CAPRISA 004

Phase IIb Trial to Assess the Safety and Effectiveness of the Vaginal Microbicide 1% Tenofovir Gel for the Prevention of HIV Infection in Women in South Africa

Trial design and conduct:
Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu-Natal, Durban, South Africa,

Coordination of Product manufacture and packaging:
CONRAD, Virginia, USA

Statistical, regulatory and operational support:
Family Health International, North Carolina, USA

Co-Principal Investigators
Quarraisha Abdool Karim, PhD, and Salim S. Abdool Karim, MBChB, PhD
CAPRISA, University of KwaZulu-Natal

Version 1.3, 29 October 2010

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PROTOCOL TEAM ROSTER

Quarraisha Abdool Karim, PhD
Co-Principal Investigator
CAPRISA, Nelson R Mandela School of Medicine, University of KwaZulu-Natal , Private Bag 7, Congella 4013, Durban, South Africa
Abdoolq2@ukzn.ac.za
T: 27-31-260 4208
F: 27-31-260 4307

Salim S. Abdool Karim, MBChB, FFPHM, PhD
Co-Principal Investigator
CAPRISA, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag 7, Congella 4013, Durban, South Africa
karims1@ukzn.ac.za
T: 27-31-260 2381
F: 27-31-260 3093

Cheryl Baxter, MSc
Research Associate
CAPRISA , Nelson R Mandela School of Medicine, University of KwaZulu-Natal , Private Bag 7, Congella 4013, Durban, South Africa
Baxterc1@ukzn.ac.za
T: 27-31-260 4559
F: 27-31-260 4566

Janet Frohlich, DipMGC, DCurVulindlela Site Operations ManagerCAPRISA Vulindlela Clinical Research Site, Mafakatini Primary Health Care Clinic, Mafakatini, Vulindlela, South Africa
frohlichj@ukzn.ac.za
T: 27-31-260 6851
F: 27-33-997 1425

Andy Gray, MSc (Pharm)
Senior Protocol Pharmacist
Department of Therapeutics and Medicines Management and CAPRISA, University of KwaZulu-Natal, Private Bag 7, Congella 4013, Durban, SA
graya1@ukzn.ac.za
T: 27-31-260 4334
F: 27-31-260 4566

Anneke Grobler, MSc
Protocol Statistician
CAPRISA, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag 7, Congella 4013, Durban, South Africa
grobler@ukzn.ac.za
T: 27-31-260 4392
F: 27-31-260 4566

Ayesha BM Kharsany, PhD
Co investigator
University of KwaZulu-Natal, Private Bag 7, Congella 4013, Durban, South Africa
kharsany@ukzn.ac.za
T: 27-31-260 4558
F: 27-31-260 4566

Leila Mansoor, PhD
Study Co ordinator
CAPRISA , Nelson R Mandela School of Medicine, University of KwaZulu-Natal , Private Bag 7, Congella 4013, Durban, South Africa
Mansoor@ukzn.ac.za
T: 27-31-260 4641
F: 27-31-260 4566

Koleka Mlisana, MBChB
Project Clinical Operations Director (eThekwini)
CAPRISA eThekwini Clinical Research Site, University of KwaZulu-Natal, Private Bag 7, Congella 4013, Durban, South Africa
mlisanak@ukzn.ac.za
T: 27-31-260 4562
F: 27-31-260 4566

Luvuyo Mtongana, MBChB
Study Clinician
CAPRISA, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag 7, Congella 4013, Durban, South Africa
mtongana@ukzn.ac.za
T: 27-31-260 4555
F: 27-31-260 4566

Sengeziwe Sibeko, MBChB, FCOG
Project Gynaecologist
CAPRISA, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag 7, Congella 4013, Durban, South Africa
sibekos@ukzn.ac.za
T: 27-31-260 4555
F: 27-31-260 4566

Douglas Taylor, PhD
Senior Protocol Statistician
Family Health International, PO Box 13950, Research Triangle Park, NC 27709, USA
dtaylor@fhi.org
T: 1-919-544 7040 ext. 327
F: 1-919-544 0207

Phase IIb trial to assess the safety and effectiveness of the vaginal microbicide 1% tenofovir gel for the prevention of HIV infection in women in South Africa

ROSTER OF ORGANISATIONS:

CONRAD
Henry Gabelnick, PhD
Executive Director,
CONRAD, 1911 Fort Myer Drive., Ste. 900, Arlington, VA 22209,
hgabelnick@conrad.org
T: 1-703-276 3904
F: 1-703-524 4744

Family Health International
Willard Cates, Jr., MD, MPH
President: FHI
Family Health International, PO Box 13950, Research Triangle Park, NC 27709, USA
wcates@fhi.org
T: 1-919-544 7040
F: 1-919-544 0207

STUDY FUNDED BY:

United States Agency for International Development
Attn: **Jeff Spieler, PhD**
Chief, Research, Technology and Utilization Division, Office of Population and Reproductive Health, Bureau for Global Health, USAID, 1300 Pennsylvania Ave, NW, Washington, DC 20523-3601
jspieler@usaid.gov
T: 1-202-712 1402
F: 1-202-216 3404

LIFELab
Attn: **Blessed Okole, PhD**
Chief Executive Officer
LIFELab
PO Box 30603
Mayville, 4058
Durban, South Africa
Blessed.Okole@LifeLab.org.za T: 27-31-261 6427
F: 27-31-261 4581

PRODUCT SUPPLIES:

Packaged Tenofovir & Placebo gel supplies provided by:

CONRAD
Attn: **Henry Gabelnick, PhD**
Executive Director, CONRAD
1611 North Kent St., Suite. 806
Arlington, VA 22209,
hgabelnick@conrad.org
T: 1-703-276 3904
F: 1-703-524 4744

Tenofovir Active Product Ingredient provided by:

Gilead Sciences
Attn: **James F. Rooney, MD**
Vice President, Medical Affairs
Gilead Sciences
333 Lakeside Drive
Foster City, CA 94404
jrooney@gilead.com
T: 650-522-5708
F: 650-522-5854

STUDY MONITOR:

Family Health International
Attn: **Amanda Troxler**
PO Box 13950
Research Triangle Park
NC 27709, USA
atroxler@fhi.org
T: 1-919-544-7040 ext. 11301
F: 1-919-544-7261

STUDY SPONSOR

Family Health International
Attn: **David Borasky(Director, Regulatory Affairs and Quality Assurance)**
PO Box 13950
Research Triangle Park
NC 27709, USA
dborasky@fhi.org
T: 1-919-544 7040
F: 1-919-544 7261

ETHICS COMMITTEES:

University of KwaZulu-Natal Biomedical Research Ethics Committee
Attn: **Douglas Wassenaar**
Faculty of Health Sciences Ethics Committee of the Nelson R Mandela School of Medicine, University of KwaZulu-Natal
Ethicsmed@ukzn.ac.za
T: 27-31-260 4495
F: 27-31-260 4410

Protection of Human Subject Committee Family Health International
Attn: **David Borasky**
Director, Office of International Research Ethics
2224 E Highway 54
Durham, NC 27713, USA
T: 1-919-544-7040 ext. 295
F: 1-919-544-0207

Phase IIb trial to assess the safety and effectiveness of the vaginal microbicide 1% tenofovir gel for the prevention of HIV infection in women in South Africa

CAPRISA 004

Signatures of Approval:

29 October 2010

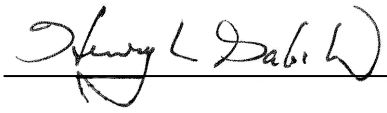
Co-Principal Investigator
Quarraisha Abdool Karim, PhD

Date of Signature

29 October 2010

Co-Principal Investigator
Salim S. Abdool Karim, MBChB, PhD

Date of Signature



29 October 2010

CONRAD
Henry Gabelnick, PhD

Date of Signature

29 October 2010

Family Health International
Willard Cates Jr, MD, MPH

Date of Signature

Phase IIb trial to assess the safety and effectiveness of the vaginal microbicide 1% tenofovir gel for the prevention of HIV infection in women in South Africa

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ABBREVIATIONS AND ACRONYMS

AE	adverse event
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal clinic
ALT	Alanine aminotransferase
AUC	Area Under the Curve
BID	Twice a day
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CDC	Communicable Disease Centre
CI	confidence interval
C_{max}	maximum concentration
CP	Community Programme
CRSG	Community Research Support Group
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
ELISA	enzyme-linked immunosorbent assay
FDA	(United States) Food and Drug Administration
FHI	Family Health International
FIV	Feline Immunodeficiency Virus
FPC	Family Planning clinic
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HEC	hydroxyethylcellulose
HIV	Human Immunodeficiency Virus
HBV	Hepatitis B Virus
HPTN	HIV Prevention Trials Network
HPV	human papillomavirus
HSV-2	herpes simplex virus-2
ICH	International Conference on Harmonization
ITT	Intention to treat
IV	intravenous
LLOQ	lower limit of quantitation
MCC	Medicines Control Council
MOP	Manual of Procedures
N-9	nonoxynol-9
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PHC	Primary Health Care
PID	Participant identification
PK	Pharmacokinetics
PSRT	Protocol Safety Review Team
RNA	Ribonucleic Acid
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	subcutaneous
SIV	Simian Immunodeficiency Virus
SOP	Standard Operating Procedures
STI	sexually transmitted infection
TDF	Tenofovir disoproxil fumarate
T_{max}	Time to maximum concentration
UNAIDS	Joint United Nations Programme on HIV/AIDS
USA	United States of America
VCT	Voluntary counselling and testing
w/w	weight per weight
w/v	weight per volume

Phase IIb trial to assess the safety and effectiveness of the vaginal microbicide 1% tenofovir gel for the prevention of HIV infection in women in South Africa

SCHEMA

Purpose: To assess the safety and effectiveness of tenofovir gel, a candidate vaginal microbicide, in sexually active women at risk for human immunodeficiency virus (HIV) infection in South Africa.

Design: Phase IIb, two-arm, double-blind, randomised, controlled trial comparing 1% tenofovir gel with a placebo gel.

Study Population: Sexually active, HIV-uninfected women aged 18 to 40 years in South Africa

Study Size: Up to 1250 women

Treatment Regimen: Participants will be provided with a supply of single-use, pre-filled applicators according to their randomisation. While in the study, participants will be asked to apply a first dose of the assigned study product, 1% tenofovir gel or placebo gel, within 12 hours prior to coitus and insert a second dose as soon as possible within 12 hours after coitus. They will be advised to use only two doses of gel in a 24-hour period.

Study Duration: Approximately 30 months in total. Accrual will require approximately 18 months and follow-up will continue until 92 incident HIV infections are observed in the study, which is expected to occur approximately 12 months after the end of the accrual period.

Primary Objective:

To evaluate the effectiveness and safety of a candidate vaginal microbicide, tenofovir gel, when applied intravaginally by women, in preventing sexually transmitted HIV infection.

Secondary Objectives:

- To assess the impact, if any, of tenofovir gel on the incidence rate of deep epithelial disruption
- To assess the impact, if any, of tenofovir gel on viral load in women who become infected with HIV during the trial.
- To assess tenofovir resistance in HIV seroconvertors in the trial
- To ascertain the impact, if any, of tenofovir gel on pregnancy rates and outcomes
- To assess the impact, if any, of product hold at study exit on HIV infection and tenofovir resistance

Ancillary Objective

- To assess the impact, if any, of tenofovir gel in preventing sexually transmitted infections, including. herpes simplex virus type 2 (HSV-2) and human papillomavirus (HPV) infections.

Study sites:

- CAPRISA Vulindlela Clinical Research Site, KwaZulu-Natal, South Africa
- CAPRISA eThekwini Clinical Research Site, Durban, South Africa

1 INTRODUCTION

1.1 Epidemiology of HIV

South Africa is experiencing one of the largest and fastest growing HIV epidemics in Sub-Saharan Africa and the world[1] About 6 million people are living with HIV/AIDS in South Africa[2]. There are many factors that contribute to the unprecedented, explosive spread of HIV in South Africa. The economically-motivated population migration with its associated disruption of conjugal stability and family units is a key factor driving the epidemic in Southern Africa[3]. Sixty percent of all infected adults acquire their infection before age 25, and young women between the ages of 20-24 years have the highest HIV prevalence and incidence rates[4]. The national HIV prevalence in prenatal women continues to rise, and in 2004, the most recent national estimate available, show that these rates have reached a new high of 29.5% (95% Confidence Interval (CI) 28.5 – 30.5). These national rates mask variations within South Africa as the prevalence of HIV varies by province, and is highest in KwaZulu Natal with a prevalence of 40.7% (95% CI 38.8 – 42.7) in 2004. This rate is matched by the HIV rates we have observed [42.7% (95% CI 38.5 - 47.0)] by replicating this survey among pregnant women at our rural clinical research site in Vulindlela, KwaZulu Natal. These trends have been increasing annually at all three levels (national; provincial; local). The majority of new infections are heterosexually transmitted with the highest incidence rates in women. Some indication of the importance of the heterosexual component of the global burden of HIV infection can be gleaned from the Joint United Nations Program on HIV/AIDS (UNAIDS) estimates at the end of 2003[5]. Of the estimated 37.8 million prevalent HIV infections, about 87% were acquired through heterosexual transmission. Further, of the 4.8 million new HIV infections and 2.9 million Acquired Immune Deficiency Syndrome (AIDS) deaths in 2003, over 85% were in persons who most likely acquired HIV infection heterosexually. These data underscore the importance of this mode of transmission.

Not only is heterosexual transmission important in driving the current major epidemic in sub-Saharan Africa but is also a major factor driving the emerging epidemics in India and China. The geographical distribution of the modes of HIV transmission, indicates that heterosexual transmission of HIV is an important mechanism of transmission beyond sub-Saharan Africa to most countries around the world and particularly in south and south-east Asia where a quarter of the new infections in 2003 occurred.

This mode of spread, which predominates in the global epidemic, is influenced by several key epidemiological factors such as age, gender, mobility, sexual partner profile, and the presence of other sexually transmitted infections (STIs). A striking characteristic of heterosexual transmission is the disproportionate burden of HIV infection in women compared to men[6]. In addition to an estimated 7-fold greater efficiency of transmission from men to women compared to women to men, social, cultural and economic factors also contribute to the excess infection observed in women compared to men[7]. Further, women acquire HIV infection at a younger age, at least 5-10 years earlier than men. Young boys aged 15-19 years have lower rates of HIV while teenage girls are already close to peak prevalence[8, 9]. In most African countries where heterosexual transmission is the major mode of transmission, a key factor in the epidemic is the high incidence rates in young women between the ages of 14 and 24 years as a result of sexual coupling with older men[10].

Notwithstanding the greater vulnerability of women, current options to reduce transmission and acquisition of HIV infection remain limited for women. There is a clear need for new technologies to prevent the sexual transmission of HIV in women. Correct and consistent use of male condoms has been shown to prevent HIV transmission[11], but women often are unable to negotiate use of condoms by their male partners[12-14]. The female condom has been marketed as an alternative barrier method, but this device is relatively costly. It requires a certain level of skill to use and acceptance by the male partner. There is clearly a need for new technologies to prevent the sexual transmission of HIV in women.

1.2 Microbicides and Developmental Pipeline

Topical microbicides are products designed to prevent the sexual transmission of HIV and other disease pathogens[12-15]. Potentially, they can be applied vaginally to prevent both male-to-female and female-to-male transmission. They also offer a female-controlled option in cases where neither male or female condom use can be negotiated. Marketed chemical spermicides, which have shown some activity against HIV and STI pathogens in vitro, have been evaluated as topical microbicides. The most notable of these was nonoxynol-

9 (N-9), which has been tested in various doses and formulations, but was shown to be ineffective in preventing HIV and possibly harmful[16-18].

Table 1 is a summary of the current stage of testing for microbicides in the developmental pipeline. Microbicides currently under development operate in one of four ways.

1) Surfactants operate by disrupting cell membranes and have a wide spectrum of activity against several microbes, spermatozoa and cell membranes. Since the testing of N-9, a newer agent, C31G (Savvy), which has a better safety profile, has been developed.
2) Vaginal defence enhancers boost the body's natural defences against infection by maintaining the naturally acidic environment of the vagina by increasing lactobacilli or by rapidly acidifying alkaline ejaculate. This process inactivates both sperm and STIs. BufferGel™ operates in this manner.
3) Entry and fusion inhibitors bind to pathogens or to healthy cells before pathogens can attach or invade them. Carraguard®, PRO 2000, and Cellulose sulfate (UsherCell™) operate in this manner. Cellulose sulfate inhibits sperm function and has recently demonstrated its contraceptive effectiveness in a 200 patient Phase II clinical study in the U.S..
4) Replication inhibitors, inhibit normal reverse transcription of viral RNA into mammalian DNA in host (infected) cells. Reverse transcriptase inhibitors are highly specific for a virus species, One such product is tenofovir gel, developed by Gilead Sciences. Tenofovir gel is designed to protect against HIV infection by inhibiting viral replication in susceptible cells.

Table 1: Developmental Pipeline of Microbicides under Evaluation

Phase				
I	**I / II**	**II**	**IIb**	**III**
• Ethanol in Emollient Gel • Dapivirine • TMC120 • UC-781 • VivaGel/SPL7013™ • CAP vaginal soft tablet (planned) • PC 815 (planned)	• Invisible Condom ™ • VivaGel®†	• 1% Tenofovir gel • Invisible Condom™ (Planned)	• BufferGel™ & PRO2000 (0.5%) • Tenofovir gel (1%) in CAPRISA 004	• PRO 2000 (0.5%;2%) • BufferGel®‡ (planned)

Table adapted from Alliance for Microbicide Development website at www.microbicide.org accessed December 17, 2008 which included the following disclaimer. "This Update is a brief overview and summary of the microbicide pipeline, produced and disseminated at the beginning of every month. As changes occur frequently, please visit www.microbicide.org for the most recent information or details, and/or contact Alliance Research Associate Stephanie Tillman (stillman@microbicide.org) for further information.".

1.3 Study Product

The study product is a gel formulation of tenofovir (PMPA, 9- [(R)-2-phosphonomethoxy)propyl]adenine monohydrate), an antiviral manufactured by Gilead Sciences, Inc., and licensed to CONRAD. Tenofovir is an adenosine nucleoside monophosphate (nucleotide) analog with potent activity against retroviruses. An orally bioavailable form of tenofovir (tenofovir disoproxil fumarate, tenofovir DF, Viread), has been approved for the treatment of HIV-1 infection since 2001 in the US and 2002 in Europe. It is estimated that over 200,000 HIV infected patients have received treatment with tenofovir DF. An intravenous (IV) formulation of this antiviral compound and an oral prodrug (Tenofovir Disoproxil Fumarate - TDF), has also been developed, approved and actively marketed by Gilead Sciences for the treatment of human immunodeficiency virus type-1 (HIV-1) infection in combination with other antiretroviral agents. Given the established safety and efficacy profile of TDF and the protective efficacy with tenofovir gel in monkey models, evaluation of tenofovir gel for prevention of HIV-1 in clinical studies is warranted.

1.3.1 Prior Research

The novel selective broad-spectrum anti-DNA virus agent, (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine (HPMPA) [19-22], served as the prototype compound for the development of a series of widely used

therapeutic acyclic nucleoside phosphonates. These include cidofovir (HPMPC) in the treatment of papilloma-, herpes-, adeno- and poxvirus infections, adefovir (PMEA) in the treatment of chronic hepatitis B virus (HBV) infections, and tenofovir (PMPA) in the treatment of acquired immunodeficiency syndrome (AIDS)[23].

Tenofovir demonstrated potent activity against a wide range of retroviruses, i.e. simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), visna-maedi virus, and murine leukaemia/sarcoma viruses and hepadnaviruses, Given that the precursors of tenofovir are active against other STIs, it is biologically plausible that tenofovir could have activity against other STIs, but further research is required.

Antiviral effects

The *in vitro* antiviral activity of tenofovir against laboratory and clinical isolates of HIV-1 was assessed in lymphoblastoid cell lines, primary monocyte/macrophage cells and peripheral blood lymphocytes. Tenofovir displayed antiviral activity *in vitro* against HIV-1 clades A, B, C, D, E, F, G and O (IC50 values ranged from 0.5 µM to 2.2 µM). In drug combination studies of tenofovir with nucleoside reverse transcriptase inhibitors (abacavir, didanosine, lamivudine, stavudine, zalcitabine, zidovudine), non-nucleoside reverse transcriptase inhibitors (delavirdine, efavirenz, nevirapine), and protease inhibitors (amprenavir, indinavir, nelfinavir, ritonavir, saquinavir), additive to synergistic effects were observed. Most of these drug combinations have not been studied in humans.

Prevention of Simian Immunodeficiency Virus transmission in animal models

Multiple studies have demonstrated the efficacy of tenofovir in preventing transmission of SIV in simian models[24]. Tenofovir has been successfully administered as pre-exposure or post-exposure prophylaxis[25, 26]. These studies have established tenofovir as a promising antiretroviral agent for the prevention of SIV infection. In one study, subcutaneous (SC) injection of tenofovir daily for 4 weeks in macaques resulted in 100% protection against acute SIV infection in a total of 25 treated animals without toxicity whether administration began 48 hours prior to IV inoculation (dose of tenofovir, 20 mg/kg in five animals, 30 mg/kg in 10 animals), 4 hours after inoculation (dose of tenofovir 30 mg/kg in five animals), or 24 hours post-inoculation (dose of tenofovir, 30 mg/kg in five animals). Evidence of SIV infection was not present in any of the treated animals monitored for up to 52 weeks, including viral load in plasma and peripheral blood mononucleocytes (PBMCs), SIV DNA in PBMCs, SIV-specific antibody, and lymph node biopsy. In contrast, each of 10 animals receiving placebo 48 hours prior to inoculation became infected [26].

The ability of tenofovir to prevent the establishment of persistent infection was investigated in a study on cynomolgus macaques (*Macaca fascicularis*). Daily SC dosing with tenofovir was initiated at varying times, as well as different durations of treatment, following intravenous inoculation with SIV. Twenty-four macaques were studied for 46 weeks after inoculation with SIV. All mock-treated control macaques showed evidence of infection within 2 weeks post-inoculation. All macaques that were treated with tenofovir for 28 days beginning 24 hours post-inoculation showed no evidence of viral replication following discontinuation of tenofovir treatment. However, extending the time to initiation of treatment from 24 to 48 or 72 hours post-inoculation or decreasing the duration of treatment reduced effectiveness in preventing establishment of persistent infection. Only half of the macaques treated for 10 days, and none of those treated for 3 days, were completely protected when treatment was initiated at 24 hours. Despite the reduced efficacy of delayed and shortened treatment, all tenofovir-treated macaques that were not protected showed delays in the onset of cell-associated and plasma viremia and antibody responses compared with mock controls[27].

In a study investigating the efficacy of tenofovir gel to prevent SIV infection, intravaginal application of 10% tenofovir gel weight per weight (w/w) administered 24 hours before, 0 hours, 24 hours after and 48 hours after intravaginal inoculation of SIV infection at 0 and 24 hours resulted in 100% protection in four female rhesus macaques, compared with evidence of infection in each of the two animals receiving placebo (vehicle only) [28]. In another study, 1% tenofovir gel (w/w) administered at three time points; 24 hours before, 15 minutes before, and 24 hours after a single intravaginal inoculation of SIV, resulted in 80% protection in five female rhesus macaques, comparable to that of the 10% tenofovir gel (w/w) group. Sixty percent protection was achieved in a third group of macaques which only received a single application of 1% tenofovir gel (w/w) 15 minutes before SIV challenge. Treated macaques were monitored for a total of 20 weeks, by virus

isolation from PBMCs. Further evidence of the efficacy of tenofovir in preventing infection following SIV exposure is provided by a study of pig-tailed macaques. Tenofovir was initiated 12, 36 and 72 hours following HIV exposure. Systemic infection was not evident in the 12 and 36 hour post exposure group (n=8) as defined by plasma viremia, cell-associated provirus, antibody response, and lymph node virus. However, breakthrough infection in one animal in the 72 hour post HIV exposure group was detected at 16 weeks [(29)].

A study investigating the protection of macaques against rectal SIV challenge showed that rectal pre-dosing with tenofovir gel has potential as a microbicide strategy. Mucosally-applied tenofovir gel was given rectally as a single dose 15 minutes or 2 hours prior to, or 2 hours after, intrarectal challenge. In the 2 control groups of macaques, 4 of 4 untreated macaques and 3 of 4 macaques given placebo gel became infected. Virus was recovered from only 1 of 6 animals receiving tenofovir gel 15 minutes prior to virus challenge. In 1 other animal in this group virus was recovered only at weeks 2 and 6. Virus was not recovered from any of the other 4 animals. Two of 3 animals receiving the drug 2 hours prior to virus challenge showed no evidence of circulating virus and in the third animal virus isolation was delayed until week 12. In the third intervention group where gel was administered 2 hours after virus challenge, 2 out of the 3 animals became infected. Interestingly, gag-specific interferon-gamma secreting T cells were detected by ELISpot in 4 of 7 animals in which virus was unrecoverable from PBMC. These T-cell responses confirm exposure to challenge virus antigens and suggest that infection did not become established despite the virus having triggered an immune response[(30)].

Such *in vivo* activity of an antiviral compound makes tenofovir a promising agent for prevention of HIV infection and available evidence shows that tenofovir gel can provide potent protection against SIV infection even, in some instances, when applied several hours after SIV challenge.

Safety of tenofovir gel

In animal efficacy and toxicity studies, tenofovir was found to be well tolerated even at high doses administered parenterally (subcutaneously or via IV infusion) or orally over prolonged periods. In rat and rabbit models, tenofovir gel (0.3-10.0%) caused minimal to mild local irritation following intravaginal administration. In rats, daily intravaginal administration of tenofovir gel (1-10% for 14 days) produced no evidence for local irritation or systemic toxicity as evidenced by no gross alterations in tissues and organs within the thoracic and abdominal cavities, and no histological lesions in the reproductive tissues (cervix, ovaries, uterine horns, vagina, vulva) or kidneys. In a 10-day rabbit vaginal irritation study, irritation was minimal in animals treated with 0.3-1.0% tenofovir gel. At higher doses (3-10%), tenofovir gel caused mild local irritation (increased leucocytic infiltrates, congestion and, in some cases, slightly increased edema) and produced average irritation scores similar to the control (Conceptrol). However, unlike Conceptrol, no animals treated with tenofovir had epithelial erosion or ulceration.

Clinical data on the safety of tenofovir gel comes from HPTN 050, a phase I safety and tolerability study among 84 low risk women who applied either 0.3% or 1% tenofovir gel once or twice daily for 14 days. The 1% tenofovir gel formulation was well tolerated in both HIV negative and HIV positive women. The majority of adverse events (AEs) reported in HPTN 050 were mild (87%) and limited to the genitourinary tract (77%). Four severe AEs were reported, but only one, lower abdominal, pain was thought to be product-related. No clinically significant systemic toxicity was observed and the tenofovir gel in this study showed a beneficial effect on vaginal microflora [(31)]. A recently completed study of 200 women from the USA and India (HPTN 059) reported that both daily and coitally dependent use of tenofovir gel was acceptable and safe[(32)].

Oral TDF is a Food and Drug Administration (FDA) category B drug. Reproduction studies have been performed in rats and rabbits at doses up to 14 and 19 times the human dose based on body surface area comparisons and revealed no evidence of impaired fertility or harm to the foetus due to tenofovir. However, there are no specific studies of the oral formulation of tenofovir in pregnant women, Therefore, while pregnancy is not a specific contraindication to use, tenofovir is not recommended for unqualified use in pregnant women. Use in pregnancy is cautioned due to the lack of human safety data in pregnancy. An Antiretroviral Pregnancy Registry has been established to monitor foetal outcomes of pregnant women exposed to oral TDF. However, this registry has very limited data relating to tenofovir which may indicate

that it is not being used sufficiently widely in pregnancy yet or it may indicate that the drug's good safety profile has resulted in few entries.

Pharmacokinetics and systemic absorption

The pharmacokinetics (PK) of intravaginal tenofovir were examined in female rabbits following administration of a single dose of 0.5 mL of tenofovir gel containing 1% weight in volume (w/v) tenofovir (5 mg tenofovir per animal; 50 µCi/kg). Concentrations of radioactivity in plasma were highest (0.010 µg-eq/mL) at the first sample time point (30 minutes post-dose) and below quantifiable limits at 24 hours [33].

In a second study, 18 female rabbits received a single intravaginal dose of 0.5 mL of tenofovir gel containing 1% w/v tenofovir (5 mg tenofovir per animal; 50 µCi/kg), while a further 18 rabbits received a single intravaginal dose of 0.5 mL of tenofovir gel containing 3% w/v 9- [(R)-2-(phosphonomethoxy)propyl]adenine monohydrate (PMPA) (15 mg PMPA per animal; 50 µCi/kg). Six animals from each group were sacrificed at each of 0.5, 4, and 24 hours post dose. The majority of the administered dose was recovered in urine (15 - 38%) or cagewash (28 - 36%), suggesting that the formulation leaked out of the vagina. Tissue concentrations of radioactivity were highest in vaginal tissue at 30 minutes post-dose, with substantial variability in actual tissue levels available (0.65 - 98.3 µg-eq/g for 1% tenofovir; 10.3 - 274 µg-eq/g for 3% tenofovir). Relatively high concentrations of radioactivity in intestinal tissues were attributed to ingestion of the formulation during grooming and poor total recovery in many animals was attributed to loss of formulation on fur and paws.

In addition, a non-blinded PK study evaluating the potential use of tenofovir in pre- and post-exposure prophylaxis[34], performed in HIV-1 infected subjects (nine men/13 women) using a 300 mg oral daily dose, showed that high extracellular and intracellular concentrations of tenofovir are achieved in the genital tract of men and women and provides encouraging data in support of tenofovir in pre- and post-exposure prophylaxis trials. After 24 hours, intracellular tenofovir genital tract to blood ratios in males for day 1 and 7 were 3.1±5.4 and 7.0±14.8, respectively. Tenofovir levels were significantly greater ($p<0.05$) in genital washes than blood at both day 1 and steady state in men and women. Tenofovir has a long half-life with intracellular levels sustained beyond 36 hours.

The systemic pharmacokinetics of 1% tenofovir gel has been evaluated in the HPTN 050 study among 25 women who applied tenofovir gel once or twice daily for 14 days. Serum plasma measurements were taken at enrolment at 0.5, 1, 2, 4, 6, 8 and 12 hours post dosing and again on day 14, 24 hours following the day 13 dose. The analysis of PK data was performed by measuring the area under the plasma concentration curve of tenofovir (AUC). Tenofovir accumulation was determined by comparing the median AUC on day 13 to the median AUC on day 0. In addition tenofovir AUC in sexually active versus sexually inactive cohorts was compared. Women on oral tenofovir prodrug (TDF) were excluded. Fourteen of 25 women (56%) had low, but detectable, serum tenofovir levels (lower limit of quantitation (LLOQ): 3.0 ng/mL) after 14 days of study gel use (Figure 1). The maximum serum tenofovir concentrations (C_{max}) ranged from 3.1 to 25.8ng/mL. For the woman with the highest observed peak level, 25.8ng/mL, this peak level occurred 2 hours following the dose; the level rapidly declined to 10.8ng/mL at 4 hours and was undetectable at 12 hours following the dose. Besides the outlier with the highest tenofovir level, the next highest C_{max} was 7.1ng/mL. No drug was detected in serum of any women at the 24 hour sampling point.

Levels for all women with measurable tenofovir levels in the blood are shown in figure 1 (14 of 25; LLOQ approximately 3.0 ng/mL [dotted line]). For reference the tenofovir level associated with the median 24 hour post-dose blood concentration following an oral 300 mg TDF dose is indicated with dashed line

Considering all women in the PK cohort, the median tenofovir C_{max} was 3.4 ng/mL (interquartile range: below LLOQ [3.0ng/ML] to 4.7ng/mL). The median C_{max} for all subjects (3.4ng/mL) corresponds to approximately 2.5% of the maximum (C_{max} at steady state = 135 ng/mL) and 7.2% of the minimum (C_{24} single dose median is approximately 47 ng/mL) blood concentrations at steady-state with 300 mg daily oral TDF dosing Figure 1.

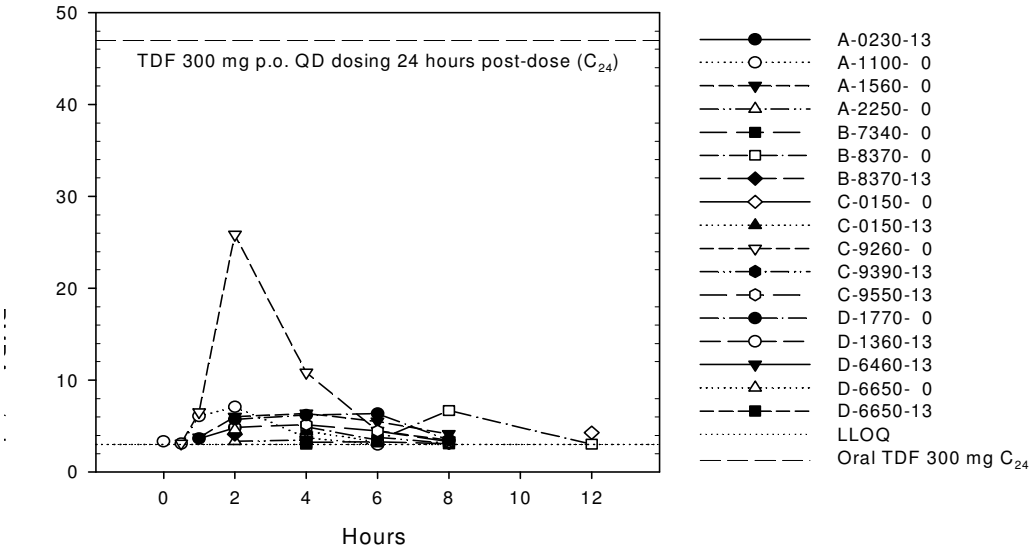

Figure 1: Tenofovir Blood Concentrations vs. Time after Vaginal Administration

Legend: First letter = Cohort; 4 digits = 4 digits of participant ID; Last digit = day of study; Cohort A – HIV uninfected/sexually abstinent; Cohort B - HIV uninfected/sexually active; Cohort C - HIV infected/sexually abstinent; Cohort D - HIV infected/sexually active

1.4 Dosing strategy of tenofovir gel

This is a Phase IIb, two-arm, randomised, double-blind, controlled trial comparing 1% tenofovir gel and a placebo gel. Despite significant advances in the microbicide field, the issue of timing of application for microbicides remains inconclusive in the absence of surrogate markers of protection or biological activity. Additionally, the timing of product insertion related to efficacy is affected by each product's mechanism of action. Hence, the definitive answers on timing of product insertion will only become available when a trial shows an efficacious product.

Two broad approaches have been proposed for the timing of insertion of tenofovir gel; daily dosing or coitally dependent dosing. There are groups of women who may benefit from each of these strategies. Daily dosing would require substantially more doses than coitally dependent use (with the concomitant increased costs), but it may be associated with higher adherence in some women. It has also been argued that daily dosing may have the converse effect of lower adherence in some women depending on various behavioural factors. With daily dosing, there may be no specific benefit to be gained from using a vaginal gel formulation as opposed to the standard oral formulation which would be more convenient and less expensive.

In the South African context, there is a specific need for a coitally-dependent microbicide (e.g. in certain young women and women with migrant partners due to the long periods without sex) to impact on high-risk sexual activity and the future course of the HIV pandemic.

Based on the available data, this trial proposes coitally-dependent application of tenofovir gel. The women will be encouraged to use the first dose of gel within 12 hours prior to coitus and insert a second dose of gel as soon as possible after coitus within 12 hours. Irrespective of the number of coital acts in a day, the women will be advised not to use more than two gel applications in a 24 hour period. The rationale for this decision, given the clear need for a coitally dependent efficacious microbicide, is based on the following::

- We hypothesize that a key mechanism of action of tenofovir gel is through its action of inhibiting viral replication in the CD4+ target cells in the lumen and in the layers of cells that make up the genital tract. Hence the achievement of adequate cellular (and tissue) concentrations of drug locally in the genital tract is considered important.
- The hypothesis that a single dose of tenofovir can prevent infection is supported by data from monkey models:
    - In macaques, 1% tenofovir gel used 24 hours before and up to 36 hours after challenge, in various dosing strategies, suggests protection.

    - A single dose of 1% tenofovir given 15 minutes before challenge suggests protection. This has been shown in macaques with both vaginal and rectal dosing followed by vaginal and rectal challenge respectively
- Tissue levels in a monkey study were elevated early as 15 minutes following administration of 1% tenofovir gel. Peak concentrations were reached between 8 and 12 hours. At 24 hours post dose, the tissue concentrations observed were well above those seen in the first 4 hours post-dose but the levels are starting to wane. At 48 hours, tissue levels were still above the level of detection.
- Based on the above data, we selected the window of up to 12 hours pre-exposure in order to maximize tissue concentrations at the time of first exposure to the virus.
- In animal models, virus replication is evident as early as 1 hour post-challenge - an animal study showed that SIV can enter the vaginal mucosa within 60 min of intravaginal exposure and is detectable in draining lymph nodes within 18 hours following intravaginal SIV exposure[35]. While cervical explant data suggest that infection may only be first detectable by p24 assays in the tissues about 30 hours after exposure to challenge virus, even when mimicking disrupted genital tract epithelium. Hence, sustained tenofovir levels may be required for at least 48 hours post-inoculation to prevent viral replication.
- To decrease the likelihood of breakthrough infection due to waning levels of tenofovir within this window of opportunity to prevent replication, we have added a second dose of gel to be administered within 12 hours of exposure. While a single dose may provide adequate levels for this 48 hour period, the second dose is considered a valuable component of the dosing strategy to provide sustained levels of tenofovir over the critical first few days post-exposure. This second dose extends in time the "theoretical" protection provided by the presence of tenofovir in genital tract cells and tissues.
- The dosing strategy has been limited to not more than two doses in one day because the available human safety data on tenofovir gel are limited to two doses per day.

2 STUDY SETTING

This study will be conducted at two Clinical Research Sites in KwaZulu-Natal, South Africa and will enrol women at high risk of HIV infection in Durban and Vulindlela.

2.1 CAPRISA Clinical Research Site: Vulindlela

The Vulindlela Clinical Research Site is situated in a rural community with approximately 400,000 residents in the KwaZulu-Natal midlands, about 150 km north-west of Durban. Primary Health Care (PHC) services are provided through seven clinics in the district. These nurse-managed services provide antenatal care, family planning, childhood immunization, STI treatment, minor ailment care, tuberculosis treatment and HIV Voluntary Counselling and Testing (VCT). The closest referral hospitals are Grey's and Edendale. The CAPRISA Clinical Research Site in Vulindlela adjoins the Mafakathini PHC Clinic.

<u>HIV incidence in Vulindlela</u>

A prospective cohort study (CAPRISA 050) has been conducted in Vulindlela with the aim of estimating rates of HIV seroincidence among women targeted for inclusion in this microbicide trial. Sexually active HIV uninfected women utilizing the Family Planning Clinic (FPC) and Antenatal Clinic (ANC) at the Vulindlela PHC facilities were enrolled in this study. Between March 2004 and April 2006 a total of 782 women were screened and HIV prevalence at baseline in this cohort was 30.2% (95%CI 26.7-33.9%). The average enrolment rate per month was 27 women. After 290 person years of follow up the HIV incidence rate in this cohort was 7.3 (95% CI 4.1-10.3)/100 person years.

<u>ANC HIV surveys</u>

The prevalence of HIV infection in pregnant women in Vulindlela has increased from 32.4% (95%CI 27.6-37.6%) in 2001 to 42.7% (95% CI 38.4-46.8%) in 2004. The age-specific prevalence for the 2001-2004 ANC survey in Vulindlela is presented in Table 2.

During this period of 4 years, most of the ANC attendees were less than 25 years of age; with 56.9% in this age range in 2001, 65.0% in 2002, 58.7% in 2003 and 65.6% in 2004. Of significance is that in 2004, 39% of the ANC attendees were younger than 19 years with the youngest being 12 years of age and the HIV prevalence in this age group was an alarming 26.7%. In 2004, the age specific prevalence was 54.7% among 20-24 year old women and 66.3% among 25-29 year old women. An increase from 31.2% (2001) to

42.9% (2002) to 66.0% (2003) was demonstrated in the 25-29 year old women. These data underscore the high and growing HIV prevalence and incidence rates in young, sexually active women under the age of 30 in this community.

Table 2: Age specific, Crude and Standardised HIV Prevalence 2001-2004 in Vulindlela ANC Attendees

	2001		2002		2003		2004	
Age group	**n/N**	**HIV Prev %(95%CI)**	**n/N**	**HIV Prev %(95%CI)**	**n/N**	**HIV Prev %(95%CI)**	**n/N**	**HIV prev %(95%CI)**
<20	6/41	14.6 (6.1-29.8)	41/ 159	25.8 (19.4-33.5)	13/67	19.4 (11.1-31.2)	54/202	26.7 (20.9-33.5)
20-24	2454	44.4 (31.1-58.5)	49/107	45.8 (36.2-55.7)	29/65	44.6 (32.5–57.4)	87/159	54.7 (46.6-62.5)
25-29	10/32	31.3 (16.8-50.2)	36/64	42.9 (32.3-54.2)	33/50	66.0 (51.1–78.4)	55/63	66.3 (55.0-76.1)
30-34	3/21	14.3 (3.8-37.4)	8/36	22.2 (10.7-40.0)	9/21	42.9 (22.6–65.6)	35/65	53.8 (41.1-66.1)
>35	3/19	15.8 (4.2-40.5)	6/23	26.1 (11.1-48.7)	8/22	36.4 (18.1–59.2)	4/41	9.8 (3.2-24.1)
Missing	67/182	36.8 (29.9-44.3)	¼	25.0 (1.3-78.1)	-	-	0/2	-
Crude prevalence	**113/349**	**32.4 (27.6-37.6)**	**141/413**	**34.1 (29.6-39.0)**	**92/225**	**40.9 (34.5-47.7)**	**235/552**	**42.6 (38.4-46.8)**
Standardised prevalence*		**27.9 (27.2-28.6)**		**35.5 (34.8-36.3)**		**43.5 (42.7-44.3)**		**47.1 (46.3-47.9*)**

* Standardised to the age structure of the National Department of Health HIV seroprevalence survey population in 2004

2.2 CAPRISA Clinical Research Site: eThekwini

The CAPRISA eThekwini Clinical Research site is located adjacent to the Prince Cyril Zulu Communicable Disease Centre (CDC), a designated PHC of the Durban City Health Department, for the diagnosis and treatment of STIs and tuberculosis. The clinic is conveniently situated in the Warwick triangle in the metropolitan region of Durban which serves as the nucleus of the public transportation with the central bus, "minibus" taxi station and rail station all within a 500 metre radius of the clinic building. This clinic is readily accessible in terms of the transport infrastructure. This clinic provides free STI and tuberculosis treatment. Annually, approximately 40 000 cases of STIs are treated at this clinic, approximately 36 000 of which are new cases. The majority of the STI patients accessing these facilities are self-referred either symptomatic with genital ulceration and/or vaginal discharge syndrome or as contacts of patients with a diagnosis of a STI and include both males and females. Given the high prevalence of HIV infection in South Africa and the strong association between STIs and HIV acquisition, these patients are at an increased risk of acquiring and transmitting HIV through sex[(36)]. Between July and December 2005, as part of a provider initiated HIV testing programme we tested 1190 women for HIV infection. The HIV prevalence was 54.7% with the highest prevalence in the 30-34 and 35-39 age groups (Table 3).

Table 3: Age-Specific Prevalence of HIV Infection Amongst Female eThekwini Clinic Attendees in Durban July 2005- December 2005

Age (years)	Number positive	Prevalence (%) (95% CI)
<u>< </u>19	50	38.2 (29.9-47.1)
20-24	182	42.6 (37.9- 47.5)
25-29	204	63.4 (57.8-68.6)
30-34	126	78.3 (70.9-84.2)
35-39	53	68.8 (57.1-78.6)
>= 40	36	50.0 (38.1-61.9)
Total	651/1190	54.7 (51.8-57.6)

<u>HIV prevalence and incidence</u>

The CAPRISA 051 study currently being conducted at the eThekwini Clinical Research site in preparation for the proposed microbicide trial (CAPRISA 004) has screened 1259 women attending the STI clinic. The HIV prevalence in this cohort was 59.3% and the average enrolment rate of HIV negative women per month is 19. After 52 person years of follow up the HIV incidence rate in this cohort is 5.8 per 100 women-years (95% CI 0-12.3). A second existing cohort of high risk women (CAPRISA 002) was established by screening a total of 776 women with an HIV prevalence of 59.4%. The retention rate among HIV-negative women who were

followed over 19 months was 87.8% and the HIV incidence rate is 7.9 per 100 person-years (95% CI 4.1-11.6). The age specific HIV incidence rate in the CAPRISA 002 cohort was highest in the 18-19 year age group, but with a wide confidence interval (Table 4).

Table 4: Age-Specific Prevalence of HIV Infection Amongst a Cohort of High Risk Women (CAPRISA 002)

Age group (years)	HIV prevalence (%)	Incident infections / new cases	Person Years	Incidence Rate (95% CI)
18-19	53.4	3	16.2	18.6 (0.0 – 37.6)
20-24	67.7	2	32.6	6.1 (0.0 – 14.5)
25-29	73.0	0	24.8	0
30-39	62.2	7	61.0	11.5 (3.0 – 20.0)
40-49	39.1	5	63.4	7.8 (0.9 – 14.1)
50+	29.0	0	20.2	0
Total	59.4	17	216	7.9 (4.1 – 11.6)

Data on the incidence and prevalence of HIV from the three CAPRISA sero-incidence studies is summarised below (Table 5).

Table 5: Summary of Three CAPRISA Sero-Incidence Studies (CAPRISA 050/051 and CAPRISA 002)

	Vulindlela (CAP050)	eThekwini (CAP051)	Durban (CAP002)
Number screened	782	1259	776
HIV prevalence	30.2	59.3	59.4
Average number enrolled per month	27	19	31
Person-years of follow up	290	52	216
Incidence / 100 person years	7.3	5.8	7.9

3. STUDY OBJECTIVES AND DESIGN

3.1 Primary Objective

- To evaluate the effectiveness and safety of a candidate vaginal microbicide, tenofovir gel, when applied intravaginally by women, in preventing sexually transmitted HIV infection.

3.2 Secondary Objectives

- To assess the impact, if any, of tenofovir gel on the incidence rate of deep epithelial disruption
- To assess the impact, if any, of tenofovir gel on viral load in women who become infected with HIV during the trial.
- To assess tenofovir resistance in HIV seroconvertors in the trial
- To ascertain the impact, if any, of tenofovir gel on pregnancy rates and outcomes
- To assess the impact, if any, of product hold at study exit on HIV infection rates and tenofovir resistance

3.3 Ancillary Objectives

- To assess the impact, if any, of tenofovir gel in preventing sexually transmitted infections including herpes simplex virus type 2 (HSV-2) and human papillomavirus (HPV) infections.

3.4 Study Overview

3.4.1 Design

This is a Phase IIb, two-arm, double-blind, randomised, controlled trial comparing 1% tenofovir gel, with a placebo gel among **1250** sexually active, women at high risk for sexually transmitted HIV infection in South Africa.

3.4.2 Accrual and Follow-up

The anticipated total study duration is approximately 30 months, with accrual requiring approximately 18 months and follow-up continuing until 92 incident HIV infections are observed, which is expected approximately 12 months after the end of the accrual period. Accrual may be altered following reviews by

the Data and Safety Monitoring Board (DSMB). The Protocol Team will be advised as to whether the study should proceed as designed, should proceed with design modifications, or should be discontinued. It is estimated that 92 HIV endpoints are very likely to accrue within 30 months of study initiation. In the event that 92 HIV endpoints are not observed within 30 months of initiation of the trial, the advice of the DSMB will be sought. Specifically, the DSMB will be requested to advise on whether the trial should be stopped for futility or continue beyond 30 months post study initiation until 92 HIV endpoints have been observed.

3.4.3 Enrolment and Randomisation

As described more fully in section 4.4, potential study participants will be screened for eligibility and eligible participants will be enrolled in the study within 30 days of screening. At each of the two sites, eligible participants who provide informed consent to take part in the trial will be assigned randomly to either tenofovir gel, or placebo gel in a 1:1 ratio.

3.4.4 Summary of Assessments

Enrolled participants will complete monthly follow-up visits for the duration of their participation (a minimum of 6 months and a maximum of 30 months) (Appendix I). At each monthly visit, participants will complete an interval medical history, HIV testing, and pregnancy testing. HIV/STI risk reduction counselling messages and condoms will be provided by counsellors trained to administer consistent prevention messages. Similarly, pregnancy prevention counselling will be provided by trained staff. The assigned study product and counselling on product adherence will be provided at monthly visits by trained staff. At each monthly visit, participants will be instructed to bring back any product. The pharmacist (or authorized designee) will record the amount of product returned before destruction. At each monthly visit participants will also undergo an interviewer-administered structured interview to ascertain key HIV risk behaviours. All scheduled and participant-initiated study visits will be documented. Study visits will take place at the CAPRISA Vulindlela Clinical Research Site and the CAPRISA eThekwini Clinical Research Site.

3.4.5 Safety Assessments

While safety is assessed as part of each monthly medical history and examination, detailed safety assessments are undertaken additionally during screening, enrolment and at months 3, 12, 24 (for those participants who have the opportunity to reach 24 months) during follow up, at study exit and additionally if indicated. At these visits, blood samples will be collected for urea, electrolytes, creatinine, liver function tests, full blood count, calcium and phosphate levels (See Appendix II for details). These visits will also include a pelvic examination including naked eye examination of the external genitalia and speculum examination of the vagina and cervix. Colposcopy will be performed if indicated. These visits will also include blood for storage of serum and plasma for the potential post-trial assessments of activity against STIs and cytobrush specimens for storage of cervical cells for potential post-trial assessments for markers of safety, risk exposure, product adherence and tenofovir resistance. For symptoms experienced between scheduled visits, the participant will be instructed to report to the Study Sites as soon as possible.

3.4.6 Outcome Assessment

The primary HIV endpoint is defined as two positive Ribonucleic Acid (RNA) Polymerase Chain Reaction (PCR) tests from independent samples obtained post-randomisation. The HIV testing algorithm at baseline, and at each monthly follow-up visits is included in Appendix III.

At screening, participants will undergo two rapid tests for HIV. Those who are negative on both tests and meet all eligibility criteria as assessed within a 30-day period since screening will be enrolled in the study.

At enrolment participants will not be re-tested for HIV, but plasma samples taken at this time will be stored for future testing among those who seroconvert early in the trial in order to confirm that seroconversion occurred post-randomisation.

At each monthly visit, participants will be tested for HIV with two rapid HIV tests. Participants with two negative rapid tests will continue follow-up in the study. If both tests are not negative i.e. either of these tests is positive or indeterminate, then the participant is considered a suspected seroconvertor. RNA PCR testing will be performed on suspected seroconvertors to confirm HIV status and a follow-up visit will be scheduled

for a week later. If the RNA PCR test is positive, a second blood sample will be drawn to confirm HIV status using RNA PCR during the scheduled visit a week later and another follow-up visit will be scheduled for a week thereafter to present results. Western blots and ELISAs will be performed on all suspected seroconvertors to provide additional confirmatory information on the presence / absence of infection. The primary HIV endpoint of HIV infection is defined as two positive PCR tests from independent samples. Participants will continue using gel product until HIV serostatus is confirmed by the algorithm in Appendix III.

Upon request, participants can be tested for HIV between scheduled study visits if they feel they have been exposed or are experiencing symptoms of HIV infection.

At the end of the study all new HIV infections will be confirmed by RNA PCR on stored plasma for quality assurance purposes and samples of plasma stored from enrolment will also be tested by RNA PCR to confirm that seroconversion occurred post-randomisation. Non-incident cases will be excluded from the analysis for the primary objective.

Participants who become infected with HIV will be offered counselling and referral, as appropriate, to the CAPRISA acute infection study (CAPRISA 002) and/or treatment programmes or other HIV/AIDS care services.

Post trial assessments – all participants will be asked to come back for one more visit 2 months after the study exit visit for outstanding results and one final HIV test and blood specimen collection for confirmatory HIV testing and potential resistance testing.

Note: Any new infections established at this visit will not be included as study endpoints. This post-trial visit is a safety visit that is part of monitoring HIV infection and tenofovir resistance following product withdrawal. All participants identified as HIV infected during this visit will be referred to the CAPRISA 002 Acute Infection study for follow-up.

All HBsAg positive patients will in addition have a blood sample collected for Hepatitis B and liver function tests. Any participants with significant changes in liver function since study exit will be referred to a health care provider for further follow-up.

3.4.7 Clinical management of non-study conditions

Participants who are found to have an STI or other treatable reproductive tract infection at a scheduled or participant-initiated visit will be provided counselling and clinical care in accordance with the South African Department of Health guidelines, free of charge. Participants with STIs will be encouraged to refer their partners for treatment.

Participants who become pregnant during the study will discontinue product use while they are pregnant. Pregnant women will be advised to continue with their follow up visits. When these participants no longer have a positive pregnancy test, the pregnancy outcome will be documented and they will be re-started on their allocated study product.

Participants who are found to be co-infected with Hepatitis B will be closely monitored. Clinical monitoring will include a review of the liver function tests done routinely through the study. Any participant needing further treatment for hepatitis B will be referred to a health care provider for further follow-up.

4 STUDY POPULATION

The study will include up to 1250-sexually active South African women at high risk for HIV infection.

4.1 Inclusion Criteria

While the HIV epidemic affects all age groups, young women have particularly high HIV incidence rates and hence the decision to focus this study on adult women up to 40 years of age. Women must meet all of the

following criteria at enrolment (by self-report, unless otherwise indicated) in order to be eligible for inclusion in the study:

- Age 18-40 years (inclusive)
- Able and willing to provide written informed consent to be screened for, and to enrol in, the study (Appendix IVa and b).
- Able and willing to provide adequate locator information for study retention purposes.
- Sexually active, defined as having had vaginal intercourse at least twice in the past 30 days prior to screening.
- HIV negative on testing performed by study staff within 30 days of enrolment (see algorithm Appendix III).
- Have a negative pregnancy test which was performed by study staff within 21 days of enrolment[a]
- Agree to use a non-barrier form of contraceptive
- Agree to adhere to study visits and procedures.

4.2 Exclusion Criteria

Women who meet any of the following criteria (by self-report, unless otherwise indicated) will be excluded from the study:

- History of adverse reaction to latex.
- Plans any of the following during the next 16 to 30 months (depending the anticipated date of study completion):
  - To travel away from the study site for more than 30 consecutive days.
  - To relocate away from the study site.
  - To become pregnant
  - To enrol in any other study of an investigational product or behaviour modification related to HIV prevention.
- Has a creatinine clearance <50ml/min, as estimated using the method of Cockcroft and Gault[(37)].
- **Has active Hepatitis B infection**
- Has a clinically apparent pelvic examination finding (observed by study staff) involving deep epithelial disruption. Otherwise eligible participants with pelvic examination findings involving deep epithelial disruption may proceed with enrolment after the findings have resolved and the inclusion/exclusion are met.
- Has in the past year participated in any research related to any vaginally applied product/s.
- Has current STI symptoms and/or other reproductive tract infection requiring treatment, as assessed by study staff. Otherwise eligible participants diagnosed during screening with infection(s) requiring treatment may be enrolled provided that treatment has commenced.
- Has any other condition that, based on the opinion of the Investigator or designee, would preclude provision of informed consent, make participation in the study unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.

4.3 Recruitment, Screening, and Enrolment

4.3.1 Sources for study participants

The high HIV prevalence and incidence rates in Vulindlela and Durban make these populations suitable for microbicide effectiveness trials as these populations; additionally these populations could derive substantial public health benefit in reduced HIV transmission from an effective microbicide. In Vulindlela, potential study participants will be recruited from among clients utilizing the primary health care clinics in Vulindlela, existing CAPRISA studies such as CAPRISA 050 and 051 as well as through community-based outreach activities, as appropriate. At the eThekwini site, potential participants will be enrolled from existing CAPRISA studies such as CAPRISA 051 and 002, from STI clients utilizing the Prince Cyril Zulu CDC and from community outreach activities in Durban. Consenting participants currently enrolled in the HIV seroincidence studies (CAPRISA 050 and 051) will be terminated from those studies and then offered the opportunity to be screened for this study.

[a] *Note: Breastfeeding is not exclusionary*

4.3.2 Cohort recruitment and accrual

Eligible participants will be enrolled over approximately 18 months. At regular intervals, the Principal Investigators in consultation with the study team will assess progress in accrual and retention at each of the two sites and may reallocate enrolment numbers and targets across the sites, as deemed necessary to achieve the goals of this trial efficiently.

4.3.3 Screening and enrolment

Eligibility for the study will be assessed in a step-wise manner at the study Screening Part 1 and Screening Part 2 Visits (described in Sections 6.2 and 6.3 respectively). Although all required procedures may be completed in two visits, additional visits may be conducted if needed or the two screening visits may be combined, where appropriate. Regardless of the number of visits required, all screening and enrolment procedures will be completed within a 30-day period. If a participant is not enrolled within 30 days of providing informed consent for screening, the participant will be re-consented for screening and the screening process will be repeated, in which case the results of the last screening prior to enrolment will be considered applicable for trial purposes.

Screening Part 1

Screening visit 1 will be completed in a step wise manner. Firstly, potential participants at the Clinical Research Sites will be invited to screen for the microbicide study and asked to provide written informed consent for screening (Appendix IVa). Potential study participants will be assigned a screening number, receive pre-test counselling, and two rapid HIV tests will be performed. . Post test counselling will be provided and those testing positive or indeterminate on at least one rapid test will be referred to an HIV/AIDS treatment programme. Participants who present with two negative test results from HIV testing conducted within 14 days of volunteering for screening for CAPRISA 004 will proceed with the screening process without repeating HIV rapid testing. HIV testing may be repeated prior to enrolment if >30 days have elapsed between the HIV rapid test result and the date of enrolment. If both HIV test results are negative, the potential participant will be invited to continue with the screening process and will be asked to provide demographic information, behavioural eligibility information, locator information, blood for creatinine levels and undergo urine pregnancy testing. Potential participants will also be evaluated by research staff for STI symptoms and will be offered syndromic treatment as per South African Department of Health guidelines. Participants deemed eligible based on the above procedures will then be invited for the Screening Part 2 visit.

Screening Part 2

Potential participants will be informed of their Screening Part 1 creatinine test results and if they continue to be eligible, they will undergo a physical and pelvic examination. Potential participants with pelvic examination findings involving deep epithelial disruption may only be enrolled after the deep epithelial disruption findings have resolved, provided they meet all other eligibility criteria. Otherwise eligible participants with clinical features suggestive of a STI may be enrolled after STI treatment is initiated.

Enrolment

Women who meet all the study eligibility criteria will be requested to provide their written informed consent for participation in the trial and thereafter enrolled in the study. Consent for specimen storage will also be sought (Appendix IVc). Blood will be drawn for haematology, liver function tests, blood chemistry tests, serology, hepatitis B assays and PBMC, serum and plasma archive (Appendix II).

At enrolment and throughout the trial, enrolled participants will be provided with:

- HIV risk reduction counselling and supplies of male condoms
- Contraception counselling and provision of contraceptive methods as needed
- Supplies of the assigned study product with counselling on product use, importance of product adherence and importance of not sharing product.
- Instructions to contact study staff with questions about the study, requests for additional counselling, requests for additional condoms and study product, requests for contraception, as needed, and/or reports of AEs.

4.4 Co-Enrolment Guidelines

Participants in this study may not take part in other concurrent research studies that would interfere with the objectives of this microbicide study. The determination of whether participation in another study would be exclusionary for a given participant will be made by the Principal Investigators. Approved co-enrolment in other concurrent protocols will be documented.

4.5 Participant Retention

The target retention rate will be 90% per annum. The Protocol Team will track retention rates and take any required action to address below-target retention rates. If volunteers do not adhere to scheduled pre-enrolment visits, screening may be discontinued at the discretion of the protocol team. Once a participant is enrolled in the study, study staff will make every reasonable effort to retain her in follow-up. This may include obtaining and checking locator data, home visits, issuing telephonic and in-person reminders of scheduled visits, and maintaining a scheduler of enrolled participants as part of a strategy to achieve the target.

4.6 Participant Withdrawal

Participants may voluntarily withdraw from the study for any reason at any time. Designated study staff also may withdraw participants from the study in order to protect their safety and/or if they are unwilling or unable to comply with required study procedures, after consultation with the Principal Investigators. Participants also may be withdrawn if the study sponsors, South African Medicines Control Council (MCC), United States (US) FDA, the University of KwaZulu-Natal Biomedical Research Ethics Committee, or FHI Protection of Human Subjects Committee (PHSC) may terminate the study prior to its planned end date.

Every reasonable effort will be made to complete a final evaluation of participants who withdraw or are withdrawn from the study. Study staff will record the reason(s) for all withdrawals in participants' study records.

5 STUDY TREATMENT CONSIDERATIONS

5.1 Product Formulation

5.1.1 Tenofovir gel

Tenofovir gel is a clear, transparent, viscous gel at concentrations of 1% (w/w) formulated in purified water with edentate disodium, citric acid, glycerin, methylparaben, propylparaben, hydroxyethylcellulose (HEC), and pH adjusted to 4-5. The gel will be administered as a 4mL dose. (See the Investigator's Brochure for detailed information on tenofovir gel)

5.1.2 Placebo Gel

The placebo gel (known as the 'universal' placebo gel) is formulated to minimize any possible effects — negative or positive — on study endpoints. It is isotonic to avoid epithelial cell swelling or dehydration. It is formulated at a pH of 4-5 but has minimal buffering capacity. When mixed with an equal volume of semen, the placebo gel induced only a trivial decrease in semen pH (from 7.8 to 7.7). The placebo gel contains HEC as a gelling agent, and its viscosity is comparable to that of tenofovir gel. HEC does not have anti-HIV properties. The gel contains sorbic acid as a preservative. Sorbic acid has no anti-HIV activity and is readily metabolized by human cells.

The placebo gel formulation was found to be non-irritating in a 10-day test for vaginal irritation in rabbits (BioSyn, Inc., personal communication). The formulation also showed minimal toxicity toward human vaginal epithelial cell monolayers in vitro (Thomas Moench, personal communication[b]). When tested in a mouse HSV-2 vaginal challenge model, the placebo afforded no protection when compared to no treatment or when compared to pre-treatment with phosphate buffered saline. The placebo also did not enhance susceptibility to HSV-2 when administered 12 hours before vaginal challenge. In contrast, N-9 and other detergent microbicides tested in the same protocol caused a 10-25 fold increase in susceptibility (Thomas Moench, personal communication[b]). In a phase I clinical trial conducted in the United States of America (USA) to

[b] Thomas Moench, MD. ReProtect, Inc, 703 Stags Head Road, Baltimore, MD 21286 USA

assess the safety of the placebo gel, no serious or unexpected AEs were reported following twice daily intravaginal application for 14 days in healthy sexually abstinent women[38]

5.2 Product Use Regimen

Trained staff will instruct participants in proper methods of storing and applying the assigned study product. This instruction will take place in advance of randomisation to minimize any potential bias. Participants will be instructed to insert one dose (the entire contents of one applicator) of product into the vagina up to 12 hours before each act of vaginal intercourse (intercourse may take place immediately after product insertion) and insert a second dose as soon as possible after coitus but within 12 hours. In the event that product is not inserted prior to coitus, the participants will be advised on the importance of inserting the post-coital dose as soon as possible within 12 hours after coitus. Irrespective of the number of coital acts in a day, participants will be advised not to use more than two applications in a 24 hour period. They will also be advised to:

- Only apply the assigned product vaginally.
- Not douche or otherwise clean the vagina, or insert other objects or vaginal products, for 2 hours after gel insertion. If a women plans to douche after coitus, she will be advised to insert the gel after douching.
- Not use other participant's study product.
- Not distribute their study product to other women.
- Not alter their study product in any way
- Properly store their study products (in a cool dry place out of direct sunlight)
- To use study product whether or not a condom is used.

As part of risk reduction counselling, participants will also be informed of the increased risk of HIV acquisition associated with anal sex compared with vaginal sex and will be encouraged not to engage in anal sex and to use a condom when anal sex cannot be avoided.

5.3 Product Management

5.3.1 Supply

CONRAD will provide the study products (tenofovir gel and placebo gel). All study gel will be produced, filled and packaged under Good Manufacturing Practices (GMP) conditions. The delivery volume, microbial limit, chemical and physical properties of the pre-filled applicators will be verified by the packager prior to shipping the clinical supplies. CONRAD will supervise the clinical supply operational procedures and review the GMP documents before authorizing shipment of the supplies to the clinical site.

The study products will be packaged in single-use opaque applicators containing approximately 4 ml of study gel. Each applicator will be individually wrapped and labelled. The products will be labelled such that the participant, investigator, monitor, data managers/analysts and sponsor (except CONRAD's Product Development Coordinator) will be blinded as to the identity of the product contained therein.

Ten individually wrapped applicators will be packaged in a box with an outside label and will be sealed with tamper-evident tape. Six randomisation groups (3 placebo and 3 tenofovir gel) will be prepared. Each gel applicator overwrap and outer box label will contain an alphanumeric variable assigned to each of the six groups, which the pharmacists on site will be able to decipher. The pharmacists on site will be blinded as to which of the three alphanumeric variables is active product and which is placebo.

The Study Pharmacist will obtain study products from CONRAD according to ordering instructions provided by CONRAD. The Study Pharmacist will maintain full accountability records in accordance with Good Clinical Practice (GCP) and legal requirements. These will include study participant logs as well as stock records for each randomisation group.

5.3.2 Storage

Drug product will be stored at controlled room temperature with excursions permitted from 15°C-30°C until required for administration. It should be stored away from direct sunlight.

5.3.3 Dispensing

Study product will be dispensed by designated study pharmacists to enrolled study participants as per Standard Operating Procedures (SOPs) in quantities expected to be sufficient until the participant's next monthly follow-up visit. In the event that a participant needs additional supplies between visits, she will be instructed to contact the study site to request additional supplies. Study participants will be asked to return all previously dispensed applicators at each visit. All returned applicators will then be disposed of in accordance with Good Pharmacy Practice.

5.3.4 Accountability

Complete records documenting receipt, inventory, dispensation, return and destruction will be maintained in accordance with Good Clinical Practice and US FDA requirements. These will include study participant logs as well as stock records for each randomisation group. Study participants will be asked to return all previously dispensed applicators at each visit. The number of used and unused applicators returned will be logged for each participant at each study visit.

5.4 Adherence Counselling

Adherence counselling will be provided to study participants upon enrolment and additionally at each study visit. Counselling will address such topics as participant-centred strategies to remember to use the products before and after sex, to ensure the availability of the products both in the home and away from home; and to identify and discuss various challenges and situations that may impede product use. Counselling also will include reminders to contact study staff with questions about product use and requests for additional supplies. For participants who have adherence problems, every effort will be made to identify adherence strategies to increase their rates of product use throughout the course of the study.

5.5 Adherence Assessment

Data on adherence to the product use regimen will be collected monthly via brief interviewer-administered instruments. These instruments will ascertain participants' frequency of sexual intercourse, condom use, product use and timing thereof in relation to coitus. The Protocol Team will monitor adherence rates over time, and adherence counselling methods will be updated and implemented systematically if needed to address lower-than expected rates. Additionally, genital specimens collected during the trial, as per procedures in the study MOP at study months 3, 12, 24 (for those participants who have the opportunity to reach 24 months), study exit and from suspected seroconvertors will be archived for potential post-trial analysis of cellular levels of tenofovir if there is utility in this potential marker of product adherence to enhance interpretation of the results of the trial.

5.6 Discontinuation of Product

Study participants will be discontinued from product use by Principal Investigators and designees in the event that they experience a Serious Adverse Event (SAE) that is judged by the study clinician or designee to be probably or definitely related to product use (see Section 7.3). Participants who become pregnant will discontinue product use and will only resume product use when their pregnancy test reverts to negative. The Principal Investigators and designees also may at their discretion discontinue product use — temporarily or permanently — among participants who:

- Experience an AE judged to be related to product use.
- Have a pelvic examination finding involving deep epithelial disruption that is not resolving.
- Are unable or unwilling to comply with required study procedures (including the strict guidelines on product sharing).
- Otherwise might be put at undue risk to their safety and well-being by continuing product use

Product use will be discontinued when a participant has been deemed to have reached the primary endpoint of HIV infection by the algorithm in appendix III.

For participants who discontinue product use, every effort will be made to complete all protocol-specified follow-up visits and procedures (except study product dispensing procedures) with these participants. The

study clinician or designee will document all changes in product application regimen, and the reason for the change, on applicable Case Report Forms (CRFs).

5.7 Concomitant Medications

Enrolled study participants may continue use of all concomitant medications, including prescription, non-prescription, traditional, and other preparations during this study, except for vaginal products. As noted in Section 5.2, participants will be encouraged to avoid douching and the use of vaginally-applied medications/preparations. All concomitant medications used by participants throughout the course of the study will be reported on applicable CRFs.

6 STUDY PROCEDURES (See Appendix I: Schedule of Evaluations)

Study staff will be trained to conduct study procedures in a standardised manner. SOPs and a Manual of Procedures (MOP) will guide this process.

6.1 Targeted |Recruitment

Study staff may conduct targeted recruitment, by focusing study outreach and recruitment efforts on women likely to be between 18 and 40 years of age. All possible efforts will be made to maintain the confidentiality of eligibility criteria, so as not to encourage artificial responses from volunteers being screened or to encourage them to change their behaviour in order to be eligible for the study.

6.2 Screening Part 1 Visit (up to day -30)

If all the required procedures in Screening Part 1 cannot be completed in a single visit, then multiple visits may be conducted if necessary. For potential participants who do not meet the study eligibility criteria, the screening process will be discontinued when ineligibility is determined.

Screening 1 will be completed in a stepwise manner. The first step includes the provision of introductory study information and obtaining written informed consent for screening procedures. HIV testing including pre and post test counselling will be done and only HIV negative participants will continue with the screening process. If participants have had an HIV test conducted within 14 days of this screening visit and are in possession of their test results, HIV testing will not be repeated as part of this screening visit but may be repeated prior to enrolment if >30 days have elapsed between HIV testing and date of enrolment. The following procedures will be completed:

6.2.1 Administrative, Behavioural, and Regulatory Procedures

- Assignment of a screening number
- Informed consent for screening
- Collection of the following:
    - Demographic information
    - Locator information
    - Behavioural eligibility information
    - Contraceptive eligibility and pregnancy-intentions assessment
    - HIV/STI risk reduction counselling and provision of condoms

6.2.2 Clinical Procedures

- Assessing for STIs and other genitourinary symptoms requiring treatment
- Blood draw

6.2.3 Laboratory Procedures

- HIV rapid testing
- Urine pregnancy testing
- Creatinine level

6.3 Screening Part 2

Screening Part 2 and Screening Part 1 may be combined. If all the required procedures in Screening Part 2 cannot be completed in a single visit, then multiple visits may be conducted if necessary.

6.3.1 Administrative, Behavioural, and Regulatory Procedures

- Update locator information
- Confirm eligibility

6.3.2 Clinical Procedures

- Focused medical and menstrual history and ascertainment of concomitant medications
- Physical examination
- Pelvic examination (and if needed, colposcopy)

6.4 Enrolment Visit (day 0)

The enrolment visit will only be commenced for participants who are found to be eligible. Written informed consent for study participation will be obtained before any enrolment (or "on-study") procedures are conducted.

6.4.1 Administrative, Behavioural, and Regulatory Procedures

- Informed consent for enrolment and stored specimens
- Assign a participant identification (PID) number
- Update locator information
- Behavioural risk assessment
- Contraceptive counselling
- HIV/STI risk reduction counselling and provision of condoms
- Product adherence counselling

6.4.2 Clinical Procedures

- Physical examination
- Pelvic examination if indicated
- Review/update pre-existing conditions
- Inventory and documentation of concomitant medications
- Blood draw

6.4.3 Laboratory Procedures

- Urine pregnancy testing if no negative pregnancy test result in last 21 days prior to enrolment
- Serum, plasma and PBMC archive
- Haematology – full blood count
- Chemistry – creatinine, urea and electrolytes, calcium and phosphate
- Liver function tests
- Hepatitis B assay

6.4.4 Pharmacy Procedures

- Provision of assigned study gel and instructions
- Update accountability log

6.5 Follow-up Visits

Monthly follow-up visits are scheduled throughout the study follow-up period on a 28-day schedule. The visit window around each of these visits is 14 days on either side. In addition to the regular monthly follow-up requirements, additional procedures are done at quarterly visits. For participants who do not complete scheduled visits within the allowable window, the visit will be considered "missed" and relevant CRFs will be completed to document the missed visit. However, for participants who miss quarterly visits, the pelvic examination and behavioural interviews specified to take place at these visits will be conducted at the participants' next visit. The same will apply to participants who miss visits 3, 12 and 24 where certain

assessments are done. Participants who become pregnant during the study will be followed as usual, but product will be withheld until they have a negative pregnancy test. For participants who become HIV infected during the study, product will be discontinued once diagnosis is confirmed by our testing algorithm. HIV seroconvertors in the trial will be maintained in follow-up until 3 months after seroconversion, when a final specimen of blood will be obtained for them for the assessment of viral load and tenofovir resistance assays.

6.5.1 Administrative, Behavioural, and Regulatory Procedures

- Locator information update:
  - At all visits and contacts
- Behavioural and product adherence assessment:
  - At all visits (note: quarterly visits will be more detailed)
  - At study exit
- HIV pre- and post-test counselling:
  - Monthly
  - At study exit
  - Additionally when clinically indicated
- HIV/STI risk reduction counselling:
  - At all visits and contacts
- Distribution of condoms:
  - At all visits and contacts
- Contraceptive counselling
  - All visits
- Product sharing and acceptability assessment
  - At study exit
- Provide test results
  - if applicable

6.5.2 Pharmacy Procedures

- Supply of assigned study product
  - Monthly (except at study exit)
  - Additionally when indicated
- Updating accountability log
  - Monthly (except at study exit)
  - Additionally when indicated
- Collection of residual unused and used study product from participant
  - Monthly
  - study exit

6.5.3 Clinical Procedures

- Interval (i.e., since last visit) medical and menstrual history including detailed intermenstrual bleeding history and AE assessment and concomitant medication review
  - Monthly
  - Additionally when indicated
- Pelvic examination (and, if needed, colposcopy)
  - Quarterly
  - Study exit
  - Additionally when indicated
- Genital specimen collection
  - Month 3, 12, 24 (for all participants having the opportunity to reach 24 months)
  - If indicated, at the time of suspected or confirmed seroconversion
  - study exit
- Blood draw
  - Month 3, 12, 24 (for all participants having the opportunity to reach 24 months) and study exit
  - Additionally when clinically indicated

6.5.4 Laboratory Procedures

- Urine pregnancy test:
  - Monthly
  - Additionally when clinically indicated
- HIV rapid tests
  - Monthly,and at study exit.
  - Additionally when clinically indicated
- HIV confirmatory RNA PCR
  - if indicated
- HIV confirmatory Western Blot and Elisa
  - if indicated
- Safety bloods (see appendix II for tests)
  - Month 3, 12, 24 (for all participants having the opportunity to reach 24 months) and study exit
  - Additionally when clinically indicated
- Hepatitis B assays
  - At study exit
- Serum, plasma and PBMC archive.
  - Month 3, 12, 24 (for all participants having the opportunity to reach 24 months) and at study exit
- Processing and storage of genital specimens
  - month 3, 12, 24 (for all participants having the opportunity to reach 24 months) and exit

6.6 Interim Contacts and Visits

Interim visits may be performed at any time during the study, for a number of reasons, which include, but may not be limited to, the following:

- For administrative reasons, e.g., a participant may have questions for study staff or may need to re-schedule a follow-up visit.
- For product-related reasons, e.g., a participant may need additional study product or want to discuss problems with adherence to product use.
- In response to AEs. When interim contacts or visits are completed in response to participant reports of AEs, study staff will assess the reported event clinically and provide or refer the participant to appropriate medical care
- For interim STI counselling and or treatment in response to STI symptoms.
- For interim HIV counselling and testing in response to presumed exposure to HIV or seroconversion symptoms.
- Contraception counselling and / or provision
- To provide participants with the results of confirmatory HIV test results, per the algorithm in Appendix III.
- For other reasons at participant request.

6.7 Study Exit Visit

6.7.1 Administrative and Behavioural Procedures

- Locator information update
- Behavioural and product adherence assessment
- HIV pre- and post-test counselling
- HIV/STI risk reduction counselling and distribution of condoms
- Product sharing and acceptability assessment

6.7.2 Clinical Procedures

- Interval medical history, AE assessment and concomitant medication review
- Pelvic examination (and, if needed, colposcopy)
- Genital specimen collection
- Safety bloods (see appendix II for tests)

6.7.3 Pharmacy Procedures

- Collection of residual unused and used study product from participant
- Updating accountability log

6.7.4 Laboratory Procedures

- HIV rapid tests
- HIV confirmatory RNA PCR
  - If indicated
- HIV confirmatory Western Blot
  - if indicated
- HIV confirmatory ELISA
- Haematology and Serum Chemistry (see Appendix II for tests)
- Hepatitis B assays
- Safety bloods (see appendix II for tests)
- Serum, plasma and PBMC archive.
- Processing and storage of genital specimens

6.8 Post trial HIV test Visit

6.8.1 Clinical Procedures

- Blood draw for tenofovir resistance testing in suspected seroconvertors
- Genital specimen collection in suspected seroconvertors

6.8.2 Laboratory Procedures

- HIV rapid tests

6.9 Final Contact

To minimise tenofovir resistance, following product hold, a final post-trial contact visit will be conducted. All participants will be scheduled to return two months after product is withdrawn at study exit for HIV testing and for potential HIV resistance testing in suspected seroconvertors. The following participants will have their follow-up from their study exit completed at this visit :

- Participants who require confirmatory HIV testing
- Participants whose final safety bloods are abnormal
- Participants who have an outstanding AE at the time of the final visit
- Participants who are pregnant
- Participants who become HIV infected during the trial
- Enrolled participants who are Hepatitis B positive

7 SAFETY MONITORING AND ADVERSE EVENT REPORTING

7.1 Adverse Events and Reporting Requirements

An AE is defined as any untoward medical or social occurrence in a clinical research participant which may or may not have a causal relationship with the study product. Study product refers to tenofovir gel and the placebo gel, and the above-listed definition of an AE will be applied to both arms of the study beginning from the time of randomisation. New information regarding symptoms or conditions that occur during the screening period, but prior to the randomisation will be recorded in the participant's medical history as pre-existing conditions. All new or worsening symptoms or conditions that occur following randomisation will be considered AEs and will be recorded on the AE CRF.

7.2 Adverse Event Reporting

Study participants will be provided contact telephone numbers and instructed to contact a study clinician to report any AEs they may experience, except for life-threatening events, for which they will be instructed to seek immediate emergency care. Depending on the severity of the event, the clinician will instruct the participant to present to the study site (for more mild events) or to a hospital casualty department (for more serious events) for immediate evaluation. With appropriate permission of the participant, records from all non-study medical providers related to AEs will be obtained and required data elements will be recorded on study CRFs. All participants reporting an AE will be followed clinically, until the AE resolves (returns to baseline) or stabilizes. AEs that are ongoing at the time of study exit will be followed up for up to 30 days after study exit and then, if not resolved, will be referred to a health care provider for further follow-up.

The Investigator must determine the severity of the AE and document it on the appropriate CRF (AE Form). Each adverse event that the participant is aware of should be graded for severity using the following scale (DAIDS severity grading system ICH 6):

- **Mild:** participant was able to perform all normal activities.
- **Moderate:** the participant had to discontinue some activities due to the adverse event.
- **Severe:** the participant was incapacitated by the adverse event and unable to perform normal activities.
- **Life-threatening** participant experienced extreme limitation in activity, significant assistance required; significant medical intervention / therapy required, hospitalisation or hospice - care probable.

An AE **does not** include:

- Pre-existing diseases or conditions present or detected prior to start of study drug administration that do not worsen.
- Medical or surgical procedures (e.g. surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an adverse event.
- Situations where an untoward medical occurrence has not occurred (e.g. hospitalization for elective surgery, social and/or convenience admissions).

The Investigator must determine the relationship of the AE to the product under investigation and document on the appropriate CRF (AE Form). For each AE, an assessment of the relatedness to the study drug will be made using the criteria and scale as outlined in the study MOP.

All AEs will be captured regardless of the association or otherwise to the study product and reported on the AE CRF in accordance with study specific procedures. All AE reports will contain at least the date the AE occurred, a brief description of the event, the relationship to study drug, the study drug action taken, the outcome, date resolved, and the seriousness of the event.

7.2 Serious Adverse Event (SAE) Reporting

An SAE includes any experience that is fatal or life-threatening, results in persistent or significant disability/incapacity, requires or prolongs hospitalization, or is a congenital anomaly. A life-threatening AE means that the participant was, in the view of the designated study staff, at immediate risk of death from the condition as it occurred. Notification of deaths will be recorded by reflecting the medical condition that led to the death on the AE CRF and also reported on the SAE report. Reporting SAEs may require additional detailed reports and follow-up, depending upon the study clinician's estimate of a causal relationship between the study product and the AE(s), and whether the AE(s) is identified in nature, severity, and frequency in the Investigator's Brochure or other risk information supplied to the Investigator.

All serious adverse events will be reported to FHI within 24 hours of the study site becoming aware of the problem. Study staff will complete a FHI SAE Report Form and submit it electronically or by fax to:

Director, RA/QA
Family Health International
PO Box 13950
Research Triangle Park, NC 27709 USA
Phone: 1-919-405-1445
Fax: 1-919-544-1308

In cases in which a SAE Report Form cannot be submitted in writing within 24 hours, the designated study staff will report the SAE via telephone and the SAE Report Form will be completed as soon as possible after the verbal report. A study SOP will outline the detailed procedures for the reporting of SAEs to the ethics committees and regulatory bodies, in fulfilment of their reporting requirements.

7.3 Safety Monitoring

Designated study staff will be responsible for continuous close safety monitoring of all study participants. The study statisticians will prepare routine study progress reports for review by the Protocol Team. In addition, the study statisticians will prepare routine study progress reports which include reports of AEs experienced by study participants (blinded to treatment assignment) for review by the Protocol Safety Review Team

(PSRT). The membership, scope of responsibility, role and modus operandi of the PSRT will be outlined in the study MOP and will include safety monitoring of enrolled participants infected with Hepatitis B. PSRT members will meet in-person and/or via teleconference regularly throughout the period of study implementation. Any deaths of study participants or other SAEs must be reviewed and a decision taken by the PSRT with regard to whether a DSMB review is warranted. In addition to monitoring performed by the PSRT, the study DSMB will review the study data during the period of study implementation. Following its review of the trial, the DSMB may recommend that the study proceed as designed, proceed with design modifications, or be discontinued.

7.4 Data, Safety, Monitoring Board

An independent Data Safety Monitoring Board will be assembled for this study. The details for the operation and responsibilities of the DSMB will be defined in a separate DSMB Operational Plan, which will be included in the MOP. The Operational Plan will delineate the composition, duties, responsibilities and procedures of the DSMB, data required at each meeting as well as the analyses that will be conducted, i.e. interim comparisons of the efficacy and safety of the study products.

Interim monitoring reports will be generated by an independent statistician (who is not otherwise involved in the study) and submitted to the DSMB (some data that go to the DSMB will be “open” and available to the study team, like grouped data listed below). All reports created by the independent statistician will be distributed to the members of the DSMB only and will not be available to any study staff. DSMB reports will be kept in a locked file with restricted access.

A full interim review of the available data will be undertaken during the conduct of the study. These interim reviews will be triggered once 22 of the anticipated primary endpoints are reached and again once 68 of the anticipated primary endpoints are reached. The data to be reviewed will include:

- Accrual data
- Baseline data
- Retention data
- Safety data
- Quality assurance data
- Effectiveness data (The type I error rate will be controlled over the two planned interim analyses plus the final analysis using O’Brien-Fleming type boundaries with the Lan-Demets spending function.)
- Any other data requested by the DSMB

After each DSMB meeting, the Chairperson will issue a written report describing all recommendations. The DSMB could recommend that the study should proceed as designed, should proceed with design modifications, or should be discontinued due to any reason (safety, efficacy, futility, etc.).

8 STATISTICAL CONSIDERATIONS

8.1 Review of Study Design

This is a two-arm, double-blind, randomised, placebo controlled trial comparing 1% tenofovir gel with a placebo gel, conducted among up to 1250 women at high risk for HIV infection in South Africa. A total study duration of approximately 30 months is planned, with accrual requiring approximately 18 months and follow-up continuing until 92incident HIV infections are observed.

8.2 Endpoints

8.2.1 Primary Endpoints

The primary study objective is to evaluate the effectiveness and safety of a candidate vaginal microbicide, tenofovir gel, when applied intravaginally by women, in preventing sexually transmitted HIV infection. The primary endpoint of HIV infection will be assessed as described in Appendix III.

8.2.2 Secondary endpoints

Consistent with the study objectives listed in section 3.2, the following secondary endpoints will be assessed:

- deep epithelial disruption observed on pelvic examination

- pregnancy rates
- pregnancy outcomes, which will be assessed in the following categories: induced abortion; spontaneous abortion; stillbirth; live birth with a congenital anomaly; or live birth without a congenital anomaly.

For women who become infected with HIV during the trial, additional secondary endpoints will be assessed:
- viral load in the first available HIV positive blood specimen, and at 3 months after the first HIV positive specimen
- Tenofovir resistance assessed by resistance assay test performed on virus isolated from the first available HIV positive blood and genital specimen and 3 months after the first HIV positive specimen.
- Tenofovir resistance assessed by resistance assay test performed on virus isolated from participants testing HIV positive at the post trial assessment visit scheduled two months after study exit following product hold

8.2.3 Ancillary endpoint

Consistent with the ancillary objective listed in section 3.3, the following ancillary endpoints will be assessed
- The presence of STIs including HSV_2 and HPV, diagnosed through retrospective analysis of stored samples, including but not limited to, antibody detection, viral nucleic acid and viral culture.

8.3 Accrual, follow-up, and sample size

The HIV incidence in the current longitudinal studies are 7.9 per 100 person years in the CAPRISA 002 study, 5.8 per 100 person years in the eThekwini CAPRISA 051 study and 7.3 per 100 person years in the Vulindlela CAPRISA 050 study. We assume that the infection rate will be slightly lower than this in the placebo arm of the study, and have therefore assumed an incidence rate of **7** per 100 person years as the rate in the placebo group.

We plan to enrol up to 1250 women over approximately 18 months and to continue follow-up until 92 incident infections are observed (unless the DSMB recommends discontinuing the trial early, see section 7.4). This number of events is expected to provide 90% power to detect a 50% effect (using a two-sided alpha=0.05 significance level test). Assuming an HIV incidence rate of 7 per 100 person years and 10% annual loss to follow-up, the required number of incident infections (92) will be reached approximately 12 months after the end of the enrolment period, resulting in a total study duration of approximately 30 months. Sample size and power were calculated using NQUERY.

The chance of detecting various other levels of effectiveness levels ranging from 30% to 60% at the two-sided, α=0.05 significance level are provided in Table 6.

Table 6: Power to Detect Different Levels of Product Effectiveness at the Two-Sided α =0.05 Significance Level Assuming 68 Events are Observed

Actual product effectiveness (reduction in risk for active gel relative to placebo gel)	**Power to detect an effect (p<0.05)**
60%	**97%**
50%	**90%**
40%	**74%**
30%	**41%**

The power to detect an effect of tenofovir gel on the first secondary safety objective, the rate of deep epithelial disruptions, is provided in Table 7.

Table 7: Power to Detect Differences in Deep Epithelial Disruption Rates (Assumes Two-Sided Alpha of 0.05, 10% Annual Loss to Follow-up, 18 months of Recruitment and Total Study Duration of 30 Months, Sample Size of up to 1250)

Baseline Rate (per 100 person years in placebo group)	Power to Detect a:		
	2-fold increase	3-fold increase	4-fold increase
1	**45%**	**80**%	**>99**%
2.5	**83%**	>99%	>99%
5	**98%**	>99%	>99%

The sample size will be re-assessed approximately 2 months before the expected enrolment of the last participant, based on the observed event rate pooled across treatment arms. If it is determined at this time that additional participants will likely be required to yield the required number of incident HIV events (i.e.92) by the end of 30 months of follow-up, then consideration will be given to enrolling additional women, up to a maximum of 1500 women.

The Principal Investigators and protocol statisticians will periodically review the overall product adherence and HIV infection rate data (pooled across treatment arms) to determine if any protocol changes with respect to duration of follow-up should be implemented. For example, if recruitment rates are lower than expected and overall adherence or infection rates decrease with longer study participation, then the protocol may be amended to limit the maximum number of months of follow-up for participants.

8.4 Random Assignment and allocation concealment

A randomisation plan describing procedures for randomisation, allocation concealment, and blinding will be established prior to initiating the trial. A summary of the procedures is as follows: Enrolled participants will be assigned at random to one of the two study treatment arms in equal proportions. However, to facilitate blinding without greatly complicating product distribution logistics, each participant will be randomly assigned on one of six different groups (designated by an alphanumeric variable e.g., A, B, C, D, E and F) in a 1:1:1:1:1:1 allocation ratio. Three groups will correspond to the placebo gel and three to tenofovir gel.

The randomisation statistician, who is not otherwise involved in the study will randomly assign three of the alphanumeric variables to placebo and three to the active gel. This information will be sent to CONRAD for drug packaging and will be stored in a locked file cabinet (paper copy) and in a secured computer directory (electronic copy) at the CONRAD office. Except for key individuals involved in drug packaging, the treatment code (e.g., which 3 alphanumeric variables correspond to active drug and which 3 correspond to placebo) will not be known to anyone else. These treatment codes may only be revealed to the DSMB for planned interim analyses. Individual treatment assignments may also be revealed to the study investigators in the event that emergency unblinding of a study participant is required (see Section 8.5 for details). Any instances of unblinding, intentional or otherwise, will be documented in the study files. .

The randomisation list used to assign individual study participants to one of the six groups will be generated by a randomisation statistician who is not otherwise involved in the study. This statistician will use a randomly permuted block design, stratified by site. Two or more pre-specified block sizes will be recorded on a formal randomisation request form, but they will not be written in the protocol or communicated to the clinical staff in order to reduce the chance of the clinical staff anticipating the assignment of the next subject. Electronic copies of the randomisation schedule and the programs used to generate the randomisation schedule will be limited in access and password protected. Paper copies of the randomisation schedule will be locked in a secure location at the CAPRISA office, where no unauthorized study staff will have access to them.

The randomisation statistician will provide the study pharmacy at each site with sealed, opaque randomisation envelopes, sequentially labelled by participant identification number (PID). These envelopes will be assigned in sequential order to eligible study participants. Upon opening the envelope the pharmacist will add his or her name and signature as well as the time and date the envelope was opened. The treatment letter to which the participant was assigned will be known only to the pharmacist.

8.5 Blinding

Both study staff and participants will be blinded to treatment assignments. Blinding will be maintained until all data are entered into the study database, all primary study endpoint data have been cleaned and verified, preliminary analyses have been performed, and the data are ready for final analysis. If study data that are not part of the primary or secondary outcomes are still outstanding at the time of the proposed locking of the database, the database could be locked, and participants treatment assignments revealed, provided that the study statisticians and Principal Investigators agree that these data are not needed to perform the primary or secondary analyses. If necessary, the DSMB Chair may be asked to consider and make a determination on this request.

It is not anticipated that unblinding will be necessary for the provision of medical treatment or to otherwise protect the safety of study participants. In the event that an Investigator is concerned that a participant might be put at undue risk by continuing product use, the Principal Investigators or designee may discontinue product use by this participant, without knowledge of the actual treatment assignment. However, in the unlikely event that a study staff member feels that specific product knowledge is necessary to protect a participant's safety, he/she will notify the Principal Investigators and the protocol statisticians to consider and jointly rule upon the request. The DSMB Chair may also be asked to consider and make a determination on this request. If deemed necessary, then the procedures outlined in the randomisation, allocation concealment and unblinding plan will be followed for requesting and documenting unblinding of individual study participants.

All study participants will be administered a brief unblinding assessment at their study exit visits in which they will be asked to report which study product they think they received. A sample of study staff will complete a similar assessment after the close of each study site.

8.6 Data Analysis

An expanded Statistical Analysis Plan (SAP), covering both the final analysis and the planned interim analysis, will be finalised before the first participant is enrolled. The following is a summary of the planned analyses. Any deviations to be made from this summary plan will be documented in the detailed SAP.

All primary analyses will be performed on an intention to treat (ITT) basis. For the ITT analysis, patients will be analysed according to the treatment arm they were randomised to, even if the participant was off product or received the product they were not assigned for a period of time while in the study. The only participants excluded from this primary analysis population will be women without a post-randomisation HIV test result, and women whose stored baseline blood samples were later found to be HIV positive. All primary and secondary analyses will be two-sided and will be performed at the 0.05 level of significance (adjusted to account for the two planned interim analyses). Additional analyses could be performed on other study populations, for example an as treated or a per protocol population. These analyses will be described in the SAP.

Any key decisions regarding the timing of outcomes, the appropriateness of test statistics or model assumptions, the eligibility of participants to be included in the various populations or any other statistical issues will be made in a blind review meeting. At the blind review at least the following people will be present: protocol statisticians; Principal Investigators; medical officer; and data manager. Only after this group documents that all data are sufficiently clean and all decisions regarding individual participant outcomes have been made will the study be unblinded to the true randomisation assignments

To assess effectiveness and safety of tenofovir gel the cumulative probability of HIV will be calculated for each treatment group using the Kaplan-Meier method. The difference in survival curves will primarily be evaluated with a logrank test, stratified by site. The type I error rate will be controlled over the two planned interim analyses, as well as the final analysis, using O'Brien-Fleming type boundaries with the Lan-Demets spending function. Secondarily, proportional hazards regression models will be used to estimate the hazard rate ratio, along with a 95% confidence interval, controlling for site and selected baseline prognostic variables (to be identified in the SAP).

Date of HIV infection will be estimated as the midpoint between the last negative HIV test date and the first confirmed positive HIV test date. Participants who do not become HIV infected before their last study visit will be censored on the day of their negative HIV test. Time to HIV infection, in days, will be computed as the difference between the estimated date of HIV infection and the randomisation date, plus one. Time to censoring will be computed as the difference between the date of censoring and the randomisation date, plus one.

Kaplan Meier estimates of the cumulative probability of deep epithelial disruption will be compared between the tenofovir gel and the placebo group using logrank tests, stratified by site. AEs occurring during the study will be summarized in frequency tables (including both the number of each type of AE and the number of distinct participants with each type of AE), by body system and by treatment group. Details of secondary analyses, including pregnancy rates and outcomes, as well as viral load levels and tenofovir resistance in HIV seroconvertors will be outlined in the SAP.

8.7 Data Management

Data will be collected on one-ply case report forms (CRFs) which will be developed by the study team. All site study staff will be trained in the correct completion of CRFs. If data entered on the CRFs are taken from an external source (e.g., laboratory reports, patient records), the source documents will be maintained in the participant's medical chart or study file at the site, and will be available for review. The CRFs will be faxed into the database management system which is DataFax version 3.7.002 (or higher) running on Sun Solaris OS 5.8 (or higher). DataFax has optical character recognition (OCR) which will read the check boxes and numerical fields on the CRFs and store them in the study database. Any fields not recognized by the OCR system will be entered manually by the Data Encoders. Data encoders will verify all data by cross-checking the faxed version to what is entered into the database.

Queries arising during validation of the data will be recorded in quality control (QC) Reports sent to the sites on a regular basis. Any queries resulting in a change to the database will be documented and attached to the original CRF. The data management centre staff will perform periodic quality control and validation checks on the data. Database files will be password-protected and access to the files will be limited to authorised study staff members only. All data will be backed up at regular intervals, and backups will be stored in secure areas with limited access.

The original CRFs and the DataFax version of the CRFs and related documents will be stored securely at the sites and both during and after the completion of the study. At all sites the forms will be stored in locked cupboards in a secure room with restricted access. Upon completion of the study, the close-out site monitoring visit and finalisation of the database for analysis, the original forms will be bound and kept for long term storage. CRFs will not be destroyed without written permission from the sponsor.

A detailed data management plan will be included in the study MOP.

9 HUMAN SUBJECTS CONSIDERATIONS

9.1 Regulatory and Ethical Review

This study will be conducted under the oversight of the South African MCC in accordance with International Conference on Harmonization (ICH) standards of Good Clinical Practice (GCP). CAPRISA will be responsible for reporting study-related information to the South African MCC.

The study also will be conducted under the oversight of the University of KwaZulu-Natal Biomedical Research Ethics Committee in South Africa and the FHI Protection of Human Subjects Committee in the USA. The study will only be initiated after it has been approved by both ethics committees. The study will be conducted in accordance with all conditions of approval by the ethics committees.

9.2 Informed Consent

Written informed consent will be obtained from each study participant in English or Zulu prior to screening and enrolment, in accordance with 21 CFR Part 50 and ICH GCP guidelines. Participants will be provided

with copies of their informed consent forms if they are willing to receive them. An impartial witness is required for the entire informed consent process in any participant who is illiterate or whose literacy is limited. Documentation of the presence of a witness will be achieved through their signature on the informed consent document. Illiterate participants will indicate their consent via use of their mark (finger/thumb print) on the informed consent documents.

9.3 Risks

The study clinical procedures are similar to those experienced by women in routine gynaecological examinations. Study participants may experience discomfort when having pelvic examinations and/or undergoing phlebotomy for this study. During phlebotomy, participants may feel dizzy or faint, and/or develop a bruise, swelling, or infection where the needle is inserted. Participants may become embarrassed, worried, or anxious when completing their HIV-related interviews and/or receiving HIV/STI counselling. They also may become worried or anxious while waiting for their HIV test results or after receiving HIV-positive test results. Trained counsellors will be available to help participants deal with these feelings.

Study personnel will make every effort to protect participant privacy and confidentiality, but it is possible that participants may disclose their HIV status to non-study participants and could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities. Participants also could have problems in their partner relationships associated with use or attempted use of condoms and/or the investigational products.

Data on participant risk behaviours and the occurrence of other potential social harms will be collected from all participants. The Protocol Team will monitor trends in risk behaviours over time based on these data, as well as the occurrence of social harms, and initiate any required follow-up action.

Available evidence on the safety and tolerability of 1% tenofovir gel from the HPTN 050 study indicates that the product is safe and well tolerated in both HIV negative (N=60) and HIV positive (N= 24) women. Administration of tenofovir gel intravaginally at 0.3% and 1% concentrations resulted in minimal local irritation and little or no systemic adverse effects were identified. Although 92% reported at least one AE, the majority (87%) were mild and limited to the genitourinary tract. The most common AEs experienced were genital pruritus (23%), applicator site bruising (17%), applicator site erythema (17%), vaginal discharge (15%), irregular menses (13%) and metrorrhagia (11%). Four severe AEs were reported, with only one, lower abdominal pain, thought to be product-related[31]. Therefore the risks associated with tenofovir gel are believed to be substantially less than those identified for systemic tenofovir use. In the HPTN 050 Phase I study of tenofovir gel, serum PK analysis in a subset of participants demonstrated that there is no clinically significant systemic toxicity. Fourteen of 24 women with PK results had low, but detectable, serum tenofovir levels.

It is not known what effect tenofovir gel could have on the HIV virus or HIV disease progression in HIV infected participants or their partners. There is a theoretical risk that tenofovir absorbed systemically from tenofovir gel could result in mutations of the HIV virus in participants who become infected with HIV during the study, or their partner, if the partner is infected with HIV. Limited resistance data from the HPTN 050 study show that no new resistance mutations evolved in plasma or cervicovaginal lavage specimens after 14 days of tenofovir gel use[31]. No participant had high level tenofovir mutations (e.g., K65R).

Some of the possible side effects of the study gel are dryness, burning, itching, cervical ulceration, abrasion, ecchymosis, erythema, sub epithelial and/or petechial haemorrhage, inflammation, or pain in the genital area.

The following side effects have been associated with the use of oral TDF[39]: upset stomach, vomiting, gas, loose stools, dizziness, abdominal pain, lack of energy, kidney damage or failure, inflammation or swelling and possible damage to the pancreas, shortness of breath, rash, low phosphate, allergic reaction, change in bone growth and strength, and exacerbations of Hepatitis B Virus (HBV) in patients co-infected with HIV and HBV. These patients will need to have hepatic function closely monitored and anti-Hep B therapy resumed if appropriate. The updated Investigators Brochure (3rd edition, dated 11 January 2008) also includes a warning for possible lactic acidosis/severe hepatomegaly with steatosis including fatalities that have been

reported with the use of nucleoside analogs. Viread is also now indicated for the treatment of chronic hepatitis B in adults at a dose of 300mg daily. Optimal duration of treatment is not known[40]. Since systemic absorption of the gel formulation is considerably lower than the oral or intravenous formulations, these side effects are unlikely in this study which will be using a gel formulation. Regardless, study participants will be monitored for AEs related to those observed for the oral formulation.

9.4 Benefits

There may be no direct benefits to participants in this study. However, participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of a safe and effective vaginal microbicide that prevents HIV infection.

Study participants will receive HIV and STI counselling and testing, a physical examination, and gynaecological assessments. Contraception will also be available to study participants. They will be provided syndromic STI treatment free-of-charge, and will be offered STI treatment for their partners. For other medical conditions identified as part of the study screening and/or follow-up procedures, participants will be referred to other sources of care available in their community. Study participants will also receive condoms and risk reduction counselling and will be reimbursed for transport and refreshment costs for each scheduled visit.

9.5 Access to HIV-related care

9.5.1 HIV counselling and testing

HIV counselling will be provided to all potential study participants who consent to undergo HIV screening to determine their eligibility for this study, and to all enrolled participants at each follow-up HIV testing time point. HIV test results will be provided with post-test counselling. Condoms will be provided to participants throughout the duration of their participation in the trial.

9.5.2 Care for participants identified as HIV-infected

Potential study participants who volunteer to undergo HIV testing as part of the study screening process may discover that they are HIV positive. Study staff will provide all HIV test results with post-test counselling. Potential study participant who have been identified as HIV positive will be referred to local AIDS treatment services. Such services include the CAPRISA–based as well as other local facilities that provide medical and psychosocial AIDS care and support.

HIV-uninfected study participants who become infected during follow-up will be referred to one of the long-term CAPRISA Acute Infection cohort studies, which have excellent provisions for care, antiretroviral therapy and support for those infected with HIV. For those who do not wish to continue in any of these studies post-seroconversion, they will be referred to their preferred AIDS care provider which could include the CAPRISA AIDS treatment programme, government or non-governmental AIDS care services for ongoing clinical management and care.

9.6 Community involvement and consultation:

The CAPRISA community programme (CP) has, through a consultative process, established CAPRISA Community Research Support Groups (CRSGs) at both the CAPRISA Sites where this study will be conducted. The CRSG membership includes local community leaders, traditional leaders, leadership of local HIV/AIDS organisations, previous study participants, local health service provider representatives and HIV positive local community members. The CAPRISA CP in partnership with the CRSG's will involve the community and local community based organisations in preparation for this trial. Specifically, the CAPRISA CP will inform, educate and mobilise the community to enhance community input into the research process. The local CRSGs in Vulindlela and eThekwini play an active role as an interface between the researchers and community members serving as advocates for the community's best interests and ensuring that the researchers are aware of any concerns within the community about the research being conducted. The CRSGs also play an important role in reviewing study educational materials, consent forms and Zulu translations of documents which will be shared with study participants.

9.7 Confidentiality

Every effort will be made to protect participant privacy and confidentiality to the extent permitted by law. Study-related information will be stored securely at the study site. All participant information will be stored in lockable file cabinets in areas with access limited to study staff. Data collection, process, and administrative forms, laboratory specimens, and other reports will be identified by a coded number only, to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link PID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.

Participants' study data, as identified by PID number only, will not be released without their written permission, except as necessary for review and monitoring by:

- Authorized study representatives
- South African MCC
- US FDA
- University of KwaZulu-Natal Biomedical Research Ethics Committee
- FHI's Protection of Human Subject Committee
- FHI Monitors and Auditors

9.8 Study Discontinuation

This study may be discontinued at any time by the South African MCC, US FDA, FHI PHSC, University of KwaZulu-Natal Biomedical Research Ethics Committee, or the Protocol Team (e.g., in response to recommendations from the DSMB or the study sponsor).

10 LABORATORY CONSIDERATIONS

The study laboratory plan will include the procedures for specimen management (e.g. chain of custody, handling, labelling and transport), assay procedures, proficiency testing and quality assurance procedures and specimen storage procedures.

10.1 Laboratory Specimens

The following types of specimens will be collected for testing:

- Urine for pregnancy testing
- Blood for haematology (full blood count [FBC]) and chemistry (liver and renal function testing, calcium and phosphate) (Appendix II).
- Blood for HIV testing by rapid tests confirmatory RNA PCR assays, Western blots and ELISAs
- Blood for HBV testing
- Blood and genital specimens from suspected seroconvertors for virus and tenofovir resistance assays
- Blood for PBMC, plasma and serum archive.
- Genital specimens for archive

All the above specimens will be collected with Good Clinical and Laboratory Practice standards and as described in the SOPs for collection of specimens.

10.2 On site testing

The study laboratory plan will detail the procedures to be followed for on-site testing as well as proficiency testing for all on-site testing (i.e. urine pregnancy tests and HIV rapid tests).

10.3 Collection and shipping of specimens

All specimens (bloods, urine and genital) will be collected according to methods described in the MOP and SOPs for proper collection, processing, labelling, and transport of specimens to the laboratories conducting the assays.

10.4 Specimen Storage for Quality Assurance and Potential Future Research Testing

Serum, plasma, PBMC and genital specimens will be stored for potential post-trial assessments for activity against STIs, markers of safety, risk exposure, product adherence and tenofovir resistance. In addition, stored plasma will be used for retrospective RNA PCR or Western blot testing to confirm whether early incident cases of HIV infection during the trial occurred post-randomisation. Where possible, stored specimens will be re-tested to assess the validity of unusual or unexpected assays results. For those participants who do not consent to long-term storage of their specimens, any residual specimens will be destroyed at the end of the study after all protocol-required and quality assurance testing has been completed.

10.5 Laboratory Quality Control and Quality Assurance Procedures

The laboratories involved in the study will follow the quality assurance and quality control procedures outlined in the study laboratory plan. For the on-site tests, the quality assurance personnel from the CAPRISA laboratory will conduct periodic visits to the Clinical Research Sites in Vulindlela and eThekwini to assess the implementation of on-site quality control procedures, including maintenance of laboratory testing equipment, use of appropriate reagents, proficiency testing records and quality checks of on-site testing procedures.

11 ADMINISTRATIVE PROCEDURES

11.1 Protocol Compliance

The study will be conducted in full compliance with the protocol. Amendments to the protocol will be required to follow an SOP which stipulates the levels of approval required prior to submission to regulatory bodies and the steps to be followed prior to implementation of a protocol amendment.

11.2 Protocol violations

A "protocol violation" is broadly defined as any departure from the procedures described in the study protocol. Protocol violations that may impact subject safety, affect the integrity of study data, affect subject's willingness to participate in the study, and/or provide evidence of wilful or knowing misconduct or non-compliance on the part of the site investigator(s) will be documented and reported, at minimum, to FHI. Protocol violations may be identified by any of the study staff or by the study monitor. The procedures for documenting protocol violations will be specified in the monitoring plan

Some examples of protocol violations include:

- Omission or inadequate administration of informed consent
- Inclusion/exclusion errors, including legal age limit
- Treatment errors: incorrect dispensation of study product
- Missing or incorrectly timed study procedures and assessments
- Failure to discontinue product use due to protocol criteria

In an emergency, the Investigator may make departures from the protocol to eliminate an apparent immediate hazard for a particular participant. In such a case, he/she will notify the Ethics Committee and FHI in writing as soon as possible and document reasons for the violation (unless solely caused by participant non-compliance such as not attending for study visits).

11.3 Quality Assurance and Study Monitoring

Quality assurance in the trial will be undertaken according to the Study Quality Assurance Plan which will be part of the study MOP. The Quality Assurance Plan will include ongoing monitoring of study progress and safety by the Protocol Team, study monitoring in accordance with ICH GCP guidelines by trained FHI monitoring staff, and independent quality assurance audits by FHI Regulatory Affairs/Quality Assurance staff and/or other outside QA contractors. The Investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, CRFs), as well as observe the performance of study procedures. The Investigators will also allow inspection of all study-related documentation by authorized representatives of the South Africa MCC, the FDA, and the study sponsors. A site visit log will be maintained at the study site to document all visits.

11.3.1 Study Monitoring

Study monitoring will be conducted by representatives of FHI who have received adequate training as monitors and specifically on this study. Monitoring shall commence shortly after enrolment of the first participants and at regular intervals thereafter. A site visit log will be maintained at the study site to document all visits. Monitor findings will be documented per FHI SOP. The Principal Investigators will be notified of the visit findings. If the monitor discovers issues related to safety, he/she is to report their findings immediately to the Principal Investigators or designee as well as to the project manager at FHI.

11.3.2 Auditing

FHI will conduct site visits and evaluation after at least 5 months of study conduct and then again about 6 months before the projected end of the study to evaluate study systems for quality control, monitoring of the study, documentation of the study, and overall control of the study.

11.4 Study Records

Complete, accurate, and current study records will be maintained and stored in a secure manner, throughout the study. All study records will be maintained for at least 5 years after the termination of the trial and extended to 2 years following the date of marketing approval for the study product for the indication in which it was studied and until there are no pending or contemplated marketing applications or at least 2 years have elapsed since the formal discontinuation of the development of the investigational product. Gilead Sciences or Conrad will inform the Principal Investigators if this storage period needs to be extended.

11.5 Use of Information and Publications

Presentation and publication of the results of this study will be governed by CAPRISA's publication policy.

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APPENDICES

I. Schedule of Study Visits and Procedures
II. Safety Laboratory Evaluations to be Performed in Study Months 1-3
IIIa. HIV Antibody Testing Algorithm for screening
IIIb. HIV Antibody Testing Algorithm for Primary Endpoint Ascertainment at Follow-up Visits
IIIc: HIV Antibody Testing Algorithm for Suspected Acute Illness
IVa. Informed Consent Form for Screening Participants
IVb Informed Consent Form for Enrolling Participants
IVc Informed Consent Form for Specimen Storage and Possible Future Research Testing

Appendix I: Schedule of Evaluations

Procedure	Screening		Enrol	Monthly Follow-up																																		Exit	
	Screening part 1 (up to 30 days)	Screening part 2 (up to 30 days)	Enrolment * (Day/month 0)	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12	Month 13	Month 14	Month 15	Month 16[b]	Month 17[b]	Month 18[b]	Month 19	Month 20	Month 21	Month 22	Month 23	Month 24	Month 25	Month 26	Month 27	Month 28	Month 29	Study exit	Final contact
	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26	V27	V28	V29	V30	V31	V32	V33
Administrative, Behavioural and Regulatory Procedures																																		
Informed consent for screening	X																																	
Obtain screening number	X																																	
Informed consent for storage			X																															
Informed consent for enrolment			X																															
Demographic information	X																																	
Locator information	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Eligibility assessment	X	X	X																															
Counselling and condom supplies	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Document concomitant medication			X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	X	
Obtain random assignment and PID			X																															
Baseline data collection			X																															
Sexual behaviour & product adherence assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Product sharing & acceptability assessment																																	X	
Clinical Procedures																																		
Physical examination		X	X																															
Demonstration of product use			X																															
Focused medical and menstrual history		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Pelvic examination		X	0	0	0	X	0	0	X	0	0	X	0	0	X	0	0	X	0	0	X	0	0	X	0	0	X	0	0	X	0	X	X	
Genital specimen collection				0	0	X	0	0	0	0	0	0	0	0	X	0	0	0	0	0	0	0	0	0	0	0	X	0	0	0	0	0	X	
Colposcopy		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Scheduled blood draws for assays in lab	X		X	0	0	X	0	0	0	0	0	0	0	0	X	0	0	0	0	0	0	0	0	0	0	0	X	0	0	0	0	0	X	
Provide test results	X	X	(X)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pharmacy procedures																																		
Provide study product & instructions			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Update accountability log			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Collection of used and unused product				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Perform laboratory evaluations:																																		
Urine pregnancy test	X		0[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
HIV serology (rapid tests)	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HIV RNA PCR, Western blot and ELISA[e]	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
PBMCs, Plasma and serum archive[c, e]			X			X									X												X						X	
Hepatitis B assays[e]			X																														X	
Creatinine level[e]	X					X									X												X						X	
Haematology[e] (full blood count) & Blood chemistry (LFT, ALT, U & E, CaPO4)			X			X									X												X						X	X[f]
Genital specimen for archive[c]				0	0	X	0	0	0	0	0	0	0	0	X	0	0	0	0	0	0	0	0	0	0	0	X	0	0	0	0	0	X	
Viral load in seroconvertors[d]																																		
Resistance testing in seroconvertors[d]																																		X
Amount of blood collected (mls):	85[e]	0	80	80[e]	80[e]	155	80[e]	80[e]	80[e]	80[e]	80[e]	80[e]	80[e]	80[e]	155	80[e]	80[e]	80[e]	80[e]	80[e]	80[e]	80[e]	80[e]	80[e]	80[e]	80[e]	155	80[e]	80[e]	80[e]	80[e]	80[e]	165	15

[a] if no negative pregnancy test result in last 21 days prior to enrolment

[b] The visit schedule for month 16, 17, and 18 visit will be repeated for each quarter after 24 months if necessary until study exit

[c] The stored serum, plasma and cytobrush specimens will be used for potential post-trial assessments for markers of safety, risk exposure, product adherence and tenofovir resistance. In addition, stored plasma will be used for retrospective RNA PCR testing to confirm whether incident cases of early HIV infection during the trial occurred post-randomisation. [d] Performed on first positive HIV rapid specimen and repeated at 3 months later.

[e] Blood volumes: Confirmatory tests (WB and ELISA) (20ml) (only if indicated), Creatinine (5 mls); Safety bloods (20 mls), HBV assays (10 mls); Storage (50 mls), 60 ml tenofovir resistance assays (only if indicated)

0 = if indicated

[f] HBsAg positive participants will have a liver function test performed

Appendix II: Safety Laboratory Evaluations

Performed at enrolment and at Study Months 3, 12, 24 and study exit

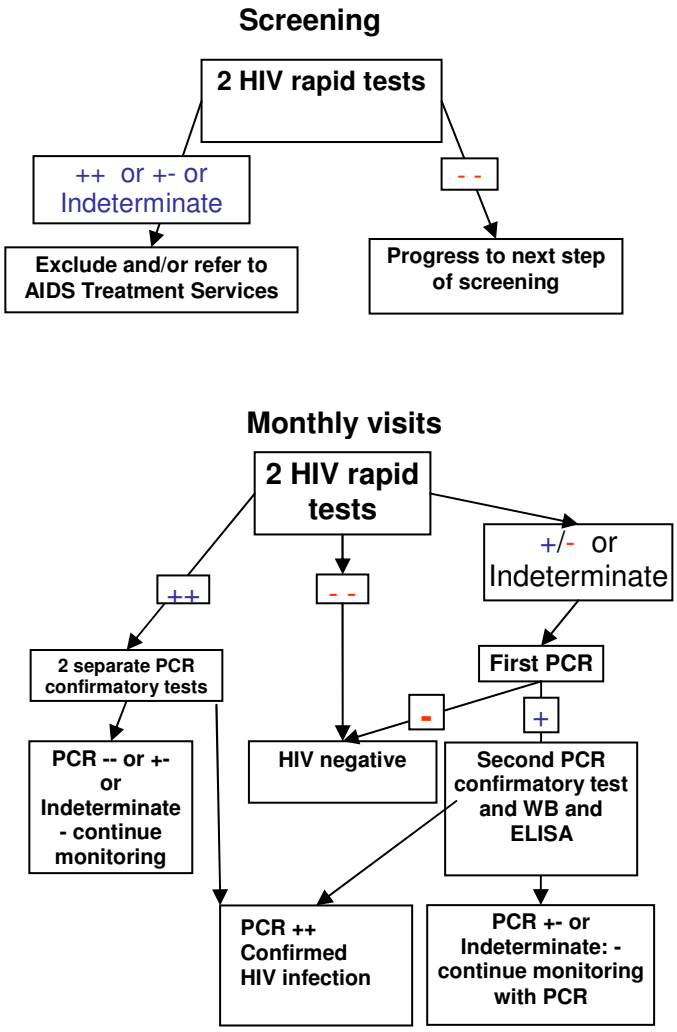
HEMATOLOGY TESTS
Full (Complete) blood count

Blood Chemistry
LIVER FUNCTION TESTS
Alkaline phosphatase
ALT
AST
Total bilirubin

RENAL FUNCTION TESTS
Urea
Creatinine
Serum electrolytes (Na+, K+, PO4-, Ca++)

Serum amylase

Appendix III: HIV Antibody Testing Algorithm



Appendix IVa: Informed Consent Form for Screening Participants (separate document)

Appendix IVb: Informed Consent Form for Enrolling Participants (separate document)

Appendix IVc: Informed Consent Form for Specimen Storage and Possible Future Research Testing (Separate document)