latino (72%), White (18%), Black (9%) and Asian (5%) race [31]. The Partners PrEP trial enrolled both males (61-64% across treatment groups) and females in Kenya and Uganda [20].

Laboratory Abnormalities: Six subjects in the TDF-containing arms of the Partners PrEP trial discontinued participation in the study due to an increase in blood creatinine compared with no discontinuations in the placebo group [20]. One subject in the TRUVADA arm of the iPrEx trial discontinued from the study due to an increase in blood creatinine and another due to low phosphorous [32].

In addition to the laboratory abnormalities described above, Grade 1 proteinuria (1+) occurred in 6% of subjects receiving TRUVADA in the iPrEx trial. Grades 2-3 proteinuria (2-4+) and glycosuria (3+) occurred in less than 1% of subjects treated with TRUVADA in the iPrEx trial and Partners PrEP trial.

Changes in Bone Mineral Density (BMD):
In clinical trials of HIV-1 uninfected individuals, decreases in BMD were observed. In the iPrEx trial, a substudy of 503 subjects found mean changes from baseline in BMD ranging from -0.4% to -1.0% across total hip, spine, femoral neck, and trochanter in the TRUVADA group compared with the placebo group, which returned toward baseline after discontinuation of treatment [33]. Thirteen percent of subjects receiving TRUVADA vs. 6% of subjects receiving placebo lost at least 5% of BMD at the spine during treatment. Bone fractures were reported in 1.7% of the TRUVADA group compared with 1.4% in the placebo group. No correlation between BMD and fractures was noted. The Partners PrEP trial found similar fracture rates between treatment and placebo groups (0.8% and 0.6%, respectively). No BMD evaluations were conducted during this trial [20].

1.11 Clinical Experience With and Safety of *Emtricitabine and Tenofovir Disoproxil Fumarate (FTC and TDF) - Truvada* in Pregnancy and Breastfeeding

Emtricitabine (FTC, Emtriva™) in animal studies
Fetal variations and malformations were not increased with FTC dosing in mice in systemic drug exposures that were 60 times higher than doses recommended in humans [34]. FTC crosses the placenta in mice and rabbits with average fetal/maternal drug concentration ratios of 0.4 in mice and 0.5 in rabbits.

Emtricitabine (FTC, Emtriva™) in human studies – pregnancy and breastmilk
FTC is classified as FDA pregnancy category B. because of limited data demonstrating safety in pregnancy. In a study of 35 pregnant women given a dose of 400 mg FTC at the onset of labor, median cord/maternal drug ratio was 0.73, indicating significant placental transfer. Median AUC after a 400 mg dose in labor was 15.5 mg*h/L, similar to levels in non-pregnant adults after a 200 mg dose. Among 18 women receiving standard FTC dosing (200 mg/day) during the third trimester, median AUC of 8.6 µg*h/mL was above the target of > 7 µg*h/mL, but only 12 of 18 women were above the target [35]. Mean cord/maternal blood ratio at delivery was 1.17. Exacerbations of HBV have been reported in patients after discontinuation of FTC [36]. Patients, who are co-infected with HBV, may have increased values on liver function tests and exacerbation of hepatitis symptoms when FTC is stopped. Usually these symptoms are self-limiting; however, serious complications have been reported. The causal relationship to FTC discontinuation is unknown.

Tenofovir Disoproxil Fumarate (TDF, Viread) in animal studies
Tenofovir and TDF administered in toxicology studies to rats and monkeys at exposures (based on AUCs) between 6- and 12-fold higher than observed in humans caused bone toxicity [37, 38]. In monkeys, the bone toxicity was diagnosed as osteomalacia, and appeared to be reversible upon dose reduction or discontinuation of tenofovir. In rats and dogs, the bone toxicity manifested as reduced bone mineral density. Chronic dosing of rats in pregnancy noted no growth or reproductive problems when TDF was

administered at doses not associated with maternal toxicity [38]. At high doses of exposure (25 times the AUC achieved with therapeutic dosing), no fetal structural changes were seen.

Chronic exposure of fetal monkeys to TDF at a high dose of 30 mg/kg (25 times the AUC levels achieved with therapeutic doses in humans) from days 20-150 of gestation did not result in gross structural abnormalities [37]. However significantly lower fetal circulating insulin-like growth factor levels were reported and were associated with body weights 13% lower than untreated controls. A slight reduction in fetal bone porosity was also observed within 2 months of maternal treatment. However, a macaque treated for over 10 years with 10 mg/kg/day of TDF has given birth over several years to three infant macaques, all of whom were normal and had no bone abnormalities at birth.
Studies of intravenous TDF administration in pregnant cynomolgus monkeys reported a fetal/maternal concentration of 17% indicating some placental transfer [39].

Tenofovir Disoproxil Fumarate (TDF, Viread) in human studies
Pharmacokinetic profile of TDF has only been described in HIV infected pregnant women when given in combination with other antiretrovirals as treatment. No data exists for TDF pharmacokinetics when given alone to HIV uninfected pregnant women as prophylaxis. TDF pharmacokinetics during the third trimester of pregnancy among 37 women receiving TDF as part of a combination treatment was reported by the IMPAACT P1026s Study team [40]. The median AUC for TDF was lower in the 2nd (1.9ug/ml) and 3rd (2.4ug h/ml) trimesters as compared to postpartum (3.0ug h/ml). The AUC postpartum was comparable to non-pregnant women. The AUC values in the P1026s study were comparable to the PANNA study (2.5 and 3,2ug h/ml) 3rd trimester and postpartum respectively [40]. Although TDF AUC exceeded the target (>1.99 ug h/ml) for 50% of women in 2nd trimester, 73% in the 3rd trimester and 84% postpartum it is likely that women weighing more than 90kg in the 3rd trimester are subject to decreased TDF exposure.. In three studies of pregnant women the cord-to-maternal blood ratio ranged from 0.60 to 0.99 indicating high placental transfer [40, 42, 43]. A dose of 600 mg of TDF in labor resulted in levels in the women similar to levels in non-pregnant adults after a 300 mg dose, suggesting higher doses are required for adequate levels during labor in term pregnant women [43]. This was confirmed in PACTG 394 and HPTN 057, which showed adequate tenofovir concentrations with 600 mg intrapartum doses and a small increase in tenofovir concentrations when the intrapartum dose was increased to 900 mg [44, 45].

TDF is designated as FDA pregnancy Category B based on animal and clinical data. In the Antiretroviral Pregnancy Registry, sufficient numbers of first trimester TDF exposures have been monitored to detect at least a two-fold increase in risk of overall birth defects but no such increase in birth defects has been observed. The prevalence of birth defects after first trimester TDF exposure was 11 (2.2%) of 491 (95% CI 1.1-4.0%) which is within the range of congenital anomalies reported in the general US population [46]. Safety data for TDF use in pregnancy is only available for HIV infected pregnant women. Low serum creatinine levels correlated strongly with lower AUC during the 3rd trimester in pregnancy. Women with target AUC had a serum creatinine level 0.1mg/dl higher than women who did not achieve target AUC. Although clearance is much more rapid during 3rd trimester when compared to postpartum, the median half-life was similarly long during pregnancy and postpartum. TDF was well tolerated by women in the P1026s study [40]. A case series found TDF to be well tolerated among 76 pregnant women, with two stopping therapy, one for rash and one for nausea. All 78 infants were healthy with no signs of toxicity, and all were HIV-uninfected [47]. Tenofovir appears to cross the placenta efficiently to achieve cord-maternal blood ratios 0.88 for P1026s and 0.82 in the PANNA study [40, 41].

In a multi-centre study of antiviral safety and efficacy in HIV uninfected pregnant women with high HBV viral load (>7 log IU/ml) in Australia; lamivudine was used from 2007 to 2010 and tenofovir disoproxil fumarate (TDF) from late 2010 [48]. Congenital abnormality rate and neonatal growth centiles were similar across cohorts.
Adverse events and pregnancy outcomes from January 1, 2011 to June 30, 2013 were evaluated in a small cohort of 17 pregnant women with HBV infection in China [49]. There were no significant changes in serum creatinine and phosphorus levels during TDF treatment. In addition, no adverse events related to TDF treatment were observed. The mean birth weight was 3226.5±331.7 g, and the mean length at birth was 50.4±1.1 cm. The growth and development of the infants was normal at birth, and no infants had birth defects related to TDF treatment. Exacerbations of HBV have been reported in patients after discontinuation of TDF [36]. Patients who are co-infected with HBV may have increased values on liver function tests and exacerbation of hepatitis symptoms when TDF is stopped. Usually these symptoms are self-limiting; however, serious complications have been reported. The causal relationship to TDF discontinuation is unknown.

FTC and TDF Fixed Dose Combination Tablet (Truvada) in Pregnancy

Gilead Sciences developed Truvada, a product containing FTC 200 mg and TDF 300 mg in a fixed-dose combination (FDC) tablet formulation that was approved by the US FDA on August 2, 2004. TRUVADA has been evaluated in a limited number of women during pregnancy and postpartum. Available human and animal data suggest that TRUVADA does not increase the risk of major birth defects overall compared to the background rate. There are no safety data for TDF/FTC when used in HIV uninfected pregnant women.

As of July 2011, the Antiretroviral Pregnancy Registry for HIV infected women has received prospective reports of 764 and 1219 exposures to emtricitabine- and tenofovir-containing regimens, respectively in the first trimester, 321 and 455 exposures, respectively, in second trimester, and 140 and 257 exposures, respectively, in the third trimester [46]. Birth defects occurred in 18 of 764 (2.4%) live births for emtricitabine-containing regimens and 27 of 1219 (2.2%) live births for tenofovir-containing regimens (first trimester exposure) and 10 of 461 (2.2%) live births for emtricitabine-containing regimens and 15 of 714 (2.1%) live births for tenofovir-containing regimens (second/third trimester exposure). Among pregnant women in the U.S. reference population, the background rate of birth defects is 2.7%. There was no association between emtricitabine or tenofovir and overall birth defects observed in the APR.

A review of obstetric records of 9445 HIV infected women in Botswana between 2009-2011 concluded that adverse birth outcomes were not any different among women who received ZDV or other 3 drug ART or women initiating TDF/FTC/EFV ((3% SB, 22% PTD and 12% SGA) [50].

The Antiretroviral Pregnancy Registry report ending Jul 2015 listed 60 cases of birth defects among 2608 first trimester exposures (2.3%; 95%CI 1.8-3.0) [51]. A prevalence similar to other antiretrovirals and furthermore the APR concluded that none of the antiretrovirals listed, including Tenofovir, contributed to the birth defects.

All serious adverse events and their relationship to TDF in pregnancy were summarised in a recent review by Wang and Kourtis from the Global AIDS Program (US CDC) [52]. The authors have concluded that from the few studies included in the review adverse events were mild to moderate and none were related to TDF. The review also includes a summary of studies exploring bone health and foetal exposure to TDF. These studies are few in number and virtually all have found no significant association between maternal use of TDF during pregnancy and infant growth or bone abnormalities [53-56].

Although one US study reported 12% lower bone mineral content (BMC) in newborns exposed to TDF in pregnancy, the key authors of this study concluded from a similar study in an African cohort of HIV infected pregnant women that maternal TDF use was not independently associated with lower newborn BMC but a triple-ARV LPVr-containing regimen may have led to reduced bone mineralization [57, 58]. There are no studies that have explored the effect of TDF on newborn BMC among HIV uninfected women. Further studies are also needed to explore the effect of TDF exposure during pregnancy on long term child growth.

SUMMARY OF PREGNANCY OUTCOMES IN STUDIES USING TDF OR TDF/FTC

Much of the data have been generated in studies involving ART in HIV infected pregnant women. The majority of these studies indicate no added adverse effect of TDF on pregnancy outcomes. The few studies of TDF use in HIV uninfected women are treatment studies to prevent mother-to-child transmission of Hepatitis B and only 2 studies of TDF as PrEP in pregnant women. However, exposure to TDF during pregnancy in the PrEP studies is limited since TDF was halted once pregnancy was diagnosed. And although adherence to PrEP was lower in one study, the pregnancy outcomes were similar.

TDF in HIV Infected Pregnant Women:

Stillbirths*:* No significant differences in stillbirths in TWO comparative studies of HIV-infected women receiving TDF-ART, nonTDF-ART or ZDV/sdNVP.

Preterm Deliveries: There were no significant differences in preterm delivery rates between regimens, although in the PROMISE randomized trial. Women randomized to both TDF-ART and non-TDF (AZT/3TC-based) ART arms had non-significantly higher PTD rates than women randomized to the AZT/sdNVP arm.

Very Preterm Deliveries: Two studies reported on very preterm delivery <34 weeks gestation (VPTD). In the PROMISE trial, the rate of VPTD was significantly higher in the women randomized to TDF ART compared to the non-TDF (AZT/3TC-based) ART; however, the VPTD rate in the TDF ART group was not significantly different from women randomized to AZT/sdNVP.

Low Birth Weight*:* All studies except one showed no significant difference in LBW rates between women receiving TDF ART and those receiving non-TDF ART. One study, the PROMISE study, reported significantly higher LBW rates in the women randomized to TDF ART or non-TDF ART when compared to AZT/sdNVP and rates were similar between the two ART regimens (TDF vs non-TDF). In the non-randomized Moodley study the rates of LBW were similar between women receiving TDF ART and AZT/sdNVP, as well as between those receiving TDF ART and non-TDF ART. One study compared LBW rates stratified by timing of TDF ART initiation, and observed no significant difference between TDF ART started preconception vs during pregnancy.

Birth Defects: Four studies in HIV-infected women on ART reported on birth defects found no significant differences in birth defects between regimens. The international Antiretroviral Pregnancy Registry (APR) reported a birth defect rate of 2.3% among 2,608 infants exposed to TDF ART, not significantly different than the US population rate of birth defects from CDC surveillance, 2.7% (29). The APR noted that this number of first-trimester TDF ART exposures were sufficient to rule-out a 1.5-fold increased risk of overall birth defects as well as a 2-fold increased risk of defects in the most common classes (genitourinary and cardiovascular). There was also no difference in the APR in birth defects between those with first TDF ART compared to second/third trimester TDF ART exposure (2.3% vs 2.1%, respectively).

TDF in HBV infected Pregnant Women:

TDF has also been used for prevention of perinatal hepatitis B virus (HBV) transmission in HBV-mono-infected pregnant women with high levels of HBV DNA (HBV DNA >log5-6 copies/mL), where the risk of perinatal HBV transmission is high even when the infant receives hepatitis B immunoglobulin and HBV vaccine. In these instances, TDF, given as a single drug, is initiated in the third trimester of pregnancy and usually (but not always) stopped 1-2 months postpartum.

Preterm Deliveries: In the HBV studies, including 143 women receiving TDF and 267 women receiving 3TC or no drug, preterm delivery rates were not significantly different between regimens.

***Low Birth Weight*:** Three studies in HBV mono-infected women, including 102 women receiving TDF compared to 215 control (3TC or no drug) women, found no significant differences in LBW rates between TDF and control regimens in any of these studies.

***Birth Defects*:** No difference in the birth defect rate between the TDF regimens and control arms.

TDF in HIV Uninfected Pregnant Women:

There were TWO PrEP trials in which women were found to be pregnant after enrolment and study drug was discontinued after diagnosis (1-2 months of exposure).

Still births: Spontaneous abortion rate was high in the PartnersPrEP and Voice studies, but not significantly different between the HIV uninfected women receiving TDF or TDF/FTC or Placebo.

Preterm Deliveries: Overall Preterm delivery rates in these HIV-uninfected women were lower than observed in the HIV-infected population, ranging between 2-9% compared to 9-25%. No significant differences were observed in both studies between TDF or TDF/FTC and the comparison control groups.

***Low birth weight**:* The LBW rates in HIV-uninfected women (ranging from 0-5%) were much lower than observed for HIV-infected women (ranging from 10-20%), as also observed for PTD rates.

The pregnancy outcomes in each of the above studies are tabulated below for each population group in a particular risk category.

FTC and TDF Fixed Dose Combination Tablet (Truvada) during BREASTFEEDING

The majority of HIV infected women in SubSaharan Africa and other low-income countries attending the public health sector will choose to breastfeed despite the risk of mother-to-child transmission (MTCT) of HIV. Protection against MTCT is widely available in the form of ARV treatment (including TDF) of the mother or prophylaxis in the baby.

Only a handful of studies have demonstrated the excretion of TDF into breastmilk and have concluded that these are relatively low doses of exposure to the infant. When women were given TDF 300mg/FTC 200mg as part of the triple drug regimen in Option B+ program in Malawi the Tenofovir concentrations in breastmilk were very low (breast milk/maternal plasma ratio = 0.08) [59]. Additional studies are needed to explore the effect of long term TDF exposure during breastfeeding on child growth and child health. Considering HIV uninfected women are encouraged to breastfeed for at least a year, studies exploring the long term effect of breastmilk exposure to TDF when used as PrEP in the mother, are needed.

TABLE: Summary of Birth Outcomes in Studies of TDF or TDF/FTC Use in Pregnancy

Study/Outcomes	**HIV Positive TDF - ART**	**HIV Positive Non TDF - ART**	**HIV Positive AZT/NVP**	**HIV Positive No ARVs**	**HIV Uninfected -TDF/TDF-FTC**	**HIV Uninfected No ARVs**
Stillbirths						
Gibbs-Africa 2012	8.0% (N=248)	4.0% (N=110)				
Moodley-Africa 2016	2.2% (N=1629)	4.2% (N=869)	1.8% (N=957)	13.5% (N=148)		2.2% (N=130)
Zash-Botswana Preconcept	4.9% (N=165)	6.4% (N=2006)				
Zash-Botswana During Preg	1.7% (N=231)	4.9% (N=243)				
Bunge-Voice PrEP					2.0% (N=66) 4.0% (N=104)	5.0% (N=100)
Spontaneous Abortions						
Mugo-Partners PreP Africa					27.7% (N=62) 42.5% (N=80)	32.3% (N=96)
Bunge-Voice PrEP					15.0% (N=66) 19.0% (N=104)	20.0% (N=100)
Preterm Deliveries <37 weeks						
Gibbs-Africa 2012	9.0% (N=248)	11.0% (N=110)				
Ransom-US 2015	18.0% (N=630)	16.0% (N=1395)				
Fowler-Africa 2014	18.5% (N=341)	19.7% (N=346)	13.5% (N=349)			
Moodley-Africa 2016	21.1% (N=1315)	24.5% (N=685)	20.1% (N=778)	32.4% (N=100)		18.5% (N=4820)
Zash-Preconcept	28.0% (N=165)	31.0% (N=2006)				
Zash-During Preg	19.5% (N=231)	19.8% (N=243)				
Malaba-Preconcept	20.1% (N=572)					
Malaba-During Preg	18.4% (N=922)					
Greenup-HBV Australia-2014					1.7% (N=58) 5.7% (N=52)	0%
Chen-HBV Taiwan-2015					7.8% (N=62)	3.6% (N=56)
Kochaksare-HBV Canada-2016					8.3% (N=23)	2.1% (N=138)
Mugo-Partners PrEP Africa-2014					2.5% (N=81) 8.7% (N=46)	7.7% (N=65)
Low birth weight <2500g						
Gibbs-Africa 2012	15.0% (N=251)	19.0% (N=115)				
Siberry US 2012	19.5% (N=449)	19.1% (N=1580)				
Pintye Africa 2015	10.0% (N=89)	7.0% (N=188)				
Fowler Africa 2015	16.9% (N=341)	20.4% (N=346)	8.9% (N=349)			
Moodley – Africa 2016	13.5% (N=1442)	15.3% (N=768)	10.0% (N=877)			11.0% (N=5265)
Malaba - Preconcep	12.2% (N=572)					
Malaba – During Pregnancy	12.0% (N=922)					
Celen – HBV 2013 Turkey					4.7% (N=21) 4.3% (N=24)	
Greenup – HBV 2014 Australia					3.4% (N=58) 1.9% (N=53)	
Kochaksaraei – HBV 2016 Canada					0% (N=23) 0% (N=128)	

Study/Outcomes	**HIV Positive** **TDF - ART**	**Conclusion**
Le Roux et al. *2016 (CROI)* *South Africa* *Comparative duration* *TDF <12 w to >22 w*	Length <12 wk TDF: 49 (47-51); 12-22 wk TDF: 49 (48-52); >22 wk TDF: 49.5 (48-51) *LAZ at ages 0-8 wk, 9-20 wk, 21-32 wk, 33-44 wk, and >44 wk: median LAZ did not vary by duration of TDF exposure at any time point*	NO effect duration of TDF ART exposure on length growth
Jao al. 2016 South Africa Comparative duration of TDF TDF <10 wk: 188 10-24 wk: 326 >25 wk: 132	□ Femur length z-score (FLZ) median (IQR): o Baseline 0.20 (-0.11, 0.63) o Throughout gestation, median 0.30 (IQR -0.03, 0.63) □ Humerus length z-score (HLZ) median IQR) o Baseline -0.10 (-0.45, 0.3) o Throughout gestation, median 0.22 (IQR -0.26, 59) *No difference in baseline or throughout gestation scores by duration TDF <10 wk, 10-24 wk, >25 wk*	No effect of duration of TDF ART exposure on fetal bone growth
Siberry et al. 2016 Malawi, S Africa, Uganda, Zimbabwe Comparative TDF: 113 Non-TDF: 118 *Zash-Botswana Preconcept*	Estimated difference LS BMC g, adjusted mean (CI) o TDF triple vs AZT triple: -0.08 (-0.16 to 0.1) (p=0.09) o TDF triple vs AZT/sdNVP: 0.01 (-0.08 to 0.1) (p=0.82) o AZT triple vs AZT/sdNVP: 0.09 (0 to 0.17) (p=0.05) □ Estimated difference WB BMC g, mean (CI) o TDF triple vs AZT triple: 1.76 (-2.43 to 5.95) (p=0.41) o TDF triple vs AZT/sdNVP: 9.73 (5.49 to 13.96) (**p<0.001**) o AZT triple vs AZT/sdNVP: 7.97 (3.97 to 11.96 (**p<0.001**)	Triple ART results in significantly lower whole body BMC than AZT/sdNVP, but not difference between TDF ART vs non-TDF ART regimens

TABLE: Summary of Fetal Abnormalities and Infant Bone and Growth Studies related to TDF or TDF/FTC Use in Pregnancy

Vigano et al. 2011 Italy Comparative TDF: 33 Non TDF: 35	BW g, median (range): TDF 2,830 (1310-3800) vs other ART 2,735 (1220-3700): BW <10%ile: TDF 12.1% vs other ART 11.4% □ Birth length cm median (range): TDF 47 (37-59) vs other ART 46.8 (40-52); BL <10%ile: TDF 27.3% vs other ART 28.6% □ Birth HC cm median (range): TDF 33 (28-35) vs other ART 33 (28-38) □ Wt <10%ile at median 23 mo: TDF 21.2% vs other ART 17.1% □ Ht <10%ile at median 23 mo: TDF 18.2% vs other ART 14.3% □ Bone US (SOS) z score median (range): TDF 0.6 (-2.4, 2.6) vs other ART 0.8 (-2.2, 4.4) (p=0.40) □ Ca, phosphorus: NS different (p=0.37, p=0.09) □ Urine Ca/Cr ratio: TDF 0.08 (0.01-0.8) vs other ART 0.05 (0.007-0.51) (**p=0.039**) □ PTH (adjusted) pg/ml median (range): TDF 9.9 (4.6-15.3) vs other ART 12.3 (6.6-18.6) (**p=0.023**) but all values wnl □ Low IGF-1: TDF 3.7% (1/27) vs other ART 4.1% (1/24) □ BAP U/L median (range): TDF 147.6 (83-216) vs other ART 124.4 (94-222) ((p=0.37) □ CTX ng/mL median (range): TDF 1.4 (0.26-3.52) vs other ART 1.11 (0.28-3.8) (p=0.95)	No difference TDF vs non-TDF ART for birth weight, length, HC, 23 mo weight, height, bone US, lab Ca, P, IGF-1, BAP, CTX. Significantly higher urine Ca/Cr ratio and lower PTH levels TDF vs non-TDF ART (but wnl)
Siberry et al. 2012 USA Comparative TDF:449 (426 infants) Non TDF:1,580	LBW: TDF 19.5% vs other ART 19.1% (aOR 0.73, p=0.14); no association with duration TDF exposure, p=0.88 □ SGA: TDF 8.3% vs other ART 8.6% (aOR 0.96, p=0.88); no association with duration TDF exposure, p=0.31 □ Birth WAZ, dynamic cohort, adjusted mean (SD): TDF-0.63 (0.05) vs other ART -0.66 (0.04) (p=0.58) □ Birth LAZ: TDF-0.25 vs other ART -0.16 (p=0.29) □ Birth HCZ: TDF -0.66 vs other ART -0.65 (p=0.83) □ Birth LAZ <-1.5 SD: TDF 8.7% vs other ART 9.0% (aOR 1.18, p=0.58) □ Birth HCAZ <-1.5 SD: TDF 13.9% vs other ART 16.8% (aOR 0.82, p=0.39) □ 1 year adjusted WAZ, mean (SD): TDF -0.09 (0.08) vs other ART -0.04 (0.06); (p=0.62) □ 1 yr adjusted LAZ, mean (SD): TDF -0.17 (0.07) vs other ART -0.03 (0.06); (**p=0.04**) □ 1 yr adjusted HCAZ, mean (SD): TDF 0.17 (0.08) vs other ART 0.42 (0.06); (**p=0.02**) □ 1 yr WAZ <-1.5 SD: TDF 11.5% vs other ART 9.5% (aOR 1.25, p=0.42) □ 1 yr LAZ <-1.5 SD: TDF 7.9% vs other ART 8.2% (aOR 0.95, p=0.87) □ 1 yr HCAZ <-1.5 SD: TDF 6.2% vs other ARV 5.8% (aOR 1.17, p=0.67)	No difference TDF ART vs non-TDF ART for LBW, SGA, birth WAZ, LAZ, HCAZ or birth z score <-1.5 SD; at 1 year, WAZ no difference but LAZ and HCAZ significantly lower with TDF than other ART; however, z score <-1.5 SD (severe delay) does not differ by regimen

TABLE: Summary of Maternal Bone Studies of TDF or TDF/FTC Use

Study/Outcomes	**HIV Positive** **TDF - ART**	**Conclusion**
Le Roux et al. *2016 (CROI)* *South Africa* *Comparative duration* *TDF <12 w to >22 w*	Length <12 wk TDF: 49 (47-51); 12-22 wk TDF: 49 (48-52); >22 wk TDF: 49.5 (48-51) *LAZ at ages 0-8 wk, 9-20 wk, 21-32 wk, 33-44 wk, and >44 wk: median LAZ did not vary by duration of TDF exposure at any time point*	NO effect duration of TDF ART exposure on length growth
Jao al. 2016 South Africa Comparative duration of TDF TDF <10 wk: 188 10-24 wk: 326 >25 wk: 132	□ Femur length z-score (FLZ) median (IQR): o Baseline 0.20 (-0.11, 0.63) o Throughout gestation, median 0.30 (IQR -0.03, 0.63) □ Humerus length z-score (HLZ) median IQR) o Baseline -0.10 (-0.45, 0.3) o Throughout gestation, median 0.22 (IQR -0.26, 59) *No difference in baseline or throughout gestation scores by duration TDF <10 wk, 10-24 wk, >25 wk*	No effect of duration of TDF ART exposure on fetal bone growth
Siberry et al. 2016 Malawi, S Africa, Uganda, Zimbabwe Comparative TDF: 113 Non-TDF: 118 *Zash-Botswana Preconcept*	Estimated difference LS BMC g, adjusted mean (CI) o TDF triple vs AZT triple: -0.08 (-0.16 to 0.1) (p=0.09) o TDF triple vs AZT/sdNVP: 0.01 (-0.08 to 0.1) (p=0.82) o AZT triple vs AZT/sdNVP: 0.09 (0 to 0.17) (p=0.05) □ Estimated difference WB BMC g, mean (CI) o TDF triple vs AZT triple: 1.76 (-2.43 to 5.95) (p=0.41) o TDF triple vs AZT/sdNVP: 9.73 (5.49 to 13.96) (**p<0.001**) o AZT triple vs AZT/sdNVP: 7.97 (3.97 to 11.96 (**p<0.001**)	Triple ART results in significantly lower whole body BMC than AZT/sdNVP, but not difference between TDF ART vs non-TDF ART regimens

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2. AIM AND OBJECTIVES

Pre-exposure antiretroviral prophylaxis comprising a daily dose of Truvada (Tenofovir/Emtricitabine) is a proven prevention strategy for same sex couples and discordant couples and now internationally recommended for individuals at substantial risk. The benefit of PrEP in high risk population groups significantly outweigh the potential threat of adverse events. The high risk population groups are MSMs, men in incarceration, commercial sex workers and adolescent girls and young women. Additional options for PrEP in young women through long acting antiretroviral (Dapivirine) prophylaxis in vaginal rings are also possible, but preclude use in pregnancy.

Truvada (the antiretroviral drug of choice for PrEP) is classified as a FDA pregnancy Category B antiretroviral. Pregnancy and lactation are distinct contraindications to initiate PrEP because of the limited evidence of its (Truvada) adverse effects on the child. Despite this classification, Truvada has been endorsed by the WHO to be used as part of a treatment combination in HIV infected pregnant women and to reduce mother-to-child transmission of HIV. Arguably the benefits of Truvada, either as prophylaxis for prevention of mother to child transmission or as part of a treatment regimen for the mother, certainly outweigh the potential risk of adverse events (minimal) in this HIV infected population. But, do the benefits of Truvada as PrEP outweigh potential risk of adverse events in HIV uninfected pregnant women?

Because younger pregnant and lactating women are disproportionately at risk for HIV infection, a targeted intervention approach for HIV uninfected pregnant women aged between 18 and 24 years would yield a higher benefit:risk ratio. Therefore it would be ethically acceptable to demonstrate the safety of Truvada as PrEP in combination with current standard of care interventions to preventing sexually transmitted infections and HIV in this most vulnerable group of pregnant women. The Intervention Package would therefore include a daily dose of Truvada (TDF/FTC), risk reduction counselling, STI screening and treatment, inviting the sexual partner to receive HCT and referral for treatment and support if he tests HIV positive and condom promotion. The Control Group of young pregnant women will receive the above package minus the antiretroviral prophylaxis - Truvada (TDF/FTC).

2.1 Aim:

To explore the safety of Truvada when used as PrEP in combination with current recommendations for prevention of sexually transmitted infections and HIV in young women at substantial risk of HIV acquisition during pregnancy and lactation.

2.2 Primary Objective:

- To compare the frequency and seriousness of specific adverse events related to TDF/FTC in women and their children in Arm A (exposure to TDF/FTC) and Arm B (no exposure to TDF/FTC) during pregnancy until breastfeeding cessation.

2.3 Secondary Objectives:

- To estimate HIV incidence in women in ARM A with ARM B at delivery and at 18 months post-delivery.
- To determine the interdependency of adherence to a once daily oral TDF/FTC regimen and standard HIV prevention methods during pregnancy and postdelivery.
- To measure antiretroviral drug (TDF/FTC) level in women and their infants in association with adherence, safety and level of protection (plasma and breastmilk)
- To detect drug resistance among women who acquire HIV infection and correlate the treatment outcomes (after initiating immediate ARV treatment) with drug resistance
- To determine mother-to-child transmission of HIV among women who acquire incident infections during pregnancy and breastfeeding and the frequency of drug resistance in their infected babies

3. SETTING

The Umlazi Research Clinic is a well-established research facility, which hosts HIV prevention research with a particular focus on the prevention of mother-to-child transmission for the past 7 years. The facility is well equipped to conduct large cohort studies as well as smaller pharmacokinetic and safety studies. Umlazi, an urban and the third largest township in South Africa is located in the EThekwini District of KwaZulu-Natal. Umlazi is geographically divided into 26 sections and each section is alphabetically named as Section A or Section D or Section H, etc. Its estimated population of 1.6 million has access to a single regional hospital, Prince Mshiyeni. With a bed state of 1200, this institution provides a comprehensive, integrated health service with elements of preventive, promotive, curative and rehabilitative health care. Approximately 1100 births are recorded per month deliveries per month. Prince Mshiyeni is the referral hospital for 17 primary health care clinics in Umlazi. Busiest clinics in Umlazi are H, D, U and K clinics. The priorities of these clinics are maternal and child care, HIV/AIDS and chronic diseases.

Umlazi Township spans 26 sections and Umlazi Clinical Research Site is located on the grounds of Prince Mshiyeni Hospital.

3.1 Population at Risk

Pregnant women seek antenatal care at any of the 17 primary health care clinics and deliver at the Prince Mshiyeni Memorial District hospital. Women range in age between 16 and 40 years, and almost half (46%) of the women who deliver at the hospital are adolescents are young women (<25 years). Approximately 25% are primigravid and the average gestational age at first visit is 28 weeks, although 48% register prior to 20 weeks of gestation. The PHC clinics have an active PMTCT program. As prescribed in the National HIV treatment guidelines, pregnant women are offered HIV counselling and testing at their first antenatal visit. A serial HIV testing algorithm using Point of Care serological tests is implemented at all public health facilities. Women who are positive on the first POC test are immediately confirmed infected if the second POC test is also reactive. If pregnant women test negative on the first POC, they receive risk reduction counselling and informed of an opportunity to repeat the test 3 monthly during pregnancy and post-delivery.
In a maternity audit conducted at the District Hospital (PMMH) during a 3 month period in 2011 and 2014, 1732 (37.3%) and 2219 (38.7%) women were HIV positive among the 4644 and 5738 deliveries respectively. A third (34%) of the HIV infected pregnant women were <25 years and 52% of women who remained HIV negative by the time of delivery were between 18 and 24 years old.

In a cohort study conducted at a PHC clinic in Umlazi, 38% (548/1446) of antenatal attendees tested HIV positive and almost half (47%) of these women were <25 years

of age. The prevalence of Trichomonas, Chlamydia and Neisseria in pregnant women was 15.6%, 17.7% and 6.4% respectively. All three STIs were significantly more common in younger women <25yrs (17.2%, 21% and 8.6% vs 12.4%, 13.2% and 3.4% respectively) than older women. Among 636 pregnant women tested for HSV-2 shedding in cervical secretions, 52 (8.18%; 95%CI 6.22-10.65) women tested positive by DNA PCR. HSV-2 shedding was detected in 10.4% of HIV positive women and 6.7% of HIV negative women.

4. SELECTION OF PARTICIPANTS

4.1 Selection and Enrollment of Subjects

4.1.1 Inclusion Criteria

- At least 18 years old.
- Confirmed HIV-1 uninfected.
- Willing to provide screening informed consent
- Currently pregnant
- Considered high risk for HIV infection
  - <25 years of age
  - Unprotected sex during pregnancy
  - HIV status of current sexual partner is positive or unknown
- Results of HBV screening (HBsAg testing) available from specimen obtained within 30 days prior to entry
- Plans to deliver in the study affiliated hospital
- Has no plans to move residence outside of the catchment area during the 18 months following delivery

4.1.2 Exclusion Criteria

- HIV infected
- >24 years of age and <18 years of age
- The following laboratory values from a specimen obtained within 30 days prior to study entry:
  - Hemoglobin <11 g/dL
  - WBC < 1500 cells/mm^3
  - ANC < 750 cells/mm^3
  - Platelets < 100,000 cells/mm^3
  - ALT > 2.5 x upper limit of normal (ULN)
  - Estimated creatinine clearance of < 70mL/min using the Cockroft-Gault equation for women: {([140 – age (years)] x [weight (kg)]) ÷ [72 x serum Cr (mg/dL)]} x 0.85
  - Hepatitis B surface antigen (HBsAg) positive
- Participation in any other study
- In labour – at onset or beyond
- Serious illness (including TB) and/or hospitalization
- Receipt of TB treatment within 30 days prior to study entry
- Fetus detected with serious congenital malformation (ultrasound not required to rule out this condition)
- History of documented structural or conduction heart defect
- Social or other circumstances which would hinder long-term follow-up, in the opinion of the site investigator
- Currently incarcerated
- Substance or alcohol abuse (a score of ≥8 on the WHO Alcohol Use).

5. RANDOMISATION

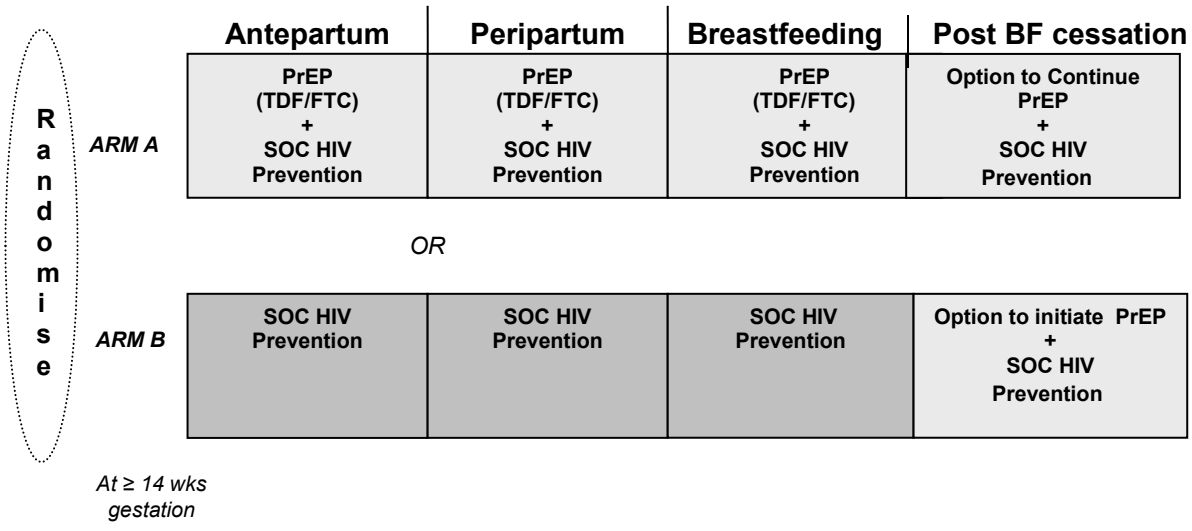
5.1 Screening and Enrollment

Women who meet preliminary eligibility criteria: HIV uninfected and at substantial risk will be screened for further eligibility criteria following a signed screening informed consent. Blood will be drawn for haematology, liver function tests, blood chemistry tests, serology and HepB sAg assay.
Women who are confirmed eligible following screening will be requested to provide their informed consent for participation in the trial and thereafter enrolled in the study. Consent for specimen storage will also be sought. Enrolled participants will undergo a physical and pelvic examination accompanied by a genital specimen collection for storage. Blood will also be drawn for plasma, dried blood spot and cell pellet storage.

5.2 Randomisation

Following baseline clinical assessments, laboratory investigations and behavioural questionnaires, participants will be randomized to one of two arms (ARM A – Immediate PrEP or ARM B –Deferred PrEP) See Figure below. All participants will receive risk reduction counselling, STI screening and treatment, an invitation to the sexual partner to receive HCT and referral for treatment and support if he tests HIV positive and condom promotion. The randomisation method is described under the statistical section.

Randomisation – Treatment Arms



SOC – Standard of Care: Current recommendations for prevention and control of STIs in all pregnant women include:

- correct diagnosis by syndrome or laboratory diagnosis
- provision of effective treatment;

- reduction in or prevention of further risk-taking behaviour through age-appropriate education and counselling;
- promotion and provision of condoms, with clear messages for correct and consistent use; and
- notification and treatment of sexually transmitted infections in sexual partners, where applicable.

In the proposed study, we will screen for STIs using serological tests (syphilis, HSV-2) and genital tract screening using PCR molecular technology (chlamydia, gonorrhoea and trichomoniasis). Until laboratory results are received (approximately 1 week), pregnant women presenting with vaginal discharge or genital ulcers (symptomatic sexually transmitted infections) will be treated syndromically.

6.0 STUDY TREATMENT CONSIDERATIONS

The drug to be used in this study is Truvada (tenofovir disoproxil fumarate/ emtricitabine) which is the antiretroviral drug to be used to prevent HIV acquisition. TRUVADA tablets are fixed dose combination tablets containing emtricitabine and tenofovir disoproxil fumarate. EMTRIVA is the brand name for emtricitabine, a synthetic nucleoside analog of cytidine. Tenofovir disoproxil fumarate (tenofovir DF) is converted in vivo to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5′-monophosphate. Both emtricitabine and tenofovir exhibit inhibitory activity against HIV-1 reverse transcriptase.

6.1 Study product formulation and storage conditions

Study Drug : Emtricitabine-Tenofovir disoproxil fumarate FTC-TDF Truvada®
Formulation: 200 mg/300 mg tablets
Appearance: Blue, capsule shaped, film-coated tablet that are debossed with “GILEAD” on one side
Storage: 25ºC (77ºF) - Excursions permitted between 15-30ºC (59-86ºF). Keep container tightly closed. Each bottle contains thirty tablets with a silica gel desiccant canister that should remain in the original container to protect the product from humidity.

6.2 Treatment Procedures

ARM A: Pregnant women allocated to ARM A will initiate Truvada on the day of randomization and will continue throughout peripartum until cessation of breastfeeding and thereafter given an option to continue PrEP until 18 months postdelivery.

ARM B: Pregnant women allocated to ARM B will only initiate Truvada at cessation of breastfeeding and thereafter given an option to initiate PrEP until 18 months postdelivery.

6.3 Treatment Schedule

The treatment schedule is a once daily uninterrupted course.

6.4 Study product procurement

The study site pharmacist will order Truvada from the manufacturer (Gilead Sciences).

6.5 Study product accountability

The study pharmacist will maintain complete accountability and storage condition records of all study products received from Gilead Sciences and subsequently dispensed to study participants. All study products will be stored at the site study pharmacy. All unused study products will be returned to Gilead Sciences after the study is completed and terminated.

6.6 Study product dispensing

The site pharmacist will dispense a 28 day supply of tablets. The aim will be to provide the participant sufficient to last beyond the next scheduled visit. Dispensing of study product will be done either directly to the study participant or to authorized study staff if the participant is unable to present in person to the study pharmacy.

6.7 Study product returns

Participants must present with all remaining study product at each study visit conducted during the duration of the trial.

6.8 Adherence counselling

Adherence counselling will be provided to study participants upon enrolment and additionally at specified intervals. Counselling will address topics such as participant-centred strategies to remember to take the tablets every day, to ensure the availability of the tablets both in the home and away from home; and to identify and discuss various challenges and situations that may impede daily tablet adherence.

6.9 Adherence assessment

Data on adherence to the daily regimen of TDF/FTC will be collected at each study visit via pilot tested interview tools and pill count. Additionally, stored plasma specimens collected during the trial at each scheduled visit from suspected HIV seroconvertors will be archived for analysis of markers of product adherence to enhance interpretation of the results of the study.

6.10 Concomitant, prohibited, and precautionary medications

The following medications are not permitted:

- Drugs containing emtricitabine or tenofovir disoproxil fumarate
- Adefovir dipoxil
- Lamivudine and other cytidine analogues
- Didanosine
- Cidofovir and other medicinal products that compete for active tubular secretion
- Drugs that reduce renal function

6.11 Treatment after HIV Seroconversion

Participants who seroconvert during pregnancy or during breastfeeding will stop Truvada and will be managed according to national guidelines. Women will receive the fixed dose combination of TDF/FTC/EFV as per national guidelines. The participant will be monitored for treatment response and drug resistance. They will continue in follow-up until end of study.

If daily Truvada is required to treat active replication of hepatitis B acquired after enrolment, that will take precedence over the randomization allocation. Participants will continue in follow-up until end of study.

6.12 HIV Prevention Post Breastfeeding Cessation

Participants in both arms will be offered PrEP post breastfeeding cessation until end of study at 18 months after the last participant enrolled has delivered. Thereafter, participants who remain at high risk will be referred to the nearest Dreams PrEP Demonstration site or the National PrEP Program site whichever is available at the time of study completion. The study is expected to be complete by June 2019. We believe that there would be at a minimum ONE PHC or NGO that will be a PrEP service delivery site in Umlazi by the time of study completion. PrEP is slated to be included in the PEPFAR-supported DREAMS initiative focused on young women and adolescent girls. PrEP is also included in the National Strategic Plan (NSP) for HIV, STIs and TB (2012-2016).

7.0 ASSESSMENTS AND FOLLOWUP PROCEDURES

7.1 Source of Study Population

HIV uninfected pregnant women attending any of the three busiest clinics in Umlazi (D, H and U Clinics) will be informed of the study. Interested volunteers will be transported to the Umlazi Research Clinic for screening. Distance between PHC clinics and the Research Clinic is approximately 5 to 10 km.

7.2 Cohort Accrual and Follow-up

Cohort accrual is expected to be completed in 12 months. Accrual to this study will be monitored by the Protocol Team in accordance with standard operating procedures. An overview of the study procedures for women and their infants are provided in Appendices 5 and 6. The women will follow the basic antenatal care schedule. For participants with early antenatal booking (<12 weeks), return visits will be scheduled for 20, 26-28, 32-34, and 38 weeks until delivery. Following delivery and a <72 hour postdelivery assessment women and their infants will be seen at 6, 10, 14 weeks and thereafter 4 weekly until 18 months postdelivery.

7.3 Pre-screening

Pregnant women attending antenatal clinics at off-site locations in Umlazi will be pre-screened by study staff to ascertain preliminary eligibility. These criteria include:

- 18≤24 years
- No major obstetric complications
- Women plan to reside in the area for the next 2 years
- Tested HIV negative at the off-site location

During these interactions, the study staff will explain the study to the potential candidates for study participation. Process information (e.g. number of potential participants contacted, number potentially eligible, number interested in further screening) will be recorded and stored at the Research Site. Participant information will not be linked to participant identifiers.

7.4 Screening

Screening and enrolment procedures will be completed within 14 days. Eligibility will be determined using a combination of biomedical and behavioural criteria. If a participant is not enrolled within 14 days of providing informed consent for screening, the participant will not be re-screened or enrolled. Screening will be completed in a step-wise manner. Firstly, potential participants will be invited to screen for the study and asked to provide informed consent for screening (Appendix 1). Potential study participants will be assigned a screening number, followed with a risk assessment (Appendix 2) and the first series of biomedical screening procedures including confirmation of HIV uninfected status. If both HIV test results are negative, the potential participant will be invited to continue with the screening process and will be asked to provide demographic information, locator information and blood for chemistry and haematological tests.

7.5 Enrolment

At screening, eligible participants will have been given a date to return to the clinic for enrollment. This date will be within two weeks of screening. If a participant does not return within 14 days of the screening visit, she will not be enrolled nor re-screened.
At the enrollment visit, if the participant meets all eligibility criteria that can be known from the screening visit, study staff will obtain informed consent for study participation (Appendix 3). Informed consent will also be obtained for blood to be stored for future HIV related research (Appendix 4). A participant may refuse this procedure and still be part of the study. All study procedures listed in the Schedule of Evaluations for the mother (Appendix 5) and for the HIV unexposed infant (Appendix 6) will follow.

There will be a clinic interview, during which baseline demographic, clinical and behavioural data will be collected on a case record form. The behavioural and lifestyle information provided by the individual together with results from the screening for sexually transmitted infections will inform the development of a personalised plan for reducing their risk. A personalised invite to the sexual partner will be provided to all participants. The invitation is intended to encourage partners to test for HIV and be referred for ART if HIV positive. Partners will also be informed of their options for HIV prevention and what is available in the public sector.
Participants will receive supplies of condoms in quantities anticipated to last until the next visit. A participant can return between scheduled visits to obtain more condoms.

7.6 Randomisation Procedures

Randomisation will be performed on the day of the enrolment visit after the eligibility checklist is confirmed. Randomisation will be performed using a computer-generated randomization list. The participant's study number, allocation and the date of randomization will be entered into the Trial Register at the Research Site.

7.7 Post-randomisation Procedures

All participants will receive support to the agreed plan to manage their personal risk and counselling about the importance of adhering to the agreed schedule of visits. A diary will be provided to record coital acts and condom use, different partners and pill taking when applicable.

For participants starting Truvada immediately, the clinician and participant will go through the instructions for use Truvada. They will be issued with 1 bottle, containing 30 tablets, and the next clinic appointment will be made for a four-week visit. They will be asked to tick the days they take the drug on the diary.

7.8 Follow-up Visit after 1 month on Truvada

This assessment will be required for women on immediate ART (1 month) and for women on deferred ART (post cessation of breastfeeding).

There will be an interview to see if the participant has experienced any side effects sufficient to interrupt Truvada, and how they are coping with the daily regimen. Urine will

be collected for analysis. A blood sample will also be collected for a HIV test, renal function test (creatinine), other routine antenatal investigations and specimen storage.

7.9 Follow-up Visits until Delivery and Post-delivery

Following enrolment, women will follow the schedule of evaluations in Appendix 5. Women will remain on their assigned treatment arm until cessation of breastfeeding and thereafter follow their choice of either continuing or discontinuing ARV PrEP through the 18 month postpartum visit. Each participant will have follow-up visits approximately every four weeks (28 days) until delivery and following 72 hour postdelivery assessment the participant will be given an appointment for 2 weeks, 6, 10, 14 weeks and thereafter 4 weekly visits until week 74 (approximately 18 months post-delivery) at the study clinic.

7.10 Follow-up Visit <72hours Post Delivery

Participants will be advised to inform the staff by sending a text message when they are admitted to the Labour Ward of the adjacent Hospital. Nursing staff from the research site will also conduct daily postnatal ward rounds to track study participants. A postnatal study visit will be conducted within 72 hours. A maternity chart audit and further clinical assessments of mother and child will be completed prior to discharge from the hospital. The maternity chart audit will specifically be done to record any complications since the last study visit through admission and during the labour process. Pregnancy outcomes will be recorded in the maternal case report form. Infant assessment and mother's intention to breastfeed will be documented in the infant's case report form. Women will be encouraged to breastfeed their infants for as long as possible. Study drug (Truvada) will be dispensed to women in the Treatment Arm. All participants will have a repeat HIV test, and blood drawn for chemistry and haematological investigations and for storage.

7.11 Follow-up Visits Post Delivery

Subsequent post-delivery visits will be scheduled for weeks 2, 6, 10, 14, 18 weeks, and thereafter monthly until 18 months postdelivery. Study product discontinuation could occur at cessation of breastfeeding for participants in the Treatment Arm who choose not to continue PrEP. And study product will be dispensed to participants in the Control Arm who choose to commence PrEP post cessation of breastfeeding. Study product will be distributed until 74 weeks and thereafter participants will be referred for ongoing PrEP at the nearest PrEP Demonstration Site at a public health clinic.

7.12 At Follow-up Visits during Product Use

At follow-up visits antenatal week 4 through week 74, study staff will:

- Update contact information (when necessary);
- Conduct pill count of product returned by participant, and document any product returned to participant;
- Collect information on adherence to the daily pill-taking regimen;
- Conduct risk reduction counseling, including pre- and post-test HIV counseling and distribute condoms;
- Conduct study product adherence counseling;
- Conduct contraceptive counseling as appropriate, re-supply contraceptive method as needed;

- Collect blood by venepuncture (10ml) and:
  - ✓ Perform HIV rapid antibody testing according to the algorithm described at the enrollment visit.
  - ✓ Test for ALT, AST, creatinine and phosphorus. If results are abnormal, repeat regularly until resolution to ≤ Grade 1 or until stabilization. Any required follow-up will be done per local standard of care.
  - ✓ Store plasma and ULPC aliquots for HIV PCR testing, QA and confirmatory laboratory testing as needed. Also store one plasma aliquot (if sufficient sample available) and one serum aliquot for potential future HIV related research;
  - ✓ Screen and treat other STIs using the syndromic management approach
  - ✓ Collect cervical/vaginal swabs for storage and subsequent screening for STIs using molecular techniques.
- Conduct adverse event assessment as per the US Division of AIDS Adverse Event Reporting and Toxicity Grading;
- Conduct physical and/or pelvic examination if clinically indicated; and
- Record medications taken since last visit.
- Complete the Final Status CRF if the participant does not require further follow-up due to risk of Hepatitis B flare and/or HIV seroconversion.

Participants will be allowed to complete each follow-up visit up to five days prior to and two (for the week 4 follow-up visit) or four (for later follow-up visits) days after their next 4-week (28 days) target visit date. If participants complete a visit outside the given window for that specific visit, the visit will be regarded an interim visit and an appointment for the next scheduled visit will be given.

7.13 Followup Visits for Infants

Infants will be assessed at birth, 26 weeks, 50 weeks and 74 weeks. In addition to a full physical examination, a blood sample will be drawn for assessing renal function (Creatinine Clearance) and Hormonal growth factors. For infants exposed to TDF/FTC an additional blood sample will be collected for a drug level assessment. At each visit the following growth parameters will be recorded: weight, height and head circumference. Infant feeding modality will be recorded at each visit. Cessation of breastfeeding is defined as 28 days after last exposure to breastmilk.

7.14 Followup Visits for Women who Seroconvert during Study Participation

This section applies to enrolled participants who test HIV antibody positive on or prior to week 74 and participants who are HIV antibody negative and HIV PCR (RNA or DNA) positive at enrollment or any follow-up visit. Prior to initiation of the clinical trial, site-specific seroconverter plans will be made in consultation with the Department of Health to ensure specific provisions for care and treatment according to the standard treatment guidelines. Study clinicians may share relevant laboratory results with the referral centre care providers provided that the participant provides verbal consent; such consent will be documented in the participant's file.

Any participant who is diagnosed as HIV positive at a follow-up visit will have her study product withdrawn at that study visit. She will receive post-test counselling. If testing of a second sample does not confirm the initially positive result, she may continue using study product. If the participant tests positive on the first sample and refuses to provide a sample for confirmatory testing, her product will be interrupted until a confirmatory test is done. Partners may come to the clinic for voluntary counselling and testing (VCT) according to site-specific SOPs. Participant will be asked if she still agrees to stay in the study until the last scheduled study visit (74 weeks). She will be referred to treatment

centres for appropriate care and treatment according to the site-specific seroconverter care plan and to ongoing HIV treatment or acute HIV infection trials where available. Effective referral will be facilitated by the study counsellors, who will provide voluntary one-on-one counselling and assistance to women in accessing health care or social support services (including help with transportation and communication with health care providers concerning health needs). The counsellors and/or other study staff will also follow up to ensure that participants have secured needed care or services and to determine any problems encountered by women referred from the trial.

When a participant tests HIV positive on the first sample according to the study algorithm, she will be counselled and asked to immediately provide a second sample (10ml in EDTA tube) for a repeat of the HIV algorithm tests as confirmation of the first positive result. According to site SOPs the following samples will also be taken either immediately after the first sample is positive or after the second sample has confirmed the first positive result:

- 10ml in EDTA tube for HIV PCR, HIV-1 viral load and resistance testing;

and,

- 2ml in BD CD4 stabilization tube or 3ml in EDTA tube for CD4 count;

A plasma aliquot will be stored (long term storage) if sufficient sample is available for potential future HIV related research (if participant consented). If the visit of the HIV diagnosis does not coincide with a visit in which secondary endpoints are assessed, another 5-10ml blood in serum tubes (dependent on the site, see site specific lab SOPs) will be collected and tested for AST, ALT, phosphorus and creatinine; a serum aliquot will be stored (long term storage) for potential future HIV related research (if participant consented). HepBsAg testing will be done if appropriate. Procedures for early product withdrawal will also be done.

Samples from her previous visit(s) will be tested by HIV PCR to better determine the infection time and length of exposure to Truvada while HIV infected. Samples from visits in which she was HIV PCR positive but HIV antibody negative will also be tested for resistance and drug levels. At her first follow-up visit after the diagnosis (week 4 post diagnosis), HIV testing according to the study algorithm will be repeated. HIV testing will stop if the first algorithm testing gives a positive result. At each monthly visit post HIV diagnosis, study staff will:

- Update contact information (when necessary);
- Update her medical history, including her medication;
- Conduct further risk reduction counselling and distribute free condoms

7.15 Followup Visits for Children Born to Women who Seroconvert During Pregnancy or Breastfeeding

All HIV exposed infants will continue with study followup until 18 months of age.

Infants born to women who seroconvert during pregnancy will have a HIV DNA PCR test performed at birth and 3 monthly thereafter until cessation of breastfeeding. These infants will also receive antiretroviral prophylaxis (according to the National PMTCT Guidelines) commencing at birth until breastfeeding cessation.

Infants being breastfed by a mother who seroconverts during breastfeeding will have a HIV DNA PCR test performed at the visit when the mother's HIV status is confirmed and 3

monthly thereafter until cessation of breastfeeding. The infant will commence antiretroviral prophylaxis according to the National PMTCT Guidelines.
Infants with confirmed HIV infection (Positive HIV DNA PCR on 2 separate specimens) will be managed according to the National Treatment Guidelines and in addition to routine haematological and chemistry tests al blood sample will be drawn for resistance testing.

7.16 Stored Specimens

Any blood specimens (i.e. sera or plasma) collected during screening, enrollment, and all follow-up visits will be cryopreserved during the clinical trial and stored at the Global Clinical Lab repository. These specimens may be used for repeat and confirmatory analyses of effectiveness, safety endpoint parameters, and drug levels. These study samples will be destroyed after the final study report has been submitted. At enrollment, participants will be asked to sign a specimen storage informed consent form for long term storage of samples (ten years after final study report) for any future testing related to HIV research that falls outside of this study. These additional specimens will be stored for a period of 10 years and thereafter destroyed.

7.17 Participant Retention

The target retention rate will be 90% per annum. During the informed consent process the participant will be informed of the planned retention procedures. This is to ensure participant awareness of all the efforts that will be undertaken to contact them in the event that they miss a scheduled study visit throughout the participant's Involvement in the study (up to 2 years for those who enroll early in pregnancy). Study participants will be informed that staff will attempt to make telephonic contact with them for missed visits or for unscheduled visits if the need arises, failing which home visits will be made.
The Protocol Team will track retention rates. Once a participant is enrolled in the study, study staff will make every reasonable effort to ensure adequate locator information is available for follow-up tracking. A missed visit in either arm will prompt a single telephonic reminder, where the participant is spoken to directly and reminded of her clinic visit. A home visit will be triggered if a second clinic visit is missed. A home visit will be considered complete when the participant is met in person. A maximum of 3 attempts will be made to complete a home visit. Retention efforts will be conducted by the same team for both arms.

7.18 Participant withdrawal

Participants may voluntarily withdraw from the study for any reason at any time. Designated study staff may also withdraw participants from the study in order to protect their safety. Participants may also be withdrawn if the South African MCC or the University of KwaZulu-Natal's (UKZN) Biomedical Research Ethics Committee (BREC) terminates the study prior to its planned end date. Every reasonable effort will be made to complete a final evaluation of participants who withdraw or are withdrawn from the study. Study staff will record the reason(s) for all withdrawals in participants' study records.

7.19 Sexual Partners

Sexual partners of study participants will be invited to attend the HCT services but not participate in the study. They will have access to HIV counselling and testing,

appropriate clinical referrals for HIV treatment, STI management and other medical conditions. Study staff will not obtain informed consent from any men, nor will they enroll or collect data from them. Whenever a participant tests positive for any STI (excluding HIV), she will be counselled to inform her partner(s) and refer him/them to a clinic for testing. Treatment for any curable STI will be offered to the male partner if he comes to the study clinic. Local guidelines on partner notification will be followed. Participants will decide on disclosing their HIV status to their partners and study staff will support her decision.

7.20 Procedures for Assessing HIV Seroconversion

Women will have a rapid HIV test performed at every visit using the serial testing algorithm as per national guidelines. In the event of a reactive rapid HIV test, a repeat sample will be obtained and reactivity confirmed by HIV RNA PCR assay. In the event of a confirmed seroconversion, a genotypic drug resistance test will be performed to identify mutations as a result of exposure to Truvada.

7.21 Procedures for Assessing Safety

Participants will be asked one month after starting Truvada and at each subsequent scheduled visit whether they have had any admissions to hospital, or suffered from any significant illness that required medical attention. The site clinician will determine whether an illness meets the criteria for Serious Adverse Event (SAE) Reporting. SAEs will be reported to the UKZN BREC, Medicines Control Council and Department of Health. Participants will also be asked whether they have experienced any side effects.

Clinical assessments during pregnancy will be conducted as per the national guidelines for maternity care.

Maternal safety assessments during pregnancy and until 74 weeks study exit: All women will have bloods drawn for chemistry and haematological investigations at screening, enrolment, 2 weeks, 4 weekly until delivery, <72 hours of delivery, 2 weeks post delivery, 6 weeks, 10 weeks, 14 weeks and 4 weekly until 74 weeks (inclusive). Chemistry tests include liver function markers (ALT/AST/Phosporous), renal function (serum creatinine/urea/sodium/potassium), and bone density markers (serum calcium and magnesium).

Bone mineral content in the mother will be measured by DXA scan at 6 weeks postpartum, 6 months, 12 months and 18 months. Both Hip and Spine DXA scans will be done at above scheduled visits.

Within 72 hours of delivery, the participant with her maternity care record (MCR) will be reviewed by the study clinician. Fetal outcome in addition to other obstetric data will be extracted from the MCR. The following definitions will be used to document fetal outcome:

NON-VIABLE FETUS (MISCARRIAGE)
Less than 28 weeks* gestation and where there is no evidence of life at delivery.
STILL BIRTH
Gestational age of 28 weeks or more and where there is no evidence of life at delivery.
NEONATAL DEATH**
Death after a live birth whatever the duration of the pregnancy.
When the gestational age is not known, a legal weight cut-off of 1000 g is used.

Maternal gestational age will be determined by clinical and obstetric assessments that will include an Ultrasound, measurement of symphysis fundal height and last menstrual date if known. Preterm delivery is defined as < 37 weeks. Moderate preterm delivery 32-35 weeks and severe preterm delivery <32 weeks.

The New Ballard method will be used to assess gestational age of neonate. In addition, neonates will be determined small for gestational age (SGA), average for gestational age (AGA) and large for gestational age (LGA).

Fetal Growth:

Fetal monitoring will include fetal growth and an ultrasound for fetal abnormalities. For fetal growth monitoring, we would measure the symphsis fundus height at each study visit, plot on graph against gestational age and compare with 10^{th}, 50^{th} and 90^{th} centiles and previous measurements. Any measurement $<10^{th}$ centile or failure to increase will be referred for an ultrasound for further fetal assessment.

Infants exposed to TDF/FTC either in-utero or during breastfeeding will have chemistry (serum creatinine) and haematological (complete blood count) at birth, 26 weeks (6 month), 50 weeks (12 months), and 72 weeks (18 months).

Infant Bone Growth:

DXA scans of the lumbar spine and whole body of infants will be performed at 6 weeks, 6 months, 12 months and 18 months to determine BMC and bone growth.

Clinical Examination of Infants:

Clinical examination of infants at birth will be conducted as per standard of care prescribed in the SA paediatric guidelines. This will include examination for physical abnormalities.

Physical examination of infants will include OFC, weight, and length to monitor growth and also include assessments for neurodevelopmental milestones at 6 weeks, 3 months, 6 months, 12 months and 18 months.

8.0 SAFETY REPORTING

All study staff will prioritize participant safety while carrying out their day-to-day roles and responsibilities. Potential safety issues requiring follow-up, treatment, and/or referral may be identified through study-related activities, including:

- Clinical evaluations: Clinical staff (nurses, medical officers) may identify medical issues requiring follow-up, treatment, and/or referral when performing protocol-specified clinical assessments, as well as when reviewing study laboratory test results.

- Laboratory evaluations: The Medical Officers will be responsible for identifying laboratory test results requiring follow-up, treatment, and/or referral when performing protocol-specified tests.

- Questionnaires: Study staff, both clinical and non-clinical, may identify medical, drug adherence and/or psychosocial issues requiring follow-up, treatment, and/or referral when administering questionnaires to participants.

- Counselling: Study staff, both clinical and non-clinical, may identify medical, drug adherence and/or psychosocial issues requiring follow-up, treatment, and/or referral when providing counseling.

- Retention Activities: Study staff, both clinical and non-clinical, may identify medical and/or psychosocial issues requiring follow-up, treatment, and/or referral when conducting participant retention activities (e.g., visit reminders, locator contacts, and other outreach).

8.1 Safety Follow-up Procedures: Medical

In accordance with their training, qualifications, and designated study responsibilities, clinical staff will assess potential medical safety issues and identify medically appropriate follow-up, treatment, and/or referral requirements.

All follow-up actions, including diagnosis, treatment, and referrals, will be documented in chart notes.

- Medical emergencies will be managed per the site Clinical Emergency Treatment and Evacuation SOP
- Other medical issues will be managed per usual standards of care for medical practice in the study site locale.
- When appropriate and applicable, clinical staff will perform additional procedures to diagnose and treat medical problems. Additional (interim) study visits will be scheduled as needed to ensure timely follow-up. All visits, procedures, findings, and actions taken will be documented in chart notes.
- When medical issues are beyond the scope of services that can be provided through the study, clinical staff will actively refer participants to appropriate non-study health care providers for further evaluation and treatment.

All medical issues will be followed to resolution or stabilization, with the current status and further action plans (if applicable) documented at each study visit until resolution or stabilization occurs.

All referrals will also be documented. At each study visit after a referral is made, the attending medical officer will actively follow-up on the referral to determine whether the participant sought the services to which the child was referred, determine the outcome of the referral, and determine whether additional referrals are needed. All follow-up actions, outcomes, and plans for next steps will be documented in chart notes.

- In the event that medical issues are reported during a study visit to non-clinical study staff members, these staff members will communicate the information to clinical staff during the visit so that clinical staff may assess the participant's report and proceed as described above.

- In the event that medical issues are reported to non-clinical study staff off-site (e.g., during outreach activities), these staff members will communicate the information - either upon return to the clinic or by phone - to the clinical/study co-ordinator who will determine the appropriate course of follow-up action.

8.2 Safety Follow-up Procedures: Psychosocial

In accordance with their training, qualifications, and designated study responsibilities, study staff will assess potential psychosocial safety issues and identify appropriate follow-up, treatment, and/or referral requirements.

All follow-up actions, including diagnosis, treatment, and referrals, will be documented in chart notes. Psychosocial emergencies will be referred to the Medical Out-Patients Department (MOPD) of the hospital on whose premises our CRS is located. Other non-emergency psychosocial issues will be managed per usual standards of care for medical practice in the study site locale.

- When appropriate and applicable, study staff will provide information and counselling to assist participants with addressing the issues. All such counselling will be documented in chart notes. Should the participant require more intense counselling or intervention strategies, they will be referred to the local hospital psychologist.

- When psychosocial issues are beyond the scope of services that can be provided through the study, study staff will actively refer participants to appropriate non-study service providers for further evaluation and assistance.

All psychosocial issues will be followed to resolution or stabilization, with the current status and further action plans (if applicable) documented at each study visit until resolution or stabilization occurs.

All referrals will also be documented. At each study visit after a referral is made, the attending medical officer will actively follow up on the referral to determine whether the mother of the participant sought the services to which the participant was referred, determine the outcome of the referral, and determine whether additional referrals are needed. All follow-up actions, outcomes, and plans for next steps will be documented in chart notes.

- In the event that psychosocial issues are reported during a study visit to study staff who are not trained/qualified to respond to the issues, these staff members will communicate the information to the clinical/study co-ordinator during the visit so that an appropriately trained staff member can assess the participant's report and proceed as described above.

- In the event that psychosocial issues are reported to study staff who are not trained/qualified to respond to the issues off-site (e.g., during outreach activities), these staff members will communicate the information — either upon return to the clinic or by phone — to the clinical/ study co-ordinator who will determine the appropriate course of follow-up action.

8.3 Documentation

Study-staff will document all safety-related participant reports (histories), observations, findings, and actions taken in response to these. In the event that study participants are hospitalized or otherwise treated by a non-study health care provider, every effort will be made to obtain copies of relevant medical records. These records will be copied and certified by the study receptionist. The clinical/study co-ordinator will oversee these efforts and all efforts will be documented in chart notes.

8.4 Internal and External Reporting

- Individual study staff members will routinely communicate participant safety information to their supervisors on a day-to-day basis. Specific case management will be discussed as needed in regular staff meetings as well as in individual staff supervision sessions and will be documented in meeting notes or minutes.

- Participant safety information, including any unanticipated problems related to study participation, will be reported to the South African Medicines Control Council and UKZN BREC.

- Related specifically to adverse event reporting:

- Event evaluation Case report forms (CRFs) will be completed per instructions on the case report form. Medical Officers will complete these CRFs.

8.5 Definitions

Adverse Event:
An adverse event (AE) is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which may or may not have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product. Study participants will be instructed to contact the study clinician to report any AEs their infants may experience.

Serious Adverse Event:
A serious adverse event (SAE) is any untoward medical occurrence that at any dose that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a

congenital anomaly/birth defect (April 1996 International Conference on Harmonisation (ICH), Good Clinical Practice: Consolidated Guidance, (ICH E6). Important medical events that may not be immediately life – threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above may also be considered to be serious. (October 1994 ICH guidance (E2A), Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

SUSAR: Suspected, Unexpected Serious Adverse Reaction:
Serious (see SAE definition above)
Related (i.e. there is a reasonable possibility that the AE may be related to the study agent)
Unexpected (see definition below)

Unexpected AE:
An AE, the nature or severity (intensity) of which is not consistent with the applicable agent information (Investigator's Brochure, package insert, or summary of agent characteristics).

9. QUALITY MANAGEMENT

The Quality Management plan includes both Quality assurance (QA) and Quality Control (QC)

9.1 Quality Assurance

QA involves periodic, systematic objective review of trial related activities to ensure that the trial is performed and the data are generated, documented and reported in compliance with good clinical practice (GCP).

The Clinical/study co-ordinator, medical officers and the Investigator will routinely review random selections of participant charts to ensure that protocol specifications related to participant safety are being followed and that appropriate clinical and psychosocial management is being provided and properly documented. The clinical/study co-ordinator, medical officers including the investigator will also review the charts of all participants experiencing serious and other expedited adverse events on a monthly basis to ensure proper management and documentation. If at any time these reviews identify areas for improvement, the clinical/study co-ordinator will follow-up with relevant study staff to ensure that any oversight or deficiency is appropriately addressed. All actions taken will be documented (e.g. in meeting minutes or training records).

9.2 Quality Control

QC processes are the activities that occur on a day to day basis to ensure that the data entered into the database is accurate and complete.

The QC team has developed a QC plan based on our own experiences during the conduct of previous clinical trials.

The site has developed specific templates for all visits which are guided by the case report forms and we have also implemented 3 different levels of the QC process.

Level 1 QC– Source documents and CRF completion
Rapid QC of source documentation is done Real-time. At this point the source documentation is checked for completeness at the end of the day but before the patient leaves the clinic. If any information obtained during patient interview is missing and needs to be addressed immediately, it is flagged by the QC assistant and the relevant staff obtains the information. Other missing information that does not need immediate attention is flagged and reviewed at a later time-point. After receipt of laboratory results (2-3 days), the CRF's are completed and QC is conducted for CRF completeness.
At level 1, all queries are resolved within (2-3 days) of the patient visit.

Level 2 QC – Detailed review of Source documentation and CRF's
At this level all information transcribed onto the CRF's are checked for accuracy and verified in the source documents. All Transcription errors are corrected at this time-point. If any information cannot be verified, the relevant staff members are asked to add a note to file to clearly explain the error or incorrect information.

Level 3 QC- Computer system generated
This level of QC occurs during data capturing. The data management system prompts for any missing information that is required. If this information is not completed on the CRF, the data team refers these queries back to the QC team who resolves them accordingly.

10. STATISTICAL CONSIDERATIONS

10.1 Method of Randomisation

Enrolled participants will be assigned at random, to one of the two study treatment arms in equal proportions. The randomisation list used to assign individual study participants to one of the two treatment arms will be generated by the protocol statistician, using randomly permuted blocks. The randomisation statistician will provide the study pharmacy with sealed, opaque randomisation envelopes, sequentially labelled by PID. These envelopes will be assigned in sequential order to eligible study participants. Upon opening the envelope the pharmacist will add her name and signature as well as the time and date the envelope was opened. This being an open label study, the treatment assignment of a participant will be known by the participant and the study staff.

10.2 Outcome Measures

Main Outcome Measure:

- Renal Function estimated by Serum Creatinine Clearance, adverse pregnancy outcomes (e.g., stillbirth, preterm delivery at < 37 weeks gestation, low birth weight <2,500 grams, and congenital anomalies) and Bone Mineral Density among women

  Bone Mineral Density and growth parameters in neonates and infants until cessation of breastfeeding.

Other Outcome Measures:

- Confirmed presence of HIV infection detected by HIV NAT positivity at scheduled 3 monthly visits until 18 months.
- Adherence –self reported use of study product and pill count
- Partners HIV status at enrollment confirmed by Two POC tests and Adherence measured at delivery and 18 months postdelivery
- Antiretroviral drug level in plasma as a marker of adherence.
- Presence of viral resistance mutations that have been selected by Truvada (K65R, M184V) in participants who acquire HIV infection
- Mother to child transmission-reactive NAT testing performed in children born to mothers who acquire HIV infection during pregnancy and post-delivery.
- **Behavioural Risk:**
  - Number of sexual partners with whom participant had unprotected intercourse during pregnancy and postnatally
  - Number of protected and unprotected acts of intercourse during pregnancy and postnatally
  - Sexually transmitted infections acquired during pregnancy
  - Sexually transmitted infections acquired postnatally
  - Facilitators and barriers to adherence.

10.3 Sample Size Calculation

In view of the limited evidence of safety data from studies of PrEP (TDF/FTC) use in pregnancy, we based our sample size on data provided in studies of ART treatment in HIV infected pregnant women. In few studies, comparisons were made to HIV uninfected pregnant women.

To compare the frequency and seriousness of adverse events in women receiving tenofovir/emtricitabine in pregnancy as opposed to women not receiving tenofovir/emtricitabine in pregnancy, we have based our sample size on the main clinically relevant pregnancy outcome.

Adverse Pregnancy Outcomes

Although all pregnancy outcomes will be compared between the intervention (immediate PrEP) and control (deferred PrEP) groups, we based our sample size on the expected preterm delivery proportion among HIV uninfected women (South Africa 18.5%, Botswana 19%). A sample size of 421 in the Immediate PrEP group and 421 in the Deferred PrEP group (i.e. 842 in total) achieves 80% power to detect a non-inferiority margin difference between the group proportions of 0.075 or 7.5%. The reference group proportion (deferred PrEP) of pre-term delivery pregnancy outcome as a main clinically-relevant adverse event is 0.185 or 18.5%.

Maternal Adverse Events (2% margin difference)

Other maternal adverse events will include abnormal chemistry (Grade $\geq$2) results. As reported in the PROMISE study, Grade 2 or higher chemistry abnormalities were significantly higher in HIV infected pregnant women randomized to TDF containing ART (3%) compared to AZT/sdNVP (1%) ($p<0.03$).

Maternal and Infant Bone Mineral Density (4-7% margin difference)

We have also used the PROMISE study findings to determine the required sample size for reporting other clinically relevant adverse events such as maternal and infant bone mineral content. In the P1084s study, a substudy of PROMISE mothers, the lumbar spine BMD declined significantly more through week 74 in the maternal TDF-ART arm compared to the infant NVP arm; mean (95% CI) percent change -2.06% (-2.90, -1.23) versus +1.09% (0.11, 2.07), mean difference -3.16% (-4.44, -1.87) (p-value< 0.001). Similarly, hip BMD declined significantly more through week 74 in the maternal TDF-ART arm compared to the infant NVP arm; mean (95% CI) percent change -5.37% (-5.99, -4.76) versus -3.05% (-3.72, -2.38), mean difference of -2.33% (-3.23, -1.42) (p-value< 0.001). We would require 150 infants exposed to maternal PrEP (TDF/FTC) and 150 infants unexposed (control group) based on 80% power to detect a pair-wise difference of 4-5% in mean WB-BMC and 6-7% in mean LS-BMC.

PASS 12 sample size software (Hintze, J. (2013). PASS 12. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com) was used to calculate the samples sizes for testing equivalence between the treatment and control groups (Blackwelder WC. "Proving the null hypothesis" in clinical trials. Controlled clinical trials. 1982 Dec 31;3(4):345-53). We assumed equal adverse events rates (proportions) across the two groups i.e. zero difference and a maximum D (difference) of 7.5% for equivalence of the two groups. The table below displays the sample sizes required to achieve a 80% power with $\alpha = 0.05$ to establish equivalency for various adverse birth outcome prevalences based on actual data from the same setting published recently (Moodley, Theron et al. "Improved Pregnancy Outcomes with Increasing Antiretroviral Coverage in South Africa." BMC Pregnancy and Childbirth 16 (2016): 35. PMC. Web. 3 Mar. 2016). Based on the largest observed adverse birth outcome prevalence for pre-term delivery yields the largest required sample size i.e. 421 per group or 842 in total based on the assumptions mentioned above.

Based on current data (maternal adverse events, maternal and infant bone mineral content depletion), all other adverse birth outcomes and maternal and infant adverse events can be housed within this sample size and will have smaller D as the relative prevalence decreases toward 0 i.e. narrower equivalence zones. Assuming a loss to follow-up of 5%, we would need 442 women in each group (884 in both arms combined) for the two year time frame of the study.

Main endpoint 1: sample size for safety in pregnancy for birth outcomes

Prevalence adverse birth outcomes among HIV-ve (in descending order)

Outcome	n/N	Prevalence	Required sample size in each group for proposed equivalence trial	Total required sample size for proposed equivalence trial
Pre-term	1096/5916	18.5%	421	**842**
LBW	651/5916	11.0%	274	548
Small for GA	429/5916	7.3%	189	378
VLBW	141/5916	2.4%	64	128
Stillbirth	130/5916	2.2%	58	116

Based on a non-inferiority trial design: A sample size of 421 in the treatment group and 421 in the control group (i.e. 842 in total) achieves 80% power to detect a non-inferiority margin difference between the group proportions of 0.075 or 7.5%. The reference group proportion (pre-term delivery birth outcome) is 0.185 or 18.5%. The treatment group proportion is assumed to be 0.26 or 26% under the null hypothesis of inferiority. The power was computed for the case when the actual treatment group proportion is 0.185 or 18.5%. The test statistic used is the two-sided Z test (unpooled). The significance level of the test was targeted at two-sided 0.05 or 5%.

Secondary Endpoint

PASS 12 sample size software (Hintze, J. (2013). PASS 12. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com) was to calculate this sample size. In order to show a 50% reduction in HIV infections, with significance level of 0.05 or 5% and 80% power, we would need to observe 66 HIV infections in total (44 in control arm [cumulative incidence of 12.72%] and 22 in the intervention arm [cumulative incidence of 6.36%]). This assumes an HIV incidence of 3 per 100 person years during the 6 months of pregnancy and 6 per 100 person years during the 18 months of breastfeeding [9]. Based on these assumptions, we would require 334 subjects in group or 668 in total. Assuming a loss to follow-up of 10%, we would need to increase this to 346 women in each group (692 in both arms combined) for the two year time frame of the study.

10.4 Analysis Plan

Full details of the proposed analyses will be described in a statistical analysis plan that will be developed once enrollment to the study begins and prior to the commencement of analyses. Hence, here we limit the description of the proposed analyses to those for the primary safety endpoint.

Analyses will use the principle of intention-to-treat (i.e., using the randomised treatment assignment, whether or not study drugs were actually taken) and will include all randomised participants. This is generally the more conservative approach for analysis of trial data. However, it has been proposed that the smaller differences commonly observed in intention-to-treat analysis have the opposite effect when testing equivalence or non-inferiority. Hence for sensitivity analysis purposes (and as a required by the FDA) we will also perform a per protocol data analysis for comparative purposes (Walker, E., & Nowacki, A. S. (2011). Understanding Equivalence and Noninferiority Testing. Journal of General Internal Medicine, 26(2), 192–196. http://doi.org/10.1007/s11606-010-1513-8).

Data will be processed and analyzed using Stata 13.0 (tataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.) and R 3.2.4 (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.).

The primary objective is to compare the frequency and seriousness of adverse events in women receiving tenofovir/emtricitabine in pregnancy and their newborn as opposed to women not receiving tenofovir/emtricitabine during pregnancy and their newborn. The number of adverse events experienced during pregnancy and post-delivery will be summarized for each of the study arms. This will also be stratified by seriousness of the adverse event. The number of adverse events will be compared between the study arms using Fisher's Exact test. Furthermore we will use the most widely used approach to test equivalence, namely the two one-sided test (TOST) procedure (Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing equivalence of average bioavailability. J Pharmacokin Biopharm. 1987;15:657–680. doi: 10.1007/BF01068419). Furthermore we will also employ a hierarchical or random effects modeling approach for a non-inferiority analysis for the primary endpoints.

A secondary objective is to compare the number of incident HIV infections in women during pregnancy alone and thereafter during breastfeeding. The analysis will be stratified by levels of PrEP adherence, partner involvement, condom usage and relationship dynamics.. The cumulative incidence and associated 95% confidence intervals will be calculated for each study arm. The number of incident events will be compared for statistical difference between the study arms using Fisher's Exact test.

11. REGULATORY AND ETHICAL ISSUES

11.1 Regulatory Compliance

The study complies with the principles of the Declaration of Helsinki (2008). It will be conducted in compliance with approval from the South African Medicines Control Council and the University of KwaZulu-Natal Biomedical Research Ethics Committee.

11.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that are transferred or transmitted off-site for processing will be identified only by a coded number to maintain subject confidentiality. All records will be kept in a secured area with access limited to authorized personnel only. All computer entry and networking programs will be performed with coded numbers only. Clinical information will not be released without written permission of the participant.

11.3 Ethical Considerations

Pre-exposure antiretroviral prophylaxis comprising a daily dose of Truvada (Tenofovir/Emtricitabine) is a proven prevention strategy for same sex couples and discordant couples and now internationally recommended for individuals at substantial risk. The benefit of PrEP in high risk population groups significantly outweigh the potential threat of adverse events. The high risk population groups are MSMs, men in incarceration, commercial sex workers and adolescent girls and young women. Additional options for PrEP in young women through long acting antiretroviral (Dapivirine) prophylaxis in vaginal rings are also possible, but preclude use in pregnancy.

Truvada (the antiretroviral drug of choice for PrEP) is classified as a FDA pregnancy Category B antiretroviral. Pregnancy and lactation are distinct contraindications to initiate PrEP because of the limited evidence of its (Truvada) adverse effects on the child. Despite this classification, Truvada has been endorsed by the WHO to be used as part of a treatment combination in HIV infected pregnant women and to reduce mother-to-child transmission of HIV. Arguably, the benefits outweigh the potential risk of adverse events (minimal) in this HIV infected population. On the other hand, in all HIV uninfected pregnant women in general, the benefit may be minimal.
Because younger pregnant and lactating women are disproportionately at risk for HIV infection, a targeted intervention approach for women in this particular group would be ethically acceptable to demonstrate the safety of the current standard approach to preventing sexually transmitted infections strengthened by the use of antiretroviral prophylaxis -Truvada (TDF/FTC). The Intervention Package would therefore include a daily dose of Truvada (TDF/FTC), risk reduction counselling, STI screening and treatment, inviting the sexual partner to receive HCT and referral for treatment and support if he tests HIV positive and condom promotion. The Control Group of young pregnant women will receive the above package minus the antiretroviral prophylaxis - Truvada (TDF/FTC).

Women participating in the PrEP study will receive compensation for travel costs incurred at each scheduled study visit.

APPENDIX 1
SAMPLE INFORMED CONSENT FORM

Immediate or Deferred Preexposure Prophylaxis for HIV Prevention: Safe Options for Pregnant and Lactating Women An Open-Label Randomised Control Study

SHORT TITLE FOR THE STUDY: Preventing HIV Infection in Young Women during pregnancy and breastfeeding using Antiretroviral Pre exposure Prophylaxis (PrEP)

SCREENING

INTRODUCTION

You and your baby are being asked to take part in this research study because:

- you are not infected with human immunodeficiency virus (HIV), the virus that causes AIDS
- you are pregnant
- you are at high risk for contracting HIV

This study is sponsored by……. The doctor in charge of this study at this site is _____. Before you decide if you want to participate in the screening tests, we would like to explain the purpose, the risks and benefits of participating, and what will be expected of you and your baby if you decide to participate. This informed consent form gives you information about the screening tests. You are free to ask any questions. After the screening has been fully explained to you and if you agree to participate, you will be asked to sign this consent form or make your mark (in front of a witness, if needed). You will be offered a copy of this form to keep.

What is PrEP?

PrEP means Pre-Exposure Prophylaxis, and it's the use of antiretroviral medications (ARV's) to reduce the risk of HIV infection in people who are HIV- negative. "Prophylaxis" means to give a drug in order to prevent a disease like HIV from occurring.

Can anyone use PrEP?

PrEP should be considered for people who are HIV-negative and at risk for HIV infection. This includes anyone who:

- Is in an ongoing relationship with an infected person
- Is not in a mutually monogamous relationship with a partner who has recently tested HIV-negative.
- For heterosexual couples where one partner has HIV and the other does not. PrEP is one of the several options to protect the uninfected partner during conception and pregnancy.
- Who is in a relationship but does not know whether the partner has tested.

Oral PrEP

It's meant to be used consistently, as a pill taken every day, and to be used with other prevention options such as condoms. PrEP has been approved in America by the US Food and Drug Administration (FDA) for HIV prevention and has shown to be effective and safe. This tablet is known as Truvada. The medication interferes with HIV's ability to copy itself in your body after you have been exposed to the virus. This tablet prevents it from establishing an infection and making you sick. Based on the data that has been collected to date the FDA announced its approval of daily oral Truvada in 2012. Such a regimen may be soon available

in South Africa for HIV negative women who are at substantially high risk of getting HIV. Pregnant women and breastfeeding women who are not HIV infected are not allowed to take Truvada because we do not have adequate safety data if Truvada is to be taken alone and for the sole purpose of preventing HIV infection. While Truvada, together with other anti-HIV medicines in South Africa, is used to treat HIV infected women during pregnancy and during breastfeeding and is known to be safe and highly beneficial in these women and their children, we don't know if Truvada will be safe and as beneficial in HIV uninfected women and their children.

Potential Benefits of PrEP use

In clinical trials with heterosexually active adults, daily oral PrEP with TDF/FTC was safe and reduced the risk of HIV acquisition by an average of 63%-75%. Higher levels of protection (≥90%) were found among persons whose drug levels in their blood indicated that they had consistently taken the medication.

The risk of HIV acquisition increases during pregnancy, as does the risk of HIV transmission to an infant born to a mother who becomes infected during pregnancy or breastfeeding. Therefore, an HIV-negative woman who believes her sexual partner tested positive for HIV or doesn't know her partner's HIV status may benefit from continuing PrEP use throughout her pregnancy and breastfeeding to protect herself and her infant.

Potential Risks of PrEP use

In PrEP studies, follow-up with persons taking medication has been conducted for an average of 1-4 years. Although no serious health risks were associated with PrEP use by HIV-uninfected adults, the long-term safety of PrEP has not yet been determined.

In PrEP trials women were taken off medication as soon as pregnancy was detected. During these trials, no health problems have been associated with PrEP use by women in early pregnancy or for their offspring. However, the long-term safety of PrEP taken by HIV-uninfected women after fetal (during pregnancy) or infant (during breastfeeding) exposure is not yet determined.

No adverse effects have been found among infants exposed to TDF/FTC when the medications were taken as part of a treatment regimen for HIV-infected women during pregnancy or during breastfeeding (for which data suggest limited drug exposure).

WHAT SHOULD YOU KNOW ABOUT SCREENING FOR THE PREP STUDY?

- Your participation in the screening is entirely voluntary.
- You may decide not to participate in the screening tests or to withdraw from the screening at any time without losing the benefits of your standard medical care.
- Even if you agree to participate in the screening, it does not mean you have agreed to participate in the research study.
- If you decide not to participate in the screening, you cannot participate in this research study, but you can still join another research study later, if one is available and you qualify.

WHAT WILL HAPPEN IF YOU AGREE TO THE PrEP STUDY SCREENING?

If you are interested in joining the PrEP Study, we will first do some screening tests to see if you are eligible.

The study staff will ask you some questions about your health and pregnancy, review your antenatal and other available health records, and do a physical examination. The study staff will draw about 1 tablespoon (15ml) of blood from you.

- We will test you for HIV to confirm your status
- We will test your blood to see how healthy you are
- We will test to see if you are infected with Hepatitis B virus

You will be asked to return to this clinic to get the results of these blood tests. These blood tests are the first step in determining if you will be able to join the study. If the screening shows that you may be eligible, you will be provided more detailed information about the PrEP Study and be asked to sign another consent form like this one to participate in the study.

If you join the PrEP Study, you will be randomly assigned by chance to one of two study groups, one group will begin PrEP immediately in pregnancy and the other group will begin PrEP after breastfeeding has stopped. You will be followed throughout your pregnancy and through labour and delivery and for 18 months after your baby is delivered. Your baby will be followed until he or she is 18 months old.

WHY MIGHT THE STUDY DOCTOR STOP MY SCREENING TESTS EARLY?
You will be withdrawn from the screening if at any time the screening tests show that you will not be able to participate in the study. You may also be withdrawn from the screening if the study is cancelled or stopped.

WHAT ARE THE RISKS OF STUDY SCREENING?
Taking blood from you may cause slight pain, swelling, and bruising at the place where the blood is taken. Drawing blood can also cause fainting or infection, but this is rare. The study doctors and staff will protect information about you and your participation in these screening tests to the best of their ability. On your screening records, a code will be used instead of your name. Only the study staff will know this code. Study staff will make every possible effort to be sure that others do not learn your HIV status. However, sometimes if you receive special treatments or attend a special clinic, it may make others wonder if you have HIV.

WHAT ARE THE POSSIBLE BENEFITS OF STUDY SCREENING?
These screening tests may or may not be of direct benefit to you. The results of the screening tests will be shared with you and with the medical staff providing your antenatal care, if you wish, as this may help them know more about what care you need. They will refer you for additional care if they find that your body's system for fighting infections is weak. If you do not know whether or not you are infected with hepatitis B, you will find out through the screening tests.

WHAT ARE THE CHOICES IF YOU DO NOT WANT TO BE SCREENED FOR THE STUDY?
You do not have to agree to be screened for this research study. If you do not agree to the screening, your care will not be affected. If you agree to take part in the screening, you can change your mind at any time without losing the benefits of your standard medical care.

WHAT ABOUT CONFIDENTIALITY?
Every effort will be made to keep personal information confidential. This personal information may be disclosed, if required by law. Any publication of this study will not use your or your baby's name or identify you or your baby personally.

The outreach workers may contact you so we need to know the best way to reach you (such as home visit or phone call). Your records may be reviewed by the ethics committee

overseeing the study at this site, local regulatory authorities, study staff, study monitors, and the drug companies supporting this study.

WILL THERE BE ANY COSTS OR PAYMENTS?
The screening procedures, physical examinations and blood tests will be done free - at no cost to you - but you will not receive any payment for having the screening tests done. You will receive R150 for your time and transport costs.

WHAT IF I DO NOT ENROLL INTO THE STUDY?
If you decide not to take part in the PrEP Study or if you do not meet the eligibility requirements for this study, we will still use some of your information from the screening visits, some demographic (e.g., age, gender), clinical (e.g., disease condition, diagnosis), and laboratory information, so that the researchers may determine whether there are patterns or common reasons why people do not join the study. Only a code number will be used for this – not your name or other information that will identify you.

WHAT HAPPENS IF EITHER MY BABY OR I ARE INJURED?
It is possible that you could experience a problem or injury that would not have occurred if you did not participate in the screening. If the study doctor determines that you have been injured as a direct result of being in the screening, you will be given immediate treatment for those injuries at no cost to you and then referred for further care if needed.

However, the study doctor may determine that your illness or injury would have happened even if you did not participate in the screening. In that case, appropriate care and/or referral will likewise be provided for any illness or injury that occurs during screening. You will not be giving up any of your legal rights by signing this consent form.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?
If you ever have questions about this study or in case you are injured as a result of participation in this research study, you should contact _____ (Tel _____)

Address:_____

If you ever have questions about your rights as a research subject or wish to report complaints/problems contact _____*(insert Ethics Committee details)*

If you have questions about this trial, you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). 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
SA Medicines Control Council
Department of Health
Private Bag X828
PRETORIA
0001

SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to take part in the screening for this study, please sign your name below.

Participant's Name (print)	Participant's Signature and Date
Name of Study Staff Member Conducting Consent Discussion (print)	Study Staff Signature and Date
Witness's Name (print) (if needed)	Witness's Signature and Date

Appendix 2
HIV RISK ASSESSMENT TOOL

PID_____ **Initials:** _____

1. How old are you?

<25	1
≥25	0

2. Are you married or living with your partner?

No	1
Yes	0

3. How old is your current partner?

≥25	1
<25	0

4. What is the HIV status of your partner?

Positive	1
Unknown	1
Negative	0

5. Does your partner have other girlfriends?

Yes	1
I do not know	1
No	0

6. Does your partner provide you with financial support?

Yes	1
No	0

7. Have you had any alcohol in the last 3 months?

Yes	1
No	0

Final Score _____

High Risk	≥3
Moderate or Low Risk	<3

Risk Assessment Tool modified from : An Empiric **HIV** Risk Scoring Tool to Predict **HIV**-1 Acquisition in African Women. Balkus JE, Brown E, Palanee T, et al. J Acquir Immune Defic Syndr. 2016 Feb 25. [Epub ahead of print] .
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Appendix 3
SAMPLE INFORMED CONSENT FORM

Immediate or Deferred Preexposure Prophylaxis for HIV Prevention: Safe Options for Pregnant and Lactating Women An Open-Label Randomised Control Study

SHORT TITLE FOR THE STUDY: Preventing HIV Infection in Young Women during pregnancy and breastfeeding using Antiretroviral Pre exposure Prophylaxis (PrEP)

ENROLLMENT

This study is sponsored by……. The doctor in charge of this study at this site is _____. This study involves research. Research is not the same as medical care. Research answers scientific questions. These answers can help find new medicines, treatments, vaccines, and even knowledge on how the human body works. Only people who want to participate will be part of this study. This informed consent form tells you about this study. You can ask questions at any time. You can discuss the study with others before deciding to join. No matter what your decision is, any other care that you get at this clinic will not change.

Researchers and study staff are asking you to join this study because you are not infected with HIV, but you are at risk of becoming infected either during this pregnancy or after. The study staff will give you a copy of this form.

1. WHY ARE RESEARCHERS DOING THIS STUDY?

As explained when you agreed to participate in the screening, the specific purpose of the PrEP Study is to look at the safety of an anti-HIV medicine used to prevent a woman from getting HIV from her sexual partner, while she is pregnant and after delivery. We want to determine if the anti-HIV drug is safe for the unborn baby and yourself during your pregnancy and during breastfeeding.

What makes me to be at higher risk for getting HIV?

- Young women who have multiple partners, or
- Have older partners, or
- Practice unprotected sex, or
- Whose partner is HIV positive, or
- Do not know their partners HIV status, or
- Have one or more of other sexually transmitted infections.

Recently, research conducted among discordant couples (i.e. one partner is HIV-infected and the other uninfected) showed that an anti-HIV medicine called Truvada (TDF/FTC) helps decrease the chance of women getting infected while they take a daily dose of the anti-HIV medicine. Truvada, which is a combination of tenofovir plus emtricitabine, has to be taken every day for a certain period while the woman is at risk. Such a regimen may be soon available in South Africa for HIV negative women who are at substantially high risk of getting HIV. Pregnant women and breastfeeding women who are not HIV infected are not allowed to take Truvada because we do not have adequate safety data if Truvada is to be

taken alone and for the sole purpose of preventing HIV infection. While Truvada, together with other anti-HIV medicines in South Africa, is used to treat HIV infected women during pregnancy and during breastfeeding and is known to be safe and highly beneficial in these women and their children, we don't know if Truvada will be safe and as beneficial in HIV uninfected women and their children.

2. WHAT WILL I NEED TO DO DURING THIS STUDY?

During this study, you will be in one of TWO study groups. Researchers will "randomize" you into one of the study groups described here. Randomization means that you are put into a study group by chance. Chance means "like flipping a coin". Neither you nor the study staff can choose your study group.

If you are in Group 1	IMMEDIATE PrEP You will start taking Truvada on the day you enrol into the study while you are pregnant and continue until you stop breastfeeding. Thereafter, you may choose to continue to take Truvada if you consider yourself to be at risk of HIV infection.
If you are in Group 2	DEFFERED PrEP You will not receive Truvada during pregnancy and throughout breastfeeding. You may choose to receive Truvada after you have stopped breastfeeding if you consider yourself to be at risk of HIV infection.

Note: No matter to which group you are allocated, you will also receive basic counselling on how to reduce your chance of becoming HIV infected, you will be provided condoms, you will receive a letter to invite your partner to be tested for HIV and referred for further care if needed, you will be tested/examined for other sexually transmitted infections and receive appropriate treatment.

Once you join this study, you will need to come to this clinic about SEVEN times to continue with you antenatal care, and after delivery you will have to visit the clinic every month until baby is 18 months old.

At most visits you will have the routine examinations, tests and procedures performed PLUS study specific examinations and tests as listed below:

Routine:

- ✓ Ultrasound examination – pregnancy screening to estimate stage of pregnancy and identify any abnormalities
- ✓ Obstetric assessment - monitoring of fetal growth and to identify any sign/s that could result in complications in pregnancy.
- ✓ Urine Collection – routine pregnancy screening for signs of infection or hypertension

Study Specific:

- ✓ Behavioural Risk assessment - study specific
- ✓ Discussion of Coital Log – study specific
- ✓ Physical Examination – study specific to assess physical well being
- ✓ Blood Collection - study specific investigations. Approximately 12 ml blood (a little more than a tablespoon) will be collected for a HIV test, Full Blood Count, Chemistry, Kidney function test, and Storage if you give us permission.
- ✓ Cervico-vaginal secretion and a swab collection – study specific investigations to examine any change in the tissue in the genital area that would give some explanation

for the HIV acquisition in pregnancy and postdelivery. This will be performed at every alternate visit during the study.

✓ DXA Scan – to be done four times postdelivery for yourself and for your baby. This is an XRAY of your bones to determine bone strength.

You will be followed throughout your pregnancy and through labour and delivery. Once your baby is born, you and your baby will continue to be followed until baby is 18 months old.

You will be seen two weeks and four weeks after you join the study; thereafter, you will be seen every four weeks while you are still pregnant. Each visit will last about 1 hour. You will have routine medical check-ups at the study clinic. It is important that you attend all of these study visits. If you do not come for a scheduled visit or if a test result comes back abnormal, the outreach worker will contact you to find out how you are doing. If at any time, you become sick you should let the study nurse or doctor know right away.

You will deliver at this hospital (Prince Mshiyeni Hospital) assisted by the hospital staff and you and your baby will be examined after birth by the research staff. You and your baby will return for a visit at 2 weeks after delivery, then 4 weekly until baby is 18 months old.

Tests and procedures at the study visits

- Medical history, questionnaire, interviews, and physical exam
  We will ask you about your medical history and about any medications you have taken since the last visit and about how well you are taking the study drug, if still on them. You and your baby will have a physical exam. We will update your contact information (for example, your address and telephone number). We may ask questions about your home life and general wellbeing. At some visits, we will also ask questions about infant feeding and nutrition.

- Blood
  Blood will be collected from you for various tests. Some tests measure whether you are tolerating the study drugs and to check on your health, and to measure the amount of study drug in your blood. But the important test is to determine if you get HIV infected. You will have approximately 10 to 32 ml (1 to 3 tablespoons) of blood taken at all visits.

  We will collect about 5 ml (1 teaspoon) from your baby at certain visits to make sure that the medications you are taking are not harming your baby.

  You will be given the results of blood tests that might affect your or your baby's health care as soon as possible, usually at the next study visit. Some of your blood and your baby's blood will be tested immediately, and some of the blood may be kept and used later for study-specified tests.

  Later, we will ask you if you are willing to have some of your blood and your baby's blood saved even after the study is over for future tests not yet specified. This stored blood might be used later to answer other research questions. You can still participate in the Study whether or not you agree to have your and your baby's blood stored after the study is completed. We will review the details with you and you will be asked to sign a separate consent form like this one if you agree to have your own and your baby's blood stored.

- Breast milk
  We will ask you to express up to 20 ml of breast milk (about 2 tablespoons), which will be kept to look for the presence of study medications.

The Tables below give you some guidance to visits in the study and what procedures to expect for yourself and your baby at these visits.

For You (Mother):

	ANTENATAL				LABOUR AND DELIVERY	POSTNATAL					
	SCREENING 1ST ANTENATAL VISIT	2ND ANTENATAL ENROLMENT ≥14W	3RD ANTENATAL 2 WEEKS	SUBSEQUENT 4 WEEKLY ANTENATAL VISITS	<72 HOURS POST DELIVERY	2 WEEKS	6 WEEKS	10 WEEKS	14 WEEKS	SUBSEQUENT 4 WEEKLY POSTNATAL VISITS	74 WEEKS/STUDY EXIT
Study Informed Consent	X	X									
Storage Informed Consent		X									
Documented HIV test result	X										
HIV Risk Assessment	X										
Medical History	X										
Hx, Signs/Sx of STIs	X		X	X	X	X	X	X	X	X	X
Pregnancy Ultrasound	X										
Obstetric Assessments	X	X	X	X							
DXA Scan Hip and Lumbar Spine							X			X*	X
Physical Exam	X	X	X	X	X	X	X	X	X	X	X
Randomisation		X									
Sexual Behaviour Questionaire	X	X	X	X	X	X	X	X	X	X	X
Drug Adherence Interview			X	X	X	X	X	X	X	X	X
Condom Usage Interview		X	X	X	X	X	X	X	X	X	X
Risk Reduction Counselling		X	X	X	X	X	X	X	X	X	X
Laboratory Investigations											
RPR Test	2ml								2mL		2mL
HIV Rapid Test (finger prick)	X	X	X	X	X	X	X	X	X	X	X
Hep B Surface Antigen	2ml						2mL				2ml
ALT/AST/Phosporous		2ml		2ml[a]				2ml		2ml[a]	
CBC	2ml		2ml	2ml[a]	2mL				2mL	2ml[a]	
Serum Creatinine	2mL		2mL	2mL		2mL		2mL	2mL	2mL	2mL
Stored EDTA Plasma, DBS /cell pellets		10mL	10mL	10mL	10mL	10mL	10mL	10mL	10mL	10mL	10mL
Stored Breast Milk (during BF only)						10mL	10mL	10mL	10mL	10mL	10mL
Cervical Vaginal Swabs		X	X	X			X	X	X	X	X
TOTAL BLOOD VOLUME	8ml	12ml	14ml	16ml	12ml	12ml	12ml	14ml	14ml	16ml	12ml

For the Baby:

	BIRTH	6 WEEKS	26 WEEKS	50 WEEKS	72 WEEK/STUDY EXIT VISIT
Physical Exam	X	X	X	X	X
Growth Parameters and neurodevelopmental milestones	X	X	X	X	X
DXA Scan Lumbar Spine and Whole Body		X	X	X	X
Laboratory Investigations					
CBC	2mL	2ml	2mL	2mL	2mL
Serum Creatinine	2mL	2ml	2mL	2mL	2mL
Stored DBS	X	X	X	X	X
TOTAL BLOOD VOLUME	4ml	4ml	4ml	4ml	4ml

3. HOW LONG WILL I BE IN THIS STUDY?

If you decide to join, you will be in this study for about 24 months including this visit.

4. HOW MANY PARTICIPANTS WILL BE IN THIS STUDY?

There will be 842 pregnant women participating in this study, 421 in the Immediate PrEP Group and 421 in the Deferred Prep Group.

5. WHAT POSSIBLE RISKS CAN I EXPECT FROM PARTICIPATING IN THIS STUDY?

In this study, study staff will take some blood from you. This procedure rarely can cause an infection. Taking blood from you may cause a low blood cell count or "anemia" and make you feel tired. You may also experience some discomfort when study staff conduct a genital examination and collect genital secretions using a soft cup, swab, syringe and a cytobrush in that order.

Taking part in this study may involve some risks and discomforts. These include possible side effects of the anti-HIV medicine (Truvada) that you may take, possible risks and discomforts from the study tests, and possible risks to your privacy. More information is given on each of these types of risks below.

Side Effects of Truvada (Anti-HIV Medicine) for Women

Some side effects are minor, while others can be severe. Some are common, while others are rare. If you join the study, the study staff will tell you about the side effects of the antiHIV medicine you will take. They will check for side effects during study visits and tell you what to do if you have any side effects.

In HIV-1 infected patients, the most common adverse effects (10 or more cases in every 100 women) are diarrhea, nausea, fatigue, headache, dizziness, depression, insomnia, abnormal dreams, and rash.

In HIV-1 uninfected individuals in PrEP trials, adverse reactions that were reported by 2 or more cases in every 100 women were headache, abdominal pain and weight decreased.

First you should know about the possible severe side effects. These effects are rare, but they can cause serious health problems.

Blood and the lymphatic system disorders *Common:* Neutropenia (1-10%)
Immune system disorders *Common:* Allergic reactions (1-10%)
Metabolism and nutrition disorders *Common:* Hypertriglyceridemia, hyperglycaemia (1-10%) *The following side effects have been reported but frequencies are unknown:* Hypophosphataemia, lactic acidosis, hypokalaemia

Other severe side effects are listed below:

- Inflammation of the pancreas. The pancreas is an organ near the stomach. When the pancreas becomes inflamed, it can cause pain in the belly, nausea, vomiting, and increased fats in the blood.

- Inflammation of the liver. The liver is an organ near the stomach. When the liver becomes inflamed, it can cause pain and swelling in the belly, nausea, and vomiting.

- Lactic acidosis, enlargement of the liver, and fatty liver, which can result in liver failure. Lactic acidosis is an imbalance in the blood that can cause weight loss, pain in the belly, nausea, vomiting, tiredness, weakness and difficulty breathing. When the liver is enlarged, it can cause pain especially on the right side of the belly, swelling in the belly, nausea, vomiting, and loss of appetite. It can also cause bleeding problems that can result in vomiting blood or dark coloured stools. Fatty liver is when healthy liver cells are replaced with fat. Sometimes it causes the liver to be enlarged, but doctors usually find out about it from tests of the blood. They occur more often in women, pregnant women, people who are overweight, and people who already have liver problems.

- Kidney damage or failure. The kidneys are organs near the middle of your back (one on each side). Doctors usually find out about kidney damage from tests of the blood. These effects can be caused by tenofovir.

Thinning of the Bones: In clinical trials in HIV-1 infected adults and in a clinical trial of HIV-1 uninfected individuals, tenofovir DF was associated with slightly greater decreases in bone mineral density (BMD) and increases in biochemical markers of bone metabolism, suggesting increased bone turnover. These studies have been mainly conducted among HIV infected women. In a recent study conducted at this clinic, BMD decline between delivery and 74 weeks postpartum was significantly greater among HIV-infected breastfeeding women receiving TDF-ART compared to women not receiving ART. There were no bone fractures reported in this study, and long term consequences of this bone decline is not known. We will be conducting similar tests on you to determine if your bone content is also affected by Truvada.

You should also know about the more common side effects, which are not severe. There are many possible mild and moderate side effects. Some people who take anti-HIV medicines have some of these effects, other people have different effects. The more common mild and moderate side effects are:

<table>
<tr>
<td>Overall Body Effects
• Overall weakness, tiredness, or feeling unwell
• Loss of appetite
• Loss of weight
• Changes in the placement of body fat, such as enlargement of the neck, stomach, and breasts and thinning of the arms, legs, and cheeks
• Numbness or tingling in the hands, arms, feet, legs, or around the mouth
• Pain in the hands or feet
• Allergic reaction
• Fever</td>
<td>Effects on Your Muscles and Bones
• Aches or pains
• Loss of muscle
• Muscle weakness
• Bone thinning or softening (which could increase the chance of breaking a bone)</td>
</tr>
<tr>
<td>Effects on Your Skin
• Rash, with or without itching</td>
<td>Effects on Your Blood
• Decreased blood cells
  • White blood cells help fight infection.
  • Red blood cells help store and transport energy through the body. Low red cells can cause weakness, tiredness, and dizziness.
• Increased bleeding if you have haemophilia</td>
</tr>
</table>

<table>
<tr>
<td>
• Yellowing of the skin
• Darkening of the palms and soles of feet

Effects on Your Head
• Headache
• Runny nose
• Yellowing of the eyes
• Blurred Vision
• Changes in the sense of taste
• Swelling of the face, lips, or tongue

Effects on Your Chest
• Cough
• Shortness of breath
• Heartburn

Effects on Your Belly
• Pain or discomfort in the belly
• Nausea
• Vomiting
• Gas
• Loose or watery stools
• Inflammation of the gall bladder. The gall bladder is an organ near the stomach. If it becomes inflamed, it can cause severe pain.
</td>
<td>
• Increased blood sugar or development of diabetes
• Increased fats in the blood that may increase the risk of heart problems
• Other changes in blood test results that may indicate problems with the muscles, kidneys, liver, pancreas, or gall bladder. The blood tests that may be affected include tests of how well these organs are working, tests of substances made by these organs, and tests of fats in the blood.

Effects on Your Mind or Mental Function
• Drowsiness
• Trouble sleeping
• Unusual dreams
• Difficulty concentrating
• Confusion
• Depression
• Agitation or anxiety
• Exaggerated feeling of well being
• Hallucinations
• Feeling of strangeness or losing touch with reality
• Dizziness
</td>
</tr>
</table>

Other Possible Risks of Truvada for Women

Risk of Resistance: All anti-HIV medicines can cause resistance. Resistance has been seen in women taking one anti-HIV medicine during pregnancy and in women taking combinations of anti-HIV medicines during pregnancy. When resistance occurs, a medicine no longer works against HIV, which can limit the choices of medicines a person can take against HIV in the future. To avoid resistance, it is important to test for HIV at all scheduled study visits and to stop taking the study drug if HIV infection is confirmed.

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Risks with Hepatitis B: Some anti-HIV medicines are active against Hepatitis B. For women who have Hepatitis B, and take anti-HIV medicines that are active against Hepatitis B, there are some risks. Usually, women with Hepatitis B are treated with at least 2 medicines that are active against Hepatitis B. For women who get 1 anti-HIV medicine that is active against Hepatitis B, the Hepatitis B could become resistant and harder to treat. In this study, women will stop study medications and receive the standard of care treatment for Hepatitis B.

Side Effects of Anti-HIV Medicines for Babies

The anti-HIV medicine that you take could affect your baby during pregnancy and after birth.

The anti-HIV medicine (Truvada) that some women in this study will take during pregnancy have been taken safely by thousands of other HIV infected women during pregnancy, and the only side effect seen in babies has been mild anaemia (low red blood cells), which got better on its own, with no treatment. Some studies have suggested higher rates of premature (early) births with the use of this type of medicine, while other studies have not. There also is less information available for tenofovir and emtricitabine, but studies giving these medicines to women at labour and their newborn babies have not found serious problems.

Babies may also receive some anti-HIV medicine taken by their mothers through breast milk but recent studies have detected negligible levels of Truvada in the breast milk. It is not known how much of effect this low exposure may have on baby. We will monitor side effects in the baby related to exposure to the low level of Truvada in the breastmilk.

Clinical trials evaluating tenofovir DF in children have noted that bone mineral density (BMD) decreases more rapidly in HIV-1 infected children who have received tenofovir DF. Total body BMD gain was less in the tenofovir DF treated HIV-1 infected pediatric subjects as compared to the control groups. However, in all pediatric trials, skeletal growth (height) appeared to be unaffected. There were no bone fractures reported. The long term effect and the consequence of BMD decline is not yet known. In this study we will monitor your baby's bone development.

The study staff will check for side effects in babies during study visits and tell you what to do if your baby has any side effects.

WHAT IF I BECOME PREGNANT AGAIN WHILE ON THE PrEP STUDY?

If you wish to become pregnant again or think you may be pregnant again at any time during the study, please tell the study staff right away and we will test you using a blood or urine test. The study staff will talk to you about your choices.

If you get pregnant again during the PrEP Study, you can continue on the study but you will be asked to stop study drugs.

If you become pregnant again during the study, and are still pregnant at your last study visit, the study staff will contact you again to find out about the outcome of your pregnancy.

Researchers want to know if the anti-HIV medicine you took during your pregnancy affected your baby. This information may help find the best HIV drugs to use in pregnant women. So, researchers will report this information to the "Antiretroviral Pregnancy Registry". This computer database will not use your name or information that can identify you.

You may face personal problems because of being in this study. Family, friends, and others may worry, get upset, or treat you unfairly. People may think that you have HIV or are likely to get it.

You may feel embarrassed when answering personal questions about sex or having a physical exam. You may feel anxious when waiting for your HIV test results. If you have these feelings, please tell the study staff so that they can find a way to help you.

Researchers and study staff try hard to protect your privacy. They also have a duty to maintain your privacy. But there is a risk that others, including your partner, may find out that you are participating in this study. Another social risk is that someone may use your personal information in a bad way. For example, someone finds out your test results and shares them with others. You could then have problems getting or keeping a job. You may

no longer have your family's or your community's support. These situations may cause you stress and embarrassment. Researchers have ways to reduce these social risks. Some of these ways include limiting access to your study records, having your study visits in private, and using codes to identify you and your samples. If you have any of these problems, please talk to the study staff, so that they can try to help you.

There may be other risks that researchers do not know now. If you feel something bad that you think is because of being in this study, you should tell the study staff. Researchers will tell you about any new risks from this study or other studies that can affect your decision to stay in this study.

6. WHAT POSSIBLE BENEFITS CAN I EXPECT FROM PARTICIPATING IN THIS STUDY?

Researchers believe that the PrEP using Truvada may have a direct benefit to you. Also, researchers may learn some information from this study that may help others.

7. WHAT OTHER CHOICES DO I HAVE?

If you choose not to participate in this study:

- You could choose to get the available local standard of care
- You may choose to do nothing

If you would like more information about the risks and benefits of each one of these choices, feel free to talk to the study staff. You can also discuss these options with your doctor. Regardless of your choice, any care that you get at this clinic outside of this study will not change.

8. CAN I CHANGE MY MIND ABOUT PARTICIPATING IN THIS STUDY?

Yes, you can change your mind at any time. Your participation in this study is completely voluntary. Tell the study staff if you are thinking about leaving or have decided to leave this study. Again, any care that you get at this clinic outside of this study will not change.

Study staff may want you to do some follow up visits and testing before you leave this study.

9. CAN RESEARCHERS TAKE ME OFF THIS STUDY EARLY?

Yes, researchers can take you off this study at any time:

- If they believe it is the best thing for you
- If you do not follow the study requirements
- If one of the groups watching over the study stops it

Study staff may want you to do some follow up visits and testing before you are off this study.

10. WHAT HAPPENS IF I BECOME HIV INFECTED DURING THIS STUDY?

If you do get infected, you and your baby will remain in the study. You will be provided counselling about your care and treatment options. You will be given anti-HIV medicines that are currently available to all HIV infected women in South Africa and your baby will also receive anti-HIV medicines to prevent him/her from getting HIV from you.

11. WHAT HAPPENS AT THE END OF THIS STUDY?

Once you finish this study, researchers cannot give you the anti HIV medicine for continued use.. If the medicine is made available by the Department of Health for women in your age group and risk category, and the drug was helping you, the study staff may be able to refer you to the nearest service point for access to Truvada as PrEP.

12. WHAT WILL HAPPEN TO MY SAMPLES AT END OF THIS STUDY?

Once this study ends, researchers may store some of your unused *blood/body fluid/tissue*. Researchers may use these samples for future research. You need to let researchers store these samples to participate in this study. There is another informed consent form that explains what researchers may do with these samples.

13. HOW WILL RESEARCHERS PROTECT THE PRIVACY OF MY INFORMATION?

Researchers have protections in place to maintain your privacy. They keep your study records in a secure place. They do not use your name in publications, meetings, or stored samples. They use a code to identify you and your samples. They do not share any information that could identify you.

14. WILL I HAVE TO PAY ANYTHING TO PARTICIPATE IN THIS STUDY?

You will not have to pay for any study lab tests, procedures, or exams required for this study.

15. WILL I RECEIVE ANY PAYMENT FOR MY PARTICIPATION IN THIS STUDY?

No, you will not receive any payment. You will need to spend some time and maybe some money to participate in this study. Researchers will reimburse you for some of these expenses. You will receive a total of R150 for transport costs, meals and for the time spent at the clinic. You will receive this reimbursement at every scheduled study visit.

16. WHO SHOULD I CONTACT IF I THINK THAT I AM HURT BECAUSE OF MY PARTICIPATION IN THIS STUDY?

It is possible that either you or your baby could experience a problem or injury that would not have occurred if you did not participate in this study. If the study doctor determines that you or your baby has been injured as a direct result of being in this study, you and/or your

baby will be given immediate treatment for those injuries at no cost to you and then referred for further care if needed.

However, the study doctor may determine that your or your baby's illness or injury would have happened even if you did not participate in this study. In that case, appropriate care and/or referral will likewise be provided for any illness or injury that occurs during the study.

17. WHAT ARE MY RIGHTS AND WHO SHOULD I CONTACT IF I HAVE QUESTIONS?

You have the right to leave this study at any time and for any reason. The study staff will continue to treat you the same no matter what you decide. You will not give up your legal rights by signing this informed consent form. You also have the right to know about any new information from this study or other studies. This information may affect your health, welfare, or decision to stay in this study. If you ever have questions about this study or in case you are injured as a result of participation in this research study, you should contact your doctor in this study – clinic tel.no. _____
Address: _____

17.1.1 If you ever have questions about your rights as a research subject or wish to report complaints/problems contact

The Administrator or Chair
Ethics committee details

17.1.2 If you have questions about this trial, you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). 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 of Medicines
Medicines Control Council
Department of Health
Private Bag X828
PRETORIA
0001

Fax: (012) 395 9201

E-mail: mogobm@health.gov.za

DO I WANT RESEARCHERS TO TELL ME ABOUT IMPORTANT FINDINGS THAT ARE NOT PART OF THE STUDY GOALS AND THAT WERE FOUND BY CHANCE?

Findings are <u>important</u> if they impact your health, welfare, or decisions about your health care.

____ (Initials) Yes, I want researchers to tell me.	____ (Initials) No, I don't want researchers to tell me.

18. HOW DO I CONFIRM MY DECISION TO BE IN THIS STUDY?

My signature below confirms:

- that I voluntarily decided to participate in this study
- that I had the opportunity to read this form or that it was read to me
- that this form was explained to me
- that I had the opportunity to ask questions

Participant's Name (print)	**Participant's Signature/Mark/Thumb Print and Date**
Study Staff's Name Obtaining Consent (Print)	**Study Staff's Signature and Date**
Witness' Name (Print)	**Witness's Signature and Date**

Appendix 4
SAMPLE INFORMED CONSENT FORM

Immediate or Deferred Preexposure Prophylaxis for HIV Prevention: Safe Options for Pregnant and Lactating Women An Open-Label Randomised Control Study

SHORT TITLE FOR THE STUDY: Preventing HIV Infection in Young Women during pregnancy and breastfeeding using Antiretroviral Pre exposure Prophylaxis (PrEP)

FACT SHEET and CONSENT FORM for SPECIMEN STORAGE

When you join this study, you will be asked to give permission for having some specimens that the doctor or nurse will take from you and your child saved in a repository. (A repository is a special laboratory with freezers where specimens like blood or tissue cells and body fluids that are taken from you during a study are kept. Your and your child's name will not be on these specimens, only a special study number. The people who run the repository laboratory will not know your or your child's name.)

Why have a repository?

Researchers can learn a lot from a study but as time goes by, the tests that they used get better or brand new tests are developed, and more can be learned with these better or new tests. When study volunteers consent to put specimens in the repository and consent to the researchers doing new tests on the specimens at some time in the future after their time in the study is ended, researchers can learn new information by being able to use the specimens. Your and your child's rights and privacy will be protected in any of these new studies.

How will my privacy and my child's privacy be protected?

The only record that you and your child participated in this study is at the clinic where it is kept separate from your and child's health records and locked away.

Your specimens and your child's specimens in the repository will not have your name or your child's name on them. The specimens will have a special study code. It will be the same code that is on your study record that is kept at the clinic. Again, none of this information will have your name or child's name on it.

How would a researcher get to use the specimens in the repository?

If a researcher wants to do a test on specimens from the repository in the future, he or she will write up the idea and it will have to be approved by a committee to make sure the research is worthwhile. If the idea is approved, then coded specimens and coded information will be given to the researcher. The researcher will not know the names, addresses, or phone numbers of the people who gave the specimens to the repository.

Would I ever be contacted in the future about research using my / our specimens?

All of the studies to be done in the future on your specimens in the repository will be for the particular reasons that you agreed to. If it is, then the research will go ahead since you would have agreed that these particular tests could be done without anyone contacting you to get your permission in the future.

If the study to be done is not like the kind of tests you agreed could be done, then the committee will decide if you need to be contacted to give permission for the new study.

I gave my permission to testing my specimen and my child's specimens in the repository, but what if I change my mind?

People always have the right to stop participating in research. So if you decide that you do not want researchers to be able to use the specimens in the repository, you can contact the clinic staff. They will tell the repository that the specimens with the study code number linked to your name and your child's name in the clinic should not be studied. These specimens can be removed from the repository and destroyed if you tell us to do that.

What type of research will be done with our specimens?

Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat or even cure diseases. In the future, some of the research may help to develop new products, such as tests and drugs. If this would happen and these tests or drugs make money, there are no plans to share that money with the people who gave the specimens.

As part of this study (Preventing HIV Infection in Young Women during pregnancy and breastfeeding using Antiretroviral Pre exposure Prophylaxis (PrEP)), you are being asked to have some (blood, genital fluid and breastmilk) taken. These specimens will go into the repository for research to be done at some time in the future so that more information can come from your participation in this Study.

You do not have to agree to store your specimens for future tests, for you to take part in this study. You will not lose any benefits to which you or your child is entitled if you decide against storing your specimens.

You will also be asked to agree that these particular tests can be done without anyone contacting you to get your permission sometime in the future. No one doing these tests would know that these specimens came from you or your child and no one would contact you or your doctor or nurse with the results from these tests that might happen in the future.

CONSENT FORM

What are the general HIV-related studies that can be done with the repository specimens?

Researchers would like to store your specimens to understand why you have become infected with HIV or why/how have you avoided becoming infected. HIV causes disease and complications, and how best to treat or prevent HIV infection and its complications. They need specimens from people who have HIV and from those who do not. Sometimes, the specimens can be used to learn something about new problems that people with HIV have like liver disease, diabetes, and heart disease. These general studies would not include any genetic testing (looking at your DNA).

Benefits: There are no direct benefits to you or your child. You will be helping researchers learn more about how to help people with HIV or at risk of HIV infection.

Risks: The specimens would be collected as part of your study visits, in the same way we normally draw blood samples. Once in the repository, there are few risks. Your name or your child's name will not be available to the repository or to the scientists who may be doing any future test. Since there are no plans to give participants the results of the tests performed on their stored specimens, you will not receive any information on your genetic makeup.

I give permission for the use of my specimens and my child's specimens for the purposes stated in the preceding section (general HIV-related tests).

Parent or Legal Guardian Signature	Witness Signature (if needed)	Date
Name of Study Staff Conducting Consent Discussion	Signature	Date

What are the special HIV-related studies that can be done with the repository specimens?

Researchers in this study would also like to store your specimens and your child's specimens to understand how HIV causes disease and complications, and how best to treat or prevent HIV infection and its complications through looking at how each person's genetic makeup (your and your child's DNA) either protects them or puts them at greater risk. It may be that researchers use some of your blood to make a "cell line". That means the blood cells can keep dividing and give an endless supply of your DNA for tests to be done in the future. This kind of information will be particularly important as scientists work toward a vaccine that could protect people from AIDS. They need specimens from people who have HIV and from those who do not.

Benefits: There are no direct benefits to your child. Your child will be helping researchers learn more about how to help people with HIV or at risk of HIV infection.

Risks: The specimens would be collected as part of your study visits. Once in the repository, there are few risks. Your name or your child's name will not be available to the repository or to the scientists who may be doing any future test. Since there are no plans to give participants the results of the tests performed on their stored specimens, you will not receive any information on your or your child's genetic makeup.

I GIVE PERMISSION for the use of my and my child's stored specimens for the purposes stated in the preceding section (special HIV-related tests).

Participant's Name (print)	**Participant's Signature/Mark/Thumb Print and Date**
Study Staff's Name Obtaining Consent (Print)	**Study Staff's Signature and Date**
Witness' Name (Print)	**Witness's Signature and Date**

What if I have more questions?

If you have any questions about the repository, about storage, or the use of your child's samples, contact the study doctor _____.

If you ever have questions about your rights as a research subject, giving consent as a research volunteer, or wish to report complaints/problems contact

The Administrator or Chair
Insert Ethics committee details

If you have questions about this trial, you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). 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 of Medicines
Medicines Control Council
Department of Health
Private Bag X828
PRETORIA
0001

Fax: (012) 395 9201 e-mail: mogobm@health.gov.za

I REFUSE to have any specimen collected from me or my child stored in the repository.

Participant's Name (print)	**Participant's Signature/Mark/Thumb Print and Date**
Study Staff's Name Obtaining Consent (Print)	**Study Staff's Signature and Date**
Witness' Name (Print)	**Witness's Signature and Date**

APPENDIX 5
SCHEDULE OF EVENTS FOR MOTHER

	ANTENATAL				LABOUR AND DELIVERY	POSTNATAL					
	SCREENING 1ST ANTENATAL VISIT	2ND ANTENATAL ENROLMENT ≥14W	3RD ANTENATAL 2 WEEKS	SUBSEQUENT 4 WEEKLY ANTENATAL VISITS	<72 HOURS POST DELIVERY	2 WEEKS	6 WEEKS	10 WEEKS	14 WEEKS	SUBSEQUENT 4 WEEKLY POSTNATAL VISITS	74 WEEKS/STUDY EXIT
Study Informed Consent	X	X									
Storage Informed Consent		X									
Documented HIV test result	X										
HIV Risk Assessment	X										
Medical History	X										
Hx, Signs/Sx of STIs	X		X	X	X	X	X	X	X	X	X
Pregnancy Ultrasound	X										
Obstetric Assessments	X	X	X	X							
DXA Scan Hip and Lumbar Spine							X			X*	X
Physical Exam	X	X	X	X	X	X	X	X	X	X	X
Randomisation		X									
Sexual Behaviour Questionnaire	X	X	X	X	X	X	X	X	X	X	X
Drug Adherence Interview			X	X	X	X	X	X	X	X	X
Condom Usage Interview		X	X	X	X	X	X	X	X	X	X
Risk Reduction Counselling		X	X	X	X	X	X	X	X	X	X
Laboratory Investigations											
RPR Test	2ml								2mL		2mL
HIV Rapid Test (finger prick)	X	X	X	X	X	X	X	X	X	X	X
Hep B Surface Antigen	2ml						2mL				2ml
ALT/AST/Phosporous		2ml		2ml[a]				2ml		2ml[a]	
CBC	2ml		2ml	2ml[a]	2mL				2mL	2ml[a]	
Serum Creatinine	2mL		2mL	2mL		2mL		2mL	2mL	2mL	2mL
Stored EDTA Plasma, DBS /cell pellets		10mL	10mL	10mL	10mL	10mL	10mL	10mL	10mL	10mL	10mL
Stored Breast Milk (during BF only)						10mL	10mL	10mL	10mL	10mL	10mL
Cervical Vaginal Swabs		X	X	X			X	X	X	X	X
TOTAL BLOOD VOLUME	8ml	12ml	14ml	16ml	12ml	12ml	12ml	14ml	14ml	16ml	12ml

*** DXA scan at 6 and 12 months**

APPENDIX 6

SCHEDULE OF EVENTS FOR HIV UNEXPOSED INFANTS

	BIRTH	6 WEEKS	26 WEEKS	50 WEEKS	72 WEEK/STUDY EXIT VISIT
Physical Exam	X	X	X	X	X
Growth Parameters and neurodevelopmental milestones	X	X	X	X	X
DXA Scan Lumbar Spine and Whole Body		X	X	X	X
Laboratory Investigations					
CBC	2mL	2ml	2mL	2mL	2mL
Serum Creatinine	2mL	2ml	2mL	2mL	2mL
Stored DBS	X	X	X	X	X
TOTAL BLOOD VOLUME	4ml	4ml	4ml	4ml	4ml