

MTN-016

**HIV Prevention Agent Pregnancy Exposure Registry:
EMBRACE Study**

Microbicide Trials Network

**Sponsored by:
Division of AIDS, US National Institute of Allergy and Infectious Diseases
US National Institute of Child Health and Human Development
US National Institutes of Health**

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MTN-016

HIV Prevention Agent Pregnancy Exposure Registry: EMBRACE Study

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LIST OF ABBREVIATIONS AND ACRONYMS

3TC	lamivudine
ACASI	audio computer-assisted self interview
AE	adverse event
AIDS	Acquired Immunodeficiency Syndrome
ALP	alkaline phosphatase
ALT	alanine transaminase
AP	anterior-posterior
ARV	antiretroviral
AST	aspartate aminotransferase
AUC	area under the curve
BID	twice a day
BMD	bone mineral density
BV	bacterial vaginosis
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CONRAD	Contraceptive Research and Development Organization
d4T	dideoxy-thymidine (stavudine)
DAIDS	Division of AIDS
DHHS	(United States) Department of Health and Human Services
DNA	deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
<i>E. coli</i>	<i>Escherichia coli</i>
EAE	expedited adverse event
EC	Ethics Committee
EFV	efavirenz
EMBRACE	Evaluation of Maternal and Baby outcome Registry After Chemoprophylactic Exposure
FDA	(United States) Food and Drug Administration
FHI	Family Health International
FTC	emtricitabine
GCP	Good Clinical Practices
HBV	hepatitis B virus
HIV	human immunodeficiency virus
HPTN	HIV Prevention Trials Network
IATA	International Air Transport Association
IGF	insulin-like growth factor
IND	investigational new drug application
IoR	Investigator of Record

LIST OF ABBREVIATIONS AND ACRONYMS (Continued)

IRB	Institutional Review Board
LC-MS	liquid chromatography-mass spectrometry
LDMS	Laboratory Data Management System
LLOQ	lower limit of quantification
LPV	lopinavir
mg	milligram
mL	milliliter
mm	millimeter
MTN	Microbicide Trials Network
NIAID	National Institute of Allergy and Infectious Disease
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
NOAEL	no-observed-adverse-effect-level
NNRTI	non-nucleoside reverse transcriptase inhibitor
OHRP	Office of Human Research Protections
PACTG	Pediatric AIDS Clinical Trials Group
PK	pharmacokinetic
PMPA	9-R-2-phosphonomethoxypropyl adenine
PMPApp	tenofovir diphosphate
PMTCT	prevention of mother-to-child transmission
PPD	Pharmaceutical Product Development Inc.
PrEP	pre-exposure prophylaxis
PSRT	Protocol Safety Review Team
PTID	participant identification number
RCC	Regulatory Compliance Center
RNA	ribonucleic acid
RT	reverse transcriptase
RTV	ritonavir
SAE	serious adverse event
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	Statistical Data Management Center
SHIV	simian/human immunodeficiency virus
SMC	Study Monitoring Committee
SOP	standard operating procedure (s)
TDF	tenofovir disoproxil fumarate
ULN	upper limit of normal
UNAIDS	United Nations Joint Programme on HIV/AIDS
ZDV	zidovudine

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EMBRACE Study**

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MTN-016

**HIV Prevention Agent Pregnancy Exposure Registry:
EMBRACE Study**

INVESTIGATOR SIGNATURE FORM

**Version 1.0
17 December 2008**

A Study of the Microbicide Trials Network (MTN)

Sponsored by:

Division of AIDS, US National Institute of Allergy and Infectious Diseases
US National Institute of Child Health and Human Development
US National Institutes of Health

I, the Investigator of Record, agree to conduct this study in full accordance with the provisions of this protocol. I agree to maintain all study documentation for a minimum of three years after submission of the site's final Financial Status Report to the US Division of Acquired Immunodeficiency Syndrome (DAIDS), unless otherwise specified by DAIDS or the Microbicide Trials Network (MTN) Coordinating and Operations Center. Publication of the results of this study will be governed by MTN policies. Any presentation, abstract, or manuscript will be made available by the investigators to the MTN Manuscript Review Committee, NICHD, and DAIDS, for review prior to submission.

I have read and understand the information in this protocol and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

Name of Investigator of Record

Signature of Investigator of Record

Date

MTN-016

HIV Prevention Agent Pregnancy Exposure Registry: EMBRACE Study

PROTOCOL SUMMARY

- Short Title:** EMBRACE (Evaluation of Maternal and Baby Outcome Registry After Chemoprophylactic Exposure)
- Protocol Co-Chairs:** Richard Beigi, MD, Samuel Kabwigu, MBChB, MMED
- Sample Size:** Approximately 500 pregnant women and approximately 300 live infants
- Study Population:**
1. Current or recent participants who become or became pregnant during HIV prevention trials, or who have or had planned exposures in pregnancy safety studies, provided pregnancy outcome was diagnosed less than 1 year from the date of the EMBRACE Screening/Enrollment Visit
 2. The infants resulting from those pregnancies, provided infants have not yet reached their 1 year birth date.
- Study Sites:** As determined by the MTN Executive Committee
- Study Design:** Prospective observational cohort investigation of prevention agent and control group exposures in pregnancy
- Study Duration:** Until May 31st, 2013, with possibility of extension
- Primary Objectives:**
1. To evaluate the prevalence of spontaneous pregnancy loss in mothers exposed to an active study agent during pregnancy as compared to that in mothers not exposed to an active study agent during pregnancy.
 2. To evaluate the prevalence of major malformations in infants of mothers exposed to an active study agent during pregnancy as compared to that in mothers not exposed to an active study agent during pregnancy.

Primary Endpoints:

- Pregnancy loss as evidenced by the following:
 - Negative urine pregnancy test performed by study staff
 - Clinically confirmed intrauterine demise at a gestation > 20 weeks.
 - Ultrasound evidence at any gestation
- Major malformation (structural abnormality with surgical, medical, or cosmetic importance) identified before one year of life

Secondary Objectives:

1. To monitor for adverse pregnancy outcomes.
2. To evaluate growth parameters in the first year of life among infants born to mothers exposed to an active study agent during pregnancy, as compared to those of mothers not exposed to an active study agent during pregnancy.
3. To provide a cohort of infants not exposed to an active study agent representing the background incidence of major malformations among babies born to women participating in HIV prevention trials.

Exploratory Objectives:

1. To monitor for select risks of prevention agents identified during pre-clinical reproductive toxicology studies by trimester(s) of exposure.
2. To evaluate the prevalence and persistence of HIV drug resistance mutations in plasma among HIV-infected infants .
3. To compare the results of developmental screening at select time points in the first year of life among infants born to mothers exposed to an active study agent during pregnancy as compared to those of mothers not exposed to an active study agent during pregnancy.

1 KEY ROLES

1.1 Protocol Identification

Protocol Title: HIV Prevention Agent Pregnancy Exposure Registry

MTN Protocol Number: MTN-016

Short Title: EMBRACE

Date: 17 December 2008

1.2 Sponsor and Monitor Identification

Sponsor: Division of AIDS (DAIDS)/National Institute of Allergy and Infectious Diseases (NIAID)/National Institutes of Health (NIH)
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2 INTRODUCTION

2.1 HIV/AIDS Prevention: Microbicides and Oral PrEP

According to the United Nations Joint Programme on HIV/AIDS (UNAIDS), approximately 33.2 million (30.6 million to 36.1 million) people worldwide were living with HIV in 2007.¹ Widespread implementation of HIV-1 prevention services, including behavioral strategies, has had only modest impact on the rate of new HIV-1 infections in most populations, thus continued efforts to identify effective preventative modalities are needed. Many different approaches are being evaluated in clinical trials including behavioral interventions, male circumcision, vaccines, chemoprophylaxis and topical microbicides. Microbicide clinical trials in HIV-uninfected participants conducted by the Microbicide Trials Network (MTN) will include Phase 1 and 2 safety trials of new compounds as well as larger, Phase 2B randomized trials. The variety of potential compounds is broad and includes agents with and without specific HIV-1 inhibitory activity. Trials conducted by the MTN will include topical microbicides, orally

administered antiretroviral agents (also referred to as chemoprophylaxis), and investigational agents with other routes of delivery.

2.2 Background

HIV prevention trials are underway in many parts of the world. The predominant target population in these trials is and will likely continue to be young, reproductive age women. In the event that HIV prevention agents demonstrate effectiveness and achieve licensure for HIV prevention, the target population will remain the same. One of the common occurrences among trial participants and the target population at large will be unintended pregnancy (estimated 5-10% of populations).^{2, 3} This will result in inadvertent exposures to microbicide and pre-exposure prophylaxis (PrEP) agents in early pregnancy, thus facilitating the opportunity to assess the effects of these inadvertent exposures on their unborn fetuses. In addition, one trial (MTN-002) has been designed to evaluate single-dose microbicide use in term gestation gravidas. Pending reassuring safety data from MTN-002, future larger trials directly assessing repeated microbicide use in pregnancy will be forthcoming. Thus, many opportunities exist for the systematic gathering and analysis of exposure data of these agents in pregnancy that can inform and augment the total body of safety information regarding HIV prevention agents.

The need for data on exposures and infant outcomes of all candidate HIV prevention agents during pregnancy makes this registry an essential component of ongoing efforts investigating the safety of these products. The intent of the registry is to collect data on pregnancy period exposure to drugs followed in the registry, potential confounding and/or relevant factors (such as maternal age, disease status during pregnancy, and gestational age at exposure, etc.), and information related to the outcome of the pregnancy. This will allow for ongoing and future analysis of the risk to pregnant women and their unborn fetuses from HIV prevention agent use.

Given that pregnancies will occur among study participants and, ultimately, users of microbicides and PrEP agents, the main purpose of the HIV Prevention Agent Exposure Pregnancy Registry (Registry) is to detect any adverse pregnancy outcome, including potential teratogenic effects involving study products in microbicide studies. Prevention agents planned for inclusion in this Registry are tenofovir (PMPA) Gel, UC781 gel, oral tenofovir disoproxil fumarate (TDF), oral emtricitabine/TDF, and their respective placebos, although other HIV prevention agents may be included in the future.

This protocol aims to serve as a reporting and data tracking mechanism for any clinical trials investigating the use of prevention agents and experience pregnancy exposures. In addition, this registry may serve as a foundation for ongoing population level surveillance in the future if licensure of one or more of these agents occurs.

2.3 Study Hypotheses and Rationale

2.3.1 Study Hypothesis

We hypothesize the following:

- Exposures to prevention agents during pregnancy will not be associated with an increased risk of spontaneous abortion or intrauterine fetal demise.
- Exposures to prevention agents during pregnancy will not be associated with an increased risk of major malformations among infants exposed *in utero*.

2.3.2 Rationale

In addition to the inevitable early pregnancy exposures that will occur, there are many other compelling reasons for continuing microbicide-specific investigations of safety in pregnancy:

1. Among pregnant and post-partum women, sexual activity, including sexual activity with multiple partners, is common. Therefore, women who may become pregnant and post-partum women may be suitable target populations for HIV prevention agent use.
2. Recent data suggest that pregnancy represents a time of potential heightened risk for the sexual acquisition of HIV.⁴
3. If microbicides become widely available, pregnant women will likely use them with or without evidence of safety. In the absence of safety data, possible recommendations to perform a pregnancy test prior to each use would create a logistical barrier to widespread use.
4. As anti-HIV microbicides might be used to augment ongoing efforts to prevent maternal-to-child transmission of HIV, there is a need for safety data that will allow for informed testing and use of these agents in this regard.

Given the growing number of microbicide trials and biomedical approaches to therapy, an increasing number of women may be exposed to HIV prevention agents during pregnancy or become pregnant while using one of these agents.

The lack of data on use and infant outcomes of microbicides during pregnancy makes this Registry an essential component of ongoing epidemiologic studies on the safety of these products. This is a non-interventional method of acquiring safety data for use in pregnancy that may support large-scale use of these products during pregnancy for HIV prevention.

This registry study will be unique in approach, in that it will also capture infant outcomes, including developmental milestones and growth, as defined by birth weight and serial measures of length, weight, head circumference, and abdominal circumference in the first year of life. Including these endpoints among the registry data will provide a richer picture of the potential impact of study product exposure on pregnancy and infant outcomes. Notably, we do not include BMD and/or bone metabolism evaluations on enrolled infants for several reasons. First, we do not believe there are sufficient data to suggest that very early gestational exposures (e.g., the 1st trimester exposures likely to be a large majority in our registry) would be associated with a detectable change in infant bone mineral density (BMD) and/or bone metabolism. Secondly, we believe that requiring routine blood draws and/or technically challenging bone imaging procedures for infants would significantly deter enrollment due to the general desire to avoid such interventions among participants in the involved countries. This registry, in its current form, is already more involved than most pregnancy exposure registries; expanding it will require greater and unwarranted investment on the part of the participants. Further, as there are very limited data to support standards for BMD and/or bone metabolism evaluations via examination of blood/serum samples or imaging studies for infants, the data generated would be of uncertain value.

Another unique feature of this protocol is its inclusion of data collected on pregnancy and infant outcomes for participants using non-active study agents. The prospective collection of these outcome data prior to the unblinding of study treatment assignment will build a natural comparison group into the study database.

The impact of study product exposure during pregnancy in the presence of HIV-infection is also unknown. While this is likely to be a rare occurrence, it would be prudent to explore the potential impact on the prevalence of HIV drug resistance mutations in the plasma of HIV-infected infants.

Potential differences in infant growth patterns between those exposed and unexposed to active study agents are readily measurable and accounted for by primary and secondary objectives in this study. Less easily discerned are potential impacts on infant development that can only be estimated using a series of infant developmental screening evaluations. There are currently no data to suggest that any product under investigation for prevention of HIV infection would negatively impact infant development. However, the evaluation of infant development may contribute to a more comprehensive view of potential long-term effects of antenatal exposure. As validated tools for this evaluation do not exist for all populations anticipated to enroll in MTN-016, the findings prompted by this exploratory objective will be considered pilot data.

2.4 Study Agents

Currently, several HIV prevention trials are focusing on one or more of the following agents: oral TDF, oral emtricitabine/TDF, tenofovir 1% gel, and UC781 gel. Therefore, background data will be included on these agents. As testing of other agents becomes more prevalent, those agents will also be included for ongoing surveillance.

2.4.1 Tenofovir Disoproxil Fumarate (TDF)

TDF is approved under the trade name Viread[®] for treatment of HIV-1 infection in adults.⁵ TDF is the oral pro-drug of tenofovir, an acyclic nucleotide analogue (9-R-2-phosphonomethoxypropyl adenine, PMPA) with activity *in vitro* against retroviruses, including HIV-1 and HIV-2, as well as hepadnaviruses. Further information on TDF is available in the current version of the Viread[®] package insert.⁵ Once absorbed, TDF is rapidly converted by diester hydrolysis to tenofovir. Tenofovir is then phosphorylated by cellular enzymes to tenofovir diphosphate (PMPApp), a competitive inhibitor of HIV-1 reverse transcriptase (RT) that terminates the growing deoxyribonucleic acid (DNA) chain. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α , β , and mitochondrial DNA polymerase γ . The strength of the TDF tablets expected to be seen in pregnancy exposures is the dose approved by the FDA for the indication of treatment of HIV-1 infection in adults (300 mg). For the treatment of HIV infection, TDF is administered once daily as one 300 mg tablet and has excellent activity against wild-type and many drug-resistant viruses.⁵

2.4.1.1 Animal Studies of Tenofovir and TDF

Toxicology

Tenofovir and TDF administered orally in toxicology studies to rats, dogs, and monkeys at exposures [based on (area under the curves) AUCs] ≥ 6 fold those observed in humans caused bone toxicity.⁵ In monkeys, bone toxicity was diagnosed as osteomalacia. Osteomalacia observed in some monkeys appeared to be reversible upon dose reduction or discontinuation of tenofovir. In rats and dogs, bone toxicity manifested as reduced BMD. The mechanism(s) underlying bone toxicity is unknown.

Four gravid rhesus monkeys were administered tenofovir subcutaneously once daily from 20 to 150 days of gestation (30 mg/kg; term: 165 ± 10 days).⁶ Fetuses were monitored sonographically, and maternal and fetal blood and urine samples were collected to assess hematological parameters, clinical chemistry, insulin-like growth factor (IGF) levels, and bone biomarkers. Fetuses were delivered by hysterotomy near term for necropsy and an evaluation of bone-related mechanical properties was performed. Results of these studies showed the following: 1) normal fetal development, although overall body weights and crown-rump lengths were less than those for age-matched controls ($P \leq .03$); 2) a significant reduction in circulating IGF-I ($P < .001$); 3) a small reduction in fetal bone porosity ($P \leq .03$); and, 4) transient alterations in maternal body weights and bone-related biomarkers during treatment. Results of these studies suggest that chronic fetal exposure to subcutaneous tenofovir at the maternal dose of 30 mg/kg throughout gestation can alter select fetal parameters and transiently affect maternal bone biomarkers.

Evidence of renal toxicity from oral TDF was noted in 4 animal species.⁷ Increases in serum creatinine, blood urea nitrogen, glycosuria, proteinuria, phosphaturia, and/or

calciuria and decreases in serum phosphate were observed to varying degrees in these animals. These toxicities were noted at exposures (based on AUCs) 2 to 20 times higher than those observed in humans. The relationship of the renal abnormalities, particularly the phosphaturia, to bone toxicity is not known.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at exposures up to approximately 16 times (mice) and 5 times (rats) those observed in humans at the therapeutic dose for HIV infection.⁵ At the high dose in female mice, liver adenomas were increased at exposures 16 times that observed in humans. In rats, the study was negative for carcinogenic findings at exposures up to 5 times the therapeutic dose used in humans. TDF was mutagenic in an *in vitro* mouse lymphoma assay, but negative in an *in vitro* bacterial mutagenicity test (Ames test). In an *in vivo* mouse micronucleus assay, TDF was negative when administered to male mice.

Reproductive Toxicity

There were no effects on fertility, mating performance, or early embryonic development when TDF was administered to male rats at a dose equivalent to 10 times the human dose, based on body surface area comparisons, for 28 days prior to mating, and to female rats for 15 days prior to mating through day seven of gestation.⁵ There was, however, an alteration of the estrous cycle in female rats. Reproduction studies performed in rats and rabbits at doses up to 14 and 19 times the human dose based on body surface area comparisons revealed no evidence of impaired fertility or fetal harm due to tenofovir. Subcutaneous administration of TDF to pregnant rhesus macaques resulted in a fetal/maternal concentration of 60%, demonstrating that TDF does cross the placenta⁸. Studies in rats have shown that tenofovir passes into breast milk.

2.4.1.2 Clinical Studies of Tenofovir Disoproxil Fumarate 300 mg Tablet

Pharmacokinetics

TDF pharmacokinetics (PK) have been evaluated in healthy volunteers and HIV-1 infected individuals.⁵ Tenofovir PK are similar between these populations and between male and female patients. Oral bioavailability of tenofovir from TDF in fasted patients is approximately 25%. *In vitro* binding of tenofovir to human plasma proteins is < 0.7%, and is independent of concentration over a range of 0.01 to 25 µg/mL. Following oral administration of one dose of TDF 300 mg to HIV-1 infected patients in the fasted state, maximum serum concentrations are achieved in 1.0 ± 0.4 hrs. Maximum serum concentration and AUC values are 296 ± 90 ng/mL and 2287 ± 685 ng-hr/mL, respectively. Approximately 70 to 80% of the intravenous dose of tenofovir is recovered as unchanged drug in urine. Tenofovir is eliminated by glomerular filtration and active tubular secretion. Following a single oral dose of TDF 300 mg, the terminal elimination half-life of tenofovir is approximately 17 hours. The PK of individual doses of tenofovir are dose proportional over a TDF dose range of 75 to 600 mg and are not affected by repeated dosing.

Safety

Gilead Study 903, a randomized, double-blind trial conducted in the United States, Europe and South America, was designed to compare the efficacy and safety of a treatment regimen of TDF, 3TC (lamivudine), and EFV (efavirenz) to a regimen of d4T (stavudine), 3TC, and EFV in 600 ARV-naïve, HIV-1 infected patients in a 144-week, double-blind phase. Patients who completed the 144-week double-blind phase on TDF were then eligible to roll over to the extension phase (weeks 144-480). In the double-blind phase, the most common (occurring in 2% or greater of tenofovir recipients) adverse events AEs emerging after treatment with TDF plus EFV and 3TC in HIV-infection, treatment-naïve adults included distresses of the whole body (headache, pain, fever, abdominal pain, back pain, asthenia), gastrointestinal tract (diarrhea, nausea, dyspepsia, vomiting), musculoskeletal system (arthralgia, myalgia), nervous system (depression, insomnia, dizziness, anxiety), respiratory system (pneumonia), and the skin (rash). The most frequently observed laboratory abnormalities were elevations in fasting cholesterol, creatine kinase, amylase, aspartate aminotransferase (AST) or alanine transaminase (ALT), hematuria, and decreased absolute neutrophil count. The frequency of all these events and laboratory abnormalities was similar or lower in the tenofovir-treated group compared to the d4T-treated group.

Follow-up data from an interim 288-week analysis of patients who enrolled in the extension phase of the study have recently been reported.¹⁰ Eighty-six patients (62% male, 70% white) initially randomized to the TDF arm continued treatment with TDF. No patient discontinued TDF due to renal events. Mean limb fat increased from 8.0 kg at week 96 to 8.8 kg at week 288. Thus, sustained TDF therapy was not associated with renal AEs or limb fat loss. Tenofovir is eliminated by the renal route, including tubular secretion. Thus, dose-interval adjustments are necessary for TDF in patients with significant renal impairment. TDF-induced nephrotoxicity has been reported in some series.¹¹ especially in patients with other medical problems or pre-existing renal dysfunction, although observational prospective studies tend to accord with Gilead Study 903 in finding an absence or low frequency of significant renal dysfunction⁷; when renal dysfunction occurs, it is generally predictable based on identifiable risk criteria.¹² One study that followed 27 HIV-infected children treated with TDF for 96 weeks found no evidence of impaired glomerular or tubular renal function.¹³

In Gilead Study 903 through 144 weeks, decreases from baseline in BMD were seen at the lumbar spine and hip in both arms of the study. At Week 144, there was a significantly greater mean percentage decrease from baseline in BMD at the lumbar spine in patients receiving TDF + 3TC + EFV (-2.2% ± 3.9) compared with patients receiving d4T + 3TC + EFV (-1.0% ± 4.6). Changes in BMD at the hip were similar between the two treatment groups (-2.8% ± 3.5 in the TDF group vs. -2.4% ± 4.5 in the d4T group). In both groups, the majority of the reduction in BMD occurred in the first 24 to 48 weeks of the study, and this reduction was sustained through Week 144. Twenty-eight percent of TDF-treated patients vs. 21% of d4T-treated patients lost at least 5% of BMD at the spine or 7% of BMD at the hip. Clinically relevant fractures (excluding fingers and toes) were reported in four patients in the TDF group and six patients in the

d4T group. In addition, there were significant alterations in biochemical markers of bone metabolism (serum bone specific alkaline phosphatase (ALP), serum osteocalcin, serum C-telopeptide, and urinary N-telopeptide) in the TDF group relative to the stavudine group, suggesting increased bone turnover. Serum parathyroid hormone levels and 1.25 Vitamin D levels were also higher in the TDF group. Except for bone specific ALP, these changes resulted in values that remained within normal range. Importantly, changes in BMD at the lumbar spine and hip noted in the first 48 weeks of the study were non-progressive through 288 weeks in the extension phase. However, the effects of TDF-associated changes in BMD and biochemical markers on long-term (> 144 weeks) bone health and the risk of future fracture are unknown.

Severe acute exacerbations of hepatitis B have been reported in patients who are co-infected with the hepatitis B virus (HBV) and HIV and have discontinued TDF. Both TDF and FTC (emtricitabine) are highly active against HBV and are recommended as part of the ART regimens for HIV/HBV co-infected individuals.^{14, 15} However, HBV exacerbations (defined as significant increase in hepatic transaminases) have been observed after stopping TDF, adefovir (a nucleotide similar to TDF), or 3TC (closely related to FTC) in approximately 20% of persons with chronic active hepatitis B.^{16, 17} Flares have typically been self-limited, but more serious liver decompensation has been reported.¹⁸ While the risk is thought to be greater among persons with clinically apparent liver disease, and may be even lower in HIV-uninfected persons, it has not been studied in detail. For this reason, it is recommended that hepatic function be monitored closely with both clinical and laboratory follow-up for at least several months in patients who are coinfecting with HIV and HBV and discontinue TDF for at least several months after TDF and/or FTC are discontinued.

Peterson et al. evaluated the safety of TDF 300 mg daily versus placebo for prevention of HIV-1 infection in women in a Phase 2 double-blind study conducted at 3 sites in West Africa.¹⁹ This study closed prematurely resulting in insufficient power to evaluate efficacy. In the primary safety analysis, with 428 person-years (p-y) of follow-up, there was no significant difference in the rate of safety endpoints (defined as grade 2 or higher serum creatinine, grade 3 or 4 transaminase elevation, or grade 3 or 4 phosphate abnormality). Among the 368 participants on TDF, none had grade 3 or 4 transaminase elevation or grade 2 or higher creatinine. One TDF recipient had self-limited grade 3 phosphate. Additional safety information from clinical studies on the TDF 300 mg tablet is available in the package insert at: http://www.gilead.com/pdf/viread_pi.pdf.

Pregnancy Outcomes

Oral tenofovir is being studied for use in prevention of peripartum maternal-to-child transmission of HIV-1 in late pregnancy. Data on the first cohort of 15 women enrolled to Pediatric AIDS Clinical Trials Group (PACTG) Protocol 394 have been presented.²⁰ Women were given an oral dose of 600 mg of tenofovir either at the onset of labor or four hours before scheduled cesarean delivery, and PK and safety in the mother and infant were evaluated. No significant adverse events in the women or infants were attributed to tenofovir. The maternal tenofovir concentrations were similar to those seen

after chronic dosing with 300 mg daily in non-pregnant individuals, despite the dose of 600 mg. Median cord blood levels were 76 ng/mL (range 0-309 ng/mL) and the median cord blood/maternal ratio was 0.69. All levels were below the level of quantitation (25 ng/mL) in the infants at 12, 24, and 36 hours of age. The study is continuing with a dose of 900 mg to the mother at onset of labor or before cesarean delivery. Another study, HIV Prevention Trials Network (HPTN) 057 is also evaluating maternal intrapartum and neonatal PK and safety of tenofovir. Both of these studies are in preparation for a large Phase 3 trial evaluating the use of oral tenofovir with emtricitabine and nevirapine for prevention of perinatal transmission and development of resistance to HIV-1. This selection is based on the expected effectiveness and safety of oral tenofovir in pregnancy.

Oral TDF, as Viread[®], or in combination with Emtricitabine (Truvada[®]) was studied in pregnant women during the third trimester, at delivery, and postpartum as part of the PACTG P1026s protocol (unpublished).²¹ The women in this cohort received 300 mg TDF daily, and intensive PK profiles were performed during the third trimester at 30 to 36 weeks gestation (n = 19) and at 6 to 12 weeks postpartum (n = 14). TDF area under the curve (AUC), C_{last} and C_{max} was lower in the third trimester (P = .020, .064, and .069, respectively) than in the same women postpartum. Third trimester C_{min} and C₀, however, did not differ from postpartum values. The results from this study also showed that chronic dosing with TDF results in umbilical cord blood concentrations at least 60% of maternal concentrations.

An unpublished study conducted with TDF-containing regimens in 76 pregnant women in the Frankfurt HIV Cohort between December 2005 and December 2007 showed that TDF should be considered for maternal-to-child-transmission (MTCT) prophylaxis.²² The women began the TDF regimen during the 24th week of pregnancy. Two women discontinued the TDF regimen due to nausea and exanthema. All 78 exposed children delivered via caesarean section were HIV-negative, and had no signs of TDF toxicity.

The Antiretroviral Pregnancy Registry is intended to provide an early signal of any major teratogenic effect associated with a prenatal exposure to the products monitored through the Registry. The Registry is a voluntary prospective, exposure-registration, observational study designed to collect and evaluate data on the outcomes of pregnancy exposures to antiretroviral (ARV) products. Data through 07/31/07 show six defects among 380 first trimester TDF exposures. This rate (1.6%) is not elevated compared to 4/263 (1.5%) after second/third trimester exposure²³ and the 2.67% background rate of defects reported by the Centers for Disease Control Metropolitan Atlanta Congenital Defect Program, or the generally accepted background rate for birth defects in the US population (approximately 3-4%).

2.4.2 Emtricitabine/Tenofovir Disoproxil Fumarate

Emtricitabine (FTC) is approved for treatment of HIV-1 infection in adults. FTC, which has activity against HIV-1 RT, is administered once daily, either as a single drug

formulation (Emtriva[®]) or in a fixed dose combination with TDF (Truvada[®]).²⁴ FTC (5-fluoro-1-(2R, 5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl] cytosine) is the synthetic negative enantiomer of a thio analogue of cytidine that differs from other cytidine analogues in that it has a fluorine in the 5-position. It is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate, which inhibits the activity of HIV-1 RT by competing with the natural substrate deoxycytidine 5'-triphosphate to become incorporated into the viral DNA, resulting in chain termination. Emtricitabine 5'-triphosphate is a weak inhibitor of mammalian DNA polymerases α , β , ϵ , and mitochondrial DNA polymerase γ . Further information on Emtriva[®] is available in the package insert.²⁵

Coformulation of FTC and TDF (Truvada[®]) has been approved by the FDA for treatment of HIV-1 infection in adults.²⁴ This once-daily, film-coated tablet contains 200 mg of FTC and 300 mg of TDF, which is equivalent to 245 mg of tenofovir disoproxil, as active ingredients. During PK studies, one Truvada[®] tablet was bioequivalent to one Emtriva[®] capsule (200 mg) plus one Viread[®] tablet (300 mg) following single-dose administration to healthy participants (n = 39). Further information on Truvada[®] is available in the current package insert.²⁴

2.4.2.1 Animal Studies of Emtricitabine

Toxicology

In long-term oral carcinogenicity studies of FTC, no drug-related increases in tumor incidence were found in mice or rats, and FTC was not genotoxic in the reverse mutation bacterial test, mouse lymphoma, or mouse micronucleus assays.²⁵

Reproductive Toxicity

FTC did not affect fertility in male rats at exposures approximately 140-fold or in male and female mice at exposures approximately 60-fold higher than human exposures at the recommended daily dose.²⁵ Fertility was normal in the offspring of mice exposed daily from before birth (*in utero*) through sexual maturity to a dose approximately 60-fold higher than recommended human dose. Reproduction studies in rats and rabbits at doses up to 14 and 19 times the human dose based on body surface area comparisons revealed no evidence of impaired fertility or harm to the fetus due to FTC.²⁶ The incidence of fetal variations and malformations was not increased in embryofetal toxicity studies performed with FTC in mice at exposures (AUC) approximately 60-fold higher and in rabbits at approximately 120-fold higher than human exposures at the recommended daily dose.

2.4.2.2 Animal Studies of Emtricitabine and Tenofovir in Combination

Toxicology

A 14-day oral gavage toxicity study comparing non-degraded and degraded FTC/TDF conducted in rats found no treatment-related effects on body weight, food consumption,

hematology, biochemistry, or urinalysis parameters.²⁷ The study found marginal increases in the weights of adrenal glands in most groups, although no gross or histological changes were identified that might account for this change in weight, and no treatment-related gross changes were observed at necropsy. Hyperplasia of the anterior duodenal mucosa overlying Brunner's glands, which was considered treatment-related, was seen at the high-dose level in 7 of the 10 rats treated with non-degraded FTC/TDF and in 2 of the 10 rats receiving degraded FTC/TDF.

A 4-week toxicity study in male dogs showed TDF at 30 mg/kg or in combination with 20 mg/kg FTC minimally increased activated partial thromboplastin time and creatinine.²⁷ Minimal tubular epithelial necrosis and slight to moderate tubular epithelial regeneration were also seen in dogs given TDF at 30 mg/kg or in combination with 20 mg/kg FTC. Renal findings were reversible after a 4-week recovery period (examined for FTC/TDF only). The no-observed-adverse-effect level of FTC/TDF is 2/3 mg/kg/day in dogs.

A fixed combination (2:3 ratio) of FTC/TDF was negative for the *in vitro* bacterial assay (Ames assay).²⁷ This combination was positive for inducing forward mutations in the *in vitro* mouse lymphoma cell assay in the presence or absence of S9 metabolic activation. Increases in mutant frequency occurred at concentrations similar to that observed in TDF alone.

2.4.2.3 Clinical Studies of Emtricitabine

Pharmacokinetics

Following oral administration, FTC is rapidly absorbed with peak plasma concentrations occurring at 1 to 2 hours post-dose. *In vitro* binding of FTC to human plasma proteins is < 4% and is independent of concentration over the range of 0.02 to 200 µg/mL. Following administration of radiolabeled FTC, approximately 86% is recovered in the urine and 13% is recovered as metabolites. The metabolites of FTC include 3'-sulfoxide diastereomers and their glucuronic acid conjugate. FTC is eliminated by a combination of glomerular filtration and active tubular secretion. Following a single oral dose of FTC, the plasma FTC half-life is approximately 10 hours. No PK differences due to race have been identified following the administration of FTC.

Safety

More than 2000 adults with HIV infection have been treated with FTC alone or in combination with other ARV agents for periods of 10 days to 200 weeks in Phase 1-3 clinical trials.²⁵ Assessment of AEs is based on data from studies FTC-301A and FTC-303 in which 571 treatment-naïve patients (FTC-301A) and 440 treatment-experienced patients (FTC-303) received FTC 200 mg (n = 580) or comparator drug (n = 431) for 48 weeks. The most common AEs that occurred in patients receiving FTC with other ARV agents in clinical trials were headache, diarrhea, nausea, and rash, which were generally of mild to moderate severity. Approximately 1% of patients discontinued participation in the clinical trials because of these events. All AEs were reported with similar frequency in FTC and control treatment groups with the exception of skin discoloration, which was reported with higher frequency in the FTC-treated

group. Skin discoloration, manifested by hyperpigmentation on the palms and/or soles, was generally mild and asymptomatic. The mechanism and clinical significance are unknown. Laboratory abnormalities in these studies occurred with similar frequency in the FTC and comparator groups.

A randomized, double-blind, double-dummy study, FTC-301A, was carried out in 101 research clinics in North America, Latin America, and Europe, to compare the efficacy and safety of FTC (200 mg once daily) with d4T when used with a background regimen of didanosine and EFV in 571 ARV-naïve persons aged 18 years or older with viral load levels greater than or equal to 5000 copies/mL.²⁹ Median follow-up was 60 weeks. Overall, subjects in the d4T group had a greater probability of an AE that led to study drug discontinuation than did those in the FTC group (15% vs. 7%, $P = 0.005$). Skin discoloration was observed in 10/286 subjects (3.5%) from the FTC group, and 1/285 subjects (0.35%) from the d4T group, and was manifested by hyperpigmentation on the palms and/or soles that was generally mild and asymptomatic. In no subject did hyperpigmentation prompt discontinuation of study drug. Importantly, the frequency of other rash events did not differ between the two treatment arms.

The FTC-303/350 study was a controlled, open label equivalence trial of 440 patients with suppressed HIV-1 infection who were randomized to continue their current treatment regimen or replace 3TC with FTC (200 mg daily).³⁰ Skin discoloration occurred in only 1.7% of subjects in the FTC group and in 1.4% of the 3TC group (difference not significant), and again, generally manifested as increased pigmentation on the palms and/or soles that was mild and asymptomatic. None of these events prompted discontinuation of study drugs.

In summary, the available data indicate that hyperpigmentation is a side effect of FTC use, and that the incidence, while likely variable, is low (3.5% or less). The mechanism and clinical significance of this finding are not known. Whether hyperpigmentation varies with skin color or is associated with other host factors is also not clear.

The safety of FTC in pregnant women and fetuses at doses used in humans is not known.²⁵ However, FTC is closely related to 3TC, a drug that is considered to be one of the preferred agents for treatment of pregnant women, and a common agent in regimens used in prevention of maternal-to-child transmission of HIV.

Exacerbations of HBV have been reported in patients after discontinuation of FTC, as noted above. Patients, who are co-infected with HBV and HIV, may have increased values on liver function tests and exacerbation of hepatitis symptoms when FTC is stopped. These findings are typically self-limiting; however, serious complications have been reported. The causal relationship to FTC discontinuation is unknown. It is recommended that persons co-infected with HBV and HIV be closely monitored with both clinical and laboratory follow-up for several months after stopping FTC treatment.²⁵

Pregnancy Outcomes

As of 7/31/07, 2 defects among 132 first trimester exposures to FTC, and 1 defect among 78 exposures during pregnancy after the first trimester had been reported.³¹ This rate is not elevated compared to the 2.67% background rate of defects reported by the Centers for Disease Control Metropolitan Atlanta Congenital Defect Program³² or the generally accepted background rate for birth defects (approximately 3-4%).

2.4.2.4 Clinical Studies of FTC and TDF in Combination (Truvada®)

Pharmacokinetics

A population PK study of FTC (200mg and 400mg) and tenofovir disoproxil was conducted in pregnant HIV infected women and their neonates in order to describe the concentration-time courses of FTC in mothers, the transfer of FTC from maternal plasma to cord plasma, neonatal elimination, and to study the influence of covariates on FTC PK.³³ The results of this study enabled the investigators to determine the optimal dose of FTC and tenofovir disoproxil in women at the onset of labor and the optimal single dose in neonates to prevent mother-to-child transmission of HIV. The study suggested that maternal administration of 400 mg prior to delivery produces levels less than the 200 mg administration in other adults. It also appears that the majority of babies were exposed because of placental transfer at approximately 80%.

A PK study was conducted to establish the bioequivalence of the FTC 200 mg/TDF 300 mg fixed-dose combination tablet relative to administration of FTC capsules and TDF tablets as their individual dosage forms.²⁴ The steady state PK of FTC and tenofovir were unaffected when FTC and TDF were administered together versus each agent dosed alone. A single FTC/TDF combination tablet and the individual dosage forms were administered to 44 healthy volunteers in a fasted state on two occasions which were separated by a 1-week washout period. Plasma PK parameters at steady-state were assessed over 48 hours post dose using a non-compartmental analysis. Results based on 39 subjects who completed the study revealed that the FTC/TDF combination tablet is bioequivalent to administration of TDF and FTC as their individual dosage forms as 90% of the confidence intervals (CIs) of the geometric mean ratios for C_{max} and AUC were within 80% to 125%.

Truvada® may be administered with or without food.²⁴ Administration of Truvada® following a high fat (784 kcal; 49 grams of fat) or a light meal (373 kcal; 8 grams of fat) delayed the time of tenofovir C_{max} by approximately 0.75 hour. The mean increases in tenofovir AUC and C_{max} were approximately 35% and 15%, respectively, when administered with a high fat or light meal, compared to administration in the fasted state. In previous safety and efficacy studies, TDF was taken under fed conditions. FTC systemic exposures (AUC and C_{max}) were unaffected when Truvada® was administered with either a high fat or a light meal. *In vitro* and clinical PK drug-drug interaction studies have shown the potential for CYP450 mediated interactions involving FTC and tenofovir with other medicinal products is low. FTC and tenofovir are primarily excreted renally by a combination of glomerular filtration and active tubular secretion. No drug-drug interactions due to competition for renal excretion have been observed; however,

co-administration of FTC/TDF with drugs eliminated by active tubular secretion may increase concentrations of FTC, tenofovir, and/or the co-administered drug. Drugs that decrease renal function may increase concentrations of FTC and/or tenofovir.

Safety and Efficacy

Several studies have assessed the safety of FTC with TDF, albeit none using the fixed-dose combination.²⁴ Four hundred and forty-seven HIV-1 infected patients have received combination therapy with Emtriva[®] and Viread[®] with either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor for 48 weeks in clinical studies. AEs and laboratory abnormalities observed in clinical trials were generally consistent with those seen in other studies in treatment-experienced or treatment-naïve patients receiving Emtriva[®] and/or Viread[®].

Study M02-418 was a Phase 3, randomized, open-label, multicenter study designed to compare lopinavir (LPV) 800 mg/ritonavir (RTV) 200 mg QD vs. LPV 400 mg/RTV 100 mg BID with the background regimen of FTC 200 mg QD and TDF 300 mg QD in ARV-naïve patients with HIV-1 RNA > 1000 copies/mL.³⁴⁻³⁶ A total of 190 patients between the ages of 19 to 75 years were enrolled; 115 to the QD arm, and 75 to the BID arm. At week 48, based on the intention to treat (ITT) (NC=F) analysis, 70% of participants in the QD regimen demonstrated HIV-1 RNA < 50 copies/mL compared with 64% of those in the BID group (95% CI: -7%; 20%). Gastrointestinal AEs were the most common cause for discontinuation. Overall, the most common AEs (> 3%) reported were diarrhea, nausea, and vomiting, with diarrhea being reported significantly more frequently in the QD group (16% vs. 5%; $P = 0.04$). The most common Grade 3/4 laboratory abnormalities (> 3%) reported were increased ALT (> 5 x upper limit of normal [ULN]), aspartate aminotransferase [AST (> 5 x ULN)], triglyceride (> 750 mg/dL), and amylase (> 2 x ULN) levels; no significant differences between the two groups were observed.³⁴

Gilead Study 934 is a Phase 3, randomized, open-label, noninferiority, multicenter study designed to compare a regimen of TDF 300 mg + FTC 200 mg + EFV QD with a regimen of ZDV 300 mg/3TC 150 mg BID (as FD Combivir[®]) + EFV QD in ARV-naïve, HIV-1-infected participants³⁷. The 48-week data demonstrated that using the time-to-loss of virologic response as the primary analysis (where missing, switch, or early termination is counted as a failure), the proportion of participants with plasma HIV-1 RNA levels < 400 copies/mL in an ITT population ($n = 487$) was 84% in the TDF + FTC group compared with 73% in the ZDV/3TC group ($P = .002$). The proportion of participants with plasma HIV-1 RNA levels < 50 copies/mL was 80% in the TDF+FTC group versus 70% in the ZDV/3TC group ($P = .020$). Significant differences were also seen between the TDF+FTC and the ZDV (zidovudine)/3TC groups in the proportion of participants with increases in CD4+ cell counts (190 and 150 cells/mm³, respectively; $P = .002$). Safety analysis, based on 511 participants who received any study medication, showed that discontinuation due to AEs occurred more frequently in the ZDV/3TC group (9%) than in the TDF + FTC group (4%) ($P = .02$).

The most common AE resulting in discontinuation related to study drug was anemia for the ZDV/3TC group (14/254) and NNRTI-associated rash (2/257) for the TDF+FTC group. Renal safety was similar in the two groups, and no participant discontinued study medication because of renal events. A significantly ($P < .001$) greater percentage of participants in the TDF+FTC arm had a lower mean increase from baseline in fasting total cholesterol levels (21 mg/dL) compared with participants in the ZDV/3TC arm (35 mg/dL). At week 48, total limb fat was significantly less in a subset of participants receiving ZDV/3TC (mean of 6.9 kg or 15.2 pounds; $n = 49$) compared with a subset of participants receiving TDF+FTC (mean 8.9 kg or 19.6 pounds; $n = 51$; $P = .03$). All participants with confirmed > 400 copies/mL of HIV-1 RNA at week 48 or early discontinuation were analyzed for genotypic resistance. Genotype data were limited to 23 participants on ZDV/3TC and 12 participants on TDF+FTC, and showed mostly M184V/I (3% in ZDV/3TC participants vs. 1% in TDF + FTC participants) and/or EFV-resistance mutations (7% in ZDV/3TC vs. 4% in TDF + FTC participants), with no participants developing the K65R mutation.

Exacerbations of HBV have been reported in patients after discontinuation of TDF and FTC, as noted above. HIV-infected persons who are coinfecting with HBV, may have increased values on liver function tests and exacerbation of hepatitis symptoms when TDF or FTC is stopped.²⁴ Usually these symptoms are self-limiting; however, serious complications have been reported. The causal relationship to TDF or FTC discontinuation is unknown. Participants coinfecting with hepatitis B (HBV) and HIV should be closely monitored with both clinical and laboratory follow-up for several months after stopping Truvada[®] treatment, as it contains both FTC and TDF. Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogues alone or in combination, including FTC, TDF, and other ARVs.

FTC/TDF is designated as a US Food and Drug Administration (FDA) use-in-pregnancy Category B drug. Additional general information about FTC/TDF can be found in the most recent Truvada[®] package insert.²⁴

2.4.3 Tenofovir 1% Gel

Tenofovir 1% gel contains 1 gm/100 ml of PMPA (9-R-2-phosphonomethoxypropyl adenine), an acyclic nucleotide analogue with activity *in vitro* against retroviruses, including HIV-1 and HIV-2, as well as hepadnaviruses.³⁸ Further information is available in the current version of the tenofovir gel investigator's brochure.²⁷ Tenofovir is an acyclic nucleotide analogue of adenosine monophosphate that requires subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV-1 RT by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α , β , and mitochondrial DNA polymerase- γ . The strength of the tenofovir gel likely to be associated with pregnancy exposure is the strength (1%) previously

tested in HPTN 050 (IND 55,690), CONRAD A04-095 (IND 73,382) and A04-099 (IND 73,382), HPTN 059 (IND 55,690), MTN-001 (IND 55,690), and MTN-002 (IND 55,690).

2.4.3.1 Animal Studies of Tenofovir 1% Gel

Pharmacokinetics

Single-dose PK of vaginally administered tenofovir gel in female rabbits has been previously examined (0.5 mL, 1% w/v tenofovir, 5 mg/animal, 50 μ Ci/kg).³⁹ Plasma radioactivity concentrations were highest at the first sample time point (0.5 hours) and below the level of quantification at 24 hours. PK parameters including the proportion of dose absorbed systemically could not be estimated, due to the very low plasma concentrations.

In a tissue distribution study using the same radiolabeled tenofovir 1% vaginal gel formulation, dose and strength as the above study, eighteen female rabbits were administered an intravaginal dose using a gavage needle.²⁷ An additional eighteen rabbits received an intravaginal dose of 3% w/v tenofovir (15 mg per animal). Analysis of vaginal tissue sections found no clear relationship between tissue concentration and dose, with no consistent pattern of distribution. Very little radioactivity was recovered in non-vaginal tissues. Concentrations in blood (0.002 to 0.047 μ g-eq/g of tissue) exemplified the variability of distribution of the product although the effect of oral absorption due to grooming behaviors of the animals may have impacted these results.

Bioavailability of tenofovir gel was evaluated in female New Zealand white rabbits in a study examining systemic and vaginal bioavailability following receipt of single and multiple intravaginal doses of 1 mL of 1% tenofovir gel or a single intravenous dose of 10 mg tenofovir. Detected tenofovir concentrations from vaginal rinses, tissue biopsies, and blood plasma varied across groups receiving single and multiple doses, and between groups receiving intravenous vs. vaginal delivery, with plasma levels 8 hours post-dose being higher among animals receiving systemic, intravenous delivery (intravenous, 10,211 ng/mL; vaginal, 3 ng/mL). Notably, however, similarities in tenofovir concentrations were detected in both tissue samples and vaginal rinses collected 8 hours post-dose among animals receiving a single intravaginal or a single intravenous administration. Single intravaginal administration (1% tenofovir gel) produced barely detectable systemic absorption within the first 30 minutes only, while multiple intravaginal dosing (twice a day (BID) for 7 or 14 days) resulted in the detection of systemic levels of tenofovir (from 71 to 239 ng/mL).

The PK, excretion and tissue distribution of ¹⁴C-PMPA were evaluated in rats following intravaginal administration of an earlier formulation of tenofovir gel containing propylene glycol.⁴⁰ Four female rats received a single intravaginal dose administered as an aqueous gel containing 20 mg tenofovir/g. Plasma concentrations of total radioactivity were highly variable; this was attributed to inconsistent retention of the formulation within the vagina, or possibly oral absorption related to grooming. The apparent C_{max} for tenofovir occurred at the earliest time point (15 minutes), suggesting that absorption

from the vagina was relatively rapid. Thereafter, plasma concentrations declined with an approximate half-life of 1.6 hours. The bioavailability of intravaginal tenofovir was estimated by comparison of the observed $AUC_{(0-24)}$ with historical AUC data for an intravenous dose of 10 mg/kg tenofovir in rats (9.71 $\mu\text{g h/mL}$). The observed systemic bioavailability of intravaginal tenofovir was 7.9%.

In the excretion and distribution study, two groups of four additional rats received a single intravaginal dose of ^{14}C -PMPA (approximately 10 mg/kg, 100 $\mu\text{Ci/kg}$) administered as aqueous gel containing 20 mg tenofovir/g. This study found that much of the dose was lost from the vaginal orifice by leakage. Vaginal tissue contained 0.1% of the dose, and less than 0.01% of the dose was recovered in the ovaries and uterus.

The PK of radiolabeled tenofovir gel was evaluated via plasma and vaginal biopsies collected from four rhesus monkeys following single-dose intravaginal tenofovir 1% vaginal gel.²⁷ Radioactivity was detected starting at 15 minutes post application, with peak concentration of tenofovir in vaginal tissue at 8 h and remaining high at 12 hours. No significant radioactivity was detected in whole blood or plasma.

Toxicology

The preclinical toxicity of tenofovir gel has been evaluated in 14-day rat and 10-day rabbit vaginal irritation and toxicity studies.^{39, 41} Daily intravaginal administration of tenofovir gel produced no vaginal irritation in rats ($\leq 10\%$ tenofovir), and minimal to mild vaginal irritation in rabbits (3% or 10% tenofovir).

14-Day Vaginal Irritation and Toxicity Study of Tenofovir Gel in Rats

Ten female Sprague Dawley rats/group received either 0% (vehicle control), 1%, 3%, or 10% tenofovir gel (2.5% HEC formulation) by intravaginal administration (0.5 mL/dose) once daily for 14 days. There were no mortalities, and no tenofovir-related clinical signs of toxicity or changes in body weight, food consumption, or absolute/relative kidney weights. Individual and mean vaginal (gross) irritation scores for all tenofovir-dosed animals sacrificed at Day 15 were graded as 0 (no erythema or edema); microscopic irritation scores for the vagina, cervix, ovaries, uterine horns, and vulva were graded as 0 (normal histology). No tenofovir-related histopathological effects on the vagina, cervix, ovaries, uterine horns, vulva, or kidneys were observed.

10-Day Vaginal Irritation Study of Tenofovir Gel in Rabbits

The potential irritant effects of tenofovir were evaluated in vaginal tissues of female New Zealand White rabbits using three different gel formulations (2.5% HEC or 1.0 to 2.0% Carbopol[®] 1342).⁴² This study consisted of eleven treatment groups (five rabbits/group) that received one of the following: 1) a sham treatment or Conceptrol[®] (positive control); 2) 0%, 0.3%, 1.0%, 3.0%, or 10.0% tenofovir formulated in the HEC gel preparation; or, 3) 0% or 3.0% tenofovir formulated in a 1.0% or 2.0% Carbopol[®] 1342 gel preparation. With the exception of the sham dose group, all rabbits received dose formulation (1.0 mL/dose) daily applied topically to the mucosal surface of the vaginal vault for 10 consecutive days. No mortalities and no tenofovir-related clinical signs of toxicity or body weight changes were observed in this study. Group composite vaginal irritation scores for the 10% tenofovir topical gel (HEC formulation), 0% tenofovir (1.0%

Carbopol® 1342 formulation), and Conceptrol® (positive control) dose groups were each rated as “mild.” Composite vaginal irritation scores rated “minimal” were observed for all other tenofovir, vehicle, or sham treatment groups, regardless of the formulation. No unacceptable level of mucosal irritation was observed in any treatment group based on the protocol-derived criteria for this animal model. Generalized erosion and/or ulceration were observed only in animals receiving Conceptrol® positive control (two of five) or the 10% tenofovir topical gel (two of five).

2.4.3.2 Clinical Studies of Tenofovir 1% Gel

Pharmacokinetics

“A Phase 1 Safety and Acceptability Study of the Vaginal Microbicide Agent PMPA Gel”, also known as HPTN 050, is a recently completed study of tenofovir vaginal gel with published data.⁴³ Eighty-four (60 HIV-uninfected and 24 HIV positive) women applied either 0.3% or 1% tenofovir gel once or twice daily for 14 days. Systemic absorption was limited (maximum serum levels 3.1-25.8 ng/mL).

Safety and Efficacy

In HPTN 050, the tenofovir 1% gel formulation was well tolerated in both HIV uninfected and HIV infected women.⁴⁴ Ninety-two percent (92%) of women reported at least one AE. The majority of these events were mild (87%) and limited to the genitourinary tract (77%). The five most reported mild genital AEs were pruritus (n = 18), erythema (n = 14), petechial/ecchymosis (n = 14), vaginal discharge (n = 13), and burning (n = 10). Four severe AEs were reported, but only one (lower abdominal pain) was thought to be product-related. Product concentration, sexual activity and HIV status were not associated with a specific AE pattern. No clinically significant systemic toxicity was observed. No serious adverse events (SAEs) were reported.

Of 76 participants in the HPTN 050 study who had bacterial vaginosis (BV) evaluation (by using Nugent’s score criteria) at both enrollment and Day 14, 30 women had asymptomatic BV at baseline and 15 of them became BV negative after 14 days of tenofovir gel use, while one out of 46 women without BV at baseline had BV detected at 14 days. Overall, 40% of the women had asymptomatic BV at baseline compared to 21% of the women after fourteen days of tenofovir gel use ($P = .0005$), suggesting that the gel did not increase subjects’ risk of developing BV.

In a male tolerance study (CONRAD A04-099/IND 73,382), tenofovir 1% gel was well tolerated in men following seven days of once daily penile exposure. There were few genital findings observed after product use, and all findings were classified as mild, small in size, and requiring no treatment. Reported symptoms were mild, of short duration, and resolved by the final visit. There were no noticeable differences between signs and symptoms of genital irritation in the circumcised compared to uncircumcised group.⁴⁵

A Phase 2 study of tenofovir 1% gel (HPTN 059) was completed in 2007. This study assessed the safety and acceptability of, and adherence to, a regimen of tenofovir gel for vaginal use in HIV-uninfected women versus a placebo gel. Exploratory objectives included measurement of vaginal flora characteristics, assessment of the effects of tenofovir gel on cytokine and chemokine expression, and the evaluation of cytokine and chemokine expression to correlate expression with evidence of inflammation, epithelial disruption, and genital symptoms. The study design was a Phase 2 four-arm, three-site, randomized, controlled trial comparing tenofovir 1 % vaginal gel used once daily and gel used prior to intercourse, to placebo gel, with 6 months tenofovir gel exposure and follow-up. The study was conducted among 200 women at three sites: Pune, India; Birmingham, Alabama, USA; and New York, New York, USA.

Participants were sexually active, HIV-uninfected women between the ages of 18 and 50, and were neither menopausal nor post menopausal. Participants were followed through six months of study gel exposure. They were randomized to either the once daily or the coitally dependent group, and received either tenofovir or placebo gel. Participants received single use unit dose tubes and single use applicators.

No statistically significant differences were seen between participants receiving active and placebo gels in complete blood count, liver function tests, or renal function tests. Among those with the most frequent exposure (daily use), no participants had pelvic exam findings involving generalized erythema or severe edema or deep epithelial disruption at any follow-up visit during the study. At the Week 24 Visit, no participants had exam findings suggestive of vaginitis, cervicitis, superficial disruption, disrupted blood vessels, or intermenstrual bleeding. Adherence to study gel was high, and was supported by PK data. Seventy-nine percent (79%) of women reporting gel use in the past 12 hours had low but detectable plasma tenofovir supporting self-reported adherence data. Daily and coital use was highly acceptable to women. These data suggest a favorable safety and acceptability profile of tenofovir gel, and support routine monitoring for genital findings among women without genital symptoms at six month intervals.⁴⁶

In HPTN 050, no new resistance mutations evolved in plasma or cervicovaginal lavage after 14 days of tenofovir gel use, but 3 women had plasma mutations associated with low level tenofovir resistance identified at both Days 0 and 14 (M41L, L210M, ±T215I/Y).

2.4.4 UC781

UC781 is a thiocarboxanilide pentenyloxy ether derivative of the carboxanilide class of NNRTIs. The chemical name for UC781 is N- [4-chloro-3- (3-methyl-2-butenyloxy) phenyl]-2-methyl-3-furancarbothioamide.

Standard genotoxicity tests conducted for the evaluation of UC781 have included an *in vitro* reverse mutation test in bacteria (Ames test, *S. typhimurium*, *E. coli*, up to 5000 µg/plate), an *in vitro* mammalian chromosome aberration in Chinese Hamster Ovary

cells (7.5 to 150 mg/mL and 3.75 to 100 mg/ml for the 4 and 20-hour exposures, respectively), and an *in vitro* micronucleus test in mice (5/sex/group, up to 2000 mg/kg).⁴⁷ Data from these analyses did not indicate any evidence of mutagenic or clastogenic potential for UC781 or its metabolites under the conditions of these studies. No carcinogenicity studies with UC781 have been performed to date.

2.4.4.1 Animal Studies of UC781

A fertility (Segment I) study in rats (100 male, 100 female, 25/sex/dosage group, up to 400 mg/kg/day orally) was performed in May 2005, that was designed to evaluate the reproductive process and detect effects on the estrous cycle, tubal transport, implantation, and development of pre-implantation stages of the embryos of female rats as well as permit detection of functional effects (i.e., effects on libido or epididymal sperm parameters) that may not be detected by histological examinations of male rat reproductive organs.⁴⁷ The final report detailing study analysis is pending.

A developmental toxicity study (Segment II) in pregnant rats (25/group, 4 groups, up to 400 mg/kg/day orally) did not associate UC781 with mortality or clinical findings.⁴⁷ No statistically significant differences in body weight were observed among the groups, though feed consumption was reduced in the 100 mg/kg/day and 400 mg/kg/day dose groups. No fetal toxic or teratogenic effects due to UC781 treatment were observed, indicating that the maternal and developmental no-observed-adverse-effect-level (NOAEL) of UC781 is greater than 400 mg/kg/day.

A Segment II study of UC781 in rabbits (107 animals, up to 33 mg/kg, intravaginally) resulted in the sporadic death of 12 rabbits, with signs of toxicity appearing only immediately prior to death.⁴⁷ Blood drug levels were below the limits of detection after the first dose on gestation day 7 and last dose on gestation day 20.

A second rabbit embryo-fetal development study was conducted using the same UC781 dose levels and concentrations as the first study, due to the mortality seen in the previous study.⁴⁷ No deaths were observed during the dosing period in the second study. No drug related fetal toxic or teratological findings were observed in either rabbit study. Two consulting expert toxicologists concluded that the mortality observed in the first study but not in the repeat study was most likely due to the handling and dosing procedure and not a direct toxic effect of UC781.

Recent data on vaginal and rectal exposure in pig-tailed macaques suggest that formulated UC781 gel is not systemically absorbed after multiple daily applications, and that the product is non-toxic to vaginal and rectal microenvironments (D. Patton, personal communication).

2.4.4.2 Clinical Studies of UC781

A Phase 1, randomized, double-blind, placebo-controlled, safety and tolerance study of three different concentrations (0.1%, 0.25%, 1%) of UC781 was conducted in a total of 48 women.⁴⁸ The 12 women in each of the UC781 and placebo groups were given a single dose of study product followed by five consecutive daily doses, and were evaluated by colposcopy after the first and sixth gel applications. A total of 37 AEs were experienced by 8 (66.7%) participants in the placebo group, 5 (41.7%) in the 0.1% UC781 group, 5 (41.7%) in the 0.25% UC781 group, and 4 (33.3%) in the 1.0% UC781 group. Only one of the eight moderate AEs was determined to have any relationship to a study product.

Twelve women each had 1 finding on colposcopy: 1 in the placebo group, 3 in the 0.1% group, 5 in the 0.25% group, and 3 in the 1% group. Only 1 woman had more than superficial epithelium changes, a woman in the 0.25% group with a deep epithelium lesion due to applicator injury

Participants' plasma samples were analyzed for UC781 at baseline and after 6 applications of placebo gel, 0.1%, 0.25%, or 1% UC781 gel. The lower limit of quantification (LLOQ) of the liquid chromatography-mass spectrometry (LC-MS) bioanalytical assay was 2.5 ng/mL. There were no detectable levels of UC781 in samples after treatment with placebo gel or 0.1% and 0.25% UC781 gels. After six daily applications, two participants who were randomized to the 1% UC781 gel group had plasma levels that were detectable but less than the LLOQ.

The key acceptability item was whether or not the subject indicated that she would buy the product if she needed protection against HIV and if the product she used were effective. All of the participants indicated that they would buy the product if proved effective with only one exception in the 0.1% UC781 group.

Another Phase I study conducted at Emory University was started in October 2005 to assess the safety and acceptability of vaginal use of 0.1% and 0.25% UC781 gel in sexually active, HIV-uninfected women, their male partners, and sexually abstinent HIV-infected women. The population will consist of 36 sexually active HIV-uninfected women and up to 36 of their male partners as well as 18 sexually abstinent HIV-infected women.

In Thailand, a completed Phase 1 study currently under analysis examined the safety and acceptability of UC781 topical vaginal microbicide in women, as well as acceptability in their male partners. This study was a single center, double-blind, placebo-controlled trial with twice-daily use of UC781 0.1%, UC781 0.25%, or placebo gel (randomized in a 1:1:1 ratio) for 14 days. The study, which enrolled 45 HIV-uninfected, sexually active couples at low risk for HIV infection, included women 18 to 50 years old and male partners of any age who were to have protected (condom) vaginal sex at least twice a week.

Safety was evaluated after 7 and 14 days of product use and incorporated interviews, physical exam including genital exam with colposcopy, lab evaluations, and completion

of a daily diary card. Male sexual partners of HIV-uninfected women were enrolled for an acceptability assessment. Acceptability in all participants was evaluated by questionnaire after 14 days of product use and by focus group after completing product use.

The primary endpoints of the study included safety and toxicity of study gel assessed weekly according to genital tract inflammation or epithelial disruption, symptoms of irritation, changes in vaginal flora, assessment of pro-inflammatory cytokines in the genital tract, and other adverse events. It also examined acceptability of the study gel among women and men as assessed by structured questions using audio computer-assisted self interview (ACASI) and focus group discussions (two among women, two among men).

The primary endpoints of the study included safety and toxicity of study gel assessed weekly according to genital tract inflammation or epithelial disruption, symptoms of irritation, changes in vaginal flora, assessment of pro-inflammatory cytokines in the genital tract, and other adverse events; and acceptability of the study gel among women and men, assessed by structured questions using audio computer-assisted self interview (ACASI) and focus group discussions (two among women, two among men).

A Phase 1 study of the safety and persistence of topical UC781 vaginal gel (0.1%) in women was recently completed in Pittsburgh and is currently in analysis. This study was a single site, randomized, double-blind, placebo controlled, parallel assignment study with a single application of UC781 or placebo gel (randomized in a 1:1 ratio). Participants were assigned to one of eight groups: UC781 or placebo gel, and either 0, 2, 4, or 8 hours of product use/presence in the vagina. This study enrolled 60 HIV-uninfected women who agreed to be sexually abstinent from Visit 1 to the completion of Visit 4.

Safety was evaluated at each study visit, which were scheduled for 24 to 48 hours, 6 to 8 days, and 25 to 35 days following study entry and included pelvic exams, vital sign measurements, and laboratory evaluations. Colposcopy was performed at select study visits. Vaginal secretions were also collected at each study visit to assess the amount of microbicide remaining in the vagina following exposure to study product.

The primary endpoints of the study included evidence of > Grade 3 toxicity for hematology, liver, or renal function and/or macroscopic evidence of damage to the cervical, vaginal, and/or vulvar epithelium, including ulceration and other lesions, severe erythema, and/or severe edema.

3 OBJECTIVES

3.1 Primary Objectives

1. To evaluate the prevalence of spontaneous pregnancy loss in mothers exposed to an active study agent during pregnancy as compared to that in mothers not exposed to an active study agent during pregnancy.
2. To evaluate the prevalence of major malformations in infants of mothers exposed to an active study agent during pregnancy as compared to that in mothers not exposed to an active study agent during pregnancy.

3.2 Secondary Objectives

1. To monitor for adverse pregnancy outcomes
2. To evaluate growth parameters in the first year of life among infants born to mothers exposed to an active study agent during pregnancy, as compared to those of mothers not exposed to an active study agent during pregnancy.
3. To provide a cohort of infants not exposed to an active study agent representing the background incidence of major malformations among babies born to women participating in HIV prevention trials.

3.3 Exploratory Objectives

1. To monitor for select risks of prevention agents identified during pre-clinical reproductive toxicology studies by trimester(s) of exposure.
2. To evaluate the prevalence and persistence of HIV drug resistance mutations in plasma among HIV-infected infants.
3. To compare the results of developmental screening at select time points in the first year of life among infants born to mothers exposed to an active study agent during pregnancy as compared to those of mothers not exposed to an active study agent during pregnancy.

4 STUDY DESIGN

4.1 Identification of Study Design

The HIV Prevention Agent Pregnancy Exposure Registry will be a prospective observational cohort investigation of women with exposures to active and non-active study agents in trials investigating agents intended for HIV prevention and the infants

resulting from those pregnancies. Parent trial participants should be offered enrollment in the Registry whenever it is determined that there has been an exposure to a study agent during pregnancy.

Optimally, participant mothers will be enrolled in MTN-016 prospectively (prior to the outcome of pregnancy being known). Participant mothers may also be enrolled in the registry after the pregnancy outcome is known, but only up until one year following the date of pregnancy outcome diagnosis. Sites are encouraged to enroll participants as early in pregnancy as possible so as to maximize the validity of the data. A mother-infant pair may be enrolled up to the time the baby is one year of age, but retrospective data, i.e., data obtained after pregnancy outcome is known, will be omitted from the primary analysis (see Section 10).

4.2 Summary of Major Endpoints

- Pregnancy loss as evidenced by the following:
 - Negative urine pregnancy test performed by study staff
 - Clinically confirmed intrauterine demise at a gestation > 20 weeks
 - Ultrasound evidence at any gestation
- Major malformation (structural abnormality with surgical, medical, or cosmetic importance) identified before one year of life

Inclusion and exclusion criteria to be applied for the identification of major malformations will be consistent with those outlined in Holmes, 1999.⁴⁹ In summary, major malformations will include structural abnormalities meeting the following criteria:

1. Having surgical, medical, or cosmetic importance
2. Ascertained up to one year of age
3. Independently confirmed according to criteria outlined in the MTN-016 SSP Manual

The following will be excluded as major malformations:

1. All birth marks
2. All minor physical features
3. Deformities that represent the normal response of fetal tissue to mechanical forces, i.e., atypical body part growth and/or appearance attributable to fetal position and/or pressure of surrounding maternal tissue(s). For example, molding of the skull, also known as positional plagiocephaly, would not be considered a major malformation.
4. Structural abnormalities detected only at autopsy or at surgery

These inclusion/exclusion criteria will be applied to reported major malformations to determine their eligibility for inclusion as primary endpoints. A subset of the MTN-016 protocol team, in collaboration with external experts if required, will review, at least

annually, all reported major malformations for eligibility as inclusion as primary endpoints.

4.3 Description of Study Population

The study population will consist of current or recent participants who become or became pregnant during HIV prevention trials, or who have/had planned exposures in pregnancy safety studies (provided pregnancy outcome was diagnosed less than one year from date of Screening/Enrollment Visit), and the infants resulting from those pregnancies (provided infants have not yet reached their 1 year birth date).

HIV-infected participants and their infants will not be excluded from this registry. This registry may be open to participants from other HIV prevention trials not part of the MTN as approved by the MTN Executive Committee.

4.4 Time to Complete Enrollment

The time to complete enrollment will be dependent upon the number of pregnancies occurring within parent trials. Registration will remain open for the duration of MTN funding with the possibility of extension.

4.5 Expected Duration of Participation

The expected duration of participation is from the Mother's Screening and Enrollment Visit (optimally as close as possible to the onset of exposure during pregnancy) until follow-up is completed on the pregnancy outcome and/or infant (if the pregnancy results in a birth). If the pregnancy results or resulted in a live birth, the upper limit of scheduled participation may be one year of follow-up for the infant. If a pregnancy does not result in a live birth, participation would end after follow-up is completed on the pregnancy outcome, typically following the Pregnancy Outcome Visit.

4.6 Sites

The study is open to sites as determined by the MTN Executive Committee.

5 STUDY POPULATION

The study population will consist of female participants who become or became pregnant during HIV prevention agent trials, or who have or had planned exposures in pregnancy safety studies, and the infants resulting from those pregnancies. Mother participants must still be pregnant, or have had a pregnancy outcome diagnosis less than one year before screening/enrollment, and infant participants must be less than one year old. The study may include HIV-uninfected and -infected participants.

Mothers may participate in EMBRACE without participation of their infants; however, infants whose mothers have not enrolled in EMBRACE will not participate.

5.1 Selection of the Study Population: Mother

Recruitment

Potential participants will be recruited for EMBRACE as soon as possible after identification of pregnancy. Participants and their infants can be enrolled for subsequent pregnancies; subsequent pregnancies of an EMBRACE participant will require a separate informed consent process to be initiated. With assistance from the SDMC, the Site Investigator of Record (IoR) or designee will identify participants who become pregnant during participation in parent microbicide trials.

Retention

Each site will establish participant retention procedures. Study site staff members at each site are responsible for developing and implementing site-specific standard operating procedures (SOPs) to target high rates of retention.

Co-Enrollment Guidelines

Co-enrollment in other trials not involving investigational agents is permitted by this protocol. Co-enrollment in EMBRACE and studies of investigational agents (other than the parent protocol) will be considered on a case-by-case basis and must be approved by the Protocol Chair(s). However, it is expected that the Protocol Chair will permit co-enrollment in studies of potential benefit to the mother or baby including prevention of mother-to-child transmission (PMTCT) and/or adult or infant treatment of HIV infection.

5.2 Inclusion Criteria: Mother

Individuals who meet the following criteria are eligible for inclusion in the study:

1. Able and willing to provide written informed consent to take part in the study
2. During participation in a parent protocol, has/had a known confirmed pregnancy, meeting at least one of the following sets of criteria in A or B:
 - A: Two consecutive monthly study visits with positive pregnancy tests
 - B: One or more of the following assessments:
 - Auscultation of fetal heart tones
 - Positive pregnancy test confirmed by clinic staff in the presence of clinically confirmed enlarged uterus
 - Positive pregnancy test confirmed by clinic staff in the presence of missed menses by participant report
 - Clinical assessment of fetal movement
 - Demonstration of pregnancy by ultrasound
3. Able and willing to provide adequate locator information, as defined in site SOPs

Note: Participants do not have to be currently enrolled or engaged in follow-up in a parent protocol to participate in EMBRACE.

5.3 Exclusion Criteria: Mother

Individuals who meet the following criteria at screening will be excluded from the study:

1. Has any condition that in the opinion of the investigator or designee, would complicate interpretation of study outcome data, make participation in the study unsafe, or otherwise interfere with achieving the study objectives
2. Pregnancy outcome was diagnosed greater than one year ago

5.4 Inclusion Criteria: Infant

Individuals who meet the following criteria are eligible for inclusion in the study:

1. Has written informed consent provided by parent(s)/guardian to take part in the study in a manner consistent with local standards, site Institutional Review Board (IRB) guidance and the US Code of Federal Regulations (CFR)
2. Born to EMBRACE participant mother from pregnancy concurrent with participation in parent study

5.5 Exclusion Criteria: Infant

Individuals who meet the following criteria at screening will be excluded from the study:

1. Has any condition that, in the opinion of the investigator or designee, would complicate interpretation of study outcome data, make participation in the study unsafe, or otherwise interfere with achieving the study objectives
2. Has reached 1 year birth date

6 STUDY PRODUCT

EMBRACE will not include the administration of any study product.

7 STUDY PROCEDURES

An overview of the study visits and evaluations schedule is presented in Appendices I and II. Presented in this section is additional information on visit-specific study

procedures. A detailed instruction guide including visit windows will be provided in the EMBRACE Study-Specific Procedures Manual, which will be available at www.mtnstopshiv.org.

Depending on the timing of screening and enrollment for the mother-infant pair, which may occur up until the time the infant is one year old, the follow-up schedule for mother and infant participants may vary. In cases where protocol-defined visits were missed because the mother or infant had not yet enrolled, such missed visits will not be considered protocol violations. Study visits for mothers and infants may or may not occur on different days. For example, the Screening and Enrollment Visit for the mother and the Newborn/Initial Visit for the infant could occur on the same day in cases where the infant is already born.

It is expected that, in most cases, all required visit procedures will be completed at one visit; however, more than one visit may be completed if needed to complete all required procedures. If a participant is being followed in her parent trial, site staff will make every effort to schedule and complete MTN-016 visits on the same day as parent protocol visits. Completion of the parent MTN protocol visit should take priority if time or other factors do not allow for both study visits to be completed on the same day.

Because laboratory testing, ultrasound, or other testing procedures may be performed at study visits, a post-visit contact may be required after a study visit to provide participants with their test results, clinically relevant post-test counseling, and/or clinically indicated treatment. Study staff may complete these contacts at the study site or at community-based locations, depending on site capacities and participant preferences. All contacts will be documented in participant study records and written documentation of test results will be provided upon request to participants and/or their primary care providers.

Scheduled visits for the mother include the following:

- Screening and Enrollment
- Quarterly
- Pregnancy Outcome

Scheduled visits for the infant include the following:

- Newborn/Initial Visit
- Month 1
- Month 6
- Month 12

7.1 Screening and Enrollment: Mother

Day 0 for the participant mother will be the day of the Screening and Enrollment Visit – Mother.

- Administrative

- Obtain written informed consent for screening and enrollment of mother
- Identification number assignment (PTID)
- Locator information
- Eligibility assessment
- Reimbursement
- Schedule next visit
- Clinical
 - Obtain medical history
 - Obtain medication history
 - Obtain pregnancy history
 - Obtain genetic screening history

7.2 Quarterly Visit: Mother

If the mother is still pregnant, a quarterly visit will occur with the following components:

- Administrative
 - Locator information
 - Reimbursement
 - Schedule next visit
- Clinical
 - Update medical history
 - Update medication history
 - Update pregnancy history, including but not limited to pregnancy-related morbidities such as hypertensive disorders of pregnancy, antenatal hemorrhage, and abnormal placentation
 - Update genetic screening history

7.3 Ultrasound Exam

If the mother is still pregnant, a minimum of one ultrasound exam should be performed. In cases where the study site already has a copy of results for an ultrasound meeting gestational age and measurement criteria recommended by the protocol, and allowing for complete documentation of the Ultrasound Results Form, the protocol defined ultrasound may be omitted.

When deemed necessary by the IoR or designee, additional ultrasounds may be ordered to confirm gestational age or provide follow-up information on potentially abnormal findings.

- For each ultrasound exam
 - Perform or refer for performance of obstetrical ultrasound
 - Complete ultrasound results form, including dating and anatomical survey data (as appropriate by gestational age).

Note: When possible, site staff should attempt to facilitate the performance of at least one obstetrical ultrasound for anatomical survey during the time period corresponding with 20 and 28 weeks gestation.

Note: Ultrasound exams should include, at a minimum, the following measurements:

- *If estimated gestational age is <14 0/7 weeks, a crown-rump length*
- *If estimated gestational age is 14 0/7 weeks or greater, a biparietal diameter (a femur length is useful but not required)*

7.4 Pregnancy Outcome Visit: Mother

- Administrative
 - Locator information
 - Reimbursement
 - Schedule next visit if indicated
- Clinical
 - Update medical history
 - Update medication history
 - Update pregnancy history, including but not limited to pregnancy-related morbidities such as hypertensive disorders of pregnancy, antenatal hemorrhage, and abnormal placentation, if applicable
 - Obtain pregnancy outcome
 - Type and number of pregnancy outcome(s)
 - Gestational age at pregnancy outcome
 - Method of calculation for gestational age at pregnancy outcome
 - If delivery, type of delivery (e.g., vaginal, vaginal forceps-assisted, vaginal vacuum-assisted, cesarean section)
 - Complications related to pregnancy outcome
 - Delivery complications (e.g., intrapartum and/or postpartum hemorrhage, non-reassuring fetal status, chorioamnionitis)
 - Other complications not related to a delivery
 - Baseline infant information (as available and per infant)
 - Number (e.g., singleton, twin, etc.)
 - Sex
 - Weight
 - Length
 - Head circumference
 - Apgar scores
 - Medical history (e.g., sepsis, respiratory distress, any abnormalities noted on infant exam)
 - Medication history

7.5 Interim Visit: Mother

- Administrative
 - Locator information
 - Schedule next visit if indicated
- Clinical
 - Update medical history if indicated
 - Update medication history if indicated
 - Update pregnancy history if applicable

7.6 Newborn/Initial Visit:

If the pregnancy results or resulted in a live-born infant, a Newborn/Initial Visit for the infant should occur during the first ten days of life when possible. If local custom, medical status, or other reason deemed acceptable by the IoR/designee, prohibits a study visit during this time period, the infant's first visit may be delayed, or the visit may occur off-site, if possible. If the timing of the Newborn/Initial Visit corresponds to the visit window for a scheduled 1-, 6-, or 12-month follow-up visit, procedures need not be duplicated within that particular visit window.

- Administrative
 - Written informed consent for screening and enrollment of infant
 - Eligibility assessment
 - Identification number assignment (if not already assigned)
 - Locator information
 - Reimbursement
 - Schedule next visit
- Clinical
 - Medical history
 - Medication history
 - Weight
 - Length
 - Head circumference
 - Abdominal circumference (preferably within 10 days, but no later than Month 1 Visit)
 - Physical exam
 - Photographic documentation of suspected or confirmed anomalies as clinically indicated (to be sent to MTN Statistical Data Management Center (SDMC))
- Laboratory
 - If clinically indicated according to the IoR/designee, FDA-approved HIV test with confirmatory tests as indicated

7.7 Months 1, 6, and 12: Infant

The first visit for an infant may also occur after the first ten days of life in cases where the mother-infant pair is not yet enrolled, provided that this first visit for the infant occurs before the infant has reached their 1 year birth date. Day 0 for the infant is on the first day of life.

Months 1, 6, and 12

- Administrative
 - Locator information
 - Reimbursement
 - Schedule next visit (1 and 6 months only; if indicated for month 12)
- Clinical
 - Update medical history
 - Update medication history
 - Weight
 - Length
 - Head circumference
 - Physical exam (see Appendix III)
 - Developmental screening assessment (6 and 12 months only)
 - If specific consent for this has been obtained, photographic documentation of suspected or confirmed anomalies as clinically indicated. Photographs should include at least the following images: Anterior-posterior (AP) and lateral of face/head, neck and upper third of thorax, standing up picture of child (front and back), hands, feet, as well as AP and lateral images of any suspected abnormal finding.
- Laboratory
 - As clinically indicated to follow a previous abnormal finding at the Newborn/Initial Visit

7.8 Interim Visit: Infant

- Administrative
 - Locator information
 - Schedule next visit if indicated
- Clinical
 - Update medical history
 - Update medication history
 - If specific consent for this has been obtained, photographic documentation of suspected or confirmed anomalies as clinically indicated. Photographs should include at least the following images: Anterior-posterior (AP) and lateral of face/head, neck and upper third of thorax, standing up picture of

child (front and back), hands, feet, as well as AP and lateral images of any suspected abnormal finding.

- Laboratory
 - As indicated to follow a previous abnormal finding
 - As indicated for confirmatory testing
 - As indicated for infants identified as HIV-infected (see Section 7.9)

7.9 Procedures for Infants of HIV-infected Mothers

Mothers who are diagnosed with HIV infection according to criteria in the EMBRACE SSP Manual may elect to have their infants tested for HIV infection. Infants diagnosed with HIV infection according to criteria in the EMBRACE SSP Manual will be tested for HIV-1 drug resistance mutations as soon as possible after diagnosis of HIV infection.

Infant testing may occur at a scheduled visit or an Interim Visit.

Testing during follow-up may include the following assays performed by the Site and/or Network Laboratory according to capacity:

- HIV-1 testing (RNA and DNA, as indicated)
- Standard genotypic resistance testing
- Additional resistance testing may include allele-specific polymerase chain reaction (PCR) for relevant drug resistant codons and single genome sequencing

7.10 Procedures for Infants with Suspected or Confirmed Major Malformation

If, at any point in the study participation, a major malformation is suspected in an infant or other pregnancy outcome, a Major Malformation Eligibility Assessment Form, and, if needed, a Major Malformation Assessment Form should be completed. These forms will be included in the EMBRACE SSP Manual at <http://www.mtnstopshiv.org>.

7.11 Specimen Collection

Each study site will adhere to the standards of good clinical laboratory practices (GCP), the HPTN-MTN Network Laboratory Manual (www.mtnstopshiv.org), DAIDS Laboratory Requirements (<http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/Laboratories.htm>), EMBRACE Study Specific procedures manual (www.mtnstopshiv.org), and site standard operating procedures for proper collection, processing, labeling, transport, and storage of specimens at the local laboratory. Specimen collection, testing, and storage at the site laboratories will be documented when applicable using the Laboratory Data Management System (LDMS). In cases where laboratory results are not available due to administrative or laboratory error, sites are permitted to re-collect specimens.

7.12 Specimen Handling

Specimens will be handled in accordance with Requirements for DAIDS Sponsored and/or Funded Laboratories in Clinical Trials (<http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/Laboratories.htm>).

7.13 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study as recommended by the CDC and NIH. All biological specimens will be transported using packaging mandated by CFR 42 Part 72. All dangerous goods materials, including diagnostic specimens and infectious substances, must be transported according to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations. This applies to both US and international sites. Biohazardous waste will be contained according to institutional, transportation/carrier, and all other applicable regulations.

7.14 Study Staff Performing Infant Procedures

Pediatric exams will be performed by a clinician who has completed some formal training in pediatrics. Developmental screening assessments will only be performed by staff members who have completed specialized training provided for the performance of developmental screening assessments.

8 ASSESSMENT OF SAFETY

EMBRACE is an observational study involving no investigational products or procedures associated with significant risk to participants. Therefore, few safety concerns are expected as a result of study participation. The study site IoR is responsible for continuous close safety monitoring of all study participants and for alerting the protocol team if unexpected concerns arise. Study sites will have written procedures for ensuring prompt reporting to the IRB/Ethics Committees (ECs), of any unanticipated problem involving risks to subjects or others.

The Manual for Expedited Reporting of Adverse Events to DAIDS will not be used for this study for the following reasons: 1) this study is observational in nature; and, 2) this study does not involve a study drug or intervention.

The study team will monitor for and track unanticipated problems definitely, probably, or possibly related to study procedures and/or to participation in the study, until participants' time of termination from the study. Study staff will provide clinically appropriate treatment and/or referrals should any such problems occur.

For EMBRACE participants who are or were enrolled in a parent study, any unanticipated problems will be reported to the DAIDS Medical Officer concurrent with problem reporting to the responsible site IRB/ECs overseeing the research according to pre-established procedures as required by 45 CFR 46. Participants co-enrolled in EMBRACE and a parent study will have SAEs and Expedited Adverse Events (EAEs) considered reportable in the parent study reported via the safety reporting system utilized by the parent study. Once a participant is no longer enrolled in the parent study, any unanticipated study-related injury will be reported to the site IRB/EC and DAIDS Medical Officer.

Participants may experience social harms—non-medical adverse consequences as a result of their participation in the study. All reports of social harm, regardless of severity, will be collected for data purposes. Social harms that are judged by the IoR to be serious or unexpected will be reported to responsible site IRB/ECs at least annually, or according to their individual requirements. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant as needed.

9 CLINICAL MANAGEMENT

9.1 Criteria for Early Termination

Participants may voluntarily withdraw from the study for any reason at any time. The principal investigators may, with the approval of the Protocol Safety Review Team (PSRT), withdraw participants to protect their safety, and/or if participants are unable or unwilling to comply with study procedures.

Early (premature) termination of study participants will occur only under certain criteria. The criteria for early termination from the study for an individual participant are:

- Request by participant to withdraw
- In the case of infants, the legal guardian declines follow-up evaluations
- Request by the principal investigator to protect the participant's safety and/or if the participant is unable or unwilling to comply with study procedures

At least one of the above criteria must be met for the participant to be terminated early from the study.

9.2 Findings Identified During Follow-up

Any infant noted to have abnormal or clinically suspicious findings on physical exam, developmental screening assessment, growth monitoring and/or testing will be provided with or referred to local providers of pediatric care. Note, all women, upon enrolling in

the study, will receive referrals for prenatal care if they are still pregnant. In the case of HIV drug resistance mutation testing, the IoR/designee will make reasonable efforts to furnish a written copy of the results to the infant's care provider, with permission of the parent(s) or guardian, as applicable. In the case of identified structural anomalies and/or potential deviations from normal health and/or development, the IoR/designee will make every effort to communicate directly with the referral entity, provided that consent has been obtained for this purpose.

10 STATISTICAL CONSIDERATIONS

10.1 Overview and Summary of Design

This is a prospective observational cohort study of pregnant women and their infants identified in HIV prevention agent trials. Infants will be followed for 12 months. Infants from multiple-birth pregnancies are eligible for enrollment. Participants and their infants can be enrolled for subsequent pregnancies and will need to be re-enrolled into EMBRACE.

A large portion of participants are expected to come from one trial: MTN-003. We expect that the MTN-003 trial population will be similar to the HPTN 035 trial population; as such, we expect that about 590 (~14%) of the 4200 enrolled women in MTN-003 will get pregnant. Given that it is difficult to anticipate the number of pregnant women generated by future and other HIV prevention trials, we will conservatively estimate the number of eligible pregnant women at 590. Based on the latest HPTN 035 data, we estimate that 350 live infants (~60% of 590) will be eligible for EMBRACE.

In an exploratory analysis, we will compare developmental endpoints across arms. The nature of this analysis cannot be specified as the protocol has not yet identified developmental assessment measures validated for African populations.

10.2 Study Endpoints

A pregnant woman is defined as a woman meeting the criteria outlined in Section 5.2.

Primary and secondary study endpoints will be collected from the pregnant women, their pregnancy outcomes, and the infants born from these women.

10.2.1 Primary Endpoints: Pregnant Women

Consistent with the primary study objectives, the following primary endpoint will be assessed for pregnant women:

- Pregnancy loss as evidenced by negative urine pregnancy test
- Clinical confirmation of intrauterine demise

- Ultrasound evidence of pregnancy loss at any gestation

10.2.2 Primary Endpoints: Infants

Consistent with the primary study objectives, the following primary endpoints (unpooled) will be assessed for infants:

- Major malformation (structural abnormality with surgical, medical, or cosmetic importance) identified before one year of life (see Section 4.2 for a more detailed description).

10.2.3 Secondary Endpoint: Pregnant Women

Consistent with the secondary study objectives, the following secondary endpoint will be assessed for women enrolled in the registry:

- Adverse pregnancy outcomes, including but not limited to ectopic pregnancy, hypertensive disorders of pregnancy, gestational diabetes, preterm delivery, and postpartum hemorrhage

10.2.4 Secondary Endpoint: Infants

Consistent with the secondary objectives, the following secondary endpoints will be assessed for infants:

- Growth parameters in the first year of life (birth, 1, 6, and 12 months):
 - Birth weight
 - Serial length, weight, and head circumference

10.3 Sample Size

As the HPTN 035 trial population will be very similar to the MTN-003 trial population, we can, based on the latest HPTN 035 data, estimate the number of eligible pregnant women from the 4200 enrolled in MTN-003 to be about 590 (~14%), and the number of live infants to be approximately 350 (~60% of 590). This is a conservative estimate as it is difficult to anticipate the number of pregnant women generated by future and other MTN trials.

Pregnant women may have elected to terminate participation in the parent study and/or do not wish to enroll in this study and/or have been lost to follow-up in the parent study. Of the 590 eligible women, we estimate that 15% of the women eligible for this study will not enroll due to one of the above reasons. Thus, we estimate that 500 pregnant women and 300 live infants (i.e., 60% of 500) will enroll in this study. We will target no more than 5% lost to follow-up of pregnant women and of mother/infant pairs.

In the absence of a contraceptive effect for any of the HIV prevention agents, we expect the number of pregnant women to be approximately balanced, producing about 100 pregnant women in each of the five arms of the MTN-003 trial. Similarly, the number of live infants should be approximately balanced, producing about 60 live infants in each of the five arms of the MTN-003 trial. The primary objective involves comparison of the placebo arms to each of the active arms. Vaginal and oral placebo arms will be pooled, therefore, each comparison with an active arm will involve about 300 pregnant women (or about 180 live infants) in a 2:1 placebo to active product ratio. Pregnant women are not randomized to the intervention arms, thus it is anticipated that a certain level of imbalance will be present that may increase or decrease power. We will make the conservative assumption that each of the three comparisons will involve 180 (placebo) and 80 (active) pregnant women or 108 (placebo) and 48 (active) live infants.

The proportion of pregnancy loss in HPTN 035 among the women with two consecutive monthly study visits with positive pregnancy tests is about 20%. Assuming that the pregnancy loss is 20%, the study will have at least 80% power to detect a minimum absolute difference of 17% (i.e., 20% vs. 37% pregnancy loss in the placebo and active arms, respectively).

Major malformations in infants are relatively rare, thus, for the purpose of power calculations, we will conservatively assume that the proportion of major malformations will be 5%. The study will, therefore, have at least 80% power to detect a minimum absolute difference of 16% (i.e., 5% vs. 21% in the placebo and active arms, respectively, about a 4-fold increase in the rate of major malformations).

Maternal mortality for African and Indian MTN sites ranges from 0.2% to 2%, while the infant mortality rate (i.e., death of infants below one year of age) ranges from 5 to 11 per 100 live births.⁵⁰⁻⁵⁷ The latter will impact the sample size available for the analysis of the secondary endpoints on the growth parameters of infants in the first year of life. Thus, up to 11% of the infants might not have complete growth parameter assessment over the first year. Therefore, comparison of infant growth in the first year might include 96 (placebo) and 43 (active) infants. Note that an 11% decrease in sample size translates into a 6% absolute decreases in power (i.e., from 80% to 74% power).

10.4 Blinding

Blinding/unblinding processes will be dictated by the parent study from which the participants come. Both study staff and participants will be blinded to the random assignments of participants assigned to study treatment groups that include a study product. However, the assignment of participants to the mode of administration (e.g., vaginal or oral interventions in MTN-003) cannot be blinded. Randomization documentation and other pharmacy records must not be accessible to study staff members who complete other study procedures with participants.

Blinding will be maintained until all data are entered into the parent study database, all study endpoint data and other data included in the final analysis have been cleaned and

verified, and the data are ready for final analysis in the parent study. Unblinding of data will only occur after the unblinding of the data in the parent study. This will be explained to participants as part of the study informed consent process.

There are no circumstances under which it is expected that unblinding will be necessary for the provision of medical treatment or to otherwise protect the safety of study participants. If an Investigator feels that specific product knowledge is necessary to protect participant safety, the Investigator will notify the Protocol Chair, Protocol Biostatistician, and DAIDS Medical Officer (or designees) to consider and jointly rule upon the request.

10.5 Participant Accrual and Retention

All women meeting criteria outlined in Section 5, including those identified as both pregnant and HIV-infected, will be recruited into this study. Once a participant has enrolled in the study, the study site will make every reasonable effort to retain her for the entire study period. A maximum of 5% loss-to-follow-up of enrolled pregnant women and babies will be targeted.

10.6 Data and Safety Monitoring Analysis

10.6.1 Study Monitoring Committee (SMC)

No Data and Safety Monitoring Board (DSMB) oversight is planned for this observational study. The MTN SMC will conduct interim reviews of study progress (pooled data only), including rates of participant accrual, retention, completion of primary and main secondary endpoint assessments, and study or lab issues. These reviews will take place approximately every 4 to 6 months, or as needed. At the time of these reviews, or at any other time, the SMC may recommend that the study proceed as designed, proceed with design modifications, or be discontinued. The SMC may consider recommending termination of this study if recruitment and retention are lower than targeted, or if study data quality is poor.

10.7 Data Analysis

When the use of descriptive statistics to assess group or site characteristics or differences is required, the following methods will be used: for categorical variables, the number and percent in each category; for continuous variables, the mean, median, standard deviation, quartiles and range (minimum, maximum). Typically, within-arm assessment of the change from the baseline measurement to a follow-up measurement will be analyzed using McNemar's test (for categorical response variables) or the paired *t*-test or Wilcoxon signed-ranks test (for continuous variables). In general, when use of formal testing to assess differences between arms is required, the following methods will be used: for binomial response variables, chi-square tests and logistic regression; for continuous variables, *t*-tests and linear regression, or nonparametric methods if data are non-Normal. To assess baseline differences between arms, participants will be

compared for baseline characteristics including demographics and laboratory measurements using descriptive statistics.

For each of the three active interventions in MTN-003, the proportion of pregnancy loss will be compared to the one observed in the pooled placebo arms (i.e., vaginal and oral placebos) using a Fisher exact test, and a two-sided level of significance. Using the same method, proportions of major malformations observed in infants will be compared.

For the secondary endpoints, the proportion of adverse pregnancy outcomes will be compared using similar methods as described above. For each of the growth parameters (i.e., birth weight, serial length, weight, and head circumference), the mean observed in the active agent and non-active agent groups will be compared using a Student's *t*-test at selected time points: birth, 1, 6, and 12 months. More generally, GEE (Generalized Estimating Equation) methods and robust variance estimates will be used to evaluate group differences over the first year. Incomplete data from infants that are lost to follow-up or terminate early in the study (including from death) will be included in these analyses if a growth parameter is available at one of the selected time points.

Finally in an exploratory analysis, we will compare developmental endpoints across arms. However, the nature of these analyses cannot be specified as the protocol has not yet identified developmental assessment measures validated for African populations.

Note that caution must be exercised in the interpretation of any difference (or lack of difference) found. Although the active agent and non-active agent groups should be comparable at baseline (in the parent studies) due to randomization, women who get pregnant in the active agent group may not be comparable to women who get pregnant in the non-active agent group (possibly due to a potential contraceptive effect of the active intervention).

11 DATA HANDLING AND RECORDKEEPING

11.1 Data Management Responsibilities

Study case report forms will be developed by the MTN SDMC in conjunction with the protocol team. Quality control reports and queries routinely will be generated and distributed by the SDMC to the study sites for verification and resolution. As part of the study activation process, each study site must identify all case report forms to be used as source documents. Data are transferred to the MTN SDMC, entered, and cleaned using the DataFax data management system.

11.2 Source Documents and Access to Source Data/Documents

All study sites will maintain source data/documents in accordance with Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials (<http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/>)

Each investigator will maintain, and store securely, complete, accurate, and current study records throughout the study.

Study records must be maintained on site for the entire period of study implementation. Thereafter, instructions for record storage will be provided by DAIDS. No study records may be moved to an off-site location or destroyed prior to receiving approval from DAIDS.

11.3 Quality Control and Quality Assurance

All study sites will conduct quality control and quality assurance procedures for EMBRACE in accordance with Requirements for Clinical Quality Management Plans at DAIDS Funded and/or Supported Clinical Research Sites (<http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/ClinicalSite/ClinQualMgmtReqs.pdf>)

11.4 Study Activation

Following IRB/EC review and approval, all study sites will complete DAIDS Protocol Registration procedures in accordance with the current DAIDS Protocol Registration Policy and Procedures Manual (http://rcc.tech-res.com/DAIDS%20RCC%20Forms/HighlightOff_PROManual_v04b.pdf).

Pending successful protocol registration, submission of all other required study activation documents to the MTN CORE, and DAIDS approval, MTN CORE staff will “activate” the site to begin study operations. Study implementation may not be initiated until a study activation notice is provided to the site by the MTN CORE.

11.5 Study Coordination

Study implementation will be directed by this protocol and further guided by the Study-Specific Procedures Manual provided by FHI, SCHARP, and the MTN NL.

12 CLINICAL SITE MONITORING

Study monitoring will be carried out by PPD Inc. (Wilmington, NC) in accordance with Requirements for On-Site Monitoring of DAIDS Funded and/or Sponsored Clinical Trials (http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/ClinicalSite/OnsiteMonitor_Reqs.pdf). Study monitors will visit the site to do the following:

- Review procedures and documentation
- Assess compliance with the study protocol, GCP guidelines, and applicable regulatory requirements (US and non-US), including US Code of Federal Regulations (CFR) Title 45 Part 46, and Title 21 Parts 50, 56, and 312.
- Perform source document verification to ensure the accuracy and completeness of study data
- Assess implementation and documentation of internal site quality management procedures
- Assess site staff training needs

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of the MTN CORE, SDMC, and NL, NIAID, and local and US regulatory authorities. A site visit log will be maintained at the study site to document all visits.

13 HUMAN SUBJECTS PROTECTIONS

The investigators will make efforts to minimize risks to participants. Before beginning the study, the investigators will have obtained IRB/EC approval. The investigators will permit audits by the NIH, MTN CORE, applicable US/local government and regulatory authorities, IRBs/ECs, or any of their appointed agents.

13.1 Institutional Review Boards/Ethics Committees

Each participating institution is responsible for assuring that this protocol and the associated site-specific informed consent documents and study-related documents (such as participation education and recruitment materials) are reviewed by an EC or IRB responsible for oversight of research conducted at the study sites. Any

amendments to the protocol must be approved by the responsible IRB/EC and DAIDS prior to implementation.

Subsequent to the initial review and approval, the responsible IRBs/ECs must review the study at least annually. Each investigator will make safety and progress reports to the IRBs/ECs at least annually and within three months after study termination or completion. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others. Study sites will submit documentation of continuing review to the DAIDS Protocol Registration Office in accordance with the DAIDS Protocol Registration Policy and Procedures Manual.

13.2 Protocol Registration

Each study site will complete protocol registration with the DAIDS Regulatory Compliance Center (RCC) Protocol Registration Office. For additional information, refer to the protocol registration documents located at <http://rcc.tech-res.com/forms.htm>. Protocol registration must occur as a condition for site-specific study activation; no participants may be screened or enrolled in this study prior to obtaining protocol registration approval and completion of all other study activation requirements. MTN CORE (FHI) staff will notify each study site when all activation requirements have been met by issuing a site-specific study activation notice. Study implementation may not be initiated until the activation notice is issued.

The study will be conducted in full compliance with the protocol. The protocol will not be amended without prior written approval by the Protocol Chairs and DAIDS Medical Officer. All protocol amendments must be submitted to and approved by the relevant IRBs/ECs and the RCC Protocol Registration Office prior to implementing the amendment.

13.3 Risk Benefit Statement

13.3.1 Risks

General

Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of questions. Participants may also feel worried while waiting for their ultrasound results.

Although study sites make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could become known as HIV-infected or at "high risk" for HIV infection). For example, participants could be

treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities.

Risks for Infants

For the infant, phlebotomy may lead to bruising, swelling, or infection (rare) at the site of the blood draw. These are considered very rare risks and occur in less than 5% of people undergoing phlebotomy.

13.3.2 Benefits

Participants in this study may experience no direct benefit. Participants may benefit from earlier than normal prenatal care, and/or referral to PMTCT services. Participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may help prevent adverse pregnancy outcomes in the future.

In addition to the benefits listed, infant participants may also have abnormalities detected as part of the evaluations in this investigation that may not have otherwise been detected. The IoR/designee will initiate referrals to local providers for ongoing evaluation and care of such infants. Infant participants may have the opportunity to access earlier directed care for certain abnormalities, which could improve prognosis depending on the condition.

13.4 Informed Consent Process

Written informed consent will be obtained from all potential study participants and from the parent(s)/guardian for infant participants, prior to the initiation of any study-related procedures. Sites may elect to use the combined mother and infant Sample Informed Consent documents (Appendix IV) or the separate mother (Appendix V) and infant (Appendix VI) Sample Informed Consent documents as applicable. Study staff will administer a comprehension checklist to potential participants and the parent(s)/guardian for infant participants, prior to obtaining written informed consent to ensure that participants and the parent(s)/guardian for infant participants, fully comprehend the nature of the study. The comprehension checklist will be included in the MTN-016 SSP Manual. In obtaining and documenting informed consent, the investigators and their designees will comply with applicable local and domestic regulatory requirements and will adhere to Good Clinical Practices (GCP) and to the ethical principles that have their origin in the Declaration of Helsinki. Study staff must document the informed consent process in accordance with the Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. Participants are provided with copies of the informed consent forms if they are willing to receive them. Each study site is responsible for developing study informed consent forms for local use, based on the templates in Appendices IV, V, VI, and VII that describe the purpose of screening and of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations. The study site

also is responsible for translating the template form into local languages, and verifying the accuracy of the translation by performing an independent back-translation.

13.5 Participant Confidentiality

All study procedures will be conducted in private, and every effort will be made to protect participant privacy and confidentiality to the extent possible. Each study site will implement confidentiality protections that reflect the local study implementation plan and the input of study staff and community representatives to identify potential confidentiality issues and strategies to address them. In addition to local considerations, the protections described below will be implemented at all sites.

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. Participants' study information will not be released without their written permission, except as necessary for review, monitoring, and/or auditing by any of the following:

- DAIDS and/or its contractors, including study monitors
- Representatives of the MTN CORE, SDMC, and/or NL
- Other government and regulatory authorities
- Site IRBs/ECs

13.6 Special Populations

This section outlines considerations made for the inclusion or exclusion of special populations in this study.

13.6.1 Pregnant Women

Pregnant women will be offered enrollment in this study in accordance with guidelines set forth in the US 45 CFR 46.

13.6.2 Children

Infants born to women participating in EMBRACE will be offered enrollment in this study in accordance with guidelines set forth in the US 45 CFR 46.

13.7 Compensation

Compensation will be provided to participants according to guidelines established by local IRBs/ECs.

13.8 Access to HIV-related Care

13.8.1 HIV Counseling and Testing

HIV testing for participant infants will be offered, as clinically indicated, including relevant pre-test and post-test counseling, by the study. Infants who have positive or indeterminate results will have limited follow-up confirmatory testing provided by the study. Referral for additional counseling related to testing or diagnosis will occur if needed or requested by the mother or guardian.

13.8.2 Care for Participants Identified as HIV-Infected

Parents or guardians (as applicable) will be provided with HIV test results for their infant in the context of post-test counseling. Parents/guardians of infants who are identified as HIV-infected during the study will be referred to available sources of medical and psychosocial care and support, and local research studies for HIV-infected infants.

13.9 Study Discontinuation

The NIAID, MTN, Office of Human Research Protections (OHRP), other applicable government or regulatory authorities, or site IRBs/ECs may discontinue this study at any time.

14 PUBLICATION POLICY

DAIDS/NIAID and MTN policies will govern publication of the results of this study. Any presentation, abstract, or manuscript will be submitted by the Investigator to the MTN Manuscript Review Committee, NICHD and DAIDS for review prior to submission.

15 APPENDICES

APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS (Mother)

	Screening/ Enrollment	Ultrasound	Quarterly	Pregnancy Outcome	Interim
Informed Consent	X				
PTID	X				
Locator	X		X	X	X
Eligibility Assessment	X				
Reimbursement	X		X	X	
Schedule Study Visit	X		X	*	*
Medical History	X		X	X	*
Medication History	X		X	X	*
Pregnancy History	X		X	*	*
Genetic Screening History	X		X		
Ultrasound (with follow-up as clinically indicated)		*			
Pregnancy Outcome				X	

* if indicated

APPENDIX II: SCHEDULE OF STUDY VISITS AND EVALUATIONS (Infant)

	Newborn/ Initial Visit	Month 1 Visit	Month 6 Visit	Month 12 Visit	Interim Visit
Informed Consent	***				
PTID	****				
Locator	X	X	X	X	X
Eligibility Assessment	X				
Reimbursement	X	X	X	X	
Schedule Study Visit	X	X	X	*	*
Medical History	X	X	X	X	X
Medication History	X	X	X	X	X
Weight	X	X	X	X	
Length	X	X	X	X	
Head Circumference	X	X	X	X	
Abdominal Circumference	**				
Physical Exam	X	X	X	X	*
Photographic Documentation	*	*	*	*	*
HIV Test with Confirmatory Test	*	*	*	*	*
Procedures for HIV-infected infants <ul style="list-style-type: none"> • HIV-1 testing • Standard genotypic resistance testing • Plasma for storage • Additional resistance testing per Section 7 	*	*	*	*	*
Developmental Screening Assessment			X	X	

* if indicated

** preferably within 10 days, but no longer than 1 month after birth

*** if consent has not been obtained during the mother's screening/enrollment visit

**** if not already assigned

APPENDIX III: COMPONENTS OF EXAMINATIONS

Neonatal Physical Exam (see MTN-016 SSP Manual for details)

Growth parameters

- Assessment of gestational age by physical parameters
- Length, weight and head circumference, with locally derived percentiles, if available
- Assessment of proportionality and symmetry
- Specific measurements where indicated by observation

General appearance

- Tone, posture, positioning, alertness, vigor, color, respiratory effort and other observations

Detailed examination

- Skin - pigmentation pattern (areas of increased or decreased pigmentation), dimples, vascular or other lesions, or excessive peeling
- Head - shape, symmetry, fontanelles
- Scalp - hair patterning and location of hair whorls
- Facial features
 - Eyes - pupils, orbits (hyper or hypotelorism) including palpebral fissure inclination and length
 - Ears - location, rotation, configuration and size, patency
 - Nose - appearance and patency of nares
 - Appearance of nasal bridge and columella
 - Mouth - appearance of upper lip, philtrum and vermilion border
 - Intra-oral examination of palate, alveolar ridges and tongue
 - Mandible - shape and symmetry
- Neck - posterior hairline, presence of sinus tracts, torticollis, redundant skin or webbing
- Chest - shape, symmetry, circumference, location of nipples, accessory nipples
- Cardiovascular - heart murmurs, pulses, blood pressure
- Lungs - symmetry of breath sounds
- Abdomen - appearance of umbilicus, muscle tone, integrity of wall, enlarged organs or masses
- Genitalia - size, appearance, palpation of testes (in males), presence of ambiguity
- Anus - location and patency
- Back - symmetry, spine, presence of sinuses or hair tufts in inter-gluteal cleft

- Extremities - proportions, appearance, range of motion (including hips), pulses, presence of reduction or duplication of segments
- Hands and feet - nails; creases (palmar, phalangeal, and flexion); joints
- Neurological - tone, response, alertness, reflexes

**APPENDIX IV: SAMPLE INFORMED CONSENT – MOTHER AND INFANT
(Screening and Enrollment)**

**MTN-016
HIV Prevention Agent Pregnancy Exposure Registry:
EMBRACE Study**

**Version 1.0
December 17, 2008**

**PRINCIPAL INVESTIGATOR: TBD
PHONE: TBD
Short Title for the Study: EMBRACE**

Introduction

MTN-016, or EMBRACE (Evaluation of Maternal and Baby outcome Registry After Chemoprophylactic Exposure) is a research study that is designed to include certain women, and, possibly their babies. Even though you may be pregnant with more than one baby, we will be using the term “baby” throughout this consent. This consent applies to you and to the offspring from this pregnancy.

You, and possibly your baby, may participate if the following are true.

1. You became pregnant while taking part in a human immunodeficiency virus (HIV) prevention study.

OR

2. You have or had planned exposure to any study agent, either active or non-active, while taking part in a pregnancy safety study.

AND

3. The pregnancy outcome is diagnosed less than 1 year from the date of the Screening/Enrollment Visit for this study (live-born babies would be less than 1 year of age).

You can include your baby in this study, if you wish, as long as you meet the criteria above. You are being asked to take part in this study so that we can look at how HIV prevention studies might affect pregnancy and baby outcomes. HIV is the virus that causes AIDS.

This study is being paid for by the United States National Institutes of Health. The person in charge of this study at this site is [INSERT NAME OF PRINCIPAL INVESTIGATOR].

Before you decide whether to take part in this study, we want to explain the purpose of the study, the risks and benefits, and what is expected of you. This consent form gives information that the study staff will discuss with you. You are free to ask questions at any time. If you agree to take part in this study, you will be asked to sign or make your mark on this form. You will be offered a copy to keep.

Why Is This Study Being Done?

The main purpose of this study is to see if using a medication to prevent HIV affects the health of pregnant women and their babies.

What Do I Have To Do If I Am In This Study?

The study is planned to continue until the year 2013, but you will only stay in the study until the outcome of your pregnancy is known (within 1 month after your pregnancy ends).

Because examination results (outlined below) may help other doctors make the best medical choices for you and your baby, study staff may give the results of your study tests to your other doctors, but they will only do this if you permit them to.

Also, if you are taking part in another study, please tell the study staff, and they will talk to you about the cases where you may and may not be permitted to be in more than one study.

Clinic Visits

This study will require some visits to the clinic. Visits will be broken up into different categories. Following the short description are more details about each visit to help you decide about participation.

Mother Visits

1. Mother's Screening and Enrollment (to see if you can and want to participate)
2. Ultrasound Visit (to check on your baby if needed)
3. Mother's Quarterly Visit (to see how you are doing and ask if there are any changes)
4. Mother's Pregnancy Outcome Visit (to give us information about you and your pregnancy)

Baby Visits

1. Newborn/Initial Visit (to allow you to enroll your baby if you like, and to allow us to get some basic information about your baby's health)
2. Baby Follow-Up Visits (to allow us to follow the health of your baby)

Mother's Screening and Enrollment Visit

This visit will continue today after you read, discuss and sign or make your mark on this form. It will take about [insert amount of time]. The study clinician will review the records from your HIV prevention study to make sure you meet the requirements for this

study. Then you will be asked some questions. The questions will be about you, where you live, and medicines you take. You also will be asked to do the following:

- Tell study staff about any health problems you might have had
- Tell the study staff about other times that you were pregnant

Ultrasound Visit

Have an ultrasound to check the growth of your baby if this applies to you and if you do not have complete results from an ultrasound taken by another doctor.

Mother's Quarterly Visits (every 3 months)

These visits will take place every 3 months. You may have fewer visits depending on how far along in your pregnancy you are when you join the study. These visits will take about [insert amount of time]. You will be asked questions about where you live and how to keep in touch with you. At these visits you will also be asked to do the following:

- Tell the study staff about any changes in your health.
- Tell the study staff about any changes in the medicines you are taking.
- Answer questions about your pregnancy.
- Have an ultrasound if you have never had one before or if the study doctor thinks you may need to have one done.

Mother's Pregnancy Outcome Visit

This will be your last visit and it will take [insert amount of time]. Any babies born will be assigned an identification number and enrolled in the study at this point, and your baby will keep coming back for your baby's visits. You will be asked questions about where you live and how to keep in touch with you. At this visit you will be asked to do the following:

- Tell the study staff about any changes in your health.
- Tell the study staff about any changes in the medicines you are taking.
- Answer questions about your pregnancy.
- Tell us about the outcome of your pregnancy.

We also ask for permission to look at your medical records, particularly the records of your labor and delivery, for medical information about you and your baby.

Newborn/Initial Visit

If your pregnancy results in a live birth, this visit should take place after the birth of your baby, but when your baby is 10 days old or younger. You will be asked questions about where you live, how to keep in touch with you and your baby, about the health of your baby and any medicines your baby might be taking. At this visit, the study staff may also do the following to make sure your baby is healthy:

- Measure the weight, length, head size, and belly size of your baby
- Perform a physical exam

- If it looks like something might be wrong with your baby, the study doctor might take a picture of your baby and share that picture with experts who may be able to see what the problem might be. If you agree to have pictures taken of your baby, you will be asked to mark your permission at the end of this consent. We can give you a copy of any of the photographs. If you do not wish to have photographs taken of your baby, you will be able to mark at the end of this consent that no photographs may be taken of your baby.

Baby Follow-Up Visits (1, 6, and 12 months)

We will ask you to bring your baby in for follow-up visits to make sure your baby is healthy. You will be asked questions about where you live, how to keep in touch with you and your infant about the health of your baby and any medicines your baby might be taking. At this visit, the study staff will also do the following to make sure your baby is healthy:

- Measure the weight, length, and head size of your baby
- Perform a physical exam
- Check how well your baby is developing (only at the 6 and 12 month visits)
- If it looks like something might be wrong with your baby, the study doctor might take a picture of your baby and share that picture with experts who may be able to see what the problem might be. If you agree to have pictures taken of your baby, you will be asked to mark your permission at the end of this consent. We can give you a copy of any of the photographs. If you do not wish to have photographs taken of your baby, you will be able to mark at the end of this consent that no photographs may be taken of your baby.

Any Time During The Study:

Please tell the study staff about any medical problems you or your baby have during the study. You can contact the study staff between regular visits to report these problems. The study staff will check you or your baby as needed and will refer you or your baby for medical care. At each study visit, the study staff will update your medical history and the medical history of your baby as well as information on where you live and how to keep in touch with you.

How Many Women and Babies Will Be In This Study?

Women who become or became pregnant during HIV prevention trials or women who enroll or enrolled in an HIV prevention trial while pregnant (as long as their pregnancy outcome was diagnosed less than 1 year ago), and babies born to these women (as long as the baby has not reached its one year birth date) can be in this study. This number is expected to be about 500 women, and about 300 babies.

How Long Will My Baby and I Be In This Study?

You will be in this study until the outcome of your pregnancy is known. If you have or had a live birth, your baby will be in this study until about the time that they turn one year old. If you have more than one baby in this pregnancy (such as twins), each baby may participate in this study.

Can the Doctor Take Me or My Baby Off This Study Early?

The study doctor may take you or your baby off the study early without your permission for any of the following reasons:

- The study is stopped or canceled.
- Staying in the study would be harmful to you or your baby.
- Other reasons that may prevent you or your baby from completing the study successfully

What Are The Risks Of This Study?

You may feel worried while waiting for your ultrasound results. Trained staff members are available to help you deal with any feelings or questions you have. You may also be uncomfortable with the personal types of questions asked.

Possible Risks to Your Privacy or Your Baby's Privacy

We will make every effort to protect your privacy and the privacy of your baby while you are in this study. All study visits – for your baby – will take place in private. However, it is possible that others may learn of your participation or the participation of your baby and, because of this, may treat you unfairly. For example, you could have problems getting or keeping a job or being accepted by your family or community. There also is a risk to your privacy or the privacy of your baby if someone else taking part in this study knows you. Efforts to protect your privacy and the privacy of your baby include only allowing medical staff to see any photographs that might be taken of your baby. If you do not feel comfortable about having any photographs taken of your baby, a doctor may write a detailed description instead, and you will still be allowed to stay in the study, and your baby, if enrolled, will also still be allowed to stay in the study.

What are the Benefits of This Study?

You may experience no direct benefit from being in this study. You or others may benefit in the future from information learned in this study. Knowledge gained from this study may help in the development of medications for the prevention of HIV infection. You may also get some personal satisfaction from being part of research on preventing HIV. You or your baby may also benefit from clinical information gained from the exams performed as part of the study.

New Information

You will be told any new information learned during this study that might affect your willingness to stay in the study.

What Other Choices Do I Have Besides This Study?

You and your baby do not have to participate in this study. The decision to not be in this study will not affect your regular care the regular care of your baby in any way.

What About Confidentiality?

We will do everything we can to protect your privacy the privacy of your baby Also, any scientific publication about this study will not use your name the name of your baby or identify you or your baby personally.

[Insert the following paragraph for US sites only] In addition to the efforts of the study staff to help keep your personal information private, we have obtained a Certificate of Confidentiality from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. The Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study to anyone you choose. Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

People who may review your records include the following: (insert Name of Site) Institutional Review Board (IRB), Ethics Committees (EC), National Institutes of Health (NIH), study staff, study monitors, and other government and regulatory authorities.

What Are The Costs To Me?

There is no cost to you for study visits, exams, or ultrasounds performed on you or your baby as a part of the study. This study will not provide or pay for others to provide routine prenatal care, delivery, postpartum, or routine baby care.

Will I Receive Any Payment?

You will receive payment for your time and effort in this study. You may also receive payment for activities [such as child care, travel cost, loss of work time - local sites to list particulars], that are affected by your participation or the participation of your baby in this study.

What Happens If I Am Injured?

If you are injured, or if your baby is injured as a result of being in this study, you will be given immediate treatment for your injuries. If your baby is injured as a result of being in this study, he/she will be given immediate treatment for injuries as well. However, you [insert if applicable: or your insurance company (or the infant's insurance company)] may have to pay for this care. This institution or the United States National Institutes of Health does not have a program to provide money for your or your infant's injuries. You will not be giving up any of your legal rights by signing this consent form.

What Will Happen If I Become Infected With HIV While In This Study?

If either you or your baby becomes infected with HIV while participating in the study, we would like you continue to come in for your study visits. We will also provide counseling and referrals to available care and support for you and/or your baby. If you become infected with HIV, it is possible that your baby will also be at risk of becoming infected with HIV. Because of this, we will ask you to complete a separate consent form to have your infant tested for HIV.

What Are My Rights?

Being in this study is completely voluntary. You may choose not to be in or to leave the study at any time, or to remove your baby from the study at any time. You and your baby will be treated the same no matter what you decide. If you choose not to be in or to leave the study, or if you choose to remove your baby from the study, neither you nor your baby will lose the benefit of services to which either of you would otherwise be entitled at this clinic. Study staff will tell you about any new information from this or other studies that may affect your or the health and welfare of your baby. At the end of the study, you will be told when study results may be available and how to learn about them.

What Do I Do If I Have Problems or Questions?

For questions about this study or a research-related injury, contact: [insert contact information]. For questions about your rights as a research participant, contact: [insert contact information]

SIGNATURES

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator.

If you have read the informed consent, or had it read and explained to you, and all your questions have been answered, and you agree to be in this study, please sign your name or make your mark below.

Please mark one of the following boxes to show whether you agree/ do not agree to have photograph(s) of your baby taken as may be requested by study staff:

- The study staff may take photograph(s) of my baby
- The study staff may **NOT** take photograph(s) of my baby

Mother/Guardian Name (print)

Mother/Guardian Signature/Mark and Date

Father's Name (print)

Father's Signature/Mark and Date
(If Reasonably Available)

Study Staff Conducting
Consent Discussion (print)

Study Staff Signature and Date

Witness' Name (print)
(As appropriate)

Witness' Signature and Date

**APPENDIX V: SAMPLE INFORMED CONSENT – MOTHER
(Screening and Enrollment)**

**MTN-016
HIV Prevention Agent Pregnancy Exposure Registry:
EMBRACE Study**

**Version 1.0
December 17, 2008**

**PRINCIPAL INVESTIGATOR: TBD
PHONE: TBD
Short Title for the Study: EMBRACE**

Introduction

MTN-016, or EMBRACE (Evaluation of Maternal and Baby outcome Registry After Chemoprophylactic Exposure) is a research study that is designed to include certain women, and, possibly their babies. Even though you may be pregnant or may have delivered more than one baby, we will be using the term “baby” throughout this consent.

You, and possibly your baby, may participate if the following are true.

1. You became or become pregnant while taking part in a human immunodeficiency virus (HIV) prevention study.

OR

2. You have or had planned exposure to any study agent, either active or non-active, while taking part in a pregnancy safety study.

AND

3. The pregnancy outcome is diagnosed less than 1 year from the date of the Screening/Enrollment Visit for this study (live-born babies would be less than 1 year of age).

You may participate in this study even if you have miscarried. You are being asked to take part in this study so that we can look at how HIV prevention studies might affect pregnancy and baby outcomes. HIV is the virus that causes AIDS.

This study is being paid for by the United States National Institutes of Health. The person in charge of this study at this site is [INSERT NAME OF PRINCIPAL INVESTIGATOR].

Before you decide whether to take part in this study, we want to explain the purpose of the study, the risks and benefits, and what is expected of you. This consent form gives information that the study staff will discuss with you. You are free to ask questions at any time. If you agree to take part in this study, you will be asked to sign or make your mark on this form. You will be offered a copy to keep.

Why Is This Study Being Done?

The main purpose of this study is to see if using a medication to prevent HIV affects the health of pregnant women and their babies.

What Do I Have To Do If I Am In This Study?

The study is planned to continue until the year 2013, but you will only stay in the study until the outcome of your pregnancy is known (within 1 month after your pregnancy ends).

Because examination results (outlined below) may help other doctors make the best medical choices for you, study staff may give the results of your study tests to your other doctors, but they will only do this if you permit them to.

Also, if you are taking part in another study, please tell the study staff, and they will talk to you about the cases where you may and may not be permitted to be in more than one study.

If you agree to participate in this study, and your pregnancy results in, or has already resulted in, a live birth, you will be asked to sign another consent form for your baby that will allow the health of your baby to be followed for the first year of life to see if your use of a medication to prevent HIV affected the health of your baby. More information about this can be found on the infant consent form.

Clinic Visits

This study will require some visits to the clinic. Visits will be broken up into different categories. Following the short description are more details about each visit to help you decide about participation.

1. Screening and Enrollment (to see if you can and want to participate)
2. Ultrasound Visit (to check on your baby if needed)
3. Quarterly Visit (to see how you are doing and ask if there are any changes)
4. Pregnancy Outcome Visit (to give us information about you and the outcome of your pregnancy, whether it resulted in a live birth or not)

Screening and Enrollment Visit

This visit will continue today after you read, discuss and sign or make your mark on this form. It will take about [insert amount of time]. The study clinician will review the records from your HIV prevention study to make sure you meet the requirements for this

study. Then you will be asked some questions. The questions will be about you, where you live, and medicines you take. You also will do the following:

- Tell study staff about any health problems you might have had
- Tell the study staff about other times that you were pregnant

Ultrasound Visit

Have an ultrasound to check the growth of your baby if this applies to you and if you do not have complete results from an ultrasound taken by another doctor.

Quarterly Visits (every 3 months)

These visits will take place every 3 months. You may have fewer visits depending on how far along in your pregnancy you are when you join the study. These visits will take about [insert amount of time]. You will be asked questions about where you live and how to keep in touch with you. At these visits you will also be asked to do the following:

- Tell the study staff about any changes in your health
- Tell the study staff about any changes in the medicines you are taking
- Answer questions about your pregnancy
- Have an ultrasound if you have never had one before or if the study doctor thinks you may need to have one done

Pregnancy Outcome Visit

This will be your last visit and it will take [insert amount of time]. If you consent to enroll your baby, a second form is required. You will be asked questions about where you live and how to keep in touch with you. At this visit you will be asked to do the following:

- Tell the study staff about any changes in your health.
- Tell the study staff about any changes in the medicines you are taking.
- Answer questions about your pregnancy.
- Tell us about the outcome of your pregnancy.

We also ask for permission to look at your medical records, particularly the records of your labor and delivery, for medical information about you and your baby.

Any Time During The Study:

Please tell the study staff about any medical problems you have during the study. You can contact the study staff between regular visits to report these problems. The study staff will check you as needed and will refer you for medical care. At each study visit, the study staff will update your medical history and information on where you live and how to keep in touch with you.

How Many Women Will Be In this Study?

Women who become or became pregnant during HIV prevention trials or women who enroll/enrolled in an HIV prevention trial while pregnant (as long as pregnancy outcome is diagnosed less than 1 year ago) will be in this study. This number is expected to be about 500 women.

How Long Will I be In This Study?

You will be in this study until the outcome of your pregnancy is known.

Can the Doctor Take Me Off This Study Early?

The study doctor may take you off the study early without your permission for any of the following reasons:

- The study is stopped or canceled.
- Staying in the study would be harmful to you.
- Other reasons that may prevent you from completing the study successfully

What Are The Risks Of This Study?

You may feel worried while waiting for your ultrasound results. Trained staff members are available to help you deal with any feelings or questions you have. You may also be uncomfortable with the personal types of questions asked.

Possible Risks to Your Privacy

We will make every effort to protect your privacy while you are in this study. Your visits here will take place in private. However, it is possible that others may learn of your participation here and, because of this, may treat you unfairly. For example, you could have problems getting or keeping a job or being accepted by your family or community. There also is a risk to your privacy if someone else taking part in this study knows you.

What are the Benefits of This Study?

Participants in this study may experience no direct benefit. You or others may benefit in the future from information learned in this study. Knowledge gained from this study may help in the development of medications for the prevention of HIV infection. You may also get some personal satisfaction from being part of research on preventing HIV.

New Information

You will be told any new information learned during this study that might affect your willingness to stay in the study.

What Other Choices Do I Have Besides This Study?

You do not have to participate in this study. The decision to not be in this study will not affect your regular care in any way.

What About Confidentiality?

We will do everything we can to protect your privacy. Also, any scientific publication about this study will not use your name or identify you personally.

[Insert the following paragraph for US sites only] In addition to the efforts of the study staff to help keep your personal information private, we have obtained a Certificate of Confidentiality from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. The Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study to anyone you choose. Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

People who may review your records include the following: (insert Name of Site) Institutional Review Board (IRB), Ethics Committees (EC), National Institutes of Health (NIH), study staff, study monitors, and other government and regulatory authorities.

What Are The Costs To Me?

There is no cost to you for study visits, exams, or ultrasounds performed as a part of the study. This study will not provide or pay for others to provide routine prenatal care, delivery, postpartum, or routine baby care.

Will I Receive Any Payment?

You will receive payment for your time and effort in this study. You may also receive payment for activities [such as child care, travel cost, loss of work time - local sites to list particulars], that are affected by your participation in this study.

What Happens If I Am Injured?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. However, you [insert if applicable: or your insurance company] may have to pay for this care. This institution or the United States National Institutes of Health does not have a program to provide money for your injuries. You will not be giving up any of your legal rights by signing this consent form.

What Will Happen If I Become Infected With HIV While In This Study?

If you become infected with HIV while participating in the study, we would like you to continue to come in for your study visits. We will also provide counseling and referrals to available care and support. If you become infected with HIV, it is possible that your baby will also be at risk of becoming infected with HIV. Because of this, we will ask you to complete a separate consent form to have your baby tested for HIV.

What Are My Rights?

Being in this study is completely voluntary. You may choose not to be in or to leave the study at any time. You will be treated the same no matter what you decide. If you choose not to be in or to leave the study, you will not lose the benefit of services to which you would otherwise be entitled at this clinic. Study staff will tell you about any new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. At the end of the study, you will be told when study results may be available and how to learn about them.

What Do I Do If I Have Problems or Questions?

For questions about this study or a research-related injury, contact: [insert contact information]

For questions about your rights as a research participant, contact: [insert contact information]

SIGNATURES

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator.

If you have read the informed consent, or had it read and explained to you, and all your questions have been answered, and you agree to be in this study, please sign your name or make your mark below.

Participant's Name (print)

Participant's Signature/Mark and Date

Study Staff Conducting
Consent Discussion (print)

Study Staff Signature and Date

Witness' Name (print)
(As appropriate)

Witness' Signature and Date

**APPENDIX VI: SAMPLE INFORMED CONSENT – INFANT
(Screening and Enrollment)**

**MTN-016
HIV Prevention Agent Pregnancy Exposure Registry:
EMBRACE Study**

**Version 1.0
December 17, 2008**

**PRINCIPAL INVESTIGATOR: TBD
PHONE: TBD
Short Title for the Study: EMBRACE**

Introduction

MTN-016, or EMBRACE (Evaluation of Maternal and Baby outcome Registry After Chemoprophylactic Exposure) is a research study of women who become or became pregnant while taking part in human immunodeficiency virus (HIV) prevention research studies, or who had planned exposures to any study agent (active or non-active) in pregnancy safety studies as long as the pregnancy outcome was diagnosed less than 1 year from the date of the EMBRACE Screening/Enrollment Visit. This study includes the babies of these women, if the parent consents and each baby is less than 1 year old. Even though you may be pregnant or may have delivered more than one baby, we will be using the term “baby” throughout this consent. You are being asked to agree to have your baby take part in this study because you have been pregnant during an HIV prevention study. HIV is the virus that causes AIDS.

This study is being paid for by the United States National Institutes of Health. The person in charge of this study at this site is [INSERT NAME OF PRINCIPAL INVESTIGATOR].

Before you decide whether you would like your baby to take part in this study, we want to explain the purpose of the study, the risks and benefits, and what is expected of you and your baby. This consent form gives information that the study staff will discuss with you. You are free to ask questions at anytime. If you agree to have your baby take part in this study, you will be asked to sign or make your mark on this form. You will be offered a copy to keep.

Why Is This Study Being Done?

The main purpose of this study is to see if using a medication to prevent HIV affects the health of pregnant women and their babies.

What Do I Have To Do If I Am In This Study?

The study is planned to continue until the year 2013, but your baby will only stay in the study until he or she turns one year old. If you decide to let your baby take part in this

study, you will be asked to bring your baby back for visits for as long as the baby is in the study. At study visits you will answer questions about your baby's health and the study doctor will check your baby's growth. Because the examination results (outlined below) may help other doctors make the best medical choices for you and your baby, study staff will give the results of your study tests to your other doctors, if you wish and with your permission.

The visits your baby will have in this study are described in detail below.

Newborn/Initial Visit

If your pregnancy results in a live birth, this visit should take place after the birth of your baby, but when your baby is 10 days old or younger. You will be asked questions about where you live, how to keep in touch with you and your baby, about the health of your baby and any medicines your baby might be taking. At this visit, the study staff may also do the following to make sure your baby is healthy:

- Measure the weight, length, head size, and belly size of your baby
- Perform a physical exam
- If it looks like something might be wrong with your baby, the study doctor might take a picture of your baby and share that picture with experts who may be able to see what the problem might be. If you agree to have pictures taken of your baby you will be asked to mark your permission at the end of this consent. We can give you a copy of any of the photographs. If you do not wish to have photographs taken of your baby, you will be able to mark at the end of this consent that no photographs may be taken of your baby.

Follow-Up Visits (Infant) (1, 6, and 12 months)

We will ask you to bring your baby in for follow-up visits to make sure your infant(s) is/are healthy. You will be asked questions about where you live, how to keep in touch with you and your baby, about the health of your baby, and any medicines your baby might be taking. At this visit, the study staff will also do the following to make sure your baby is healthy:

- Measure the weight, length, and head size of your baby
- Perform a physical exam
- Check how well your baby is developing (only at the 6 and 12 month visits)
- If it looks like something might be wrong with your baby, the study doctor might take a picture of your baby and share that picture with experts who may be able to see what the problem might be. If you agree to have pictures taken of your baby, you will be asked to mark your permission at the end of this consent. We can give you a copy of any of the photographs. If you do not wish to have photographs taken of your baby, you will be able to mark at the end of this consent that no photographs may be taken of your baby.

Any Time During The Study:

Please tell the study staff about any medical problems you might have with your baby during the study. You can contact the study staff between regular visits to report these problems. The study staff will check your baby as needed and will refer you for medical care. At each study visit, the study staff will update the medical history of your baby as well as information on where you and your baby live and how to keep in touch with you.

How Many Babies Will Be In this Study?

Babies who are born to women who become or became pregnant during HIV prevention trials or to women who enroll or enrolled in an HIV prevention trial while pregnant (as long as the baby has not reached its one year birth date) will be in this study. This number is expected to be about 300 babies.

How Long Will My Baby Be In This Study?

Your baby will be in this study until about the time he or she turns one year old. If you have more than one baby in this pregnancy (such as twins), each of those babies may participate in this study.

Can the Doctor Take My Baby Off This Study Early?

The study doctor may take your baby off the study early without your permission for any of the following reasons:

- The study is stopped or canceled.
- Staying in the study would be harmful to your baby.
- Other reasons that may prevent your baby from completing the study successfully

What Are The Risks Of This Study?

You may be uncomfortable with the personal types of questions asked.

Possible Risks to Your Baby's Privacy

We will make every effort to protect the privacy of your baby while you are in this study. Visits here will take place in private. However, it is possible that others may learn of the participation of your baby here and, because of this, may treat you unfairly. For example, you could have problems getting or keeping a job or being accepted by your family or community. There also is a risk to the privacy of your baby if someone else taking part in this study knows you. Efforts to protect you and the privacy of your baby include only allowing medical staff to see any photographs that might be taken of your baby. If you do not feel comfortable about having any photographs taken of your baby, a doctor may write a detailed description instead, and your baby will still be allowed to stay in the study.

What are the Benefits of This Study?

Participants in this study may experience no direct benefit. You or others may benefit in the future from information learned in this study. Knowledge gained from this study may help in the development of medications that are safe for use in pregnant women, for the prevention of HIV infection. You may also get some personal satisfaction from being part of research on preventing HIV.

New Information

You will be told any new information learned during this study that might affect your willingness to let your baby stay in the study.

What Other Choices Does My Baby Have Besides This Study?

You do not have to have your baby participate in this study. The decision to not have your baby participate in this study will not affect the regular care of your baby in any way.

What About Confidentiality?

We will do everything we can to protect the privacy of your baby. Also, any scientific publication about this study will not use the name of your baby or identify your baby personally.

[Insert the following paragraph for US sites only] In addition to the efforts of the study staff to help keep your personal information private, we have obtained a Certificate of Confidentiality from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. The Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study to anyone you choose. Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

People who may review your records include the following: (insert Name of Site) Institutional Review Board (IRB), Ethics Committees (EC), National Institutes of Health (NIH), study staff, study monitors, and the government and other regulatory authorities.

What Are The Costs To Me?

There is no cost to you for study visits or exams of your baby. This study will not provide or pay for others to provide routine infant care.

Will I Receive Any Payment?

You will receive payment for your time and effort in this study. You may also receive payment for activities affected by the participation of your baby in this study, [such as child care, travel cost, loss of work time - local sites to list particulars].

What Happens If My Baby Is Injured?

If your baby is injured as a result of being in this study, your baby will be given immediate treatment for any injuries. However, you [insert if applicable: or your insurance company (or the baby's insurance company)] may have to pay for this care. This institution or the United States National Institutes of Health does not have a program to provide money for your or your baby's injuries. You will not be giving up any of your legal rights by signing this consent form.

What Will Happen If My Baby Becomes Infected With HIV While In This Study?

If your baby becomes infected with HIV while participating in the study, we would like you to continue to bring your baby in for study visits. We will also provide counseling and referrals to available care and support for your baby. We will ask you to complete a separate consent form to have your baby come to the clinic for HIV-related visits.

What Are My Rights?

Being in this study is completely voluntary. You may choose not to let your baby be in or to leave the study at any time. You and your baby will be treated the same no matter what you decide. If you choose not to let your baby be in or to leave the study, you and your baby will not lose the benefit of services to which you would otherwise be entitled at this clinic. Study staff will tell you about any new information from this or other studies that may affect your or the health or welfare of your baby. At the end of the study, you will be told when study results may be available and how to learn about them.

What Do I Do If I Have Problems or Questions?

For questions about this study or a research-related injury, contact: [insert contact information]

For questions about your rights as a research participant, contact: [insert contact information]

SIGNATURES

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator.

If you have read the informed consent, or had it read and explained to you, and all your questions have been answered, and you agree to let your baby be in this study, please sign your name or make your mark below.

Please mark one of the following boxes to show whether you agree/ not agree to have photograph(s) of your baby taken as may be requested by study staff:

- The study staff may take photograph(s) of my baby
- The study staff may **NOT** take photograph(s) of my baby

Mother/Guardian Name (print)

Mother/Guardian Signature/Mark and Date

Father's Name (print)

Father's Signature/Mark and Date
(if reasonably available)

Baby's Name (print)

Date

Study Staff Conducting
Consent Discussion (print)

Study Staff Signature and Date

Witness' Name (print)

Witness' Signature and Date

APPENDIX VII: SAMPLE INFORMED CONSENT – INFANT TESTING

MTN-016 HIV Prevention Agent Pregnancy Exposure Registry: EMBRACE Study

Version 1.0
December 17, 2008

PRINCIPAL INVESTIGATOR: TBD
PHONE: TBD
Short Title for the Study: EMBRACE

Introduction

With your permission, your baby is enrolled in the EMBRACE study, which is funded by the United States National Institutes of Health. A baby of an HIV-infected mother is at risk of getting the HIV infection through the womb, at the time of labor and delivery, or through breast milk. Because your baby is at risk for HIV infection, you are being asked to agree to additional testing for your baby.

The person in charge of this study at this site is [INSERT NAME OF PRINCIPAL INVESTIGATOR].

Before you decide whether to have additional testing for your baby, we want to explain the purpose of these tests, the risks and benefits, and what is expected of you. This consent form gives information that the study staff will discuss with you. You are free to ask questions at any time. If you agree to take part in this study, you will be asked to sign or make your mark on this form. You will be offered a copy to keep.

Laboratory Assessments for Babies

There are two kinds of HIV tests done as part of this study. You may choose to have these tests done for your baby as part of this study.

The first test is to find out (or confirm) if your infant has HIV infection. The test for HIV of your baby would mean that a small blood sample about XX drops [or mL] [SITES TO INSERT LOCAL EQUIVALENT] would be taken from your baby. It may be necessary to test again at other times depending on the results of the first tests and on whether the baby is being breastfed.

If your baby is HIV infected, another test of the HIV virus will be available for your baby, as well. This is a test to see whether the HIV virus in your baby has any resistance to the medications used to treat HIV. Today and at other study visits for your baby, we will need a blood sample of about XX mL [SITES TO INSERT LOCAL EQUIVALENT] for

this test. Some samples will be collected and stored in your own country before being shipped to a central laboratory in the US for testing at a later date.

Depending on the type of test used, these test results may or may not be useful for your baby's healthcare provider to make decisions about your baby's medical care. With your permission, these results may be shared with your baby's healthcare provider.

What Are The Risks Of These Tests?

When blood is drawn, there may be bruising, swelling, or infection (rare) where the needle goes in to draw the blood. You may feel worried while waiting for test results for your baby.

What Will Happen If My Baby Is Infected With HIV?

If tests show that your baby is infected with HIV, we will make certain that your baby is receiving appropriate available medical care. Care and treatment of HIV is not provided through EMBRACE. The results of the HIV resistance tests may be made available to your baby's healthcare provider.

Possible Risks to Your Privacy or Your Baby's Privacy

We will make every effort to protect your privacy and the privacy of your baby while you are here for testing, and your baby's blood will be obtained in a private place. However, it is possible that others may learn that your baby is having blood drawn here and, because of this, may treat you unfairly. For example, you could have problems getting or keeping a job or being accepted by your family or community. There also is a risk to your privacy or the privacy of your baby if someone else taking part in this study knows you.

What are the Benefits of These Tests?

You may experience no direct benefit from these extra tests. The doctors who are taking care of your baby may be able to use this information to help choose the best treatment. You or others may benefit in the future from information learned in this study. Knowledge gained from this study may help in the development of medications for the prevention of HIV infection. You may also get some personal satisfaction from being part of research on preventing HIV.

New Information

You will be told any new information learned during this study that might affect your willingness to let your baby stay in the study.

What Other Choices Do I Have Besides This Study?

You and your baby do not have to participate in these extra tests. The decision to not have these extra tests will not affect your regular care or the regular care of your baby in any way.

What About Confidentiality?

We will do everything we can to protect your privacy and the privacy of your baby. Also, any scientific publication about this study will not use your name or the name of your infant or identify you or your baby personally.

[Insert the following paragraph for US sites only] In addition to the efforts of the study staff to help keep your personal information private, we have obtained a Certificate of Confidentiality from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. The Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study to anyone you choose. Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

People who may review your records include the following: (insert Name of Site) Institutional Review Board (IRB), Ethics Committees (EC), National Institutes of Health (NIH), study staff, study monitors, and government and other regulatory authorities.

What Are The Costs To Me?

There is no cost to you for these extra tests. This study will not provide or pay for others to provide routine prenatal care, delivery, postpartum, or routine infant care.

Will I Receive Any Payment?

Because you and your baby are participants in EMBRACE, compensation is being provided through that study. [Sites to insert if applicable: You will be compensated for your time and effort in this study.]

What Happens If My Baby Is Injured?

If your baby is injured as a result of being in this study, they will be given immediate treatment for injuries as well. However, you [insert if applicable: or your insurance company (or the baby's insurance company)] may have to pay for this care. This institution or the United States National Institutes of Health does not have a program to provide money for your or your baby's injuries. You will not be giving up any of your legal rights by signing this consent form.

What Are My Rights?

Having these extra tests is completely voluntary. If you choose not to have these extra tests, neither you nor your baby will lose the benefit of services to which either of you would otherwise be entitled at this clinic.

What Do I Do If I Have Problems or Questions?

For questions about this study or a research-related injury, contact: [insert contact information]. For questions about your rights as a research participant, contact: [insert contact information]

SIGNATURES

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator.

If you have read the informed consent, or had it read and explained to you, and all your questions have been answered, and you agree to be in this study, please sign your name or make your mark below.

Mother/Guardian Name (print)

Mother/Guardian Signature/Mark and Date

Father's Name (print)

Father's Signature/Mark and Date
(If Reasonably Available)

Study Staff Conducting
Consent Discussion (print)

Study Staff Signature and Date

Witness' Name (print)
(As appropriate)

Witness' Signature and Date

REFERENCES

1. UNAIDS. 2007 AIDS Epidemic Update: Global Summary. http://www.unaids.org/en/HIV_data/2007EpiUpdate/default.asp accessed December 18, 2007.
2. Ranjit, N., Bankole, A., Darroch, J. E. & Singh, S. Contraceptive failure in the first two years of use: differences across socioeconomic subgroups. *Family Planning Perspectives* 33, 19-27 (2001).
3. Trussell, J. Contraceptive failure in the United States. *Contraception* 70, 89-96 (2004).
4. Gray, R. H. et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet* 357, 1149-53 (2001).
5. Gilead & Sciences. Viread® (tenofovir disoproxil fumarate) tablets. Package Insert (March 2006).
6. Tarantal, A. F., Castillo, A., Ekert, J. E., Bischofberger, N. & Martin, R. B. Fetal and maternal outcome after administration of tenofovir to gravid rhesus monkeys (*Macaca mulatta*). *Journal of Acquired Immune Deficiency Syndromes: JAIDS* 29, 207-20 (2002).
7. Fux, C. A., Christen, A., Zraggen, S., Mohaupt, M. G. & Furrer, H. Effect of tenofovir on renal glomerular and tubular function. *AIDS* 21, 1483-5 (2007).
8. Van Rompay, K. K. et al. Administration of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) for prevention of perinatal simian immunodeficiency virus infection in rhesus macaques. *AIDS Research & Human Retroviruses* 14, 761-73 (1998).
9. Subbarao, S. et al. Chemoprophylaxis with tenofovir disoproxil fumarate provided partial protection against infection with simian human immunodeficiency virus in macaques given multiple virus challenges.[see comment]. *Journal of Infectious Diseases* 194, 904-11 (2006).
10. Cassetti, I. et al. The safety and efficacy of tenofovir DF in combination with lamivudine and efavirenz through 6 years in antiretroviral-naive HIV-1-infected patients. *HIV Clinical Trials* 8, 164-72 (2007).
11. Gitman, M. D., Hirschwerk, D., Baskin, C. H. & Singhal, P. C. Tenofovir-induced kidney injury. *Expert Opinion on Drug Safety* 6, 155-64 (2007).
12. Nelson, M. R. et al. The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. *AIDS* 21, 1273-81 (2007).
13. Vigano, A. et al. Renal safety of tenofovir in HIV-infected children: a prospective, 96-week longitudinal study. *Clinical Drug Investigation* 27, 573-81 (2007).
14. Nunez, M. & Soriano, V. Management of patients co-infected with hepatitis B virus and HIV. *The Lancet Infectious Diseases* 5, 374-82 (2005).
15. Schmutz, G. et al. Combination of tenofovir and lamivudine versus tenofovir after lamivudine failure for therapy of hepatitis B in HIV-coinfection.[see comment]. *AIDS* 20, 1951-4 (2006).

16. Levy, V. & Grant, R. M. Antiretroviral therapy for hepatitis B virus-HIV-coinfected patients: promises and pitfalls.[see comment][erratum appears in Clin Infect Dis. 2006 Nov 15;43(10):1376]. Clinical Infectious Diseases 43, 904-10 (2006).
17. Thio, C. L., Sulkowski, M. S. & Thomas, D. L. Treatment of chronic hepatitis B in HIV-infected persons: thinking outside the black box.[erratum appears in Clin Infect Dis. 2005 Nov;41(9):1371]. Clinical Infectious Diseases 41, 1035-40 (2005).
18. Lim, S. G., Wai, C. T., Rajnakova, A., Kajiji, T. & Guan, R. Fatal hepatitis B reactivation following discontinuation of nucleoside analogues for chronic hepatitis B. Gut 51, 597-9 (2002).
19. Peterson, L. et al. Tenofovir disoproxil fumarate for prevention of HIV infection in women: a phase 2, double-blind, randomized, placebo-controlled trial. PLoS Clin Trials 2, e27 (2007).
20. Rodman, J., Flynn, P., Shapiro, D. & et, a. I. in 13th Conference on Retroviruses and Opportunistic Infections (CROI) (Denver, CO, 2006).
21. Burchett, S. et al. in 15th Conference on Retroviruses and Opportunistic Infections (Boston, MA, 2008).
22. Haberl, A. et al. in 15th Conference on Retroviruses and Opportunistic Infections (Boston, MA, 2008).
23. APR. The Antiretroviral Pregnancy Registry: Interim report. 1/1/89-7/31/07. www.APRegistry.com.
24. Gilead, Sciences & Inc. Truvada® (emtricitabine and tenofovir disoproxil fumarate) tablets. Package Insert. May 2007.
25. Gilead, Sciences & Inc. Emtriva® (emtricitabine) tablets. Package Insert. April 2008.
26. Szczech, G. M., Wang, L. H., Walsh, J. P. & Rousseau, F. S. Reproductive toxicology profile of emtricitabine in mice and rabbits. Reproductive Toxicology 17, 95-108 (2003).
27. Gilead, Sciences & Inc. Investigator's Brochure. Emtricitabine/Tenofovir Disoproxil Fumarate Tablets. Human Immunodeficiency Virus Type 1 Infection. Second Edition.
28. Garcia-Lerma, J. G. et al. in Conference on Retroviruses and Opportunistic Infections (Los Angeles, CA, 2006).
29. Saag, M. S. et al. Efficacy and safety of emtricitabine vs stavudine in combination therapy in antiretroviral-naive patients: a randomized trial.[see comment]. JAMA 292, 180-9 (2004).
30. Benson, C. A. et al. A randomized study of emtricitabine and lamivudine in stably suppressed patients with HIV. Aids 18, 2269-76 (2004).
31. www.apregistry.com/forms/interim_report.pdf accessed April 30.
32. www.cdc.gov/ncbddd/bd/macdp.htm accessed April 30.
33. Hirt, D. et al. in 15th Conference on Retroviruses and Opportunistic Infections (Boston, MA, 2008).
34. Gathe, J. & Podzamczar, D. in 11th Conference on Retrovirus and Opportunistic Infections (CROI) (San Francisco, CA).

35. Molina, J. M. et al. Pharmacokinetics of emtricitabine, didanosine and efavirenz administered once-daily for the treatment of HIV-infected adults (pharmacokinetic substudy of the ANRS 091 trial). *HIV Medicine* 5, 99-104 (2004).
36. Podzamczer, D., Gathe, J. & Johnson, M. in 9th European AIDS Conference (Warsaw, Poland, 2003).
37. Gallant, J. E. et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV.[see comment]. *New England Journal of Medicine* 354, 251-60 (2006).
38. Gilead & Sciences. Investigator's Brochure: Tenofovir Gel (GS-1278). Third Edition.
39. Creek, M. R. Pharmacokinetics and tissue distribution study of PMPA (GS-1278) following intravaginal administration in female New Zealand White Rabbits. SRI Report No. B010-97.
40. Lund, B. & Weber, S. Pharmacokinetics and distribution of intravaginal [¹⁴C] PMPA (GS-1278) following single dose administration to rats. Gilead Sciences Report No. 96-DDM-1278-004.
41. Fairchild, D. 14-day vaginal tissue irritation and toxicity study of PMPA in rats. Report No. M016-97.
42. Rush, R. E. A vaginal irritation study in rabbits with PMPA (GS-1278) topical gel. . Gilead Sciences Report No. 96-TOX-1278-003.
43. Smith, D. et al. Antiretroviral resistance is not an important risk of the oral tenofovir prophylaxis trial in Botswana: a simple mathematical modelling approach. XVI International AIDS Conference, August 13-16, 2006 Toronto, Canada, Abstract THAX0105 (2006).
44. Palmer, S., Kearney, M., Maldarelli, F. & et, a. I. Multiple, Linked Human Immunodeficiency Virus Type 1 Drug Resistance Mutations in Treatment-Experienced Patients Are Missed by Standard Genotype Analysis. *JCM* 43, 406-413 (2005).
45. CONRAD. Protocol #A04-099 (Study Report), Male Tolerance Study.
46. Hillier, S. Safety and acceptability of daily and coitally dependent use of 1% tenofovir over six months of use. *Microbicides 2008*. New Delhi, India.
47. Biosyn & Inc. Investigator's Brochure: UC-781 Vaginal Gel. Version 2.0 February 2006.
48. Schwartz, J. L. et al. A randomized six-day safety study of an antiretroviral microbicide candidate UC781, a non-nucleoside reverse transcriptase inhibitor. *Sexually Transmitted Diseases* 35, 414-9 (2008).
49. Holmes, L. B. Need for inclusion and exclusion criteria for the structural abnormalities recorded in children born from exposed pregnancies.[see comment][comment]. *Teratology* 59, 1-2 (1999).
50. http://www.afro.who.int/home/countries/fact_sheets/malawi.pdf. (Accessed August, 2008.).
51. http://www.afro.who.int/home/countries/fact_sheets/southafrica.pdf. (Accessed August, 2008.).
52. http://www.afro.who.int/home/countries/fact_sheets/uganda.pdf. (Accessed August, 2008.).

53. http://www.afro.who.int/home/countries/fact_sheets/zambia.pdf. (Accessed August, 2008.).
54. http://www.afro.who.int/home/countries/fact_sheets/zimbabwe.pdf. (Accessed August, 2008.).
55. http://www.paho.org/English/DD/AIS/cp_840.htm. (Accessed August, 2008.).
56. http://www.searo.who.int/LinkFiles/India_CHP_india.pdf. (Accessed August, 2008.).
57. http://www.who.int/whosis/mort/profiles/mort_amro_usa_unitedstatesofamerica.pdf. (Accessed August, 2008.).