

MTN-028

**Phase 1 Pharmacokinetic Trial of Two Intravaginal Rings (IVRs) Containing
Different Dose Strengths of Vicriviroc (MK-4176) and MK-2048**

Microbicide Trials Network

Funding Agencies:

**Division of AIDS, US National Institute of Allergy and Infectious Diseases
US *Eunice Kennedy Shriver* National Institute of
Child Health and Human Development
US National Institute of Mental Health
US National Institutes of Health**

Grant Numbers:

UM1AI068633, UM1AI068615, UM1AI106707

DAIDS Protocol #: 12022

IND Sponsor:

DAIDS

Pharmaceutical Collaborator:

Merck & Co.

IND# 123,134

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Version 1.0

February 12, 2015

MTN-028

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**Phase 1 Pharmacokinetic Trial of Two Intravaginal Rings (IVRs) Containing
Different Dose Strengths of Vicriviroc (MK-4176) and MK-2048**

LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ALT	alanine transaminase
API	Active pharmaceutical agent
ART	antiretroviral therapy
ARV	antiretroviral
ASM	American Society for Microbiology
AST	aspartate aminotransferase
ASTM	American Society for Testing and Materials
AUC	area under the curve
BID	bis in die, twice a day
BRWG	Behavioral Research Working Group
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHC	combination hormonal contraceptive
C _{max}	maximum concentration
CMRB	Clinical Microbicide Research Branch
CNS	Central nerve system
CPX	Ciprofloxacin
CRF	case report form
CTA	Clinical Trial Agreement
CVF	cervical vaginal fluid
CWG	Community Working Group
DAERS	DAIDS Adverse Event Reporting System
DAIDS	Division of AIDS
DNA	deoxyribonucleic acid
EAE	expedited adverse event
EEG	Electroencephalography
ENR	Enrollment
EVA	ethylene vinyl acetate
FDA	(US) Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
FRCP	Federal Rules of Civil Procedure
GCP	Good Clinical Practices
GEE	generalized estimating equations
GLP	Good laboratory practice
GMP	Good Manufacturing Practices
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEENT	Head, Eye, Ear, Nose and Throat

HHS	Health and Human Services
HIV	Human Immunodeficiency Virus
HSV	Herpes simplex virus
hu-PBL	human peripheral blood lymphocytes
IATA	International Air Transport Association
ICF	informed consent forms
ICRC	International Committee of the Red Cross
IND	Investigational New Drug
INR	International normalized ratio
IRB	Institutional Review Board
IUD	intrauterine device
IVR	Intravaginal Ring
KOH	potassium hydroxide
LDMS	Laboratory Data Management System
LLOQ	lower limit of quantification
LOC	Leadership and Coordinating Center
MPI	maximum calculated percent inhibition
MSD	Merck Sharp & Dohme
MTN	Microbicide Trials Network
NAAT	nucleic acid amplification test
NGR	next generation rings
NIAID	National Institute of Allergy and Infectious Diseases
NICHHD	<i>Eunice Kennedy Shriver</i> National Institute of Child Health and Human Development
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NOAEL	no-observed-adverse-effect-level
OBT	Optimized Background Therapy
OHRP	Office for Human Research Protections
OSS	Overall susceptibility score
PBMC	Peripheral blood mononuclear cells
PEP	post-exposure prophylaxis
PK	pharmacokinetics
PPD	Pharmaceutical Product Development
PrEP	Pre-Exposure Prophylaxis
PRO	Protocol Registration Office
PSRT	Protocol Safety Review Team
PTID	participant identification
RE	Regulatory Entity
RNA	Ribonucleic acid
RSC	Regulatory Support Center
RTI	reproductive tract infection
SAE	serious adverse event
SCR	Screening
SDMC	Statistical Data Management Center
SMC	Study Monitoring Committee
SSP	study specific procedures
STI	sexually transmitted infection

UPMC	University of Pittsburgh Medical Center
USA	United States of America
UTI	urinary tract infection
VCV	Vicriviroc
WHO	World Health Organization

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MTN-028

**Phase 1 Pharmacokinetic Trial of Two Intravaginal Rings (IVRs) Containing
Different Dose Strengths of Vicriviroc (MK-4176) and MK-2048**

INVESTIGATOR SIGNATURE FORM

Version 1.0

February 12, 2015

A Study of the Microbicide Trials Network

Funded by:

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

IND Holder:

DAIDS

I, the Investigator of Record, agree to conduct this study in full accordance with the provisions of this protocol. I will comply with all requirements regarding the obligations of investigators as outlined in the Statement of Investigator (Form FDA 1572), which I have also signed. I agree to maintain all study documentation for at least two years following the date of marketing approval for the study product for the indication in which it was studied. If no marketing application is filed, or if the application is not approved, the records will be retained for two years after the investigation is discontinued and the US Food and Drug Administration is notified. Publication of the results of this study will be governed by MTN policies. Any presentation, abstract, or manuscript will be submitted to the MTN Manuscript Review Committee, DAIDS, Merck & Co. and other entities for review prior to submission, as required by the MTN Publication Policy.

I have read and understand the information in the Investigator's Brochure(s), including the potential risks and side effects of the products under investigation, and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

Name of Investigator of Record

Signature of Investigator of Record

Date

MTN-028

Phase 1 Pharmacokinetic Trial of Two Intravaginal Rings (IVRs) Containing Different Dose Strengths of Vicriviroc (MK-4176) and MK-2048

PROTOCOL SUMMARY

Short Title:	PK Trial of Two MK-2048A IVRs of Varying Dose Strengths
Clinical Phase:	Phase 1
IND Sponsor:	DAIDS
Protocol Chair:	Albert Liu, MD, MPH
Sample Size:	Approximately 18 women
Study Population:	Healthy, HIV-uninfected, sexually abstinent women between the ages of 18-45
Study Site:	US site selected by the MTN Executive Committee
Study Design:	Single-site, single-blind, two-arm, randomized trial (2:1)
Study Duration:	Approximately 5 weeks per participant, with approximately 6-9 months for planned accrual
Study Products:	Two ethylene-vinyl acetate intravaginal rings (IVRs) containing a combination of the active ingredients; Vicriviroc (VCV) (MK-4176) and MK-2048, have been formulated with different dose strengths: <ol style="list-style-type: none">1. MK-2048A IVR (Low Dose): 91 mg of VCV (MK-4176) + 10 mg of MK-20482. MK-2048A IVR (Original Dose): 182 mg of VCV (MK-4176) + 30 mg of MK-2048
Study Regimen:	Participants will be randomized to the study products in a 2:1 ratio. Participants will insert one IVR to be used for a period of approximately 28 days, followed by approximately 7 days of no study product use.

Primary Objectives:

Pharmacokinetics

- Assess local and systemic pharmacokinetics of VCV (MK-4176) and MK-2048 during and after 28 days of use of two MK-2048A IVRs containing different dose strengths

Safety

- Assess and compare the safety of two MK-2048A IVR formulations containing different dose strengths of VCV (MK-4176) and MK-2048 during and after 28 days of use by healthy, HIV-uninfected, sexually abstinent women

Primary Endpoints:

Pharmacokinetics

- Assessment of vaginal fluid, blood plasma, and cervical tissue concentrations of VCV (MK-4176) and MK-2048 during and after IVR use

Safety

- Genitourinary events Grade 1 or higher as defined by the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, Dec 2004 (Clarification dated August 2009), Addendum 1, (Female Genital Grading Table for Use in Microbicide Studies) judged to be related to study product
- Adverse events Grade 2 or higher as defined by the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, Dec 2004 (Clarification dated August 2009)

Secondary Objective:

Assessment of Remnant Content in IVRs

- Assess remnant content in returned IVRs

Secondary Endpoint:

Assessment of Remnant Content in IVRs

- Residual drug levels measured in IVRs

Figure 1: MTN-028 Study Visit Schedule

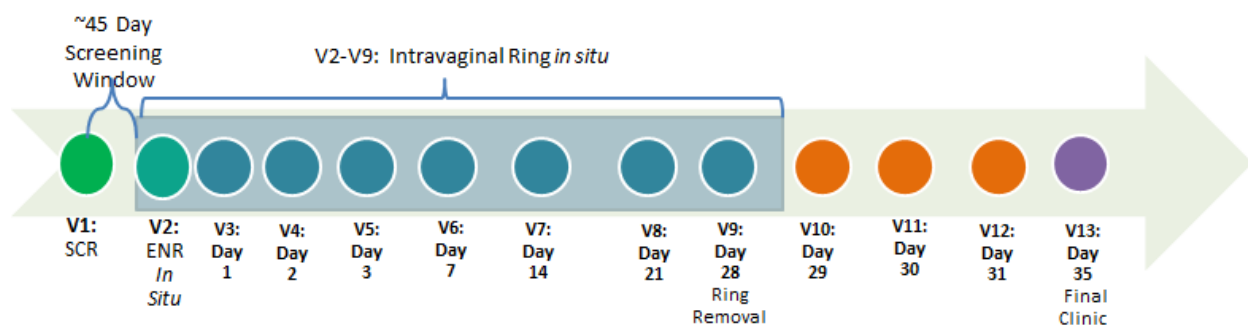


Table 1: PK Specimen Collection Schedule

STUDY VISIT	PK Specimen Collection
Screening	None
Enrollment	Blood (hr 1, 2, 4, 6), Vaginal fluid (hr 0,1, 2, 4, 6)
Day 1	Blood, Vaginal fluid
Day 2	Blood, Vaginal fluid
Day 3	Blood, Vaginal fluid
Day 7	Blood, Vaginal fluid
Day 14	Blood, Vaginal fluid
Day 21	Blood, Vaginal fluid
Day 28	Blood (hr 0, 1, 2, 4, 6), Vaginal fluid (hr 0, 1, 2, 4, 6), Cervical tissue (hr 0)
Day 29	Blood, Vaginal fluid
Day 30	Blood, Vaginal fluid
Day 31	Blood, Vaginal fluid
Day 35	Blood, Vaginal fluid

1 KEY ROLES

1.1 Protocol Identification

Protocol Title: Phase 1 Pharmacokinetic Trial of Two Intravaginal Rings (IVRs) Containing Different Dose Strengths of Vicriviroc (MK-4176) and MK-2048

Protocol Number: MTN-028

Short Title: PK Trial of Two MK-2048A IVRs of Varying Dose Strengths

Date: February 12, 2015

1.2 Funders, Sponsor and Monitor Identification

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2 INTRODUCTION

2.1 Human Immunodeficiency Virus (HIV) Prevention and Intravaginal Rings

The HIV/AIDS epidemic remains one of the world's most significant public health challenges. With approximately 2.3 million individuals newly infected each year, the development of safe and efficacious coitally-dependent and coitally-independent HIV prevention strategies remains a global health priority.¹ Extensive research efforts to develop novel vaginal and rectal antiretroviral (ARV) formulations and delivery systems are ongoing.² Undeniably linked to establishing the safety and efficacy of products in development is participant adherence to drug regimen. As products advance to late stage clinical trial development, the critical importance of participant adherence is evident. Across several large Phase 3 pre-exposure prophylaxis (PrEP) clinical trials, moderate to high participant adherence coupled with highly effective ARV products led to the extension of Truvada®'s licensure to include PrEP,^{3, 4} However, in trials testing the same drug, due to low participant adherence no efficacy was demonstrated.^{5, 6}

Sustained drug delivery devices, such as IVRs, may offer an ideal method of drug delivery. IVRs are discreet, coitally independent, offer continuous delivery of drug and are acceptable.⁷ IVRs have previously been developed and approved as delivery methods for various medications. For example, NuvaRing®, a contraceptive IVR made of ethylene vinyl acetate (EVA) copolymers (with a 28% w/w core and a 9% w/w skin layer) and magnesium stearate, in which 2.7 mg of ethinyl estradiol and 11.7 mg of etonogestrel are dispersed, has been found to be both effective and acceptable to women. In an acceptability study involving 1,950 NuvaRing® users, 45.5% of women cited that their reason for liking the IVR was "not having to remember anything".⁸ In a randomized controlled trial testing two alternative delivery systems for combined hormonal contraceptive, women overwhelmingly preferred the IVR to oral contraceptives ($P < .001$).⁹ It is likely that products that can be applied less frequently, such as a vaginal ring that is replaced every 28 days, may be more acceptable to users, resulting in higher user-adherence and may lead to increased effectiveness. IVRs that need to be replaced every 28 days may have benefits over dosage forms that need to be used more frequently, as well as offer a wider choice of microbicide formulations for women if proven effective.

2.2 Rationale

MTN-028 is a Phase 1 clinical trial designed to assess the pharmacokinetics of two IVRs containing different dose strengths of the combination of VCV (MK-4176) and MK-2048. The combination IVR (MK-2048A IVR) is novel as it combines two different classes of antiretroviral agents - a CCR5-receptor antagonist, VCV (MK-4176), with an integrase inhibitor, MK-2048.

The rationale for a combination microbicide IVR is based on combining antiretroviral drugs with different mechanisms of action to increase the protection and limit the emergence of resistant viral strains. Highly active antiretroviral therapy (ART) and the use of antiretroviral combinations in the prevention of mother-to-child HIV transmission have demonstrated the benefit of ARV combinations in the treatment and prevention of HIV infection. Although the effectiveness of the drug combination planned for this safety trial has not yet been evaluated clinically, both VCV and MK-2048 are highly potent ARVs.

VCV is a CCR5-receptor antagonist that is very potent against CCR5-tropic viruses, which are the predominantly transmitted strains, and is active against viruses resistant to other drug classes. Two formulations of VCV currently are in development: VCV (MK-4176) is the free-base form of VCV used for the development of VCV-based IVRs, and the maleate VCV form, MK-7690, used for the development of the VCV-based oral formulation. Development of resistance to VCV (MK-7690) (i.e., tablet formulation) in the Phase 3 trials was uncommon, thereby making it an attractive microbicide candidate. However, it is not active against viruses using co-receptors other than CCR5.

MK-2048 is an HIV-1 integrase inhibitor that is highly potent against both wild type HIV-1 and raltegravir-resistant isolates. The combination of two highly potent antiretrovirals not currently being used for HIV-1 treatment may serve as an ideal HIV prevention combination. MTN-027 will be the first trial testing vicriviroc (MK-4176) VR, containing 182 mg vicriviroc (MK-4176); the MK-2048 VR, containing 30 mg MK-2048; the MK-2048A VR, containing 182 mg vicriviroc (MK-4176) and 30 mg MK-2048; and the Placebo VR (1:1:1:1). This randomized (1:1:1:1) PK and safety study will enroll approximately 48 healthy, adult women who are HIV-uninfected, non-pregnant, sexually abstinent, and using adequate contraception.

MTN-027 will contribute safety comparisons of each study product as compared to placebo, as well as PK data. It is important to note that while the MTN-027 and MTN-028 trials are ongoing, they will be treated as companion trials and safety data from both trials will be reviewed/considered by the PSRT(s).

MTN-028 will provide additional data regarding the required dosage of MK-4176 and MK-2048 necessary to achieve sufficient vaginal fluid concentrations for antiviral activity. The development goal is to make rings that would lead to 50-100X IC₉₅ locally for both API's in sheep studies. The original dose formulation achieved this. The single compound rings being tested in MTN-027 have equivalent loading to the combination ring being used in that study (the original dose combination IVR). The rationale for the assessment of the low and original doses for the IVR in MTN-028 is to provide local and systemic PK data of different dose formulations of the combination ring, to inform development of the final product, including a multipurpose technology ring where dosages may need to be adjusted to optimize release profiles of multiple agents. It is not anticipated that the low dose ring will be the exact formulation to move forward; it is instead being used to obtain PK-data at lower release rates in order to inform further formulation development. The original and low dose ring drug loadings were chosen to

provide the low and high end of the dosing spectrum, as the final product will likely have a dosage somewhere between.

2.3 MK-2048A IVR

2.3.1 Description

MK-2048A IVRs

The MK-2048A IVR is a novel combination product (drug/device) designed to offer sustained-release of the combination of two active ingredients—the antiretrovirals VCV (MK-4176) and MK-2048.¹⁰ The MK-2048A IVR consists of a closed-ring fiber having two coaxial layers: a core layer and a skin layer. MK-2048A IVR is an ethylene-vinyl acetate copolymer (EVA) 28 core loaded with VCV (MK-4176) surrounded by an EVA 28 skin layer loaded with MK-2048. The MK-2048A IVR is a smooth, flexible, white to off-white, opaque IVR, with an outer diameter of 54 mm and a cross-sectional diameter of 4 mm. Further information is available in the MK-2048 and MK-2048A Investigator Brochures (IBs).^{11,10}

Active Drug Ingredient: VCV - Free Base (MK-4176)

VCV (MK-4176) is the VCV free-base formulation used for development of VCV as a microbicide administered in an IVR. VCV (MK-4176) is chemically described as 5-({4-[(3S)-4-{2-methoxy-1-[4-(trifluoromethyl)phenyl]ethyl}-3-methylpiperazin-1-yl]-4-methylpiperidin-1-yl}carbonyl)-4,6-dimethylpyrimidine. Further information is provided in the MK-2048A IB.¹¹ The maleate salt formulation of VCV, known as MK-7690, represents the oral formulation of VCV. Further information on VCV (MK-7690) is available in the Vicriviroc Maleate IB.¹²

Active Drug ingredient: MK-2048

MK-2048 is a second-generation HIV-1 integrase inhibitor that belongs to a novel class of compounds with antiretroviral activity. MK-2048 is chemically described as (6S)-2-(3-chloro-4-fluorobenzyl)-8-ethyl-10-hydroxy-N,6-dimethyl-1,9-dioxo-1,2,6,7,8,9-hexahydropyrazino[1',2':1,5]pyrrolo[2,3-d]-pyridazine-4-carboxamide. MK-2048 is a white crystalline non-hygroscopic powder that is highly soluble across the physiological pH range (pH 2 to 8).¹⁰ Further information is available in the MK-2048 and MK-2048A IBs.^{11,10}

2.3.2 Mechanism of Action

Vicriviroc (VCV) (MK-4176)

VCV is a potent *in vitro* CCR5 entry inhibitor of HIV-1. It inhibits HIV infection by acting as a potent *in vitro* inhibitor of HIV-1 infection of human cells, where in primary PBMC infection assays the overall geometric mean IC₅₀ and IC₉₀ values for VCV were 0.61 nM and 6.1 nM. Originally developed by Schering-Plough, VCV binds to a small hydrophobic pocket between the transmembrane helices near the extracellular surface of the CCR5 receptor, preventing the binding of gp120 to the target cell thereby preventing the virus from entering the target cell.^{12,13}

MK-2048

MK-2048 is a second-generation HIV-1 integrase inhibitor. HIV-1 integrase is an enzyme required for the catalysis of the insertion of the HIV-1 DNA into the genome of the host cell. Integration is required for stable maintenance of the viral genome as well as efficient viral gene expression. MK-2048 inhibits the insertion of HIV-1 DNA into host cells, thus preventing viral replication.¹⁰

2.3.3 Strength of Study Products

The two formulations of the MK-2048A IVRs differ in dose strengths, please find details below:

- Low Dose: 91 mg of VCV (MK-4176) + 10 mg MK-2048
- Original Dose: 182 mg of VCV (MK-4176) + 30 mg MK-2048

2.3.4 Product Storage and Handling

The data generated for MK-2048A supports the shipping condition of 2 to 8 °C with allowable excursions of -20 °C to 40 °C.

Temperature excursions of -20 °C to 2 °C for 5 days are allowable (>5 days would be assessed on a case-by-case basis). The 5 day excursion of -20 °C to 2 °C is justified based upon sound scientific rationale that the physical and chemical stability of MK-2048A will not change in this temperature range over the 5 day period.

Temperature excursions of >8°C to 30 °C for 7 days are allowable. The 7 days excursion to 30 °C is justified given the acceptable stability data at 40°C/75% room humidity for 6 months.

Temperature excursions of >30 °C to 40 °C for 1 day are allowable. The 1 day excursion to 40°C is justified given the acceptable stability data at 40°C/75% room humidity for 6 months. (Unpublished data supplied by Merck & Co.)

2.4 In vitro and Ex Vivo Studies

2.4.1 In Vitro and Ex Vivo Studies of VCV (MK-4176)

VIROLOGY

Anti-HIV Activity

VCV maleate (MK-7690) inhibited the replication of genotypically diverse HIV primary isolates in human peripheral blood mononuclear cells (PBMCs), with geometric mean inhibitory concentration 50% of viral inoculum inhibited (IC₅₀) and inhibitory concentration 90% of viral inoculum inhibited (IC₉₀) concentrations of 0.61 nM and 6.1 nM, respectively.¹¹ The *in vitro* antiviral effect was additive to synergistic when used in combination with other classes of ARV agents, including a monoclonal antibody against

the CCR5 receptor. *Note:* Preliminary data showed that the VCV free-base and maleate salt forms of VCV (MK-4176 and MK-7690, respectively) had similar activity in *in vitro* antiviral infection assays with CCR5-tropic virus.¹¹

In vitro and *ex vivo* anti-HIV activity studies conducted to support the development of VCV for a microbicide indication are summarized in Table 2. ARVs that are delivered intravaginally and are intended to be active against male-to-female transmission of HIV during sexual activity are exposed to a variety of factors that may influence their efficacy. *In vitro* assays were conducted to assess these factors and included antiviral activity in the presence of vaginal and seminal fluid simulants, a simulated pH shift and in the presence of whole semen (Table 2). *In vitro* anti-viral activity of VCV (MK-4176) was not affected by any of the factors. Approximately 30-fold more drug was needed to protect against cell-associated virus transmission as compared to cell-free. VCV (MK-4176) was not able to prevent CCR5-mediated cell fusion likely due to the creation of a viral synapse.¹⁴ Because VCV (MK-4176) is specific for CCR5, it was confirmed not to be effective against CXCR4 virus.

Table 2: *In vitro/Ex vivo* studies of VCV (MK-4176)/VCV (MK-7690)

<i>In Vitro</i> Efficacy	
CCR5-tropic cell-associated virus transmission	EC ₅₀ , 360 nM TC ₅₀ , >1 μM TI, >2.8
CCR5-tropic cell-free entry inhibition in TZM-bl	EC ₅₀ , 0.09-11.3 μM TC ₅₀ , >100 μM TI, >8,85-11.11
CXCR4-tropic cell-free entry inhibition	EC ₅₀ , >100 μM TC ₅₀ , >100 μM TI, I
CCR5-tropic fusion inhibition	EC ₅₀ , >100 μM TC ₅₀ , >100 μM TI, I
CCR5-tropic cell-free entry inhibition in the presence of semen in TZM-bl	EC ₅₀ , 2.58 nM TC ₅₀ , >200 nM TI, >77.52
CCR5-tropic cell-free entry inhibition in human PBMC	EC ₅₀ , 1.56 nM TC ₅₀ , >500 nM TI, >2293
CCR5-tropic cell-free entry inhibition in the presence of simulated seminal fluid in human PBMC	EC ₅₀ , 1.12 nM TC ₅₀ , >500 nM TI, >446
CCR5-tropic cell-free entry inhibition in the presence of simulated vaginal fluid in human PBMC	EC ₅₀ , 0.548 nM TC ₅₀ , >500 nM TI, >912
CCR5-tropic cell-free entry inhibition at pH 4.5 in human PBMC	EC ₅₀ , 9.17 nM TC ₅₀ , >200 nM TI, >21.81

Ex Vivo Efficacy	
API (VCV) antiviral activity in human ectocervical explant	Partial protection at 100 μ M, 1/6 explants
API (VCV) antiviral activity in human colorectal explant	Full protection at 10 μ M, 6/6 explants
IVR segments (VCV IVR) antiviral activity in human ectocervical explant	Full protection, 6/6 explants
Other Assays	
Lactobacillus toxicity	CC ₅₀ , > 1 mM

API= Active pharmaceutical agent; CC₅₀= 50% cytotoxic concentration 50%; CCR5= C-C Chemokine receptor type 5; EC₅₀= 50% effective concentration; I= Indeterminate; IVR= Intravaginal ring; PMBC= peripheral blood mononuclear cells; TC₅₀= Toxic concentration 50%; TI= therapeutic Index. The TI could not be established based on the calculated EC50 and TC50 values.

Ex Vivo studies were conducted using both polarized and non-polarized tissue explant models, including a human cervical tissue explant model which allows for exposure of only the surface of the epithelium to IVR segments and virus in a multi-day challenge assay. VCV (MK-4176) was tested as the free drug substance [i.e., active pharmaceutical ingredient (API)] and as drug formulated into IVR segments at a dose relevant to IVR product (i.e., VCV 182 mg) (Table 2). More VCV (MK-4176) was needed to block HIV-1 infection of polarized ectocervical and colonic tissue compared to PBMCs or other cell lines (Dezzutti, unpublished data). However, protection at the 100 and 10 μ M concentrations was below the amount of VCV (MK-4176) anticipated to be released daily from an IVR (2.8 mM). An ectocervical tissue explant model, as described above, was used to determine the activity of the IVR segments containing VCV (MK-4176). For the IVR segment testing, a multi-day challenge assay was used.¹⁵ Full protection (6/6 explants) was observed (Table 2 and Table 6). Other studies were conducted, such as the effect of VCV (MK-4176) on vaginal *Lactobacilli*, bacteria important to a healthy vaginal environment which serves as a natural defense against HIV infection. VCV (MK-4176) was not toxic to any of vaginal *Lactobacilli* species tested (Table 2).

Resistance

Several *in vitro* studies have shown that VCV (MK-7690) resistance is predominantly caused by mutations in the variable domain 3 of gp120 (V3). However, mutations in other variable loops of *env* can contribute to resistance. Mutations in V2 (V169M) and V3 (L317W) emerged after 4 passages of an R5-tropic subtype B primary isolate of USA origin¹⁶ in the presence of VCV (MK-7690), and I840Y in gp120 was selected after an additional 12 passages. I408T conferred a 2-fold increase in IC₅₀ and the triple mutant had a maximum calculated percent inhibition (MPI) of 94%. The passaged virus remained R5-tropic with decreased infectivity.¹⁷ Site directed mutational analysis showed that the G516V change in gp41 accompanied by M518V or F519I is critical for VCV (MK-7690) resistance in this isolate.¹⁸ Virus passaged in CD4+ T cells or PBMCs developed mutations that in combination caused >20,000-fold VCV (MK-7690) resistance after 6-20 passages. These combinations included K305R/A316V/G321E, V535M/G514V and/or F519L, and G516V/M518V/ F519I.^{19, 20}

Cross-resistance

Several primary HIV-1 isolates were independently passaged in PBMC cultures in the presence of increasing concentrations of VCV (MK-7690) until phenotypic resistance

emerged. Resistant isolates were evaluated for susceptibility to different CCR5 antagonists in PBMC infection assays. As indicated in Table 3, VCV-resistant isolates were also cross-resistant to other small molecule CCR5 antagonists.

Table 3: Summary of Cross-Resistance Profiles for VCV-Resistant Mutants

Inhibitor Class	Inhibitor	CC 1/85	CC101.6	RU570	JV1083
CCR5 Inhibitor	Vicriviroc	R	R	R	R
	Maraviroc	R	R	R	R
	Aplaviroc	R	R	R	R

R = Resistant; S = Sensitive

Phenotypically resistant variants, with the exception of one CC 1/85 culture, remained CCR5-tropic, as demonstrated by the continued ability to replicate in CCR5- but not CXCR4-expressing U-87-CD4 cell lines.

Condom Compatibility

Condom compatibility studies have not been conducted with the IVR. However, studies with high and low dose preparations of VCV (MK-4176) (2000 µM and 200 µM) dissolved in modified vaginal fluid simulant were conducted according to ASTM D7661 on the following types of condoms:

- Non-lubricated, latex condoms (Durex, LifeStyles, Trojan ENZ)
- Polyisoprene condom (LifeStyles SKYN)
- Polyurethane condom (Trojan Supra)

All types of condoms met the acceptance criteria established in the protocol for the mean values of the treated samples after treatment with compounds. The results of the condom compatibility testing indicated that VCV (MK-4176) has no deleterious effects on the integrity of condoms at any of the concentrations tested.

2.4.2 *In Vitro* and *Ex Vivo* Studies of MK-2048

VIROLOGY

Anti-HIV Activity

MK-2048 is a potent and selective inhibitor of HIV-1 integrase catalyzed strand transfer with an IC₅₀ of 7 nM. MK-2048 exhibits >5000-fold selectivity to the human DNA polymerases α, β, and γ (IC₅₀ ≥ 50µM) and ≥ 2000-fold selectivity against other HIV-1 phosphoryl transferases (RNase H and reverse transcriptase IC₅₀ > 15µM).¹⁰ MK-2048 exhibits potent inhibition in multiple-cycle HIV-1 replication assays performed in the presence of 10% fetal bovine serum (IC₉₅ = 11 ± 6 nM) or 50% human serum (IC₉₅ = 41 ± 26 nM). These antiviral potencies are comparable to many effective agents currently licensed for the treatment of HIV-1 infection (e.g., indinavir—IC₉₅ of 50 nM). When tested in a single-cycle HIV infectivity assay, MK-2048 showed potency (IC₅₀ range = 1.4 to 3.6 nM) against 23 viruses with integrase genes obtained from a broad range of clinical HIV isolates. Many of these isolates were highly resistant to licensed ARV drugs.

These findings support the expectation that MK-2048 should be broadly active against primary HIV isolates.¹⁰

In vitro and *ex vivo* anti-HIV activity studies conducted to support the development of MK-2048 for a microbicide indication are summarized in Table 4. *In vitro* anti-viral activity of MK-2048 was not affected by the presence of vaginal and seminal fluid simulants, a simulated pH shift or the presence of whole semen (Table 4). *Ex Vivo* studies were conducted using both polarized and non-polarized tissue explant models, including a human cervical tissue explant model which allows for exposure of only the surface of the epithelium to drug/IVR segments and virus in a multi-challenge assay.

MK-2048 was tested as the free drug substance (i.e., API) and as drug formulated into IVR segments at a dose relevant to IVR product (i.e., 30 mg MK-2048). *Ex vivo* testing of the MK-2048 API in polarized mucosal tissues showed more than 50% of the ectocervical tissues were protected from infection at 100 µM and 100% of colonic tissues were protected at 1 µM (Table 4). These MK-2048 concentrations are lower than the expected 649 µM/day anticipated to be released from the IVR. Activity of IVR segments containing MK-2048 was determined using an ectocervical tissue explant model (as described above). For the IVR segment testing, a multi-challenge assay was used.¹⁵ Full protection (6/6 explants) was observed (Table 4 and Table 6). MK-2048 was mildly toxic to vaginal *Lactobacilli* species tested (Table 4).

Table 4: *In vitro/Ex vivo* studies of MK-2048 supporting a microbicide indication

<i>In Vitro</i> Efficacy	
CCR5-tropic cell-associated virus transmission	EC ₅₀ , >1 µM TC ₅₀ , >1 µM TI, I
CCR5-tropic cell-free entry inhibition in TZM-bl	EC ₅₀ , 180 nM TC ₅₀ , >100 µM TI, 555.56
R4-tropic cell-free entry inhibition	EC ₅₀ , 150 nM TC ₅₀ , > 100 µM TI, >666.67
CCR5-tropic fusion inhibition	EC ₅₀ , 20.57 µM TC ₅₀ , > 100 µM TI, >4.86
CCR5-tropic cell-free entry inhibition in the presence of semen in TZM-bl	EC ₅₀ , 0.628 nM TC ₅₀ , >200 nM TI, >318.47
CCR5-tropic cell-free entry inhibition in human PBMC	EC ₅₀ , 0.083 nM TC ₅₀ , >500 nM TI, >6053
CCR5-tropic cell-free entry inhibition in the presence of simulated seminal fluid in human PBMC	EC ₅₀ , 0.269 nM TC ₅₀ , >500 nM TI, >1859
CCR5-tropic cell-free entry inhibition in the presence of simulated vaginal fluid in human PBMC	EC ₅₀ , 0.753 nM TC ₅₀ , >500 nM TI, >664
CCR5-tropic cell-free entry inhibition at pH 4.5 in human PBMC	EC ₅₀ , 1.24 nM TC ₅₀ , >200 nM TI, > 161.29

Ex Vivo Efficacy	
API (MK-2048) antiviral activity in human ectocervical explant	Partial protection at 100 µM, 4/6 explants
API (MK-2048) antiviral activity in human colorectal explant	Full protection at 1 µM, 6/6 explants
IVR segment (MK-2048 IVR) antiviral activity in human ectocervical explant	Full protection, 6/6 explants
Other Assays	
Lactobacillus toxicity	CC ₅₀ , 0.870 – 1.61 mM

API= Active pharmaceutical agent; CC₅₀= 50% cytotoxic concentration 50%; CCR5= C-C Chemokine receptor type 5; EC₅₀= 50% effective concentration; I= Indeterminate; IVR= Intravaginal ring; PMBC= peripheral blood mononuclear cells; TC₅₀= Toxic concentration 50%; TI= therapeutic Index; The TI could not be established based on the calculated EC50 and TC50 values.

Resistance

In vitro selection experiments with MK-2048 initiated with virus from clinical isolates selected the N155H mutation in integrase. Q148H/K/R with other mutations isolated from patients failing raltegravir-containing ART were found to have reduced susceptibility to MK-2048.²¹⁻²³ In another *in vitro* resistance study, the G118R and E138K mutations have also been found to be selected by MK-2048 and to cause resistance to MK-2048 but not to raltegravir or elvitegravir, two related drugs.²⁴

Cross-resistance

HIV-1 variants selected for resistance to integrase strand transfer inhibitors harbor mutations in the integrase active site, such as T66I, L74M, F121Y, V151I, S153Y, M154I, and N155S.^{24, 25} Various integrase inhibitors select for similar, but not identical, mutations in integrase. *In vitro*, mutation in the integrase active site (N155S) was found to reduce the potency of MK-2048 by 3-fold. However, the activity of MK-2048 was not effected by single or combinations mutations at other integrase sites including T66I/L74M/V151I, F121Y, T66I/S155Y or T66I/M154I.²⁵

2.4.3 *In vitro* and *Ex vivo* Studies of VCV (MK-4176) and MK-2048 in Combination

VIROLOGY

Anti-HIV Activity

Limited data is currently available on the antiviral activity of MK-2048 and VCV (MK-4176) in combination. Preliminary studies indicate at least additive antiviral activity of the two compounds when tested at equimolar concentrations relative to single drug alone in a standard antiviral assay (Table 5).¹¹

Table 5: Antiviral Activity of free drug VCV (MK-4176) and MK-2048 Alone or in Combination

Viral Isolate	VCV alone (IC ₅₀ nM)	MK-2048 only (IC ₅₀ nM)	VCV + MK-2048 (IC ₅₀ nM)
JR-FL	11.7	7.7	8.9
ASM 57	7.1	4.9	6.1

Ex vivo anti-HIV activity studies conducted to support the development of the combination of MK-2048/VCV (MK-4194) for a microbicide indication are summarized in Table 6. A polarized ectocervical tissue explant model which allows for exposure of only

the surface of the epithelium to drug and virus (as described above) was used to determine the activity of the MK-2048A combination IVR as well as IVRs containing VCV (MK-4176) or MK-2048 alone (see Tables 2 and 4). IVR segments contained drug doses relevant/proportional to planned IVR products (30 mg MK-2048 and 182 mg VCV). For the IVR segment testing, a multi-challenge assay was used.¹⁵ Full protection (6/6 explants) was observed against 4 independent HIV challenges over 11 days in explants cultured with the IVR segments of the combination MK-2048A IVR as well as the IVR segments of the single agent IVRs (containing either MK-2048 or VCV (MK-4176) only).

Table 6: Ex vivo studies of IVRs containing MK-2048 and/or VCV or placebo

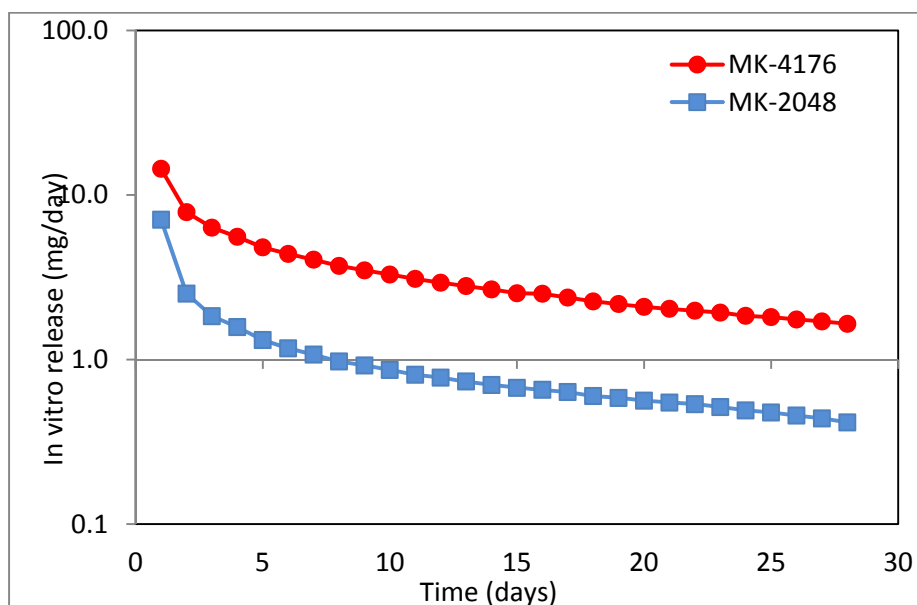
Ex Vivo Efficacy: Antiviral activity in human ectocervical explant model	
IVR segments (MK-2048A combination IVR)	Full protection, 6/6 explants
IVR segments (MK-2048 IVR)	Full protection, 6/6 explants
IVR segments (VCV IVR)	Full protection, 6/6 explants
IVR segments (placebo)	No Protection, 0/6 explants

2.4.4 In Vitro IVR Drug Release Studies: MK-2048A combination IVR

To evaluate the extent and rate of drug release from the EVA polymer IVRs, MK-2048/VCV combination IVRs were incubated at 37°C in 100 mL *in vitro* release medium (0.05% (V/V) acetic acid in sodium dodecyl sulfate solution 1.0% (m/V)). Samples for drug determination were collected every 24 hours and release medium was refreshed directly after each sampling.

A typical *in vitro* drug release profile of VCV (MK-4176) and MK-2048 over 28 days from the MK-2048A combination IVR (182 mg VCV (MK-4176) + 30 mg MK-2048) is shown in Figure 2. An initial burst release of both compounds was observed during the first 2 days of incubation with peak release rates of approximately 14 and 7 mg/day for VCV (MK-4176) and MK-2048, respectively. Following this initial burst, drug release stabilized between days 5 to 28, with release rates ranging between 4.8 to 1.65 mg/day for VCV (MK-4176) and 1.3 to 0.415 mg/day for MK-2048. Assuming a total vaginal fluid volume of 10 ml, the predicted fluid levels of VCV (MK-4176) and MK-2048 on day 28 would be approximately 300 µM and 90 µM respectively; concentrations many fold above the *in vitro* antiviral IC₉₅ for each compound.¹¹

Figure 2: In vitro release of VCV (MK-4176) and MK-2048 from MK-2048A combination IVR



2.5 Nonclinical Studies

2.5.1 Non Clinical Studies of VCV (MK-7690)

Animal studies of the safety of VCV maleate form [VCV (MK-7690)] when administered via the oral route are available in the VCV (MK-7690) IB and MK-2048A IB.^{11, 12} Animal study data were conducted in multiple species and supported the safety of MK-7690 for further investigation in human trials. No treatment related effects on renal, respiratory or cardiovascular systems were observed. Central nervous system (CNS) studies showed that seizures were observed in some animals. Seizures in animals were self-limiting and preventable with standard anti-convulsant therapy. The plasma concentration in the most sensitive species (dog), below which no seizure occurred, was 4670 ng/mL. This concentration is substantially higher (>170X) than plasma levels measured in sheep dosed intravaginally with MK-2048A IVR or VCV (MK-4176) only IVR for 28 days.

A penile irritation study in male rabbits was conducted to evaluate the potential local toxicity to male partners of women using IVRs containing VCV (MK-4176). Hourly application of 0.2 mL of gels containing 0.231%, 0.461%, 0.922%, and 2.5% VCV (MK-4176) for 4 hours over 3 days resulted in no local or systemic toxicity.

A battery of biocompatibility tests was performed using extracts from the MK-2048A combination IVR containing both VCV (MK-4176) and MK-2048, as well as IVRs containing VCV (MK-4176) or MK-2048 alone. The following biocompatibility studies were conducted and did not identify any significant safety concerns in any of the IVRs.¹¹

- **Cytotoxicity**
 - Cytotoxicity study using the elution method; extract in minimal essential medium (37°C for 24 hours)
- **Sensitization**
 - Guinea pig maximization study; extracts in 0.9% sodium chloride solution (50°C for 72 hours) and cotton seed oil (50°C for 72 hours)
- **Genotoxicity**
 - Bacterial reverse mutation study; extracts in 0.9% sodium chloride solution (50°C for 72 hours) and dimethyl sulfoxide (50°C for 72 hours)
 - Mouse lymphoma assay; extracts in 0.9% sodium chloride solution (50°C for 72 hours) and dimethyl sulfoxide (50°C for 72 hours)
- **Irritation and subacute/subchronic toxicity**
 - Rabbits dosed intravaginally, daily, for 35 days; extracts in 0.9% sodium chloride solution (50°C for 72 hours) and cotton seed oil (50°C for 72 hours)

2.5.2 Non Clinical Studies of MK-2048

Animal studies of the safety of MK-2048 agents when administered via the oral route are available in the MK-2048A IB and MK-2048 IB.^{10, 11} Animal study data were conducted in multiple species and supported the safety of MK-2048 for further investigation in human trials. No treatment related effects on renal, respiratory or cardiovascular systems were observed in various animal models. In CNS studies, no treatment-related effects were observed.

A penile irritation study in male rabbits was conducted to evaluate the potential local toxicity to male partners of women using the various IVRs containing ARVs used in the study. The hourly application of 0.2 mL of gels containing 0.059%, 0.117%, and 0.234% MK-2048 for 4 hours over 3 days resulted in no local or systemic toxicity.

2.5.3 Non Clinical Studies of VCV (MK-4176) and MK-2048 Combination

Animal studies of the safety of MK-2048 and VCV (MK-7690) when tested as single agents via the oral or intravenous route are available in the MK-2048 IB, VCV (MK-7690) and MK-2048A IBs.¹⁰⁻¹²

Systemic and local toxicities during 28-day continuous intravaginal use of VCV (MK-4176) and MK-2048 as single agents or in combination were conducted using various animal species, administered as an IVR or via a gel formulation (Table 7).¹¹

IVR Study: Sheep Model

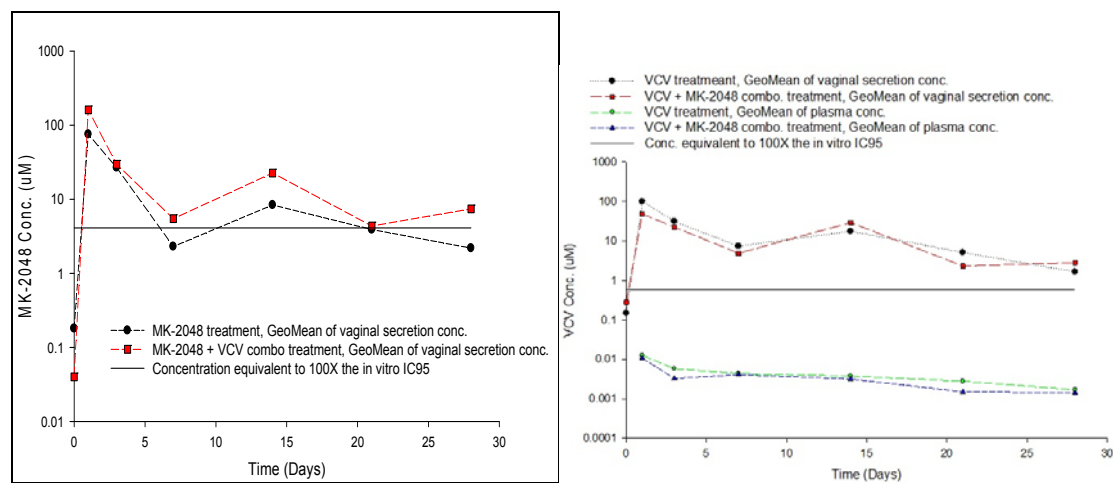
The potential local effects, systemic toxicity and toxicokinetics of placebo and antiretroviral containing IVR products (including VCV (MK-4176) IVR, MK-2048 IVR and the MK-2048A combination IVR) were evaluated in virginal Dorset sheep model for 28 days. These were the same IVR formulations to be used in the MTN-027 study. Two feasibility studies were conducted: a non-GLP study (study no. S11727) and a GLP study (study no. S12284).

In a non-GLP study conducted in virginal Dorset sheep (Study S11727), IVR containing VCV (MK-4176), MK-2048, or a combination of both (182 mg of VCV (MK-4176) and/or 30 mg MK-2048) were inserted intravaginally (Figure 3). Pharmacokinetic analysis of plasma indicated low systemic exposures of VCV ($C_{max} \leq 0.013 \mu\text{M}$) and no detectable levels of MK-2048 at all-time points during the 28-day study (LLOQ 0.0005 to 0.001 μM). The maximal concentrations of MK-2048 and VCV in local vaginal secretions were achieved a day after ring insertion. These concentrations were highly variable across animals and corresponded to a geometric mean of 75.3 μM and 160.7 μM for MK-2048 in the single-component and combination ring respectively and 99.3 μM and 49.1 μM for VCV (MK-4176) in the single-component and combination ring, respectively.

Twenty eight days after insertion of the IVRs, the geometric mean concentrations for MK-2048 were 2.19 μM and 7.41 μM in the single-component and combination ring respectively. For VCV (MK-4176), the concentrations 28 days after insertion were 1.66 μM and 2.84 μM in the single-component and combination IVR respectively. Remnant analysis of the IVRs recovered at the conclusion of the 28-day study indicated that 12.3 and 10.2 mg of MK-2048 were released from the single-component and combination IVRs respectively, at the conclusion of the study. For VCV (MK-4176), the amounts released were 111 and 105.7 mg, respectively.

The presence of the IVR was associated with mixed cell inflammation of the vaginal epithelial and sub-epithelial tissues and hypertrophy of the vaginal epithelium. However these changes were observed in all treatment groups, including the placebo IVR group, and therefore cannot be attributed to the presence of compounds in the IVRs. Of note, no sham treatment group was included in this non-GLP study. Therefore, the effect of manipulation of the animals during dosing/examination procedures could not be evaluated and compared to the findings in the IVR-treated animals. To better differentiate handling and manipulation effects from IVR related effects, a sham treated group was included in the GLP sheep study described below.

Figure 3: Plasma and vaginal fluid concentrations for MK-2048 (A) and VCV (MK-4176) (B) following insertion of IVRs containing MK-2048 (30 mg) and/ VCV (MK-4176) (182 mg) to virginal Dorset sheep



(A)

(B)

In the GLP sheep study (SN12284) placebo, single ARV agent and combination agents IVRs, were used to evaluate the potential local effects, systemic toxicity and toxicokinetics in virginal Dorset sheep for 28 days in comparison to non-treated (sham) animals. Histopathological results showed that the vaginal mucosa among non-treated (sham) animals and animals exposed to the placebo IVR was comprised of normal squamous epithelium, sometimes exhibiting parakeratosis of the most superficial layer lining the vaginal lumen. The immediately subjacent propria-submucosa contained variable amounts of mixed cellular infiltrates composed of mononuclear cells (lymphocytes, monocytes) mixed with neutrophils. These cellular infiltrates also extended into the overlying stratified squamous epithelium. The vaginal and cervical tissues among animals exposed to IVRs containing VCV, MK-2048 or both had similar morphology as described for animals of non-treated and placebo controls. Because of the presence of similar findings in the sham, placebo and VCV/MK-2048 treated groups, it can be concluded that the microscopic observations in the vagina, cervix, uterus, or ovaries were not related to the intravaginal placement of the IVRs. There were also no findings attributable to VCV (MK-4176), MK-2048, or the combination of the two compounds among sheep evaluated at end of treatment on Day 28 or at end of recovery on Day 38. In this study, no systemic toxicity was observed in the sheep after 28 days of exposure to the IVRs.

MK-2048 plasma levels were below the LLOQ (0.0108 μM) at all-time points over 28 days of exposure to the IVR containing MK-2048 alone or in combination with VCV. VCV (MK-4176) was detected in the plasma at low levels with a C_{max} of 0.050 μM (3 times LLOQ of the analytical method: 0.0187 μM) achieved by 4 hours after IVR insertion. The plasma levels rapidly declined to below LLOQ thereafter.

The combination IVR contained 182 mg VCV (MK-4176) and 30 mg MK-2048. Remnant content analysis of the rings used in the 28-day study in sheep demonstrated an *in vivo* total exposure of 106 mg MK-4176 and 16 mg MK-2048. This *in vivo* release in sheep results in a 5.2 mg/kg and 0.5 mg/kg total cumulative 28 day dose in sheep for MK-4176 and MK-2048 respectively.

Repeat Dose Toxicity Studies: Gel Administration

Although studies using the IVRs in the sheep model allowed evaluation of toxicity of the drug product *in vivo*, no MK-2048 and very low levels of VCV (MK-4176) were detected in the plasma of treated animals. Therefore, to achieve higher systemic exposures than those observed with the drug product in sheep the compounds were formulated as gels to evaluate the potential local effects, systemic toxicity and toxicokinetics in Sprague-Dawley rats and New Zealand white rabbits when administered intravaginally for 28 days. Details of each study are provided below and summarized in Table 7.

In summary, there were no significant local or systemic findings following repeat administration of up to 2.5% VCV (MK-4176) and 0.234% MK-2048 up to 28 days in intravaginal toxicity studies in rats using gels. In intravaginal toxicity studies in rabbits there were no significant systemic findings or histomorphological findings in the vaginal proximal mucosa determined the NOAEL at 0.231% of VCV (MK-4176) following repeat daily administration up to 28 days. There were no significant local or systemic findings following repeat administration up to 0.234% MK-2048 for 28 days. Although findings were reported at the higher dose levels for VCV (MK-4176) in the rabbit they were not reproduced in the equivalent studies in the rat and sheep model, therefore we consider these to be idiosyncratic for this model. The absence of relevant changes in distal mucosa and stratified squamous epithelium of the rabbit and the absence of changes in the rat and sheep models, which are more representative of vaginal tissues in women, are more relevant findings.

Table 7: Non-clinical safety/toxicity studies of VCV (MK-4176), MK-2048 and the combination of VCV (MK-4176) + MK-2048 via topical administration

Species	Dosage of ARV	Route/Mode of Administration	Duration	Study ID
Sheep ^a	Placebo VCV (MK- 4176) only, MK-2048 only VCV (MK- 4176) + MK-2048 combination VCV ^b : up to 18 mg/day ^c MK-2048 ^d : up to 7mg/day	IVR	28 days	S11727 ^e
Sheep	Placebo VCV (MK- 4176) only MK-2048 only VCV (MK- 4176) + MK-2048 combination VCV ^f : up to 18 mg/day ^c MK-2048 ^g : up to 7 mg/day	IVR	28 days 10 day recovery	S12284
Rabbit	VCV (MK- 4176): 2.305 – 25 mg/rabbit/day ^h MK-2048: 0.59 – 2.34 mg/rabbit/day	Gel formulation applied vaginally	10 days	1726-020
Rabbit	VCV: 2.305 – 25 mg/rabbit/day MK-2048: 0.59 – 2.34 mg/rabbit/day	Gel formulation applied vaginally	28 days 10 day recovery	1726-027

Species	Dosage of ARV	Route/Mode of Administration	Duration	Study ID
Rabbit	VCV: 1.844 – 20 mg/rabbit/day MK-2048: 0.472 – 1.872 mg/rabbit/day	Gel formulation applied to penis	Gel applied hourly for 4 hours for 3 days, 2 day recovery	S12392
Rat	VCV: 0.2305 – 2.5 mg/rat/day MK-2048: 0.059 – 0.234 mg/rat/day	Gel formulation applied vaginally	28 days 10 day recovery	1726-026

^aStudy was performed under non-GLP quality standards.

^bTotal VCV present in the VCV only and VCV + MK-2048 combination IVR was 182 mg. The average daily drug present in local vaginal secretions over 28 days ranged between 1.66 and 99.31 μ M.

^cDaily release rate determined *in vitro*.

^dTotal MK-2048 present in the MK-2048 only and VCV + MK-2048 combination IVR was 30 mg. The average daily drug present local vaginal secretions over 28 days ranged between 2.19 and 160.67 μ M.

^eFinal report assembled by Merck under the report designation: MK-2048 non-clinical report PK003.

^fTotal VCV present in the VCV only and VCV + MK-2048 combination IVR was 182 mg. The average daily drug present in local vaginal secretions over 28 days ranged between 0.28 and 79.24 μ M with IVR present.

^gTotal MK-2048 present in the MK-2048 only and VCV + MK-2048 combination IVR was 30 mg. The average daily drug present in local vaginal secretions over 28 days ranged between 13.86 and 1036.3 μ M with IVR present.

^hExpressed as daily dose mg/animal for daily vaginal/penile gel administration.

A battery of biocompatibility tests was performed using extracts from the MK-2048A combination IVR containing VCV (MK-4176) and MK-2048, as well as IVRs containing each of the drugs alone. The biocompatibility studies did not identify any significant safety concerns.

2.5.4 Non Clinical Studies of IVR Components

Since the ring is applied intravaginally, a program of specific toxicological studies was performed that paid special attention to local and systemic toxicity of its chemical components (i.e., EVA copolymer and potential leachables) *in vivo*, and to their toxic potential *in vitro*. Part of the evidence for the safe use of the IVR with respect to the EVA copolymer and potential leachables was obtained from studies performed with the EVA-containing radiopaque Multiload® (intrauterine device) and extracts prepared thereof. Other relevant data on the potential toxicity of the IVR constituents were derived from studies for the development of the contraceptive implant Implanon® and the vaginal ring Nuvaring®, both manufactured by N.V. Organon, now part of Merck. Detailed information about the non-clinical toxicology studies are provided in the MK-2048A IB.¹¹

The placebo IVRs were used to evaluate the potential local effects, systemic toxicity and toxicokinetics in virginal Dorset sheep for 28 days in a feasibility non-GLP study (study no. S11727) and GLP study (study no. S12284) (see Table 7). In the non-GLP feasibility study, the IVRs were generally well tolerated. The presence of the IVR was associated with mixed cell inflammation of the vaginal epithelial and sub-epithelial tissues and hypertrophy of the vaginal epithelium. Similar changes in animals that received the placebo IVR compared to those that received IVRs containing drug eliminates the drugs alone or in combination as the sole cause of the microscopic

observations. In the GLP study, no systemic toxicity was observed in the sheep model after 28 days of exposure to the placebo IVR.

These data support the use of the EVA copolymer as a delivery vehicle for VCV (MK-4176) and MK-2048.

2.6 Clinical Studies

2.6.1 Clinical Studies of VCV (MK-7690)

The pharmacokinetics, safety and tolerability of oral VCV maleate (MK-7690) have been evaluated in clinical studies (Phase 1 through Phase 3). Oral administration of VCV in healthy subjects, subjects with end stage renal disease, subjects with mild/moderate hepatic failure and patients with HIV or HIV/HCV coinfection, was generally well tolerated, without identification of an exposure-related toxicity.

Pharmacokinetics

Following oral administration of VCV (MK-7690), maximum plasma concentrations were reached approximately 1 to 2 hours after VCV administration (dosed alone or in the presence of ritonavir). C_{max} and AUC increased in a linear fashion with an increase in VCV dose. C_{max} concentrations at VCV 150 mg, in the presence of ritonavir 100 mg, are the highest attained to date (2470 ng/mL); this dose was well tolerated with no safety concerns detected.

VCV (MK-7690) is extensively metabolized by CYP3A4. Unchanged parent drug was the major component of drug-derived radioactivity in plasma. The carboxylic acid metabolite of VCV was the predominant circulating metabolite in human subjects after multiple oral doses of VCV monotherapy (50 mg twice daily for 14 days).

Safety

The highest doses of VCV (MK-7690) administered to HIV-seronegative healthy subjects have been single doses of up to 300 mg alone and multiple doses up to 250 mg QD alone for 7 days and 100 mg with ritonavir for 10 days. Pooled safety data from seronegative subject studies examined treatment-emergent adverse events (TEAEs) from studies where VCV (MK-7690) was administered from 10 mg as a single-dose to 250 mg QD alone or 150 mg with ritonavir QD. Of the 729 HIV-seronegative healthy subjects treated with VCV (MK-7690), 19 (3%) discontinued due to an AE compared to none of the subjects who received placebo. The most frequently reported TEAEs were headache (14%) and diarrhea (10%) after any VCV (MK-7690) administration (alone or in combination). A relationship to VCV (MK-7690) dose was not apparent. The majority of TEAEs, regardless of treatment, were mild to moderate in severity. In general, there were no remarkable or unexpected safety concerns, or trends in clinical laboratory tests associated with VCV (MK-7690) across the studied drug combinations. Neurological assessments of safety using continuous EEG monitoring to evaluate the potential for VCV to induce aberrant EEG waveform activity were conducted in two placebo-controlled dose-escalating studies in HIV-negative healthy subjects (P03161 and

P04873). Results of the healthy subject EEG studies and the absence of drug-related seizures in Phase 2/3 patient studies confirmed the assertion that concentrations of VCV that might lead to seizures in humans are significantly higher than those administered for clinical treatment.¹²

Phase 1b/2a HIV Infected Subjects: Safety and Activity

No dose-related increase in the number of AEs was reported among HIV-infected or HIV/HCV co-infected subjects administered VCV (MK-7690) in the Phase 1b/2a studies P02726 (a 14-day, rising-multiple-dose study in HIV-infected subjects) and P04416 (a 28-day safety study in HIV/HCV co-infected subjects). Most adverse events were mild and unrelated to study drug.¹¹

Two subjects in P02726 exhibited detectable X4 viral isolates in the 50 mg cohort. The mixed/dual R5/X4 viral phenotype was identified in one subject prior to dosing and after 14 days of dosing in the second. The second subject experienced a >1.5 log₁₀ reduction in viral ribonucleic acid (RNA) and did not have a decline in CD4+ cell (T helper cell) count. By Day 28, 14 days after the last VCV (MK-7690) dose, no CXCR4 virus was detected.¹¹

The mean change from baseline in HIV RNA for all three dose cohorts in the P02726, rising, multiple-dose study in HIV-infected subjects demonstrated potent viral suppression of 0.9 to 1.6 log₁₀ on treatment with VCV (MK-7690). After 10, 25, and 50 mg VCV (MK-7690) BID, 46%, 77%, and 82% of subjects, respectively, reached or surpassed a 1.0 log₁₀ reduction in viral load by Day 14.¹¹

Phase 2 and 3 Safety/Tolerability and Activity

As of May 2010, more than 1300 HIV-infected subjects took part in Schering-sponsored Phase 2 or 3 trials, with the majority (80%) of the HIV-infected participants being treatment-experienced (n=1207) at the time of enrollment. Most TEAEs were mild and not related to VCV (MK-7690). The most commonly reported AEs were diarrhea, nausea, headache, upper respiratory infection, nasopharyngitis, and fatigue, with no clear difference in incidence between VCV (MK-7690) recipients and control groups. Of the treatment-naïve subjects (n=309), a total of 30 subjects experienced serious adverse events (SAEs); 17 of the 177 (9.6%) treated with VCV (MK-7690), and 13 of the 132 (9.8%) in the control group. Among the treatment-experienced subjects (n=1488) a total of 210 experienced SAEs (including 171/1092 subjects (16%) receiving VCV (MK-7690) and 39/396 subjects (10%) receiving placebo).

Resistance

In two phase 3 studies (Study P04405 and P04889), viral isolates from study subjects at selected time points (Baseline, Weeks 2, 24, and 48, or at the time of virologic breakthrough) were tested for susceptibility to VCV (MK-7690). For both studies, an assessment of the Overall Susceptibility Score (OSS) was made using the PhenoSense GT assay at Screening, at Week 48 or at the time of virologic failure. Only four subjects exhibited a significant change in susceptibility to VCV (MK-7690) (manifested as a decrease in maximum calculated percent inhibition [MPI]) over the course of therapy

and these changes were not detected until Week 12 (Subject 1803) or later. Two of the four subjects had an OSS of 3, the third had an OSS of 2, and final subject (Subject 1803) had an OSS of 1. X4-using virus was not detected at the time of study discontinuation in any of the four subjects with phenotypic resistance to VCV (MK-7690).

As above, viral isolates from the four subjects who exhibited reduced susceptibility to VCV (MK-7690) were subjected to a clonal analysis. Although a comparison of baseline and end of treatment samples (12 clones/time point) revealed sequence evolution throughout the gp160 glycoprotein during the time of treatment, there was no consistent pattern of genotypic changes across subjects.¹²

Several clinical studies have also evaluated VCV (MK-7690) resistance. Two studies examined VCV (MK-7690) resistance in R5-tropic isolates from treatment failures but found no consistent pattern of resistance to VCV (MK-7690) from treated patients with virologic failure.^{26, 27} One subject from a clinical trial infected with subtype D HIV-1 developed resistance to VCV (MK-7690) via multiple changes in gp120, including Q315E and R321G in the V3 loop, and E328K and G429R in C4.²⁸ One of 29 subjects in a Phase 2b study of VCV (MK-7690) infected with subtype C HIV-1 also harbored V3 loop mutations.²⁹

Effectiveness

Based upon the analysis of a Phase 2 study (Study P03672) which evaluated VCV (MK-7690) dosages of 20 mg and 30 mg QD, the 30-mg dose was selected for the Phase 3 studies (Studies P04405 and P04889). These Phase 3 trials were designed to confirm the efficacy of the 30 mg VCV (MK-7690) QD dosage in combination with Optimized Background Therapy (OBT) including a ritonavir-boosted protease inhibitor (PI/r) in a larger cohort of HIV-infected, treatment-experienced subjects. Following completion and full analysis of the Phase 3 VCV (MK-7690) studies, it became clear that VCV (MK-7690) 30 mg QD did not provide incremental benefit to subjects with three or more active drugs in their background ART regimen. However, a greater proportion of subjects achieved virologic suppression (HIV RNA <50 copies/mL) in the VCV (MK-7690) group than in the control group, when VCV (MK-7690) was administered with a background ART regimen containing two or fewer active agents, similar to the Phase 2 study (Study P03672). Clinical development of VCV (MK-7690) was subsequently terminated.¹²

2.6.2 Clinical Study of MK-2048

Safety

One Phase 1 clinical trial of MK-2048 in an oral formulation has been conducted.^{10, 11} Merck enrolled 16 men in a double-blind, randomized, placebo-controlled, alternating panel, rising single-dose study to evaluate the safety, tolerability and PK of MK-2048 in healthy male participants between the ages of 18 to 45 years. Each participant received either a single oral dose of MK-2048 or placebo per treatment period over a course of 4 periods, with a 7 day washout interval between periods. Total duration of

follow-up for each was 8 weeks. Dose escalation (50, 100, 200, 400, 800, and 1200 mg) continued sequentially proceeding to the highest dose. Blood samples were collected for determination of MK-2048 plasma concentrations for 120 hours post-dose in each treatment period.

Safety was monitored throughout the study by repeated clinical and laboratory evaluation. MK-2048 was generally well-tolerated after single dose administration. There were no serious clinical adverse experiences or discontinuations due to an adverse event. Adverse events were generally transient and mild in intensity. Only one adverse event was reported by more than one subject: mild headache.^{10, 11}

Pharmacokinetics

In the aforementioned trial, plasma was analyzed at specified time points for determination of MK-2048 concentrations. At fasted doses up to 1200 mg, peak plasma concentrations of MK-2048 occurred within 1.5 hour post dose, and concentrations declined rapidly from C_{max} in a single log-linear phase with a half-life of approximately 1 to 1.5 hr. Plasma concentrations were largely undetectable after 12 and 24 hours post dose at all doses. AUC and C_{max} increased approximately dose proportionally over this dose range (50 to 1200 mg), although there was some variability between panels. MK-2048 failed to achieve target plasma concentrations, as the samples were largely undetectable (assay limit of quantitation = 2 ng/mL = 4.3 nM) after 12 and 24 hours post-dose at all doses. Due to these results further development of the oral product was discontinued.^{10, 11}

2.6.3 Clinical Studies of Similar IVR Delivery Systems and Placebo IVR

The vaginal delivery system used for the MK-2048A ring has a comparable copolymer composition and the same dimensions as the approved vaginal contraceptive ring, NuvaRing®.¹⁰ NuvaRing® is an estrogen/progestin combination hormonal contraceptive (CHC) indicated for use by women to prevent pregnancy. The first marketing authorization for NuvaRing® was obtained in 2001 and since market introduction in 2002, estimated worldwide exposure exceeds 8.7 million woman-years. Clinical and marketing experience with NuvaRing® indicates that the vaginal delivery system is well-accepted, as the majority of women have not had any problems with the use of NuvaRing®.

In clinical studies with NuvaRing®, an increase in local vaginal side-effects has been reported, including vaginitis (13.2%), leukorrhoea (5.6%), device-related problems (5.0%), and vaginal discomfort (2.9%). Device-related problems mainly concerned expulsion (3.1%), coital problems (1.1%), and foreign body feeling (0.7%). Vaginal discomfort predominantly concerned vaginal dryness (0.6%). Comparative studies indicated that these events were all more common for NuvaRing® users than for combined oral contraception users, which is obvious for device-related problems, but maybe less so for the other types of events. When relationship to the contraceptive method, as judged by the investigator, was taken into account the incidence of vaginitis was 4.3% for NuvaRing® as compared to 1.0-2.1% for combined oral contraception. A

similar trend, but at lower absolute incidences, was observed for leukorrhea, while vaginal dryness was uncommon altogether.

There was no placebo IVR used in the clinical development program for NuvaRing®. Merck has continued development of next generation rings (NGR) for contraception and has advanced candidates into Phase 2 clinical trials.

The design and manufacture of the NGR is based on the NuvaRing technology, with both rings utilizing an ethylene vinylacetate (EVA) copolymer. A placebo IVR has been administered to 90 subjects in a single clinical study in the NGR program. MK-8342B Protocol 057 was a multi-center, randomized, placebo-controlled, dose finding study to evaluate the treatment effect of MK-8342B compared to placebo in female subjects aged 18-50 years who had been diagnosed with primary dysmenorrhea. Each subject in the placebo arm (N=90) used the placebo IVR for 21 consecutive days followed by 7 days without the ring, over two consecutive treatment cycles. The adverse events reported for the placebo IVR included headache (7.8%), acne (5.6%), abdominal pain/discomfort (4.4%), breast pain/discomfort (3.3%), diarrhea (1.1%), nausea (1.1%), vaginal discharge (1.1%) and anxiety (1.1%). There were no severe adverse events in subjects that received placebo IVR. All events were reported as resolved by the completion of the trial.

2.7 Study Hypothesis and Rationale for Study Design

Based on *in vitro*, *in vivo*, and *ex vivo* studies, VCV (MK-4176) and MK-2048 show promise as a topical microbicide agent to prevent HIV-1 infection. The safety and acceptability of these agents alone and in combination will be evaluated in the MTN-027 trial, however the optimal dose of MK-4176 and MK-2048 to achieve sufficient vaginal fluid concentrations for antiviral activity while minimizing toxicity is unknown. Two different formulations of the MK-2048A combination IVR have been developed and will be evaluated in MTN-028 in an effort to perform *in vitro* and *in vivo* modeling to further optimize the VCV and MK-2048 ring release profiles for future studies, including the potential development of a combination antiretroviral/contraceptive ring.

MTN-028 participants will be randomized in a 2:1 ratio to receive an ethylene vinylacetate (EVA) copolymer IVR containing either 91 mg VCV (MK-4176) + 10 mg MK-2048 (Low Dose) or 182 mg VCV (MK-4176) + 30 mg MK-2048 (Original Dose); to be used for a period of 28 days, followed by 7 days with no study product. Approximately 12 participants will receive the low dose and 6 participants will receive the original dose. Vaginal and blood concentrations of VCV and MK-2048 will be measured during and after 28 days of administration of these two different ring formulations. We will also conduct assessments of local and systemic safety. The study products evaluated in this trial have potential adverse effects, and tolerance may vary depending on formulation. PK and safety data from this trial will be coupled with data from the twelve participants who are expected to enroll and be randomized to the MK-2048A IVR (Original Dose) ring in MTN-027 to optimize VCV and MK-2048 ring development in future studies.

3 OBJECTIVES

3.1 Primary Objectives

Pharmacokinetics

- Assess local and systemic pharmacokinetics of vicriviroc (MK-4176) and MK-2048 during and after 28 days of use of two MK-2048A IVRs containing different dose strengths

Safety

- Assess and compare the safety of two MK-2048A IVR formulations containing different dose strengths of VCV (MK-4176) and MK-2048 during and after 28 days of use by healthy, HIV-uninfected, sexually abstinent women

3.2 Secondary Objective

Assessment of remnant content in IVRs

- Assess remnant content in returned IVRs

4 STUDY DESIGN

4.1 Identification of Study Design

MTN-028 is a single-site, single-blind, two-arm, randomized trial (2:1).

4.2 Summary of Major Endpoints

Primary Endpoints:

Pharmacokinetics

- Assessment of vaginal fluid, blood plasma, and cervical tissue concentrations of MK-4176 and MK-2048 during and after IVR use

Safety

- Genitourinary events Grade 1 or higher as defined by the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, Dec 2004 (Clarification dated August 2009), Addendum 1, (Female Genital Grading Table for Use in Microbicide Studies) judged to be related to study product
- Adverse events Grade 2 or higher as defined by the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, Dec 2004 (Clarification dated August 2009)

Secondary Endpoint:

Assessment of remnant content in IVRs

- Residual drug levels measured in IVRs

4.3 Description of Study Population

The study population will include 18 healthy 18-45 year old women (inclusive) who are HIV-uninfected, non-pregnant, sexually abstinent and using adequate contraception, as described in Sections 5.2 and 5.3.

4.4 Time to Complete Accrual

The approximate time to complete study enrollment is expected to be 6-9 months.

4.5 Study Groups

Two study groups are planned. All study groups will be assigned to complete a total of 13 visits.

The two study groups are as follows:

1. MK-2048A IVR (Low Dose): 91 mg of VCV (MK-4176) + 10 mg of MK-2048
2. MK-2048A IVR (Original Dose): 182 mg of VCV (MK-4176) + 30 mg of MK-2048

4.6 Expected Duration of Participation

The expected duration for participants is approximately 5 weeks of follow-up, not including the 45 day screening window. No study data will be collected after the 35 Day Final Clinic/Early Termination Visit unless the participant is pregnant or has an ongoing AE at the 35 Day Final Clinic/Early Termination Visit. Participants who are pregnant at the 35 Day Final Clinic/Early Termination Visit may be followed as per Section 9.7, Pregnancy. Participants who have AEs at the 35 Day Final Clinic/Early Termination Visit that have not resolved or stabilized will be followed beyond the 35 Day Final Clinic/Early Termination Visit until a clinically acceptable resolution of the AE(s) is confirmed and documented. Clinical acceptability of resolution will be determined by the site Investigator of Record (IoR) in consultation with the Protocol Safety Review Team (PSRT).

4.7 Site

US site selected by the MTN Executive Committee will participate in MTN-028.

5 STUDY POPULATION

5.1 Selection of the Study Population

The inclusion and exclusion criteria in Sections 5.2 and 5.3 will be utilized to ensure the appropriate selection of study participants.

5.1.1 Recruitment

Participants will be recruited from a variety of sources including family planning and gynecology clinics, colleges and universities, online websites, faith communities, as well as community-based locations such as community-based organizations and street-based outreach. Participants also will be referred to the study from other local research projects and other health and social service providers serving the target study population. Recruitment strategies will be guided by input from the site's Community Advisory Group. Recruitment materials will be approved by site Institutional Review Boards/Ethics Committees (IRBs/ECs) prior to use. Site community representatives should advise on these materials before they are submitted to the IRB/EC for review.

5.1.2 Retention

Once a participant is enrolled/randomized in MTN-028, the study site will make every effort to retain the participants in follow-up to minimize possible bias associated with loss-to-follow-up. The study site will establish and follow standard operating procedures (SOPs) for participant retention. A retention rate of 95% will be targeted.

5.2 Inclusion Criteria

Participants must meet all of the following criteria to be eligible for inclusion in the study.

- 1) Born female

Note: Participants who were female at birth, who now identify as male, will not be excluded so long as they are not on female-to-male transition therapy.

- 2) Age 18 through 45 years (inclusive) at Screening, verified per site SOPs
- 3) Able and willing to provide written informed consent to be screened for and take part in MTN-028
- 4) Able and willing to provide adequate locator information, as defined by the site SOPs
- 5) HIV-uninfected, based on testing performed by study staff at Screening and Enrollment (per applicable algorithm in Appendix II) and willing to receive results

- 6) In general good health at Screening and Enrollment, as determined by the site IoR or designee
- 7) At Screening, participant states willingness to abstain from receptive sexual activity (including penile-vaginal intercourse, anal intercourse, receptive oral intercourse, finger stimulation, and the use of sex toys) for the 5 days prior to the Enrollment Visit and for the duration of study participation
- 8) Per participant report, using an effective method of contraception at Enrollment, and intending to continue the use of an effective method for the duration of study participation. Effective methods for MTN-028 include: hormonal methods (except contraceptive IVRs), intrauterine device (IUD) inserted at least 28 days prior to enrollment, engages in sex exclusively with women, sterilized (self or partner), and/or sexually abstinent for the past 90 days
- 9) Women over the age of 21 (inclusive) must have documentation of a satisfactory Pap within the past 3 years prior to Enrollment consistent with Grade 0 according to the Female Genital Grading Table for Use in Microbicide Studies Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009), or satisfactory evaluation with no treatment required of Grade 1 or higher Pap result. If otherwise eligible women do not have documentation of a recent Pap test, one may be performed at the Screening Visit.
- 10) Per participant report at Screening and Enrollment, agrees not to participate in other research studies involving drugs, medical devices, or vaginal products for the duration of study participation
- 11) Per participant report at Screening, regular menstrual cycles with at least 21 days between menses

Note: This criterion is not applicable to participants who report using a progestin-only method of contraception at Screening, e.g., Depo-Provera or levonorgestrel-releasing IUD nor to participants using continuous combination oral contraceptive pills, as the absence of regular menstrual cycles is an expected, normal consequence in this context.

- 12) At Screening, participant states a willingness to refrain from inserting any non-study vaginal products or objects into the vagina, including but not limited to, spermicides, female condoms, diaphragms, contraceptive IVRs, vaginal medications, menstrual cups, cervical caps (or any other vaginal barrier method), douches, lubricants, sex toys (vibrators, dildos, etc.) for the 5 days prior to Enrollment and for the duration of their study participation

5.3 Exclusion Criteria

Women who meet any of the following criteria will be excluded from the study:

- 1) Participant report of any of the following at Screening or Enrollment:
 - a. History of adverse reactions to any of the components of the study products
 - b. Non-therapeutic injection drug use in the 12 months prior to Screening and Enrollment
 - c. Post-exposure prophylaxis (PEP) for HIV exposure within 6 months prior to Enrollment
 - d. Pre-exposure prophylaxis (PrEP) for HIV prevention within the 6 months prior to Enrollment
 - e. Regular use and/or anticipated regular use during the period of study participation of CYP3A inducer(s) and/or inhibitor(s)
 - f. Use and/or anticipated use during the period of study participation of female-to-male transition therapy
 - g. Chronic and/or recurrent candidiasis
 - h. Gonorrhea, chlamydia and/or syphilis diagnosis in the 6 months prior to Enrollment
 - i. Last pregnancy outcome 90 days or less prior to Screening
 - j. Currently breastfeeding
 - k. Has had a hysterectomy
 - l. Intends to become pregnant within the next 3 months
 - m. Has plans to relocate away from the study site area in the next 3 months
 - n. Current sexual partner is known to be HIV-positive at Screening or Enrollment
- 2) Reports participating in any other research study involving drugs, medical devices, or vaginal products within 60 days or less prior to enrollment
- 3) At Screening or Enrollment, as determined by the IoR/designee, any significant uncontrolled active or chronic cardiovascular, renal, liver, hematologic, neurologic, gastrointestinal, psychiatric, endocrine, respiratory, immunologic disorder or infectious disease
- 4) Has any of the following laboratory abnormalities at Screening:
 - a. Aspartate aminotransferase (AST) or alanine transaminase (ALT) Grade 1 or higher*
 - b. Calculated creatinine clearance less than 60 mL/min by the Cockcroft-Gault formula, where creatinine clearance (female) in mL/min = $(140 - \text{age in years}) \times (\text{weight in kg}) \times (0.85) / 72 \times (\text{creatinine in mg/dL})$
 - c. Hemoglobin Grade 1 or higher*
 - d. Platelet count Grade 1 or higher*
 - e. White blood count Grade 2 or higher*
 - f. Positive HBsAg test result

- g. Positive Anti-HCV test result
- h. International normalized ratio (INR) > 1.5 × the site laboratory upper limit of normal (ULN)

Note: Otherwise eligible participants with an exclusionary test result (other than HIV, HBV or HCV) can be re-tested during the screening process. If a participant is re-tested and a non-exclusionary result is documented within 45 days of providing informed consent for screening, the participant may be enrolled.

*As per the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December, 2004 (Clarification dated August 2009)

5) Pregnant at either Screening or Enrollment

Note: A documented negative pregnancy test performed by study staff is required for inclusion; however a self-reported pregnancy is adequate for exclusion from screening/enrollment into the study.

6) Diagnosed with urinary tract infection (UTI) at Screening or Enrollment

Note: Otherwise eligible participants diagnosed with UTI during screening will be offered treatment. If within the 45 day screening window treatment is complete and symptoms have resolved the participant may be enrolled.

7) Diagnosed with pelvic inflammatory disease, reproductive tract infection (RTI) or a sexually transmitted infection (STI) requiring treatment per current Centers for Disease Control and Prevention (CDC) guidelines (<http://www.cdc.gov/std/treatment/>) at Screening or Enrollment

Note: With the exception of gonorrhea, chlamydia and/or syphilis, otherwise eligible participants diagnosed with a RTI during screening will be offered treatment. If within the 45 day screening window treatment is complete and symptoms have resolved, the participant may be enrolled.

8) At Enrollment, has a clinically apparent Grade 1 or higher pelvic exam finding (observed by study clinician or designee) per the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009), Addendum 1, Female Genital Grading Table for Use in Microbicide Studies

Note: Cervical friability bleeding associated with speculum insertion and/or specimen collection judged to be within the range of normal according to the clinical judgment of the IoR/designee is considered expected non-menstrual bleeding and is not exclusionary.

9) At Screening, severe pelvic relaxation such that either the vaginal walls or the uterine cervix descend beyond the vaginal introitus with valsalva maneuver or has pelvic anatomy that compromises the ability to adequately assess vaginal safety

- 10) Has any other condition that, in the opinion of the IoR/designee, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving study objectives.

5.4 Co-enrollment Guidelines

As indicated in Section 5.2, participants should not take part in other research studies involving drugs, medical devices, or vaginal products after the Screening Visit and while taking part in MTN-028. Participation in the following types of studies may be allowed at the discretion of the IoR/designee:

- Participants may take part in ancillary studies approved by MTN-028 Protocol Chair
- Participants who become infected with HIV may take part in observational and/or interventional studies for HIV-positive persons

Should any participant report concurrent participation in contraindicated studies after enrolling in MTN-028, the IoR/designee must immediately notify the MTN-028 Management Team and consult the PSRT regarding ongoing product use and other potential safety considerations associated with co-enrollment.

6 STUDY PRODUCT

6.1 Regimen

Each participant will be randomized (2:1) to one of two study regimens:

Table 8: Study Regimen

Group	N	Group Description
A	12	Low Dose: MK-2048A IVR containing the combination of 91 mg of VCV (MK-4176) + 10 mg of MK-2048
B	6	Original Dose: MK-2048A IVR containing the combination of 182 mg of MK-4176 + 30 mg of MK-2048

Each participant will be randomized to receive either an IVR containing 91 mg VCV (MK-4176) + 10 mg MK-2048 or an IVR containing 182 mg VCV (MK-4176) + 30 mg MK-2048. The IVR will be worn for approximately 28 consecutive days. It will be inserted into the vagina by the participant (or clinician, if necessary) at the Enrollment Visit and removed by participant (or clinician, if necessary) on Study Visit Day 28. The participant will be followed for approximately 7 days following IVR removal.

6.2 Administration

Study participants will be given detailed instructions in the clinic on proper IVR insertion and removal procedures. Hands should be thoroughly washed before and after study IVR insertion and/or removal. Additional details on administration (IVR insertion, removal, procedures in the event of expulsion or loss) will be provided.

6.3 Study Product Formulation

6.3.1 Study IVRs

Both IVRs are smooth, flexible, white to off-white, and opaque. The IVR dimensions are as follows: 54 mm and 4.0 mm, outer diameter and cross-sectional diameter, respectively. The IVRs consist of a closed-ring fiber having two coaxial layers: a core layer containing MK-4176 and a skin layer containing MK-2048. The EVA 28 core layer contains 10% MK-4176 and the EVA 28 skin layer contains 30% MK-2048 for the 182 mg VCV + 30 mg MK-2048 ring. The original dose ring is slightly more opaque in appearance than the low dose ring. The EVA 28 core layer contains 5% MK-4176 and the EVA 28 skin layer contains 10% MK-2048 for the 91 mg VCV + 10 mg MK-2048 ring.

The IVR is designed to provide sustained release of drug over a 28-day period.

6.4 Supply and Accountability

6.4.1 Supply

MSD in Oss, The Netherlands, will manufacture the study IVRs under Good Manufacturing Practices (GMP). The MTN-028 pharmaceutical collaborator will package, label and ship all of the study IVRs directly to the Pharmacist of Record (PoR) at the study site.

6.4.2 Storage and Dispensing

IVRs should be stored at 2-8°C. IVRs are dispensed from the pharmacy only to enrolled study participants or to clinic staff on behalf of the participant, upon receipt of a written prescription from an authorized prescriber. If an IVR is dispensed from the pharmacy to clinic staff, then a clinic staff member will subsequently provide the IVR to the study participant in the clinic. A small zip bag will be dispensed from the pharmacy with each IVR. In the event that the study participant must remove the IVR outside the clinic setting, this bag is available for storage of the used IVR.

6.4.3 Accountability

The site PoR is required to maintain complete records of all study IVRs received and subsequently dispensed. All unused study products must be returned to MTN LOC Pharmacist after the study is complete unless otherwise instructed by the MTN LOC Pharmacist. Accountability procedures to be followed will be provided in the MTN-028 Pharmacy Study Product Management Procedures Manual.

6.4.4 Retrieval of Study Product

Table 9: Retrieval of Study Product

Collection Timepoint	Retrieve Study Product Within
Permanent discontinuation or temporary hold due to potential HIV seroconversion	24 hours
Permanent discontinuation for any other reason or IoR discretion	5 working days
Temporary hold for reasons with expected duration of greater than 7 days	7 working days
End of Study	2 working days

If a study product hold extends for 7 days or more, and product has not been retrieved as of the seventh day, study staff members must make every effort to retrieve study product as soon as possible.

It is not necessary to retrieve study products from participants for whom study product use is being temporarily held for less than 7 days. However, study products may be retrieved from such participants, to protect their safety, if there is concern that the participant may not comply with clinic staff instructions to refrain from study product use for the duration of the temporary hold.

For all study product holds requiring retrieval of study product(s), if the study product(s) are not retrieved within the timeframe stated in the table above, the MTN-028 PSRT must be informed.

The IVR must be worn for approximately 28 consecutive days at a time. If prolonged use of the study IVR has occurred, attempts must be made to contact the participant and retrieve the study product and the PSRT must be informed.

For each participant, all IVRs remaining in the participant's possession must be retrieved at/by Visit Day 28. If the participant does not bring her study product to this visit, study staff must arrange to retrieve the IVR within 2 business days. If the IVR is not retrieved within that timeframe, the MTN-028 PSRT must be informed.

6.5 Concomitant Medications

Enrolled study participants may use concomitant medications during study participation. All concomitant medications reported throughout the course of the study will be

recorded on case report forms designated for that purpose. All prescription medications, over-the-counter preparations, vitamins, nutritional supplements, and herbal preparations will be recorded on forms for concomitant medications.

6.6 Prohibited Medications and Practices

Several concomitant medications/practices will not be permitted. Participants are asked to avoid using certain CYP3A inhibitors and CYP3A inducers. These medications are not recommended because VCV (MK-4176) is a CYP3A substrate. A listing of CYP3A inhibitors and inducers to be avoided are provided in the MTN-028 SSP Manual available at www.mtnstopshiv.org. Please note single dose oral fluconazole for the treatment of vaginal fungal infections is permitted. Participant use of female-to-male transition medications is prohibited. Effects of female-to-male transition medications can include vaginal dryness and thinning of the epithelium which may impact product safety, confound adverse event determination and may impact pharmacokinetic parameters.

Prohibited non-study intravaginal products and other devices include, but are not limited to, spermicides, female condoms, diaphragms, contraceptive intravaginal rings, vaginal medications, menstrual cups, cervical caps, douches, lubricants, and sex toys (e.g., vibrators, dildos, etc.). While tampon use is not restricted, participants will restrict the use of tampons during the first week of study participation (starting at the enrollment visit) and 24 hours prior to each clinic visit following Enrollment. Participants are expected to be sexually abstinent for the duration of their trial participation and for 5 days preceding Enrollment, i.e., no receptive intercourse (vaginal, anal, oral and finger stimulation). These medications and practices are restricted to protect the integrity of the lower genital tract and reduce the possibility of adverse events due to agents other than the study product.

Participants will be counseled to avoid such use and practices. Participant use of prohibited medications and engagement in prohibited practices will be documented.

Additional information for participants who report the use of prohibited medications can be found in Section 9.3.

7 STUDY PROCEDURES

An overview of the study visit and evaluations schedule is presented in Appendix I. Presented in this section is additional information on visit-specific study procedures. Detailed instructions to guide and standardize procedures are provided in the MTN-028 SSP Manual available at www.mtnstopshiv.org.

7.1 Pre-screening

As part of participant outreach and recruitment strategies, study staff can pre-screen potential study participants at either on-site or off-site locations. During these interactions, study staff may explain the study to potential participants and ascertain elements of presumptive eligibility (e.g., willingness to use an IVR, willingness to be sexually abstinent for the duration of study participation, etc.), to be confirmed at an on-site screening visit. Process information (e.g., number of potential participants contacted, number presumptively eligible) may be recorded and stored at the study site in the absence of written informed consent from potential participants, provided the information is collected in such a manner that it cannot be linked to participant identifiers, unless a waiver is granted from the local IRB. Procedures and documentation will comply with local IRB requirements.

7.2 Visit 1- Screening

Screening can take place up to 45 days prior to Enrollment. Multiple visits may be conducted to complete all required procedures, if necessary. Written informed consent will be obtained before any study procedures are initiated. For participants who do not meet the eligibility criteria, screening will be discontinued once ineligibility is determined. Detailed information regarding visit windows will be described in the MTN-028 SSP Manual.

Table 10: Visit 1- Screening Visit

Visit 1- Screening Visit		
Component	Procedures	
Administrative and Regulatory	<ul style="list-style-type: none"> • Obtain written informed consent • Assess consent form comprehension • Assign participant ID (PTID) • Collect locator information • Collect demographic information • Assess eligibility • Provide reimbursement for study visit • Schedule next visit* 	
Behavioral	<ul style="list-style-type: none"> • Provide counseling <ul style="list-style-type: none"> – HIV pre- and post-test – Protocol requirements (To include adherence, product use and contraceptive counseling, STI and risk reduction counseling, as needed) 	
Clinical	<ul style="list-style-type: none"> • Collect medical and menstrual history • Collect concomitant medications • Perform physical examination • Perform pelvic examination • Provide available test results • Treat or prescribe treatment for UTIs/RTIs/STIs or refer for other findings* 	
Laboratory	Urine	<ul style="list-style-type: none"> • Collect urine <ul style="list-style-type: none"> – Human Chorionic Gonadotropin (hCG) – Dipstick urinary analysis (UA) – Urine culture*
	Blood	<ul style="list-style-type: none"> • Collect blood <ul style="list-style-type: none"> – Complete blood count (CBC) with differential and platelets – HIV-1 serology – HBsAg – Coagulation (INR) – Anti-HCV – Chemistries (AST, ALT, Creatinine) – Syphilis serology
	Genital	<ul style="list-style-type: none"> • Collect pelvic specimens <ul style="list-style-type: none"> – Rapid test for Trichomonas – Vaginal fluid pH* – Potassium hydroxide (KOH) wet mount for candidiasis* – Saline wet mount for bacterial vaginosis (BV)* – Nucleic Acid Amplification Test (NAAT) for <i>Neisseria gonorrhoeae</i> and <i>Chlamydia trachomatis</i> (GC/CT) – Collect Pap test*

* If indicated

7.3 Visit 2- Enrollment (Day 0)

Menses must not coincide with Study Visits 2-6 (Days 0, 1, 2, 3, 7), therefore participant's menstrual cycle must be considered when scheduling Visit 2- Enrollment Visit (Day 0).

Table 11: Enrollment Visit- Visit 2 (Day 0)

Enrollment Visit- Visit 2 (Day 0)	
Component	Procedures
Administrative and Regulatory	<ul style="list-style-type: none"> • Review/update locator information • Confirm eligibility • Review informed consent and confirm participant is still interested in continued study participation • Randomization • Provide reimbursement for study visit • Schedule next visit*
Behavioral	<ul style="list-style-type: none"> • Provide counseling <ul style="list-style-type: none"> – HIV pre- and post-test – Protocol requirements
Clinical	<ul style="list-style-type: none"> • Review/update medical and menstrual history • Review/update concomitant medications • Document pre-existing conditions • Perform physical examination • Perform pelvic examination • Provide available test results • Treat or prescribe treatment for UTIs/RTIs/STIs or refer for other findings*
Laboratory	Urine <ul style="list-style-type: none"> • Collect urine <ul style="list-style-type: none"> – hCG – Dipstick UA* – Urine culture*
	Blood <ul style="list-style-type: none"> • Collect blood <ul style="list-style-type: none"> – CBC with differential and platelets – HIV-1 serology – Chemistries – Plasma archive – PK (Post ring insertion at time points: Hours 1, 2, 4, 6) – Syphilis serology*
	Genital <ul style="list-style-type: none"> • Collect pelvic specimens <ul style="list-style-type: none"> – Vaginal fluid for PK (Post ring insertion at time points: Hours 0, 1, 2, 4, 6) – Vaginal fluid pH* – Gram stain – KOH wet mount for candidiasis* – Saline wet mount for BV* – Rapid test for Trichomonas* – NAAT for GC/CT*
Study Product Supply	<ul style="list-style-type: none"> • Participants will receive study IVR, study IVR use instructions and will be instructed to self-insert the study IVR, followed by pelvic exam to check placement

* If indicated

7.4 Follow-up Visits

The following procedures will occur on Days 1, 2, 3, 7, 14, 21, 28, 29, 30, 31.

7.4.1 Visits 3-8 (Days 1, 2, 3, 7, 14, 21)

Table 12: Visits 3-8 (Days 1, 2, 3, 7, 14, 21)

Component		Procedures
Administrative and Regulatory		<ul style="list-style-type: none"> • Review/update locator information • Provide reimbursement for study visit • Record/update AEs • Schedule next visit
Behavioral		<ul style="list-style-type: none"> • Adherence Assessment • Provide modified counseling <ul style="list-style-type: none"> – HIV pre- and post-test counseling* – Protocol requirements*
Clinical		<ul style="list-style-type: none"> • Review/update medical and menstrual history • Review/update concomitant medications • Perform modified physical examination • Perform pelvic examination • Provide available test results • Treat for UTIs/RTIs/STIs or refer for other findings*
Laboratory	Urine	<ul style="list-style-type: none"> • Collect urine <ul style="list-style-type: none"> – hCG^{rw}* – Dipstick UA* – Urine culture*
	Blood	<ul style="list-style-type: none"> • Collect blood <ul style="list-style-type: none"> – PK – HIV-1 serology* – Syphilis serology*
	Genital	<ul style="list-style-type: none"> • Collect pelvic specimens <ul style="list-style-type: none"> – Collect vaginal fluid for PK analysis – Vaginal fluid pH* – Gram Stain[◆] – Rapid test for Trichomonas* – KOH wet mount for candidiasis* – Saline wet mount for BV* – NAAT for GC/CT*

* If indicated ◆ Day 3 only, ^{rw} Day 14 only

7.4.2 Visit 9 (Day 28) Ring Removal Visit

The following procedures will occur at Visit Day 28 or the Ring Removal Visit.

Table 13: Visit 9 (Day 28) Ring Removal Visit

Follow-up Visit 9 (Day 28) Ring Removal Visit		
Component	Procedures	
Administrative and Regulatory	<ul style="list-style-type: none"> • Review/update locator information • Provide reimbursement • Record/update AEs • Schedule next visit 	
Behavioral	<ul style="list-style-type: none"> • Adherence Assessment • Provide counseling <ul style="list-style-type: none"> – HIV pre- and post-test* – Protocol requirements* 	
Clinical	<ul style="list-style-type: none"> • Review/update medical and menstrual history • Review/update concomitant medications • Perform modified physical examination • Perform pelvic examination • Provide available test results • Treat or prescribe treatment for UTIs/RTIs/STIs or refer for other findings* 	
Laboratory	Urine	<ul style="list-style-type: none"> • Collect urine <ul style="list-style-type: none"> – hCG – Dipstick UA – Urine culture*
	Blood	<ul style="list-style-type: none"> • Collect blood <ul style="list-style-type: none"> – CBC with differential and platelets – Chemistries – PK (Post ring removal at time points: Hours 0, 1, 2, 4, 6) – HIV-1 serology* – Syphilis serology*
	Genital	<ul style="list-style-type: none"> • Collect pelvic specimens <ul style="list-style-type: none"> – Collect vaginal fluid for PK analysis (Post ring removal at time points: Hours 0, 1, 2, 4, 6) – Cervical tissue (hr 0) – Vaginal fluid pH* – Gram stain – KOH wet mount for candidiasis* – Saline wet mount for BV* – Rapid test for Trichomonas* – NAAT for GC/CT*
Study Product		<ul style="list-style-type: none"> • Collect IVR

* If indicated

7.4.3 Visits 10-12 (Days 29, 30, 31)

Table 14: Visits 10-12 (Days 29, 30, 31)

Follow-up Visits 10-12 (Days 29, 30, 31)		
Component	Procedures	
Administrative and Regulatory	<ul style="list-style-type: none"> • Review/update locator information • Provide reimbursement for study visit • Record/update AEs • Schedule next visit 	
Behavioral	<ul style="list-style-type: none"> • Provide modified counseling <ul style="list-style-type: none"> – HIV pre- and post-test* – Protocol requirements* 	
Clinical	<ul style="list-style-type: none"> • Review/update medical and menstrual history • Review/update concomitant medications • Perform modified physical examination • Perform pelvic examination • Disclosure of available test results • Treat or prescribe treatment for UTIs/RTIs/STIs or refer for other findings* 	
Laboratory	Urine	<ul style="list-style-type: none"> • Collect urine* <ul style="list-style-type: none"> – hCG* – Dipstick UA* – Urine culture*
	Blood	<ul style="list-style-type: none"> • Collect blood <ul style="list-style-type: none"> – PK – HIV-1 serology* – Syphilis serology*
	Genital	<ul style="list-style-type: none"> • Collect pelvic specimens <ul style="list-style-type: none"> – Vaginal fluid pH* – Collect vaginal fluid for PK analysis – Rapid test for Trichomonas* – KOH wet mount for candidiasis* – Saline wet mount for BV* – NAAT for GC/CT*

* If indicated

7.4.4 Visit 13 (Day 35) Final Clinic/Early Termination Visit

If clinically important test results are found following Visit 13 (Day 35), participants will be contacted in an effort to schedule an additional visit or receive test results over the phone.

Table 15: Visit 13 (Day 35) Final Clinic/Early Termination Visit

Visit 14 (Day 35) Final Clinic/ Early Termination Visit		
Component	Procedures	
Administrative and Regulatory	<ul style="list-style-type: none"> • Review/update locator information • Provide reimbursement for study visit • Record/update AEs • Schedule next visit* 	
Behavioral	<ul style="list-style-type: none"> • Provide counseling <ul style="list-style-type: none"> – HIV pre- and post-test – Protocol requirements* 	
Clinical	<ul style="list-style-type: none"> • Review/update medical and menstrual history • Review/update concomitant medications • Perform physical examination • Perform pelvic examination • Disclosure of available test results • Treat or prescribe treatment for UTIs/RTIs/STIs or refer for other findings* 	
Laboratory	Urine	<ul style="list-style-type: none"> • Collect urine <ul style="list-style-type: none"> – hCG*
	Blood	<ul style="list-style-type: none"> • Collect blood <ul style="list-style-type: none"> – CBC with differential and platelets* – HIV-1 serology – Chemistries – PK – Syphilis serology*
	Genital	<ul style="list-style-type: none"> • Collect pelvic specimens <ul style="list-style-type: none"> – Vaginal fluid pH* – Vaginal fluid for PK analysis – Gram stain – Rapid test for Trichomonas* – KOH wet mount for candidiasis* – Saline wet mount for BV* – NAAT for GC/CT*
Study Product		<ul style="list-style-type: none"> • Collect IVR ▲

* If indicated, ▲ If Early Termination Visit and not already performed

7.5 Follow-up Procedures for Participants Who Permanently Discontinue Study Product

7.5.1 Participants Who Become Infected with HIV-1

If a participant becomes infected with HIV-1 after the Enrollment Visit, she will be referred to local care and treatment services and may return to the research clinic for additional counseling and other support services, as needed. Continued study participation would be of no added benefit, thus follow-up visits will be discontinued and the participant will be considered terminated from the study. Participants who seroconvert after randomization may be offered additional laboratory testing (such as HIV RNA and HIV drug resistance testing), as clinically indicated per discussions between IoR and LC. Please reference the MTN-028 SSP Manual for additional details (www.mtnstopshiv.org).

7.5.2 Participants Who Become Pregnant

If a participant becomes pregnant, she will be referred to local health care services and may return to the research clinic for additional counseling, as needed. Continued study participation would be of no added benefit to the participant, thus follow-up visits and procedures will be discontinued and the participant will be considered terminated from the study. A participant who is pregnant at study termination will continue to be followed until the pregnancy outcome is ascertained. See Section 9.7 for additional details. For additional details regarding obtaining pregnancy outcome, please reference the MTN-028 SSP (www.mtnstopshiv.org).

7.5.3 Participants Who Permanently Discontinue Study Product for Other Reasons

For participants who permanently discontinue study product use for any other clinician initiated reason other than HIV seroconversion or pregnancy, site investigators may, after consultation with the PSRT and MTN-028 Management Team, decide to discontinue study follow-up visits and procedures. However, participants who permanently discontinue study product use due to an AE must continue to be followed until the resolution or stabilization of the AE is documented.

In the event study follow-up is continued, participants will have the protocol-specified weekly visits through Day 35, specifically those visits at Day 7, Day 14, Day 21, Day 28 and Day 35. Protocol-specified procedures will continue except the following:

- Pelvic exams*
- Collection of blood for safety assessments*
- Protocol requirements counseling will be modified

**Unless required for AE follow-up*

The above procedures should be collected/conducted at the visit in which study product is discontinued and omitted thereafter, unless the participant was previously on a temporary hold.

The MTN-028 Management Team, in consultation with the MTN Pharmacology Core, may provide real-time guidance to the site regarding a modified study visit schedule, in an effort to ensure that PK samples are collected at the appropriate time points. Participants' duration of use and timing of study product permanent discontinuation will be factored into the modified schedule. See SSP for additional details.

7.6 Follow-up Procedures for Participants Who are on a Temporary Clinical Study Product Hold

All protocol-specified study visits and procedures will continue except the following:

- Pelvic exams*
- Provision of product use/protocol adherence counseling

**Unless required for AE follow-up*

The collection of samples for PK should be collected/conducted at the visit in which study product is temporarily held and omitted thereafter. Completion of these procedures will resume at the visit following resumption of study product use.

The MTN-028 Management Team, in consultation with the MTN Pharmacology Core, may provide real-time guidance to the site regarding a modified study visit schedule, in an effort to ensure that PK samples are collected at the appropriate time points. Participants' duration of use and timing of study product permanent discontinuation will be factored into the modified schedule. See SSP for additional details.

7.6.1 Interim Visits

Interim visits may be performed at any time during the study and any procedures may be conducted. All interim contacts and visits will be documented in participants' study records and on applicable CRFs.

7.7 Pharmacokinetics

All enrolled participants randomized will undergo PK specimen collection procedures. These collections will occur at study visits as described in the table below. Blood, vaginal fluid, and cervical biopsies will be collected to assay for VCV (MK-4176) and MK-2048 concentrations.

Blood and pelvic PK specimens should be collected within approximately one hour of each other in the sequence listed below, if a single time point collection is planned. Participants will report ring adherence, including details regarding any ring expulsions

including the date and approximate length of time that the ring was out of the vagina. Study staff will record this information. These data will be collected at study visits in which PK assessments are scheduled to occur, see Table 16 below. Staff will also record all PK specimen collection times. The SSP will provide information regarding the study visit windows.

Table 16: PK Specimen Collection Schedule

STUDY VISIT	PK Specimen Collection
Screening	None
Enrollment	Blood (hr 1, 2, 4, 6), Vaginal fluid (hr 0,1, 2, 4, 6)
Day 1	Blood, Vaginal fluid
Day 2	Blood, Vaginal fluid
Day 3	Blood, Vaginal fluid
Day 7	Blood, Vaginal fluid
Day 14	Blood, Vaginal fluid
Day 21	Blood, Vaginal fluid
Day 28	Blood (hr 0, 1, 2, 4, 6), Vaginal fluid (hr 0, 1, 2, 4, 6), Cervical tissue (hr 0)
Day 29	Blood, Vaginal fluid
Day 30	Blood, Vaginal fluid
Day 31	Blood, Vaginal fluid
Day 35	Blood, Vaginal fluid

7.8 Product Use/Adherence Counseling

Participants will receive study IVR adherence counseling at the Enrollment Visit and modified counseling at additional follow-up visits. Site staff will counsel participants to refrain from removing the ring (except as directed) and from using prohibited medications and practices as described in Section 6.6. Site staff will also provide counseling for re-insertion in case of ring removal/expulsion.

7.9 Clinical Evaluations and Procedures

Physical exams will include the following assessments:

- General appearance
- Weight
- Vital signs
 - Temperature
 - Pulse
 - Blood pressure
 - Respirations
- Height *
- Abdomen*
- Head, Eye, Ear, Nose and Throat (HEENT) Examination*
- Lymph nodes*
- Neck*

- Heart*
- Lungs*
- Extremities*
- Skin*
- Neurological*

**may be omitted after the Enrollment Visit*

Additional clinical assessments may be performed at the discretion of the examining clinician in response to symptoms or illnesses present at the time of the exam.

Pelvic exams will be conducted per guidelines for naked eye inspection described in the WHO/CONRAD Manual for Standardization of Colposcopy for the Evaluation of Vaginal Products, Update 2004, available at <http://www.conrad.org/publications-13.html>. The required sequence of procedures and specimen collection performed during pelvic exams will be specified in the MTN-028 SSP Manual.

7.10 Laboratory Evaluations

Local Laboratory

- Urine
 - hCG
 - Dipstick UA
 - Urine culture
- Blood
 - Syphilis
 - HIV serology
 - HBsAg
 - INR
 - Anti-HCV
 - CBC with differential and platelets
 - Chemistries
 - Creatinine clearance
 - AST
 - ALT
- Vaginal
 - pH
 - Rapid test for Trichomonas
 - Saline wet mount for BV
 - KOH wet mount for candidiasis
 - Chlamydia and gonorrhea
- Cervical
 - Pap test

Laboratory Center

- Blood
 - Plasma archive
 - Standardized and specialized HIV-1 resistance tests
 - Blood PK

- Genital
 - Gram stain assessment
 - Vaginal fluid for PK
 - Cervical tissue for PK

Merck & Co. Designated Laboratory

- Study Product
 - Remnant content assessment

7.11 Specimen Collection and Processing

The site will adhere to the standards of good clinical laboratory practice in accordance with current DAIDS Laboratory Requirements and the MTN-028 Study Specific Procedures Manual (www.mtnstopshiv.org) for proper collection, processing, labeling, transport, and storage of specimens at the local laboratory. Specimen collection, testing, and storage at the site laboratories will be documented when applicable using the Laboratory Data Management System (LDMS).

In cases where laboratory results are not available due to administrative or laboratory error, the site is permitted to re-draw specimens. Further, as part of quality control, researchers may need to look at short pieces of non-coding repetitive DNA sequence (3-7 base pairs) from blood in the event of sample mix-up. This test will only let researchers know the number of times this short segment is repeated and not specific genes or specific sequences of base pairs. This sequence element does not contain any information about genes, therefore researchers will not be able to identify if participants are predisposed to specific diseases or any other genetic information based on this information. This test will be an important tool for distinguishing whether two samples collected at the same or different time points are likely from the same person. The test will only be used as part of a sample investigation with the knowledge of the site in situations where a known or suspected sample mix-up has occurred. No genetic testing (limited or genome-wide) is planned on leftover samples that are stored for the purposes of future research.

7.12 Specimen Handling

Specimens will be handled in accordance with current requirements for DAIDS Sponsored and/or Funded Laboratories in Clinical Trials. (<http://www.niaid.nih.gov/labsandresources/resources/daidsclinrsrch/documents/labpolicy.pdf>)

7.13 Biohazard Containment

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

8 ASSESSMENT OF SAFETY

8.1 Safety Monitoring

The site IoR is responsible for continuous close safety monitoring of all study participants and for alerting the Protocol Team if unexpected concerns arise. A subgroup of the Protocol Team, including the Protocol Chair, DAIDS Medical Officer, Protocol Safety Physicians, and SCHARP Clinical Affairs Safety Associates and other team designees will serve as the PSRT. The MTN Statistical Data Management Center (SDMC) prepares routine AE and clinical data reports (blinded to treatment assignment) for review by the PSRT, which meets via conference call approximately once per month or as needed throughout the period of study implementation to review safety data, discuss product use management, and address any potential safety concerns.

8.2 Clinical Data and Safety Review

A multi-tiered safety review process will be followed for the duration of this study. The study site investigators are responsible for the initial evaluation and reporting of safety information at the participant level and for alerting the PSRT if unexpected concerns arise. Participant safety is also monitored at the Network level through a series of routine reviews conducted by the SDMC Clinical Affairs staff, the PSRT and study sponsors. Additional reviews may be conducted at each of these levels as dictated by the occurrence of certain events.

MTN SDMC Clinical Affairs staff will review incoming safety data on an ongoing basis. Events identified as questionable, inconsistent, or unexplained will be queried for verification. Adverse event reports submitted in an expedited manner to the DAIDS Safety Office will be forwarded to the DAIDS Medical Officer and SDMC Clinical Affairs staff for review.

The PSRT will meet approximately every month via conference call to review clinical data reports generated by the MTN SDMC. The content, format and frequency of the clinical data reports will be agreed upon by the PSRT and the SDMC in advance of study implementation. In addition to the routine safety data reviews, the PSRT will convene on an ad hoc basis to make decisions regarding the handling of any significant safety concerns. If necessary, experts external to the MTN representing expertise in the fields of microbicides, biostatistics, HIV acquisition and medical ethics may be invited to join the PSRT safety review. A recommendation to pause or stop the trial may be made by the PSRT at this time or at any such time that the team agrees that an unacceptable type and/or frequency of AEs has been observed.

The Study Monitoring Committee (SMC) will review participant safety data as part of their regular reviews (see Section 10.7.1), since no Data and Safety Monitoring Board oversight is planned for MTN-028. The SMC may recommend that the study proceed as designed, proceed with design modifications, or be discontinued. Members of the SMC will be independent investigators with no interest (financial or otherwise) in the outcomes of this study. If at any time a decision is made to discontinue enrollment and/or study product use in all participants, DAIDS will notify the FDA and the Site IoR will notify the responsible IRB expeditiously.

8.3 Adverse Events Definitions and Reporting Requirements

8.3.1 Adverse Events

An AE is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which does not necessarily have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product. This definition is applied to all study groups, and is applied to all groups beginning at the time of enrollment (i.e., once a participant has been randomized). The term “investigational product” for this study refers to all study products.

Study participants will be provided instructions for contacting the study site to report any untoward medical occurrences they may experience. In cases of potentially life-threatening events, participants will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where a study clinician is based, and to request that the clinician be

contacted upon their arrival. With appropriate permission of the participant, whenever possible, records from all non-study medical providers related to untoward medical occurrences will be obtained and required data elements will be recorded on study CRFs. All participants reporting an untoward medical occurrence will be followed clinically until the occurrence resolves (returns to baseline) or stabilizes, including adverse events noted at the Final Clinic/Early Termination Visit.

Study site staff will document in source documents and the appropriate AE Log CRF all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. AE severity will be graded per the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009) and the Female Genital Grading Table for Use in Microbicide Studies (Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009)), except that asymptomatic BV and asymptomatic candidiasis will not be reportable AEs. In addition, changes in genital bleeding judged to be related to a woman's contraceptive use will not be considered an AE. In cases where a genital AE is covered in both tables, the Female Genital Grading Table for Use in Microbicide Studies will be the grading scale utilized.

8.3.2 Serious Adverse Events

SAEs will be defined by the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, January 2010), as AEs occurring at any dose that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event that may not result in death, be immediately life-threatening, or require hospitalization but may jeopardize the participant or require intervention to prevent one of the outcomes listed in the definition above.

8.3.3 Adverse Event Relationship to Study Product

Relatedness is an assessment made by a study clinician of whether or not the event is related to the study agent. Degrees of relatedness will be categorized according to current DAIDS-approved guidelines. Per the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, January 2010), the relationship categories that will be used for this study are:

- *Related:* There is a reasonable possibility that the AE may be related to the study agent(s)
- *Not Related:* There is not a reasonable possibility that the AE is related to the study agent(s)

8.4 Expedited Adverse Event Reporting Requirements

8.4.1 Adverse Event Reporting to DAIDS

Requirements, definitions and methods for expedited reporting of Adverse Events (AEs) are outlined in Version 2.0 of the DAIDS Expedited Adverse Event (EAE) Manual, which is available on the Regulatory Support Center (RSC) website at <http://rsc.tech-res.com/safetyandpharmacovigilance/>.

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted via the DAIDS EAE Form. For questions about DAERS, please contact DAIDS-ES at DAIDS-ESSupport@niaid.nih.gov. Site queries may also be sent from within the DAERS application itself.

Where DAERS has not been implemented, the site will submit expedited AEs by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website, <http://rsc.tech-res.com/safetyandpharmacovigilance/>. For questions about EAE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

8.4.2 Reporting Requirements for this Study

The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study. The study agents for which expedited reporting are required are the low dose and original MK-2048A IVRs.

8.4.3 Grading Severity of Events

The most current Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, Dec 2004 (clarification dated August 2009), and the Female Genital Grading Table for Use in Microbicide Studies (Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004, clarification dated August 2009) will be used and are available on the RSC website at <http://rsc.tech-res.com/safetyandpharmacovigilance/>.

8.4.4 Expedited AE Reporting Period

The expedited AE reporting period for this study begins at enrollment (i.e., randomization) and continues through the participant's termination from the study.

After the protocol-defined AE reporting period, unless otherwise noted, only Suspected, Unexpected Serious Adverse Reactions (SUSARs) as defined in Version 2.0 of the EAE Manual will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

8.5 Pregnancy and Pregnancy Outcomes

Pregnant women are excluded from this study.

Pregnancy-related data will be collected using pregnancy CRFs for all pregnancies detected during the study. Pregnancy outcomes will not be expeditiously reported to Merck or the DAIDS Medical Officer (MO) unless there is an associated AE in the pregnant participant that meets expedited reporting criteria or the pregnancy results in a congenital anomaly meeting the Manual for Expedited Reporting of EAEs to DAIDS (Version 2.0, January 2010) guidelines for expedited reporting.

8.6 Regulatory Requirements

Information on all reported AEs will be included in reports to the FDA and other applicable government and regulatory authorities. Site IoRs/designees will submit AE and any relevant safety information in accordance with local regulatory requirements.

8.7 Social Harms Reporting

Although the study site will make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others and that social harms may result. Social harms that are judged by the IoR/designee to be serious or unexpected will be reported to the PSRT and responsible site IRBs according to their individual requirements.

9 CLINICAL MANAGEMENT

Guidelines for clinical management and temporary product hold/permanent discontinuation of study product are outlined in this section. In general, the IoR/designee has the discretion to hold study product temporarily at any time if s/he feels that continued product use would be harmful to the participant or interfere with treatment deemed clinically necessary. Unless otherwise specified below, the IoR/designee should immediately consult the PSRT for further guidance on resuming study product, continuing the hold temporarily, or progressing to permanent discontinuation of study product. The IoR/designee will document all temporary product holds and permanent discontinuations on applicable CRFs.

9.1 Grading System

AE severity grading is described in Section 8.3.1.

9.2 Dose Modification Instructions

No dose modifications will be undertaken in this study.

9.3 General Criteria for Temporary Hold and Permanent Discontinuation of Study Product

Temporary Hold

The IoR/designee must consult the PSRT on all temporary product holds for further guidance on resuming product use, continuing the temporary hold, or progressing to permanent discontinuation. If the underlying reason for the temporary hold later resolves, the IoR/designee should consult the PSRT to resume product use at that time. A participant will be temporarily held from product use by the IoR/designee for any of the following reasons:

- Report of use of prohibited medications and medications to be avoided as described in Section 6.6; product use may resume when the participant reports no longer taking the prohibited medication, provided other reasons for temporary product hold/permanent discontinuation do not apply.
- Participant is unable or unwilling to comply with required study procedures, or otherwise might be put at undue risk to their safety and well-being by continuing product use, according to the judgment of the IoR/designee.

Permanent Discontinuation

A participant will be permanently discontinued from product use by the IoR/designee for any of the following reasons:

- Exposure to or acquisition of HIV infection; for those who acquire HIV study product should be held beginning immediately upon recognition of the first reactive rapid HIV test

- Pregnancy
- Breastfeeding
- Participant is unable or unwilling to comply with required study procedures, or otherwise might be put at undue risk to their safety and well-being by continuing product use, according to the judgment of the IoR/designee.

9.4 Temporary Product Hold/Permanent Discontinuation in Response to Adverse Events

Grade 1 or 2

In general, a participant who develops a Grade 1 or 2 AE as defined by the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009) or the Female Genital Grading Table for Use in Microbicide Studies, Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009) regardless of relationship to study product that is not specifically addressed in Section 9.5 below may continue product use. If the IoR/designee opts to temporarily hold study product, the PSRT must be notified.

Grade 3

For participants who develop a Grade 3 AE as defined by the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009) or the Female Genital Grading Table for Use in Microbicide Studies, Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009) that is judged by the IoR/designee to be unrelated to study product, a temporary product hold must be initiated and the PSRT must be notified.

The study product must be permanently discontinued for participants who develop a Grade 3 AE judged by the IoR/designee to be related.

Grade 4

For participants who develop a Grade 4 AE as defined by the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009) or the Female Genital Grading Table for Use in Microbicide Studies, Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009) regardless of relationship to study product, study product must be permanently discontinued.

9.5 Other Clinical Events

Genital Tract Infections

Management of sexually transmitted infections (STIs) and other forms of vaginitis and cervicitis will be in accordance with current CDC guidelines (<http://www.cdc.gov/std/treatment/>). When clinically appropriate, investigators should use oral or parenteral medications when at all possible to avoid intravaginal medication

use. Observed single dose treatment should be provided whenever possible, per clinician discretion.

- Study IVR need not be held in the event of an STI/RTI requiring treatment, unless other temporary product hold/permanent discontinuation guidelines apply.
- Should the IoR/designee determine that a temporary hold is warranted, consultation with the PSRT is required.

Note: One exception, per management guidelines below, consultation with the PSRT is not required for the initial management of deep epithelial disruption (ulceration)

If a suspected finding is reported by a participant between scheduled visits, an interim visit may be scheduled at the discretion of the site investigator.

Management of genital events observed at scheduled or interim visits will be in accordance with the following:

Superficial epithelial disruption (abrasion/peeling)

- Continue study IVR use
- Perform naked eye evaluation
- Re-evaluate in 3-5 days
- If condition worsens, temporarily hold study IVR use and consult the PSRT; otherwise continue study IVR use

Deep epithelial disruption (ulceration)

- Temporarily hold study IVR for deep epithelial disruption
- Re-evaluate in 3-5 days and resume study IVR use if resolved
- If resolved at that time, may resume study IVR use. If unresolved at this second reevaluation, continue temporary product hold, consult with PSRT regarding permanent discontinuation, and provide care per local standard. If unresolved at 3-5 days, re-evaluate within 2-3 days.
- If there is reoccurrence with no identified etiology, continue temporary product hold and consult the PSRT regarding permanent discontinuation

Localized erythema or edema: area of less than 50% of vulvar surface or combined vaginal and cervical surface

- Continue study IVR use
- Perform naked eye evaluation
- If asymptomatic, re-evaluate at next regularly scheduled visit
- If symptomatic, re-evaluate in 3-5 days
- If worsened significantly, temporarily hold study IVR use and consult the PSRT; otherwise continue study IVR use

Generalized erythema or severe edema: area of more than 50% of vulvar surface or combined vaginal and cervical surface affected by erythema

- Temporarily hold study IVR
- Perform naked eye evaluation
- Re-evaluate in 3-5 days and resume study IVR use if resolved
- If unresolved at 3-5 days, re-evaluate within 2-3 days. If resolved at that time may resume use. Safety follow up by phone after IVR use has resumed may be performed if indicated. If unresolved at this second reevaluation, continue temporary product hold, consult with PSRT regarding permanent discontinuation, and provide care per local standard

Unexpected genital bleeding

- Continue study IVR use
- Perform naked eye evaluation
- If determined to be due to deep epithelial disruption, refer to guidelines above; otherwise continue study IVR use

Genital petechia(e)

- Continue study IVR use
- Perform naked eye evaluation
- No further evaluation or treatment is required

Genital ecchymosis

- Continue study IVR use
- Perform naked eye evaluation
- No further evaluation or treatment is required

9.6 HIV-1 Infection

A participant who has a positive test for HIV must have study product permanently discontinued and will be terminated, as per Section 7.5.1.

9.7 Pregnancy

All study participants are required to be sexually abstinent during MTN-028 participation.

Pregnancy testing will be performed at scheduled study visits and participants will be encouraged to report all signs or symptoms of pregnancy to study staff. The IoR/designee will counsel any participant who becomes pregnant regarding possible risks to the fetus according to site SOPs. The IoR/designee also will refer the participant to all applicable services; however, the site is not responsible pregnancy-related care costs.

A participant who becomes pregnant during the course of the study will have study product discontinued and will be terminated from the study, as per Section 7.5.2. Participants will continue to be followed until the pregnancy outcome is ascertained (or, in consultation with the PSRT, it is determined that the pregnancy outcome cannot be ascertained). Pregnancy outcomes will be reported on relevant CRFs; outcomes meeting criteria for EAE reporting also will be reported on EAE forms.

9.8 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. The IoR/designee also may withdraw participants from the study to protect their safety and/or if they are unwilling or unable to comply with required study procedures, after consultation with the PSRT. Participants also may be withdrawn if Merck & Co., NIAID, MTN, government or regulatory authorities, including the FDA and Office for Human Research Protections (OHRP), or site IRBs/ECs terminate the study prior to its planned end date. Every reasonable effort is made to complete a final evaluation of participants who withdraw or are withdrawn from the study prior to completing follow-up. Study staff members will record the reason(s) for all withdrawals in participants' study records. In the event that participants who voluntarily withdraw from the study wish to re-join the study, they may resume product use (if applicable) and follow-up through their originally scheduled study exit date, pending consultation with the PSRT.

10 STATISTICAL CONSIDERATIONS

10.1 Overview and Summary of Design

This is a single-site, single-blind, two-arm, 2:1 randomized trial to assess the PK and safety of IVRs containing different dose strengths of vicriviroc (MK-4176) and MK-2048 when used for 28 days by healthy, HIV-uninfected, sexually abstinent women. A total of approximately 18 women will be randomized.

10.2 Study Endpoints

Primary Endpoints

Consistent with the primary study objective to assess local and systemic pharmacokinetics of vicriviroc (MK-4176) and MK-2048 during and after 28-days of use of two MK-2048A IVRs containing different dose strengths, the following primary endpoint will be assessed:

- Assessment of vaginal fluid, blood plasma, and cervical tissue concentrations of MK-4176 and MK-2048 during and after 28 days of use of an IVR

Consistent with the primary objective to assess and compare the safety of two MK-2048A IVR formulations containing different dose strengths of VCV (MK-4176) and MK-2048 during and after 28 days of use by healthy, HIV-uninfected, sexually abstinent women, the primary safety endpoints are:

- Evidence of a Grade 1 or higher genitourinary events as defined by the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, Dec 2004 (Clarification dated August 2009), Addendum 1, (Female Genital Grading Table for Use in Microbicide Studies) judged to be related to study product
- Evidence of a Grade 2 or higher adverse events as defined by the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, Dec 2004 (Clarification dated August 2009)

Secondary Endpoint

Consistent with the secondary study objective to assess remnant content in returned IVRs the following endpoint will be assessed:

- Residual drug levels measured in IVRs

10.3 Sample Size and Power Calculations

The proposed total sample size is approximately N=18 women randomized into 2 arms in a 2:1 ratio. This sample size is based upon the size of similar Phase 1 pharmacokinetic studies of vaginal microbicide products.

Pharmacokinetic endpoints will include mean concentration-time course (AUC from day 1 to 30), assessed in vaginal fluid, blood plasma, and cervical tissue for each study drug. Based on the data from a recent safety and pharmacokinetic trial assessing delivery of dapivirine from a vaginal ring in healthy women, the coefficient of variation for the pharmacokinetic endpoint AUC assessed in vaginal fluid and blood plasma is estimated at 0.35, and 0.30.³⁰ Table 17 shows the standard errors and 95% confidence intervals of the estimate of AUC in each arm for VCV and MK-2048.

Table 17. Standard errors and 95% confidence interval of the pharmacokinetic endpoint AUC in each arm based on the standard deviations estimated from the preliminary study.

Samples	Low dose arm (n=12)			Original dose arm (n=6)		
	Standard deviation	Standard error	95% Confidence interval	Standard deviation	Standard error	95% Confidence interval
Plasma VCV (pg.h/ml)	268996	77652	(619481, 923879)	537992	219634	(1112877, 1973843)
Vaginal fluid VCV (µg.h/ml)	35308	10193	(98213, 138168)	70616	28829	(179877, 292886)

	Low dose arm (n=12)			Original dose arm (n=6)		
Plasma MK-2048 (pg.h/ml)	29560	8533	(68075, 101525)	88680	36203	(183441, 325359)
Vaginal fluid MK-2048 (µg.h/ml)	3880	1120	(10793, 15183)	11640	4752	(29650, 48278)

A safety event is defined as a participant having at least one safety endpoint. Table 18 lists the probabilities of observing various numbers of participants with an event in both arms. If the true rate is 5%, the probability of observing at least one participant out of 12 participants in the low dose arm is 0.46, and the probability of observing at least one participant out of 6 participants in the original dose arm is 0.27. The probability of observing at least one participant with an event is higher if the true event rate is higher.

Table 18 The probability of observing a number of participants of having a safety event given the true event rate and the sample size in the group

	Low dose arm (n=12)			Original dose arm (n=6)		
True event rate	0 event	1 or more events	2 or more events	0 event	1 or more events	2 or more events
1%	0.886	0.114	0.006	0.941	0.059	0.001
5%	0.540	0.460	0.118	0.735	0.265	0.033
10%	0.282	0.718	0.341	0.531	0.469	0.114
15%	0.142	0.858	0.557	0.377	0.623	0.224
20%	0.069	0.931	0.725	0.262	0.738	0.345
25%	0.032	0.968	0.842	0.178	0.822	0.466

This is a Phase 1 study and therefore not powered to detect small differences in rates of safety endpoints between two arms. Given power of 0.8 and two-sided p-value of 0.05, we are only able to detect the largest difference that can be observed: 1/12 vs. 1/6.

10.4 Participant Accrual, Follow-up and Retention

Based on previous studies of vaginal products with similar eligibility requirements, the accrual of 18 eligible participants will take approximately 6-9 months. The target retention rate for each study visit is 100%. Therefore, once a participant is enrolled in the study, every effort will be made to retain the participant during the entire study duration. However, additional enrollment may occur to compensate for the potential data loss due to loss of follow-up and/or on temporary hold or permanent product discontinuation. The site will target retention of 95% of enrolled participants over the follow-up period.

10.5 Randomization

Participants will be randomized in a 2:1 ratio to the two arms of the study at the single site. Randomization scheme will be generated and maintained by the MTN SDMC.

10.6 Blinding

Participants will be blinded to the treatment assignment. All IVRs will be individually packaged and labeled.

There are no circumstances under which it is expected that unblinding of the participant will be necessary for the provision of medical treatment or to otherwise protect the safety of study participants. If, however, an Investigator feels that specific product knowledge is necessary to protect participant safety, the Investigator will notify the PSRT to consider and rule upon the request.

10.7 Data and Safety Monitoring and Analysis

10.7.1 Study Monitoring Committee

No Data and Safety Monitoring Board oversight is planned for this study. The MTN SMC will conduct interim reviews of study progress, including rates of participant accrual, retention, completion of primary and main secondary endpoint assessments, study or lab issues, and, in a closed report, safety data by arm of the study. These reviews will take place approximately once during study implementation and as needed. At the time of this review, or at any other time, the SMC may recommend that the study proceed as designed, proceed with design modifications, or be discontinued.

10.7.2 Primary Analysis

When the use of descriptive statistics to assess group characteristics or differences is required, the following methods will be used: for categorical variables, the number and percent in each category; for continuous variables, the mean, median, standard deviation, quartiles and range (minimum, maximum).

To assess the adequacy of the randomization, participants in each arm will be compared for baseline characteristics including demographics and laboratory measurements using descriptive statistics. Due to the small sample size, formal comparisons will not be done.

Pharmacokinetic Analysis

Blood and CVF will be analyzed for the primary PK parameters (AUC) and described using descriptive statistics. A non-compartmental analysis will be used to calculate AUC.

Safety Analyses

A list of AEs as defined as endpoints will be tabulated by arm and treatment period. The difference of AE rates between the two study arms will be assessed by Fisher's exact test.

10.7.3 Missing Data

We are targeting a retention rate of 95% over the 35 day study period. Based on previous MTN trials, we expect to have minimal missing data. In any situation with missing data, appropriate secondary analyses will be performed to adjust for variables that may be related to the missingness mechanism. If missing data rates are higher than anticipated (over 10%), covariates that are related to missingness in likelihood-based regression models will be included. A sensitivity analysis to assess the potential impact of the missing data will also be performed. These analyses will include imputing the data under the most extreme scenarios of information missingness, such as assuming everyone missing has an extreme value of the missing variable, and less informative imputation approaches.

11 DATA HANDLING AND RECORDKEEPING

11.1 Data Management Responsibilities

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

11.2 Source Documents and Access to Source Data/Documents

The study site will maintain source data/documents in accordance with current DAIDS policies. (<http://rsc.tech-res.com/policiesandregulations/>)

Each IoR/designee will maintain, and store securely, complete, accurate and current study records throughout the study. In accordance with U.S. regulations regarding testing investigational products, the IoR/designee will maintain all study documentation for at least two years following the date of marketing approval for the study products being tested for the indication in which they were studied. If no marketing application is filed, or if the application is not approved, the records will be retained for two years after the investigation is discontinued and the US FDA is notified.

Study records must be maintained on site for the entire period of study implementation. Thereafter, instructions for record storage will be provided by DAIDS. No study records

may be moved to an off-site location or destroyed prior to receiving approval from DAIDS.

11.3 Quality Control and Quality Assurance

The study site will conduct quality control and quality assurance procedures in accordance with current DAIDS policies. (<http://rsc.tech-res.com/policiesandregulations/>)

12 CLINICAL SITE MONITORING

Study monitoring will be carried out by Pharmaceutical Product Development, Inc. (PPD) (Wilmington, NC) in accordance with current DAIDS policies. Study monitors will visit the site to do the following:

- Review informed consent forms, procedures, and documentation
- Assess compliance with the study protocol, Good Clinical Practices (GCP) guidelines, and applicable regulatory requirements (US and non-US), including CFR Title 45 Part 46 and Title 21 Parts 50, 56, and 312
- Perform source document verification to ensure the accuracy and completeness of study data
- Verify proper collection and storage of biological specimens
- Verify proper storage, dispensing, and accountability of investigational study products
- Assess implementation and documentation of internal site quality management procedures

The IoR/designee 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 IoR/designee also will allow inspection of all study-related documentation by authorized representatives of the MTN LOC, Merck & Co., SDMC, LC, NIAID, FDA, OHRP, IRBs/ECs and other local and US regulatory authorities. A site visit log will be maintained at the study site to document all visits.

13 HUMAN SUBJECTS PROTECTIONS

Site investigators will make efforts to minimize risks to participants. Participants and study staff members will take part in a thorough informed consent process. Before beginning the study, the IoR/designee will have obtained IRB approval and the protocol will have been submitted to the FDA. The IoR/designee will permit audits by the NIH, Merck & Co., the FDA, OHRP, MTN LOC, IRBs/ECs, SDMC, and other local and US regulatory authorities or any of their appointed agents.

13.1 Institutional Review Boards

Each participating institution is responsible for assuring that this protocol, the associated site-specific informed consent forms, and study-related documents (such as participant education and recruitment materials) are reviewed by an IRB responsible for oversight of research conducted at the study site. Any amendments to the protocol must be approved by DAIDS and the responsible IRBs prior to implementation.

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

13.2 Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, the site must have the protocol and the protocol consent forms approved, as appropriate, by their local IRB and any other applicable regulatory entity (RE). Upon receiving final approval, the site will submit all required protocol registration documents to the DAIDS Protocol Registration Office (PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) will be reviewed and approved by the DAIDS PRO and the site will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB and any other applicable RE approval(s) for an amendment, the site should implement the amendment immediately. The site is required to submit an

amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received. Site-specific ICF(s) *will not* be reviewed and approved by the DAIDS PRO and the site will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

13.3 Study Coordination

DAIDS holds the Investigational New Drug (IND) applications for this study. Assignment of all sponsor responsibilities for this study will be specified in a Clinical Trials Agreement (CTA) executed by NIAID and Merck & Co.

Study implementation will be directed by this protocol, which may not be amended without prior written approval from the Protocol Chair and DAIDS Medical Officer. Study implementation will also be guided by a common study-specific procedures manual that provides further instructions and operational guidance on conducting study visits; data and forms processing; specimen collection, processing, and shipping; AE assessment, management and reporting; dispensing study products and documenting product accountability; and other study operations. Standardized study-specific training will be provided to the site by the MTN LOC, SDMC, LC and other designated members of the Protocol Team.

Close coordination between protocol team members is necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. The PSRT will address issues related to study eligibility and AE management and reporting as needed to assure consistent case management, documentation, and information-sharing. Rates of accrual, adherence, follow-up, and AE incidence will be monitored closely by the team as well as the SMC.

13.4 Risk Benefit Statement

13.4.1 Risks

General

Phlebotomy may lead to excessive bleeding, discomfort, feelings of dizziness or faintness, and/or bruising, swelling and/or infection.

Pelvic examination and vaginal fluid collection may cause mild discomfort and/or vaginal bleeding or spotting. Cervical biopsies carry the risk of discomfort or pain during the procedure and for a few hours afterwards. Participants may have mild vaginal spotting

(bleeding) for one or two days, and will be instructed to avoid sexual intercourse and product use for the duration of the study. While abstinence is a requirement of this study, if participants are sexually active they may also be at increased risk for STIs and HIV acquisition, if exposed. There is a small risk of infection and heavier bleeding. Participants will be instructed to contact the clinic if symptoms are bothersome, if heavy bleeding is noted (soaking through a pad in an hour or less) or if the participant develops any abnormal odor or discharge from the vagina.

Disclosure of HIV and STI status may cause worry, sadness or depression. Disclosure of HIV-positive status has been associated with depression, suicidal ideation, and denial as well as social isolation. Trained counselors will be available to help participants deal with these feelings.

Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of questions when discussing sexual behaviors.

Partner notification in response to diagnosed STI or HIV infection could have problems in their relationships with their sexual partners. Participants also could have problems in their partner relationships associated with maintenance of study-required abstinence.

Use of the study IVR may lead to vaginal symptoms, including irritation, increased discharge, and discomfort (including with vaginal intercourse). As with any vaginally retained product, the possibility of toxic shock syndrome, although rare, exists.

With any drug, there is the potential risk for an allergic reaction. The most commonly reported symptoms associated with allergic reactions are:

- Rash
- Dizziness
- Itching
- Muscle aches
- Nausea
- Fainting
- Facial flushing
- Chest tightness
- Cough
- Hives
- Fever
- Shortness of breath

The following side effects have been associated with the use of oral VCV in patients being treated for HIV. These side effects may or may not be associated with the use of VCV when the drug is placed into a vaginal ring and worn by HIV-negative women:

- Hepatocellular events (Liver problems)

- Ischemic cardiovascular events (Heart problems, such as a heart attack)
- Dyslipidemias (High blood lipid or cholesterol levels)
- Herpes simplex virus (HSV) infections
- Upper respiratory infections
- Seizures
- Malignancies

The most common AEs associated with VCV are: diarrhea, nausea, headache, upper respiratory infection, nasopharyngitis, and fatigue.

However, there was no clear difference in incidence of side effects or AEs between VCV recipients and control groups.

The following side effect has been associated with the use of oral MK-2048. This side effect may or may not be associated with MK-2048 when formulated in an intravaginal ring.

- Mild headache

13.4.2 Benefits

Participants in this study may experience no direct benefit. 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 safe and effective interventions to prevent HIV acquisition. Participants also may appreciate the opportunity to contribute to the field of HIV prevention research.

Participants will receive HIV/STI risk reduction counseling, HIV and STI testing, physical examination, pelvic examination, and routine laboratory testing related to blood, liver, and kidney function. Participants may be provided or referred for STI treatment in accordance with CDC guidelines. 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. Some volunteers may have the opportunity to access expedient treatment and decreased morbidity due to early diagnosis and treatment of abnormalities identified during tests, examinations and referrals.

13.5 Informed Consent Process

Written informed consent will be obtained from each study participant prior to screening. Written informed consent also will be obtained for long-term specimen storage and possible future testing, although consent for long-term specimen storage is not required for study participation. In obtaining and documenting informed consent, the IoR and their designees will comply with applicable local and US regulatory requirements and will adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Study staff must document the informed consent process in accordance with the Requirements for Source Documentation in DAIDS Funded and/or Sponsored

Clinical Trials (<http://rsc.tech-res.com/policiesandregulations/>). Participants will be provided with copies of the informed consent forms if they are willing to receive them.

In addition to informed consent forms, the Protocol Team will work with study staff and community representatives to develop appropriate materials about the study and a standardized approach to the informed consent process to be implemented at the study site, which will be detailed in the study-specific procedures manual.

The informed consent process will cover all elements of informed consent required by research regulations. In addition, the process specifically will address the following topics of importance to this study:

- The unknown safety and unproven efficacy of the study products
- The need to abstain from sexual intercourse, regardless of study treatment group
- The importance of participants in both study groups to the success of the study
- The importance of adherence to the study visit and procedures schedule
- The potential medical risks of study participation (and what to do if such risks are experienced)
- The potential social harms associated with study participation (and what to do if such harms are experienced)
- The real yet limited benefits of study participation
- The distinction between research and clinical care
- The right to withdraw from the study at any time

13.6 Participant Confidentiality

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

All study-related information will be stored securely at the study site. All participant information will be stored in locked areas with access limited to study staff. All laboratory specimens, study data collection, and administrative forms will be identified by 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 local databases will be secured with password protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participants' ID numbers to identifying information will be stored in a, locked file in an area with limited access. Participants' study information will not be released without their written permission, except as necessary for review, monitoring, and/or auditing by the following:

- Representatives of the US Federal Government, including the US FDA, the US OHRP, NIH, and/or contractors of the NIH, and other local and US regulatory authorities
- Representatives of Merck & Co.
- Study staff
- Site IRBs/ECs

The MTN has a Certificate of Confidentiality from the US Department of Health and Human Services that is applicable to this study. This Certificate protects study staff from being compelled to disclose study-related information by any US Federal, State or local civil, criminal, administrative, legislative or other proceedings. It thus serves to protect the identity and privacy of study participants.

13.7 Special Populations

13.7.1 Pregnant Women

Women who test positive for pregnancy at Screening or Enrollment Visits will not be eligible to participate in this study. Should a woman test positive for pregnancy after Enrollment, study product will be permanently discontinued and participants will be withdrawn from the study, per Section 7.5.2. During the informed consent process, women will be informed that the study IVR is not a method of contraception and the effects of the study IVR on a developing human fetus are unknown.

All potential participants are required by the eligibility criteria for Screening and Enrollment to be currently sexually abstinent for the duration of study participation and using effective contraception.

13.7.2 Children

The NIH has mandated that children be included in research trials when appropriate. This study meets “Justifications for Exclusion” criteria for younger children as set forth by the NIH. Specifically, “insufficient data are available in adults to judge potential risk in children” and “children should not be the initial group to be involved in research studies.” This study does not plan to enroll children under 18 years old.

13.8 Compensation

Pending IRB approval, participants will be compensated for time and effort in this study, and/or be reimbursed for travel to study visits and time away from work. Site specific reimbursement amounts will be specified in the site specific informed consent forms.

13.9 Communicable Disease Reporting

Study staff will comply with local requirements to report communicable diseases including HIV-1 identified among study participants to health authorities. Participants will be made aware of reporting requirements during the informed consent process.

13.10 Access to HIV-related Care

13.10.1 HIV Counseling and Testing

HIV test-related counseling will be provided to all potential study participants who consent to undergo HIV-1 screening to determine their eligibility for this study, and to all enrolled participants at each follow-up HIV-1 testing time point. Testing will be performed in accordance with the algorithm in Appendix II. Counseling will be provided in accordance with standard HIV counseling policies and methods at the site and additionally will emphasize the unknown efficacy of the study products in preventing HIV-1 infection. In accordance with the policies of the NIH, participants must receive their HIV-1 test results to take part in this study.

13.10.2 Care for Participants Identified as HIV-Positive

An individual who has been identified as infected with HIV-1 will be managed or referred for management according to the local standard of care. Should a participant test positive for HIV after Visit 2, follow-up procedures will be performed as per Section 7.5.1. Please refer to Section 9.6 for additional details.

13.11 Study Discontinuation

This study may be discontinued at any time by NIAID, the MTN, Merck & Co., the US FDA, the OHRP, other government or regulatory authorities, or site IRBs/ECs.

14 PUBLICATION POLICY

DAIDS/NIAID and MTN policies and a CTA between Merck & Co. and NIAID will govern publication of the results of this study. Any presentation, abstract, or manuscript will be submitted by the investigator to the MTN Manuscript Review Committee, DAIDS, NIAID, NIMH, and Merck & Co. for review prior to submission.

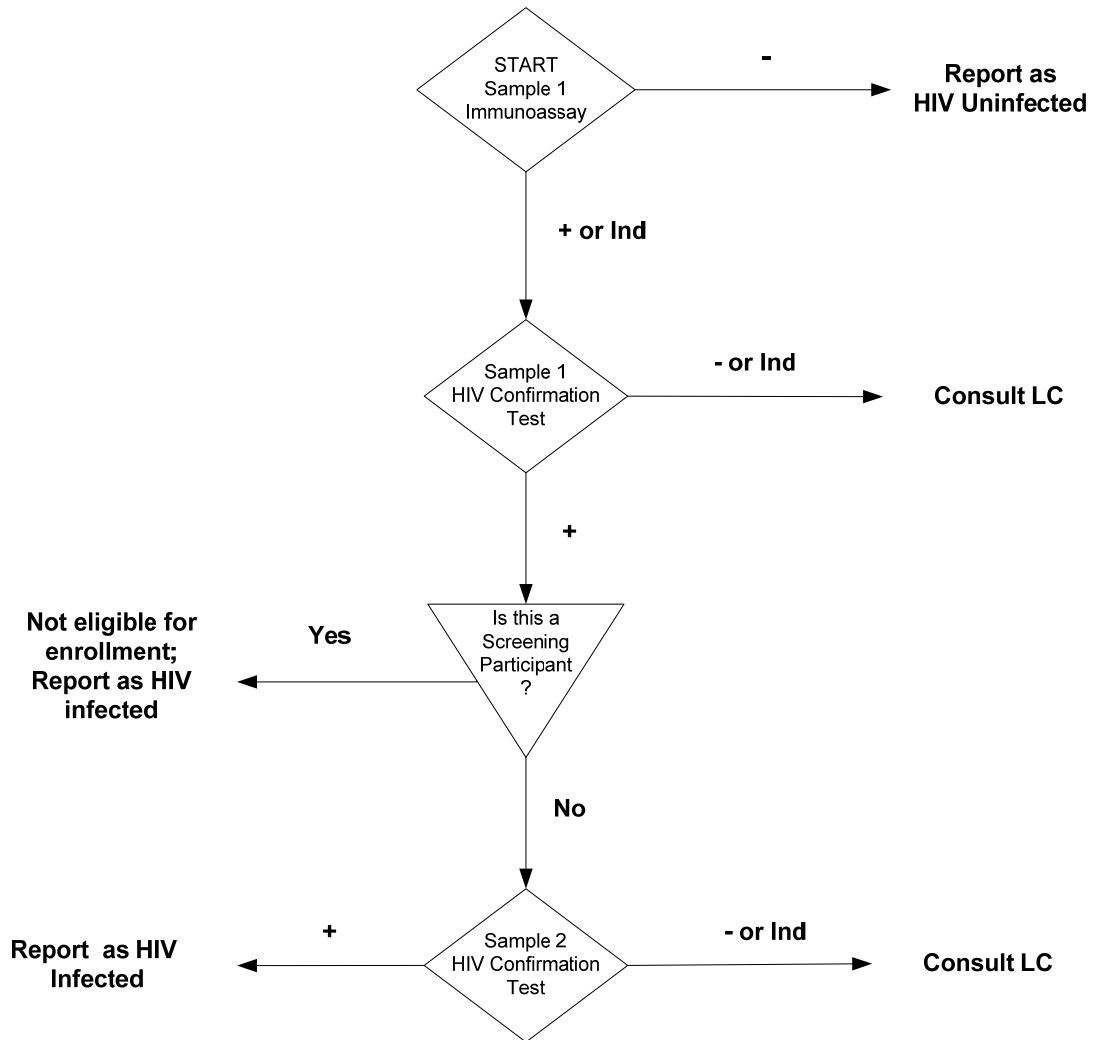
15 APPENDICES

APPENDIX I: SCHEDULE OF STUDY VISITS AND PROCEDURES

	SCR	ENR	VISIT Days 1, 2, 3, 7, 14, 21	VISIT Day 28	VISIT Days 29,30, 31	DAY 35 Final Clinic/ Term.
ADMINISTRATIVE AND REGULATORY						
Informed consent(s)	x					
Assess informed consent comprehension	x					
Assignment of PTID	x					
Locator information	x	x	x	x	x	x
Demographic information	x					
Eligibility assessment	x					
Eligibility confirmation		x				
Review IC and confirm participant is interested in continued study participation		x				
Randomization		x				
Reimbursement	x	x	x	x	x	x
Record/ update AEs			x	x	x	x
Schedule/verify next visit	*	*	x	x	x	*
BEHAVIORAL						
Adherence Assessment			x	x		
HIV pre- and post- test counseling	x	x	*	*	*	x
Protocol requirements counseling (To include adherence, product use and contraceptive counseling, STI and risk reduction counseling, as needed)	x	x	*	*	*	*
CLINICAL						
Medical and menstrual history	x	x	x	x	x	x
Concomitant medications	x	x	x	x	x	x
Document pre-existing conditions		x				
Physical examination (full or modified)	x	x	x	x	x	x
Pelvic examination	x	x	x	x	x	x
Provide available test results	x	x	x	x	x	x
Treat or prescribe treatment for UTI/RTI/STIs or refer	*	*	*	*	*	*
LABORATORY						
Urine	<i>Collect Urine</i>					
	hCG	x	x	*	x	*
	Dipstick UA	x	*	*	x	*
	Urine culture	*	*	*	*	*
Blood	<i>Collect Blood</i>					
	CBC with differential and platelets	x	x		x	*
	HIV-1 serology	x	x	*	*	x
	HBsAg	x				
	INR	x				
	Anti-HCV	x				
	Chemistries (Creatinine, AST, ALT)	x	x		x	x
	PK- Blood		x	x	x	x
	Syphilis serology	x	*	*	*	*
Plasma archive		x				
Cervical/ Vaginal	<i>Collect Pelvic Specimens</i>					
	Vaginal fluid pH	*	*	*	*	*
	Rapid Trichomonas test	x	*	*	*	*
	KOH wet mount for candidiasis	*	*	*	*	*
	Saline wet mount for BV	*	*	*	*	*
	PK- Vaginal fluid		x	x	x	x
Vaginal NAAT GC/CT	x	*	*	*	*	

<i>Collect Pelvic Specimens (Continued)</i>						
Collect Pap test	*					
Cervical tissue				x		
Gram stain		x	♦	x		x
STUDY PRODUCT						
Participants will receive study IVR, study IVR use instructions and will be instructed to self-insert the study IVR, followed by pelvic exam to check placement		x				
Collect IVR				x		▲
* If indicated. ▲ If Early Termination Visit and not already performed, ♦ Day 3 only, ☐ Day 14 only						

APPENDIX II: ALGORITHM FOR HIV ANTIBODY TESTING



Ind: Indeterminate test results
LC: Laboratory Center

**APPENDIX III: SAMPLE INFORMED CONSENT FORM (SCREENING,
ENROLLMENT, LONG-TERM STORAGE AND FUTURE TESTING)**

**SAMPLE INFORMED CONSENT FORM
DIVISION OF AIDS, NIAID, NIH**

MTN-028

**Phase 1 Pharmacokinetic Trial of Two Intravaginal Rings (IVRs) Containing
Different Dose Strengths of Vicriviroc (MK-4176) and MK-2048**

Version 1.0

February 12, 2015

PRINCIPAL INVESTIGATOR: [Site to insert]

PHONE: [Site to insert]

Short Title for the Study: PK Trial of Two MK-2048A IVRs of Varying Dose
Strengths

INFORMED CONSENT

You are being asked to take part in this research study because you are healthy, HIV-negative, assigned female sex at birth and between the ages of 18 and 45 years old. Approximately 18 people will participate at this study site in the United States. This Microbicide Trials Network (MTN) study is sponsored by the US National Institutes of Health (NIH). The study products in this clinical trial include two vaginal rings containing different doses of a combination of two anti-HIV medications, vicriviroc and MK-2048. A vaginal ring is a flexible plastic ring that is inserted into your vagina, and you will be asked not to remove the ring for approximately 28 days. The study products are supplied by Merck. At this site, the person in charge of this study is **[INSERT NAME OF PRINCIPAL INVESTIGATOR]**.

Before you decide if you want to join this study, we want you to learn more about it. This consent form gives you information about the study. Study staff will talk with you and answer any questions you may have. Once you read and understand the study and its requirements, you can decide if you want to join. If you do decide to take part in the trial, you will sign your name on this form. A copy of this document will be offered to you. Signing this consent form does not mean you will be able to join the study. You must first complete the screening tests and exams to see if you are eligible.

It is important to know that your participation in this research is your decision and taking part in this study is completely voluntary (see Your Rights as a Research Participant/Volunteer for more information).

WHAT IS THE PURPOSE OF THIS STUDY?

The main purpose of this research study is to find out how the two different dose combinations of study drugs enter and exit the body and to better understand the safety of the study product when the ring is inserted into the vagina for approximately 28 days. This study will provide important information on the best dose of these medications in a vaginal ring formulation, and help develop vaginal rings in the future, including combination vaginal rings that can also be used to prevent pregnancy.

STUDY PRODUCTS

All of the study drugs have been previously tested for HIV treatment. HIV is the virus that causes AIDS. Now researchers would like to know if these drugs can work to prevent HIV. To do this, they first need to better understand what effect these drugs have on the body, including the vagina.

The study drugs in the vaginal rings work in different ways to potentially prevent HIV:

- Vicriviroc works by preventing HIV from connecting to cells. By preventing the virus from connecting to the cell, HIV is unable to enter the cell and begin reproducing. Vicriviroc has been studied for HIV treatment in several clinical trials and has been found to be safe.
- MK-2048 blocks the mixing of HIV into the body's cells, specifically into the DNA, which is the body's genetic material. By preventing this step, the medication prevents HIV from taking hold in cells and begin reproducing. Oral tablets of MK-2048 were studied in one clinical trial involving 16 men and were generally well-tolerated.

This study is not testing to see if the study drugs prevent HIV infection. Researchers do not yet know if one or both of the two drugs will work in humans to protect against HIV.

There are only two known effective ways to prevent HIV: condoms and/or the use of pre-exposure prophylaxis (PrEP). PrEP is a new HIV prevention method in which people that do not have HIV take an oral tablet to reduce their risk of becoming infected. Study staff can provide you with additional information about PrEP if you are interested in learning more.

While the study drugs have been tested before in humans, this is the first time vicriviroc and MK-2048 are being tested in a vaginal ring formulation. This is also the first time vicriviroc and MK-2048 are being tested together in humans. Another Microbicide Trials Network study, MTN-027, is testing vaginal rings that contain vicriviroc, MK-2048, a combination of the two study drugs (See below, *original dose*) and a ring that contains no study product, i.e., a placebo ring. MTN-027 is a

companion trial to this study, and it will also look at the safety of the rings and measure how the study drugs enter and exit the body.

STUDY GROUPS

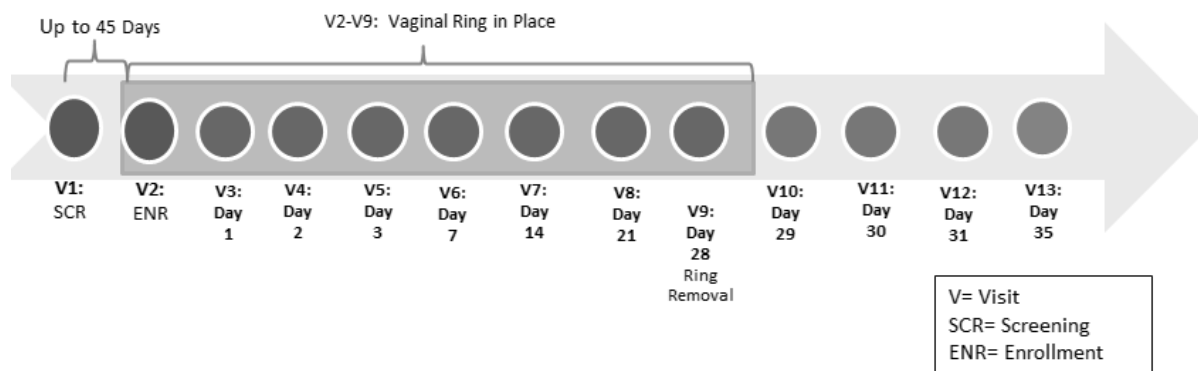
All of the eligible participants will be randomized to one of two vaginal ring study groups:

- A combination ring containing low dose strengths of vicriviroc and MK-2048, or
- A combination ring containing the original dose strengths of vicriviroc and MK-2048

Approximately twelve participants will be assigned to the low dose study group and approximately six participants will be in the original dose study group. Participants will be assigned to a group by random chance (like throwing dice). This is a single-blind study, which means you will not know which group you are in until the study is completed. The study clinician who provides the vaginal ring to participants may know the dose strength of the ring you receive. Participants will have a ring inserted into their vagina and be asked not to remove it for approximately 28 days.

Both study groups are important. No matter which study group you are in, you must remember that we do not know if the drugs contained within the rings work to protect you from getting HIV.

WHAT WILL HAPPEN DURING THE MTN-028 STUDY VISITS?



Screening Procedures:

The MTN-028 study includes a total of 13 study visits, including the Screening Visit which is taking place today after you sign this informed consent form. Visits will take place here at this study clinic and at **[SITE TO INCLUDE ALTERNATE LOCATION]**.

The procedures done at this visit (the screening visit) will take about **[SITE TO INSERT TIME]**.

- Study staff will ask you where you live and other questions about you, your medical health (including what medications you are taking), menstrual history, your sexual practices and your understanding of the study requirements.
- Study staff will:
 - Perform a physical exam.
 - Talk with you about the requirements of the study, including but not limited to:
 - Keeping the vaginal ring in place and not removing it between visits;
 - Being sexually abstinent for the duration of this study. This means no receptive penile-vaginal intercourse, anal intercourse, receptive oral intercourse, finger stimulation, or the use of sex toys.
 - Avoiding the insertion of any non-study products or objects into the vagina for the 5 days prior to Enrollment and for the duration of your study participation. These include but are not limited to: spermicides, female condoms, diaphragms, contraceptive vaginal rings, vaginal medications, menstrual cups, cervical caps (or any other vaginal barrier method), douches, lubricants, sex toys (vibrators, dildos, etc.), or fingers. Participants will be asked to avoid using tampons during the first week of study participation (starting at the enrollment visit) and for 24 hours prior to each clinic visit following enrollment.
 - Test your urine for pregnancy and other conditions.
 - If you are pregnant you cannot join this study.
 - Study staff will talk with you about ways to avoid becoming pregnant.
 - Take a blood sample **[SITE TO INSERT AMOUNT]**.
 - To test the health of your blood, liver and kidneys.
 - To test for infections, including HIV, hepatitis B, hepatitis C, syphilis, etc.
 - You will be told your test results as soon as they are available. You will talk with the study staff about the meaning of your results, how you feel about them, and hear about ways to prevent HIV and other sexually transmitted infections. Sometimes HIV tests are not clearly positive but also not clearly negative. In that case, we will do more tests until we are sure of your status. To participate in the study you must receive the results of your HIV test. If the test shows you have HIV, you cannot join the study. We will refer you to available sources of medical care and other services you may need. The study staff will tell you about other studies you may be eligible for, if any.
 - Perform a pelvic examination:
 - The study clinician will use a speculum, a plastic or metal instrument used to separate the walls of the vagina. The study clinician will check your vagina and cervix (the tissue that attaches the vagina to the uterus) for signs of infection and other problems. They will also take

some fluids to test for sexually transmitted infections and diseases (commonly known as STIs or STDs) and other problems.

- The study staff may also collect samples from your cervix for a “Pap test” or “Pap smear”. Study staff will inform you of the results of your Pap test. It takes about **[SITE TO INSERT AMOUNT OF TIME]** before Pap test results are ready. If you are 21 years of age or older and have a written report confirming a normal Pap test in the past 3 years, or if you had an abnormal Pap test but during follow-up were told no treatment was required, you will not need to have a Pap test taken at this screening visit. The results of your Pap test may affect whether or not you can join the study.
- Give you treatment or refer you for treatment for infections passed through sex, if needed.
- Inform you about other services, if needed.
- Provide you with the results of your tests, when available. It is expected that all of your results will be available by **[SITE TO SPECIFY TIMEFRAME]**.
- Schedule your next visit to enroll in the study, if you are willing and eligible.

If you decide not to join the study, blood collected at this visit will not be kept or used for any tests other than those listed above.

Enrollment and Follow-up Procedures:

At your Enrollment visit (the visit where you enter the study) you will:

- Answer questions to confirm you are able to join the study.
- Be randomly assigned to one of two study groups. You will not know which group you are in until the study is completed. The study clinician who provides the vaginal ring to participants may know the dose strength of the ring you receive. Participants in all study groups will have the same study visit schedule.
- Discuss any health or medical problems you may have had in the past or are currently experiencing.
- Have a blood sample collected **[SITE TO INSERT AMOUNT]** to test the health of your blood, liver and kidneys and to test for infections that typically are passed through sex, including HIV. You will be made aware of these results when they are ready. We also will collect blood in case there is a question about your lab results in the future. Finally, blood will be collected at multiple time points to measure the amount of study drug in your body over time, specifically 1, 2, 4, and 6 hours after the ring has been in place. An intravenous cannula (IV tube) may be placed, flushed and kept in place for the blood draws during the 6 hours after ring insertion.
- Test your urine for pregnancy
- Have a vaginal fluid sample collected at multiple time points to measure the amount of study drug in your vagina over time. This will be collected when the ring is inserted and approximately 1, 2, 4, and 6 hours after the ring has been in

place. You will be asked to collect these samples via swab(s), like a Q-tip. Study staff will provide you with more information as to how and where to place the swab and how to collect the fluid.

- Receive and insert the study ring. Study staff may help you insert the study ring if you cannot do it on your own. All participants will have an exam to ensure the ring is inserted correctly.
- This visit will take longer than most because we will check your blood and vaginal fluids over a period of 6 hours.

At most study visits, including the Enrollment visit, you will:

- Provide and/or update study staff with your contact information (i.e. where you live and how we can contact you).
- Tell study staff about your health, any changes in your health and/or any other problems.
- Talk with study staff about the following:
 - The rules of the study and how to follow the rules, including how to properly use the vaginal ring; for example, how to clean and reinsert the ring if it falls out anytime between your visits to the clinic. Another important rule of the study is sexual abstinence. We ask that you not have any receptive sexual activity (including receptive penile-vaginal intercourse, anal intercourse, receptive oral intercourse, finger stimulation, and the use of sex toys) for the 5 days prior to Enrollment and for the duration of your study participation. **If you do not think you can be sexually abstinent for 40 days then you should not join this study.**
 - Sexually transmitted infections (STIs), HIV, HIV/STI testing, and ways to avoid getting HIV and other infections passed through sex.
- After you begin using the vaginal ring, you will be asked to have blood and vaginal fluid samples collected at all clinic visits to measure the amount of study drug in your body. When the ring is removed at Day 28, you will have blood and vaginal fluid samples collected at multiple time points over a 6 hour period. An intravenous cannula (IV tube) may be placed, flushed and kept in place for the blood draws during the 6 hours after ring removal. This visit, like your Enrollment visit, will be longer.
- You will also have a urine sample collected at this visit to test for infections and other conditions.
- Have a physical exam.
- Have a pelvic exam.
 - The study clinician will use a speculum. Study staff will ask if you are experiencing symptoms of an infection. They will check your vagina and cervix for signs of problems due to the ring or infection. They will also take samples to test for bacteria and organisms in the vagina and, if necessary, look for any other problems.
 - A small amount of vaginal fluid will be collected via swab(s), like a Q-tip. This vaginal fluid will be used for research purposes only, i.e. to measure the amount of study drug in your body.

- When the vaginal ring is removed on Day 28, the study clinician will take 2 small tissue samples from your cervix, each about the size of a grain of rice. A speculum will be inserted and biopsy forceps will be used. These tissue samples will be used to measure the amount of the study drug in your cervical tissue and understand the drug's effects on the body.
- Study researchers will keep the ring at the end of the study and will run additional tests on it for research purposes only.
- Receive treatment or be referred for treatment for issues that the study staff may find.
- Receive test results, if available.
- Schedule your next visit.

At some visits you will provide a sample of blood to:

- Check the health of your blood, liver and kidneys.
- Test for HIV infection

At some visits you will provide a urine sample to test for pregnancy.

At the Final Clinic Visit, you will:

- Answer questions about your experience using the ring, including whether or not the ring was removed or fell out of your vagina. A staff member may ask you these questions.

Additional Visits and Procedures

It may be necessary for you to have additional visit(s) and/or to have additional samples collected if any of the above procedures need to be repeated due to issues with sample processing, testing or shipping, and/or if you are experiencing any symptoms or changes in your physical condition. If clinically important test results are found following the Final Clinic Visit, you will be contacted in an effort to schedule an additional visit or receive test results over the phone.

Additional testing may be performed as part of quality control.

If you become infected with HIV

As a requirement of this study you are asked to **NOT** engage in any sexual activity. Your participation in this study will not cause HIV infection. However, there is always a chance that through sexual activity or other activities you may become HIV-positive. In the unlikely event that you become HIV-positive, study staff will give you counseling and refer you for medical care and other available services. You will continue to be counseled while you are in this study. Tests may be performed to see if you have HIV drug resistance. This will allow doctors to know what HIV drugs would be best for the treatment of your type of HIV. If the HIV tests indicate you may be infected with HIV, you will stop using the vaginal ring. If HIV infection is confirmed, you will stop your participation in this study. You may be referred to other research studies.

RISKS AND/OR DISCOMFORTS

Whenever your blood is drawn, you may have:

- Excessive bleeding
- Discomfort
- Feelings of dizziness or faintness
- Bruising, swelling and/or infection

During pelvic exams and vaginal fluid collection you may feel discomfort or pressure in your vagina and/or pelvis. Due to the pelvic exam you may also have vaginal bleeding or spotting, which will stop shortly after the examination. Cervical biopsies carry the risk of discomfort or pain during the procedure and for a few hours afterwards. Participants may have spotting (bleeding) for one or two days. There is a small risk of infection and heavier bleeding from cervical biopsies. You may also be at increased risk for STIs and HIV acquisition, if exposed. You will be encouraged to call the clinic to report any problems after the collection, especially if heavy bleeding is noted (soaking through a pad or tampon in an hour or less) or if you develop any abnormal vaginal odor or discharge.

Study Rings

The study rings can cause some side effects. We do not yet know all the side effects of the rings. Some, but not all, participants who used rings in other studies have had:

- Discharge from the vagina
- Irritation and discomfort

With any product inserted vaginally, it is possible you could experience toxic shock syndrome. Toxic shock syndrome is a rare but serious illness caused by poisons (toxins) released by some types of *Staphylococcus aureus*, a common bacteria. The likelihood of this occurring is rare.

Study Drugs

The following side effects have been associated with the use of vicriviroc in patients being treated for HIV. These side effects may or may not be associated with the use of vicriviroc when the drug is placed into a vaginal ring and worn by HIV-negative women:

- Hepatocellular events (Liver problems)
- Ischemic cardiovascular events (Heart problems, such as a heart attack)
- Dyslipidemias (High blood lipid or cholesterol levels)
- Herpes simplex virus (HSV) infections
- Upper respiratory infections
- Seizures
- Malignancies (Cancerous tumors)

The most common side effects associated with vicriviroc are: diarrhea, nausea, headache, upper respiratory infection, nasopharyngitis (infectious swelling or

redness of the nose and throat), and fatigue.

However, there was no clear difference in the rate of side effects among people who received vicriviroc compared with those who did not receive this medication.

The following side effect has been associated with the use of oral MK-2048. This side effect may or may not be associated with MK-2048 when it is placed into the vaginal ring:

- Mild headache

Currently, no risk information is available for these two drugs combined.

It is also possible that you may have an allergic reaction to the study product. Signs of allergic reaction may include: rash, dizziness, itching, muscle aches, nausea, fainting, facial flushing, chest tightness, cough, hives, fever, shortness of breath.

Other Possible Risks

You may become embarrassed and/or worried when discussing your sexual practices, ways to protect against HIV and other infections passed through sex, and your test results. You may be worried while waiting for your test results. If you have HIV or other infections, learning this could make you worried. Trained study counselors will help you deal with any feelings or questions you have.

We will make every effort to protect your privacy and confidentiality during the study visits. Your visits will take place in private. However, it is possible that others may learn of your participation here and, because of this, may treat you unfairly or discriminate against you. For example, you could have problems getting or keeping a job, or being accepted by your family or community. Finding out you are HIV-positive could cause depression and/or suicidal thoughts. Finding out your HIV or STI status could also cause problems between you and your partner. If you have any problems, study counselors will talk with you and/or your partner to try to help resolve them.

It is possible that you and/or your partner(s) may experience problems in your relationships associated with maintenance of the study-required abstinence.

Sexual Practices, Pregnancy, and Breastfeeding

The vaginal rings being tested in this study are not birth control methods. You must agree to use an effective method of birth control such as birth control pills or another hormonal-based method (except for vaginal rings), or an intrauterine device (IUD), unless you or your partner have been sterilized (i.e., no longer able to have babies), you have sex with others having female genitalia exclusively, and/or you have been sexually abstinent for more than 90 days. You must also agree to not insert anything into your vagina for the duration of this study; this means that you may not have sex of any kind for the duration of this study. Sex for this study is defined as receptive penile-vaginal intercourse, anal intercourse, receptive oral intercourse, finger stimulation, and the use of sex toys. You must also agree to not use tampons during

the first week of study participation (starting at the enrollment visit) and for 24 hours prior to each clinic visit following enrollment.

We do not know what effect the study drugs have on pregnancy, including the effect of the study drug on the fetuses of persons who use the vaginal ring when pregnant, or the babies of persons who use the vaginal ring when breastfeeding. Because of this, anyone who is pregnant or breastfeeding may not join this study. Participants who join the study must agree to be sexually abstinent and use an effective method of contraception. Participants who join this study will have pregnancy tests while in the study.

If you become pregnant during the study, study staff will refer you to available medical care and other services you or your baby may need. The study does not pay for this care. You will stop using the ring and you will exit the study. We will also contact you to find out about your pregnancy and the outcome of your pregnancy. The outcome of your pregnancy is important to study staff; therefore your pregnancy will be followed until the results of your pregnancy are known.

BENEFITS

No one knows if the study ring will prevent HIV infection. Although you may not experience any direct benefit from participation in this study, information learned from this study may help in the development of ways to prevent the spread of HIV in the future. You will receive pelvic exams and counseling and testing for HIV and STIs. You will also have tests to check the overall health of your liver, kidneys, and blood cells.

This study cannot provide you with general medical care, but study staff will refer you to other available sources of care.

You will be counseled and tested for HIV and STIs. If you are infected with HIV, you will be referred for medical care, counseling, and other services available to you. Medical care for HIV infection will not be part of this study. If you have an STI diagnosed, you will receive medicine or a referral, if needed. You can bring your partner here for counseling and referral for testing and treatment for STIs if needed.

NEW INFORMATION

You will be told of any new information learned during this study that might affect your willingness to stay in the study. For example, if information becomes available that shows that the ring may be causing bad effects, you will be told about this. You will also be told when study results may be available, and how to learn about them.

WHY YOU MAY STOP TAKING THE STUDY DRUG EARLY OR BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT

You may be removed from the study early without your permission if:

- The study is cancelled by the US FDA, US NIH, Merck (the company that supplies the vaginal rings), the US Office for Human Research Protections

(OHRP), MTN, the local government or regulatory agency, or the Institutional Review Board (IRB) or Ethics Committee (EC). An IRB is a committee that watches over the safety and rights of research participants

- The Study Monitoring Committee (SMC) recommends that the study be stopped early (the SMC reviews the progress of the study and the kinds of effects that people report while they are participating in the study)
- You are found to be infected with HIV
- You become pregnant
- You are not able to reliably keep appointments
- Other reasons that may prevent you from completing the study successfully

The study clinician will ask you to stop using the study vaginal ring but continue to come in for your follow-up visits and procedures if:

- A study clinician decides that using the vaginal ring would be harmful to you
- You require a treatment that you may not take while using the study ring
- You have a bad reaction to the study ring

If a study clinician asks you to stop using the ring, you may be asked to continue to come in to the clinic for visits and undergo some of the procedures described above, including the physical examination, vital signs, and blood tests. You will stop using the ring until the study clinician decides it is safe for you to start using it again, if possible.

In the event that you are removed from or choose to leave this study, you will be asked to return your vaginal ring and complete a final evaluation. If you do not have the vaginal ring with you at the time of your contact with staff, staff members will make every effort to assist you in returning the ring as soon as possible. **[SITE TO SPECIFY ALLOWANCES FOR SPECIAL CIRCUMSTANCES]**

COSTS TO YOU

[SITE TO COMPLETE ACCORDING TO SITE CAPACITY]: There is no cost to you for study related visits, the vaginal ring, physical/pelvic examinations, laboratory tests or other procedures. Treatments available to you from the study site for infections passed through sex will be given to you free of charge or you will be referred for available treatment for the duration of the study.

REIMBURSEMENT

[SITE TO INSERT INFORMATION ABOUT LOCAL REIMBURSEMENT]: You will receive **[SITE TO INSERT AMOUNT \$XX]** for your time, effort, and travel to and from the clinic at each scheduled visit. You may receive **[SITE TO INSERT AMOUNT \$XX]** for any visits which occur in between your normally scheduled visits.

CONFIDENTIALITY

Efforts will be made to keep your information confidential. However, it is not possible to guarantee confidentiality. Your personal information may be disclosed if required

by law. The study staff may use your personal information to verify that you are not in any other research studies. This includes studies conducted by other researchers that study staff may know about. Any publication of this study will not use your name or identify you personally.

Your records may be reviewed by:

- Representatives of the US Federal Government, including the US Food and Drug Administration (FDA), US Office for Human Research Protections (OHRP), NIH and/or contractors of NIH, and other local and US regulatory authorities
- Merck, the company that supplies the vaginal rings
- Study monitors
- Site IRB/EC
- Study staff

[SITE TO INCLUDE/AMEND THE FOLLOWING]: [LOCAL/STATE/NATIONAL] regulations require study staff to report the names of people who test positive for HIV and other infections passed during sex to the ***[LOCAL HEALTH AUTHORITY]***. Outreach workers from the ***[LOCAL HEALTH AUTHORITY]*** may then contact you about informing your partners, since they also should be tested. If you do not want to inform your partners yourself, the outreach workers will contact them, according to the confidentiality guidelines of the ***[HEALTH AUTHORITY]***.

The researchers will do everything they can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have obtained a Certificate of Confidentiality from the US Federal Government. This Certificate protects study staff from being forced to tell people who are not connected with this study, such as the court system, about your participation or information you give for study purposes. However, if the study staff learns of possible child abuse and/or neglect or a risk of harm to you or others, they will be required to tell the proper authorities. This Certificate does not prevent you from releasing information about yourself and your participation in the study.

RESEARCH-RELATED INJURY

[SITE TO SPECIFY INSTITUTIONAL POLICY]: It is unlikely that you will be injured as a result of study participation. If you are injured, the ***[INSTITUTION]*** will give you immediate necessary treatment for your injuries. You ***[WILL/WILL NOT]*** have to pay for this treatment. You will be told where you can receive additional treatment for your injuries. The U.S. National Institutes of Health (NIH) does not have a mechanism to pay money or give other forms of compensation for research related injuries. You do not give up any legal rights by signing this consent form.

YOUR RIGHTS AS A RESEARCH PARTICIPANT/VOLUNTEER

[SITE TO SPECIFY INSTITUTIONAL POLICY]: Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. If you choose not to participate or to leave the study, you will not lose the benefit of services to which you would otherwise be entitled at this clinic. If

you want the results of the study after the study is over, let the study staff members know.

PROBLEMS OR QUESTIONS

If you ever have any questions about the study, or if you have a research-related injury, you should contact ***[INSERT NAME OF OF THE INVESTIGATOR OR OTHER STUDY STAFF]*** at ***[INSERT TELEPHONE NUMBER AND/OR PHYSICAL ADDRESS]***.

If you have questions about your rights as a research participant, you should contact ***[INSERT NAME OR TITLE OF PERSON ON THE IRB/EC OR OTHER ORGANIZATION APPROPRIATE FOR THE SITE]*** at ***[INSERT PHYSICAL ADDRESS AND TELEPHONE NUMBER]***.

CONSENT FOR LONG-TERM STORAGE AND FUTURE TESTING OF SPECIMENS

There might be a small amount of blood, vaginal fluid, cervical tissue and cervical fluid left over after we have done all of the study related testing. We would like to ask your permission to store these leftover samples and related health information for use in future studies. This health information may include personal facts about you such as your race, ethnicity, sex, medical conditions and your age range. If you agree, your samples and related health data will be stored safely and securely at facilities that are designed so that only approved researchers will have access to the samples. Some employees of the facilities will need to have access to your samples to store them and keep track of where they are, but these people will not have information that directly identifies you. You can still enroll in this study if you decide not to have leftover samples stored for future studies. If you do not want the leftover samples stored, we will destroy them. The type of testing planned for your leftover specimens is not yet known. However, no genetic testing on either a limited set or the full set of genes is planned for leftover specimens that are stored for the purposes of future research. It is important that you know that any future testing or studies planned for these specimens must be approved by an Ethics Committee or Institutional Review Board before they can be done. You can withdraw your consent for the storage and future testing of specimens at any time by providing your request in writing to the person in charge of this study. However, researchers will not be able to destroy samples or information from research that is already underway.

Initials and Date

I DO agree to allow my biological specimens and health data to be stored and used in future research studies.

Initials and Date

I DO NOT agree to allow my biological specimens and health data to be stored and used in future research studies.

SIGNATURES- VOLUNTARY CONSENT

[INSERT SIGNATURE BLOCKS AS REQUIRED BY THE LOCAL IRB/EC]: If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to the study, please sign your name or make your mark below.

Participant Name (print)	Participant Signature/Mark	Date
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Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date
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Witness Name (print)	Witness Signature	Date
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