Safety and Pharmacokinetics of Two Vaginal Film Formulations Containing the Integrase Inhibitor MK-2048 (FAME103)

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A RANDOMIZED, DOUBLE BLINDED STUDY OF THE SAFETY AND PHARMACOKINETICS OF TWO VAGINAL FILM FORMULATIONS CONTAINING THE INTEGRASE INHBITOR MK-2048

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A randomized, double blinded study of the safety and pharmacokinetics of two vaginal film formulations containing the integrase inhibitor MK-2048

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Protocol Title: A randomized, double blinded study of the safety and pharmacokinetics of two vaginal film formulations containing the integrase inhibitor MK-2048

DAIDS #: DAIDS-ES # 38680

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I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

_____

Protocol Chair (PRINT NAME)

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Signature of Protocol Chair

_____

Date (Month/Day/Year)

A randomized, double blinded study of the safety and pharmacokinetics of two vaginal film formulations containing the integrase inhibitor MK-2048:
FAME 103

LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse event
AIDS	Acquired Immunodeficiency Syndrome
ARV	Antiretrovirals
BV	Bacterial vaginosis
CVL	Cervicovaginal lavage
CT	*Chlamydia trachomatis*
DAIDS	Division of AIDS
EAE	Expedited adverse event
GC	Neisseria gonorrhoeae
HIV	Human Immunodeficiency Virus
HSV-1, HSV-2	Herpes Simplex Virus type 1, type 2
IRB	Institutional Review Board
ITT	Intent-to-treat
N-9	Nonoxynol-9
NGU	Nongonococcal urethritis
NIAID	National Institute of Allergy and Infectious Disease
NIH	National Institutes of Health
PCR	Polymerase chain reaction
PSRT	Protocol Safety Review Team
RSC	Regulatory Support Center
SOP	Standard operating procedure(s)
STI	Sexually transmitted infection

A randomized, double blinded study of the safety and pharmacokinetics of two vaginal film formulations containing the integrase inhibitor MK-2048: FAME 103

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A randomized, double blinded study of the safety and pharmacokinetics of two vaginal film formulations containing the integrase inhibitor MK-2048: FAME 103

PROTOCOL SUMMARY

Short Title: FAME 103

Protocol Chair: Katherine Bunge, MD MPH

Protocol Co-chair: Sharon Hillier, PhD

Sample Size: Approximately 48

Study Population: HIV-uninfected women, 18 – 45 years old

Study Sites: UPMC Magee-Womens Hospital, Pittsburgh, PA

Study Design: A phase I, 2 arm, single site, randomized clinical trial

Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Screen	Enroll Day 0	Day 3	Day 5	Day 7	Day 10	Day 14	Day 28

Study Duration: Accrual of approximately 48 participants is expected to take 9 months. The expected duration of study participation for each participant will be approximately 5-10 weeks. This includes the screening period. After enrollment, the study duration is 4 weeks

Study Products: One dose MK-2048 vaginal film (2" x 2" size). Participants will be randomized to one of two formulations- high and low ammonio methacrylate copolymer type B (Eudragit®). Both films contain 30mg of MK-2048.

Study Regimen: All participants will be randomized to receive a single dose of a vaginal film containing MK-2048 (either the high Eudragit® or low Eudragit® formulation). The film will be inserted by a clinician on the day of enrollment. Vaginal swabs and plasma will be collected at days 0, 3, 5, 7, 10, 14 and 28 for all participants. On the day of enrollment, some participants may elect to participate in immediate post insertion sample collection (vaginal swab and plasma) at one or two time points within 6 hours of insertion. Genital biopsy samples and a rectal swab will be obtained on day 7, and cervicovaginal lavage will be collected at screening and days 14 and 28.

Primary Objectives:

- To assess the safety of a single dose of two formulations of an MK-2048 vaginal film which is intended for extended release of the antiretroviral drug

Endpoints:

- Grade 2 or higher Adverse Events

Secondary Objectives:

- To describe the pharmacokinetics of MK-2048 drug at 0, 3, 5, 7, 10, 14, and 28 days after film insertion
- To describe the acceptability of two formulations of the MK-2048 vaginal film and identify qualities associated with higher or lower degrees of acceptability

Secondary Endpoint:

- MK-2048 concentrations in plasma, cervical tissue homogenate, CVL, rectal and vaginal swab eluents
- Self-reported assessment of qualities of the experience with the films drawn from existing survey on microbicidal films, and general acceptability ratings drawn from market research methodology

Exploratory Objectives:

- To compare the effects of two formulations of the MK-2048 vaginal film on the vaginal microbiota and glycome pre and post study product use
- To describe the pharmacokinetics of Eudragit® polymer to ensure that polymer persistence is proportional to drug release.
- To describe the protective effect of MK-2048 film against HIV in an *ex vivo* biopsy challenge model using one cervical tissue biopsy

Exploratory Endpoints:

- Vaginal Microenvironment
    - Quantitative vaginal cultures and quantitative PCR for selected microbiota from vaginal swab
    - Nugent score from vaginal swab
    - Levels of anti-HIV viral activity in CVL
    - Levels of glycoproteins and mucins in CVL
    - Levels of biomarkers of innate immunity in CVL
- Eudragit® pharmacokinetics
    - Eudragit® levels in CVL
- Ex vivo challenge: HIV infection of cervical tissue as measured by HIV-1 p24 replication by ELISA and confirmed either by immunohistochemistry or quantitative PCR assessment of integrated provirus in HIV-1 exposed cervical biopsies

1 KEY ROLES

1.1 Protocol Identification

Protocol Title: A randomized, double blinded study of the safety and pharmacokinetics of two vaginal film formulations containing the integrase inhibitor MK-2048

Short Title: FAME 103

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2 INTRODUCTION

2.1 HIV prevention and microbicides

Each year millions of people worldwide are newly infected with HIV, most recently 1.7 million people in 2017[1]. Sexual transmission accounts for the majority of new HIV infections worldwide. Women are disproportionately impacted by the sexual transmission of HIV [2]. This disproportionate impact arises from a combination of biologic, social, and economic factors. Women at risk face formidable challenges in protecting themselves from HIV infection, including power differentials, gender norms, and economic dependence.

Topical microbicides are agents designed to prevent or substantially reduce the acquisition and transmission of HIV when applied to the genital or gastrointestinal mucosa. In addition to preventing HIV infection, topical microbicides may satisfy two critical needs: affordability and female control. Topical microbicides also have the potential for high acceptability for both male and female partners. Acceptability and utility of topical microbicides can be maximized by the development of an array of dosing formulations. For all dosing formulations, safety, stability, and efficacy must be established.

Vaginal films are an attractive dosing option for several reasons. In terms of manufacturing, films are inexpensive, scalable, physically and chemically stable, uniform with regards to product content, and amenable to combinations of active ingredients. In terms of use, films are discreet, portable, and easy to store. Vaginal films can deliver fixed doses of microbicidal agents with minimal mess and without an applicator. The small volume of vaginal films may also result in less dilution of endogenous antiviral or antibacterial properties of vaginal fluids compared to vaginal gels. In studies of spermicidal film, women have found vaginal films to be acceptable[3-5]. When Vaginal Contraceptive Film (VCF) was compared to foaming tablets containing nonoxynol-9 (N-9), contraceptive film was preferred in three different countries [3]. Ninety-seven percent of 59 Mexican women, 60 percent of 52 Dominican women, and 86 percent of 51 Kenyan women reported liking contraceptive film. In particular, they cited the following favorable characteristics: ease of use, general feel or comfort, additional lubrication, and lack of side effects [3]. Over eighty percent of Cameroonian sex workers, who participated in a large randomized placebo-controlled trial of N-9 film, reported that they would use the film were it found to be effective against HIV[4]. In Zambian focus groups, placebo vaginal film was preferred over placebo vaginal tablets and soft-gel capsules after each product was used once daily for one week [5]. In a study evaluating the acceptability of placebo microbicidal film, soft gel capsule and tablet, African women were instructed to use each of the three products once daily for 7 consecutive days. The film and soft-gel capsule were chosen significantly more often than the tablet as the preferred dosage form (39%, 37% and 25%, respectively). In this particular study, film and soft gel were preferred because of faster dissolving time and easier insertion[5]. In other studies, participants found vaginal films to be less messy than foaming tablets or gel[3,6]. The Quatro study was a randomized crossover study conducted in Zimbabwe and South Africa to assess relative preferences of four vaginal placebo dosage forms. Two hundred participants were asked to use pre-coitally inserted film, vaginal table and gel once per week for a month, and a monthly ring (for the entire month) during a four-month crossover period. Participants subsequently chose one preferred product for the final study month. Preferences varied within and across countries, and there was no clear favorite reiterating the need for a variety of products to meet different women's preferences Just under half of the women in Zimbabwe chose the vaginal film and ranked it their favorite product; in South Africa, the vaginal tablet was the most preferred[7].

2.2 Integrated Preclinical/Clinical Program

The Film Antiretroviral Microbicide Evaluation (FAME) program brings together an inter-disciplinary group of research scientists from four academic institutions: University of Pittsburgh, University of Washington, Carnegie Mellon University, and the University of Colorado. The long-term goal of the program is to develop and evaluate an extended delivery vaginal film containing an integrase inhibitor which could provide protection from HIV for one week or more following a single application. The first iteration of the FAME IPCP established proof of concept that vaginal films containing the antiretrovirals (ARVs) dapivirine and tenofovir could deliver drug to target tissues as effectively as gel formulations of those same ARVs. The second iteration of the program featured FAME-101, a clinical trial which established proof of concept for the extended release placebo vaginal film. In FAME-101, 64 participants received a single dose of the extended release vaginal film and were randomized to the timing of follow-up (3,7,10 or 14 days). The extended release placebo film was well tolerated and visible in 56% of the participants randomized to follow-up at 7 days and 25% randomized to follow up at 14 days. The goal of the second iteration of the FAME IPCP is to leverage this extended release film platform to establish proof of concept that an extended release formulation of an antiretroviral drug could provide coverage for 7 days or longer after a single application. An extended release formulation could address the challenges associated with daily use of products which have undermined adherence, and therefore effectiveness, in clinical trials. For this proof of concept study, the integrase inhibitor MK-2048 will be evaluated in the extended release film formulation although the extended release film format could be used as a delivery method for a wide range of antiretroviral drugs.

2.3 Rationale

This is a phase I randomized trial assessing the safety and acceptability of two formulations of MK-2048 vaginal film. As with all phase 1 studies, the primary objective is to evaluate the safety of the product. However, the overarching objective of this study is to provide proof of concept that an ARV can be delivered in an extended release film formulation to provide drug delivery for 7 days or more after a single application. Whether or not Merck elects to pursue development of MK-2048, the extended release film formulation could provide a novel means to deliver antiretroviral drugs vaginally.

This study is intended to provide proof of concept that an extended release film could provide 7 days of drug delivery. Despite the shift in HIV prevention biomedical interventions towards long-acting methods, there is still a demand for short acting methods. Some women prefer shorter acting interventions because they don't want long-term systemic exposure to medication. With a 7-day product which women control themselves, it would be possible to provide protection at times of increased risk without necessarily being coitally dependent. In a survey of 300 women conducted as part of the FAME program, 73% of women preferred a prevention method that would be available "when needed" and more women reported that seven days of protection would be preferable to one day so that a woman might use the product once and not have to worry about redosing for a week (Downs, unpublished data).

MK-2048 is a potent second-generation integrase inhibitor that was advanced as a clinical candidate in 2005. However, in a single dose oral Phase 1 study, MK-2048 failed to achieve target plasma concentrations and further development was discontinued. Importantly, MK-2048 remains an attractive choice for topical microbicide applications because it is highly potent against both wild type HIV-1 and raltegravir-resistant isolates, it is not currently being used for treatment and it has demonstrated a good safety profile in preclinical and clinical studies[7]. Free drug in ectocervical and colorectal explants demonstrated anti-viral activity. Full protection (6/6 explants protected) was noted in the colorectal model at 1 μM; partial protection (4/6 explants

protected) was noted in the polarized ectocervical model at 100 µM. In an ex vivo challenge model conducted in eleven Chinese rhesus macaques, the level of virus growth in the tissue biopsies of MK-2048 gel-treated animals (n=5) was lower than that observed in tissues from the naïve (n=3) and placebo-treated animals (n=3). (DAIDS K-28 Gel Study, study identification #: 14004-MK-2048 Biopsy Challenge Study dated February 20, 2019). Of note, protection was not noted in the ex vivo challenge model performed in the MTN-027 study of the intravaginal ring containing 30 mg of MK-2048 which released drug over 28 days of use.

The current study will compare two extended release films each containing 30 mg of MK-2048 to be released over 7 days of use. The two films having either high (12.8%) or low (6.4%) Eudragit® formulations differ with respect to dissolution rate and spreadability. In *in vitro* dissolution testing, high Eudragit® film dissolves slower than the low Eudragit® film and shows decreased spreadability. Macaque data included in the investigators' brochure includes data from pig-tailed macaques which support continued release of MK-2048 over a period of 7 days. [8]

The safety of the film polymer has been established in a previous clinical trial (FAME-101) and the safety of vaginal MK-2048 has been established in preclinical animal studies as well as two clinical trials (MTN-027 and MTN-028). The established safety record of these products supports the direct comparison design of this trial without the need for a placebo arm.

The primary objective of the proposed study will be to evaluate the safety of two formulations of MK-2048 vaginal film which differ with respect to rate of dissolution.

Secondary objectives include evaluating the pharmacokinetics of the two film formulations as well. Understanding the immediate release profile of the films and how long the drug remains in the vagina will be integral to the study.

In addition, the products' acceptability and the films' impact on the vaginal microenvironment, cellular immunity, inflammation and innate anti-HIV activity will also be assessed.

Because the primary objective is safety and one of the secondary objectives is pharmacokinetics, the active vaginal film will be inserted by a clinician on the day of enrollment.

2.4 Safety Data: Placebo Eudragit® platform

The Eudragit® film has a proven safety record.

Pre-Clinical Safety Data

Safety evaluation of the extended release placebo film was performed in a nonhuman primate model. Six pigtailed macaques were exposed to two films per week for two weeks, which is double the proposed human dosing (intended dosing is one film per week). In total, four films were delivered to each macaque over fourteen days. The vaginal microbiota, vaginal pH, and number of PMNs on gram stained vaginal smears were assessed before the first film placement and after the fourth film placement. The integrity of the cervicovaginal tissues was assessed by colposcopy at each film placement. Of note, there was residual film visible in the vagina with each new film placement. No adverse events were noted by colposcopy; microbiota remained stable with no effect noted on the beneficial H202 producing microorganisms; vaginal pH remained within normal range; and PMN counts remained within the normal range even after more frequent administration than intended for use (Patton, personal communication). The entire study report is presented in Appendix IV.

Clinical Safety Data

FAME-101 was a safety and acceptability study of the high (12.8%) Eudragit® placebo vaginal film. Sixty-four HIV uninfected, healthy women were enrolled. At enrollment the participants were randomized to timing of the follow-up visit. Participants returned either 3,7,10 or 14 days after film placement depending on their randomization and then all participants returned for a final safety assessment three weeks after enrollment. Safety assessments, acceptability parameters, and changes to the vaginal microenvironment were assessed at each visit. The persistence of the film by visual examination was noted as well.

Eighty-four participants were screened and 64 enrolled. The median age was 26.5 years; 56% were white and 27% non-Hispanic black. The primary endpoint was Grade 2 or higher AEs. There were 6 Grade 2 events; these included bacterial vaginosis, vaginal itching, pelvic cramping (2), friable cervix and adnexal pain. Of these, only bacterial vaginosis and vaginal itching were attributed to study product use. Forty-seven AEs were reported amongst 64 participants. Thirty-one participants (48.5%) reported at least one AE; 22 reported at least one urogenital event. For the women randomized to the 3 and 7 day follow up visit approximately 50% of the participants had visible film on speculum exam (44% and 56%). In summary, the high Eudragit® film was very well tolerated with no concerning safety signals.

2.5 Safety and PK Data: MK-2048

Pre-Clinical Safety and PK Data

MK-2048 was formulated as a gel to evaluate the potential local effects, systemic toxicity and toxicokinetics in rats and rabbits when administered intravaginally for 28 days. Gels containing 0.059%, 0.117%, and 0.234% MK-2048 were developed for daily topical and vaginal administration. In the 4-week rat study (study no. 1726-026) there was no evidence of systemic toxicity attributable to MK-2048 following daily intra-vaginal administration for 28 days. MK-2048 appearance in plasma was rapid, with mean maximum concentrations of 0.38 to 0.76 µM (0.234% gel dose) that were achieved by 0.5 to 2 hours post dose. However, MK-2048 disappeared rapidly from plasma with mean trough concentrations (i.e., C24h) that were <LLQ (LLOQ = 0.0108 µM) at all doses. The compound was well tolerated as there were no untoward local effects in vaginal tissues. Because of the absence of any local or systemic toxicity in the rat model, the high dose gel of 0.234% MK-2048 is considered the no observed adverse effect level (NOAEL).

Ten-day (study no. 1726-020) and 28-day (study no. 1726-027) Good Laboratory Practices (GLP) intravaginal studies were conducted in female rabbits in which gels containing 0.059%, 0.117%, and 0.234% MK-2048 were administered daily. In the initial 10-day vaginal irritation study, changes within female reproductive tissues occurred with similar incidence and severity in rabbits receiving gels containing MK-2048 compared to placebo gel. Because of the absence of a dose-effect relationship and the absence of a difference between the placebo and the MK-2048-treated groups, the dose of 0.234% is considered the NOAEL for MK-2048[9].

In the 28-day GLP rabbit study (study no. 1726-027) there was no evidence of systemic toxicity following intra-vaginal administration of MK-2048. There were no test article-related gross or microscopic findings in tissues. Administration of MK-2048 gels at the higher concentrations of 0.234% and 0.117% had subtle effects on the distal mucosa characterized by leukocyte infiltration and/or focal erosion of distal mucosa. Therefore, the low concentration of MK-2048 at 0.059% is the NOAEL, which is the equivalent of a 5.5 mg/kg total cumulative dose corresponding to a dose multiple of 11. Although findings were reported at the higher dose levels for MK-2048 in the proximal mucosa of rabbits, the proximal mucosa in the rabbit is a unique tissue and is not representative of vaginal tissues in other species, including women. In summary, the absence of relevant changes in distal mucosa and stratified squamous epithelium of the rabbit and the

absence of changes in both the rat and sheep models, which are more representative of vaginal tissues in women, are more relevant findings.

Data generated in macaque nonclinical studies provide evidence of local tolerability of MK-2048 extended release films in the vagina. Human 2"x2" sized extended release films were produced for animal studies. Films were cut to 1"x1" sized extended release film so as to be compatible with pigtail macaques. In six macaque studies, in which 23 animals received extended release MK-2048 films, there were no safety concerns[8].

The highest concentration administered to macaques during nonclinical studies of MK-2048 extended release film was 25 mg. This is comparable to 100 mg of human dose based on 4x scaling between macaque and human microbicide products. This has been a general conversion factor (2-4x) used among researchers in this field based on anatomical scaling. At this dose, no local toxicity in vaginal tissue nor adverse events during the follow-up of non-human primates were reported by colposcopy analysis of vaginal tissues. Macaques exposed to extended release film loaded with 7.5 or 25 mg of MK-2048 experienced good local tolerability (observations with colpophotograpy) with no tissue adverse responses.

The observation of the presence of the extended release film in the vagina using colpophotography documented the appearance of film product as long as it is distinct from vaginal exudate. The presence of the film was observed for a week or more in the majority of animals in all nonclinical studies performed in macaques. Notably, film product was retained during menstruation. Further, in the coital study, the films remained in place even after animals had coitus.

The administration of MK-2048 extended release film *via* intravaginal route in macaques led to drug plasma levels similar to those observed with ring studies. These plasma concentrations have not led to any safety issues and were much lower than those observed in rat nonclinical study.

To compare to these results, no systemic toxicity was reported in oral safety studies demonstrating MK-2048 plasma concentrations of 0.443 - 3.1 ng/mL are unlikely to evoke any safety issues[8].

Clinical Safety and PK Data

MTN-027 was a multi-site, single-blind, four-arm, randomized, placebo-controlled Phase 1 safety and PK trial of the vicriviroc (VCV)(MK4176) vaginal ring containing 182 mg vicriviroc (MK-4176); the MK-2048 vaginal ring, containing 30 mg MK-048; the MK-2048A vaginal ring, containing 182 mg vicriviroc (MK-176) and 30 mg MK-2048; and the Placebo vaginal ring[10]. The study enrolled 48 healthy, 18-45 year old women who were HIV-uninfected, non-pregnant, sexually abstinent, and using adequate contraception. Women were randomized to one of four study regimens in a 1:1:1:1 ratio. The vaginal ring was worn for approximately 28 consecutive days. The primary objectives were to assess the safety and to determine the concentration of drug in vaginal fluid, plasma and cervical tissue after 28 days of continuous use, followed by 7 days off product. Safety was evaluated as the proportion of women with related genitourinary AEs and proportion of women with any grade 2 or higher AEs. Secondary objectives were to evaluate acceptability and adherence over 28 days of use.

Thirteen Grade 1 and 2 Grade 2 related genitourinary AEs were observed in 9 women. Of the Grade 1 AEs, vaginal burning, pruritis, and application site erythema and pain were noted. Both Grade 2 related genitourinary AEs were due to vulvovaginal candidiasis. There were no statistically significant differences in the number of participants with related genitourinary AEs or any Grade 2 AEs between the placebo arm and treatment arms. There were no Grade 3 or higher AEs.

MK-2048 peak concentrations, from both the single and combination IVR, were substantially higher in vaginal fluids than in plasma (30× higher) and rectal fluid suggesting less risk for systemic toxicity Plasma MK-2048 concentration with the MK-2048-alone vaginal ring achieved peak concentrations more rapidly when compared to the combination vaginal ring (Tmax median 27 and 47 hours, respectively), followed by a similar decline in concentration after VR removal (median terminal decay half-life of 3 and 2 hours, respectively). The antiviral activity of MK-2048 was not correlated with tissue-associated drug concentrations in an ex vivo HIV-1 challenge assay.

MTN-028 was a phase 1, single-blind, 2-arm PK trial of 2 combination vaginal rings containing different dose strengths of VCV and MK-2048[11]. Eighteen women were randomized 2:1 to a low-dose ring, containing 91 mg of VCV and 10 mg of MK-2048, or the original-dose ring, containing 182 mg of VCV and 30 mg of MK-2048. Participants used the rings continuously for 28 days and were followed up for approximately 7 days after ring removal.

There were a total of 2 grade 1 and 3 grade 2 product-related genitourinary AEs observed in 5 women. These AEs occurred in 3 participants (23%) in the low-dose arm (application site pain, vulvovaginal pruritis, and dysmenorrhea), and 2 (33%) in the original-dose arm (diarrhea and vaginal discharge). There were an additional 7 unrelated grade 2 AEs (5 in the low-dose arm; 2 in the original-dose arm); no grade ≥3 AEs were reported. There were no statistically significant differences in the number of participants with product-related genitourinary AEs (3 of 13 vs 2 of 6 participants) or any grade ≥2 AEs (6 of 13 vs 3 of 6) between the low-dose and original-dose arms, respectively ($P > .99$).

The original dose ring treatment arm offered higher plasma AUC, cervical tissue concentration, and greater drug released (based on residual drug levels). Both arms had similar CVF AUCs. Plasma and CVF concentrations of MK-2048 decreased rapidly after ring removal. The extended release films containing 30 mg of MK-2048 will deliver this drug over a shorter period of time (7 days) vs the 28 day ring, effectively delivery a higher level of drug daily than was possible using the ring. The preclinical data from the pigtailed macaques suggest that levels of MK-2048 in the tissues and vaginal fluid can be achieved after film administration which are similar to those which have been reported to correlate with protection for other integrase inhibitors such as raltegravir.[12]. For that reason, the proposed study will have value for the HIV prevention field in providing additional data on topical delivery of integrase inhibitors as well as the use of extended release film formulations for vaginal delivery of ARVs.

2.6 Summary

The current study is a proof of concept study to determine whether an extended release film could deliver drug for seven days. Two film formulations which differ by dissolution and spreadability will be compared. It is appropriate to advance these two formulations of MK-2048 film into a clinical trial for safety given that the individual ingredients used in the film have a history of safe use in the vaginal compartment. The extended release platform may prove to be an effective drug delivery system with the potential to be used with many medications

3 OBJECTIVES

3.1 Primary Objectives:

- To assess the safety of a single dose of two formulations of an MK-2048 vaginal film which is intended for extended release of antiretroviral drugs.

3.2 Primary Endpoints:

- Grade 2 or higher Adverse Events

3.3 Secondary Objectives:

- Pharmacokinetics: To describe the pharmacokinetics of MK-2048 drug at 0, 3, 5, 7, 10, 14, and 28 days after film insertion

- Acceptability: To describe the acceptability of two formulations of the MK-2048 vaginal film and identify qualities associated with higher or lower degrees of acceptability

3.4 Secondary Endpoint:

- MK-2048 concentrations in plasma, tissue homogenate, CVL, and rectal and vaginal swab eluents, comparing the two film types

- Acceptability: Self-reported assessment of qualities of the experience with the films drawn from existing survey on microbicidal films, and general acceptability ratings drawn from market research methodology

3.5 Exploratory Objectives:

- To compare the effects of two formulations of the MK-2048 vaginal film on the vaginal microbiota and glycome pre and post study product use
- To describe the pharmacokinetics of Eudragit® polymer to ensure that polymer persistence is proportional to drug release.
- To describe the protective effect of MK-2048 film against HIV in an *ex vivo* biopsy challenge model using cervical tissue

3.6 Exploratory Endpoints:

- Vaginal Microenvironment
    - Quantitative vaginal cultures and quantitative PCR for selected microbiota from vaginal swab
    - Nugent score from vaginal swab
    - Levels of anti-viral activity in CVL
    - Levels of glycoproteins and mucins in CVL
    - Levels of biomarkers of innate immunity in CVL
- Eudragit® pharmacokinetics
    - Eudragit® levels in CVL
- Ex vivo challenge: HIV infection of cervical tissue as measured by HIV-1 p24 replication by ELISA and confirmed either by immunohistochemistry or quantitative PCR assessment of integrated provirus in HIV-1 exposed cervical biopsies

4 STUDY DESIGN

4.1 Identification of Study Design
This is, single site, double blinded study.

4.2 Summary of Major Endpoints
Grade 2 or higher Adverse Events

4.3 Description of Study Population
The study population will be HIV-uninfected women who meet criteria outlined in Section 5.

4.4 Time to Complete Accrual
Accrual is expected to be completed in approximately 9 months.

4.5 Study Groups
All participants will be randomized to one of two MK-2048 films: a high Eudragit® film or a low Eudragit® film.

4.6 Expected Duration of Participation
The duration of study participation per woman is expected to be approximately 5-10 weeks. This includes the screening period.

4.7 Site
There is a single study site: UPMC Magee-Womens Hospital, Pittsburgh, PA.

5 STUDY POPULATION

5.1 Selection of the Study Population
The inclusion and exclusion criteria in this section will be utilized to ensure the appropriate selection of study participants.

5.2 Recruitment
Participants will be recruited from a variety of sources including the ambulatory clinic of Magee-Womens Hospital and the surrounding population. Participants will also 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 used that have been shown to be successful in several prior studies of topical microbicides. Recruitment materials will be approved by the University of Pittsburgh Institutional Review Board.

5.3 Retention
Once a participant is enrolled, the study site will make every effort to retain her in follow-up to minimize possible bias associated with loss-to-follow-up. The site will implement the following procedures to enhance retention:

- Thorough explanation of the study visit schedule and procedural requirements during the informed consent process and re-emphasis at each study visit.
- Thorough explanation of the importance of both groups to the overall success of the study.
- Collection of detailed locator information at the study screening visits, and active review and updating of this information at each subsequent visit.
- Use of appropriate and timely visit reminder mechanisms.

- Immediate follow-up on missed visits.

Study sites will use a participant tracking mechanism to facilitate visit scheduling and timely identification and follow-up on missed visits.

5.4 Inclusion Criteria

Women must meet all the following criteria to be eligible for inclusion in the study:

1) Age 18 through 45 years (inclusive) at screening

2) Able and willing to provide written informed consent.

3) Willing to use an effective method of birth control throughout the duration of the study. Examples of effective methods include: hormonal methods (other than NuvaRing®), IUD, bilateral tubal ligation, same sex partner, partner with a vasectomy, abstinence (defined as no vaginal sex for one month prior to screening).

4) Able and willing to provide adequate locator information

5) HIV-uninfected based on testing performed by study staff at screening (per algorithm in Appendix II)

6) In general good health as determined by the site clinician

7) Agree to be sexually abstinent, including use of sex toys, from V2 (Enrollment) until V7 (7 days after the biopsy visit) and 48 hours prior to all study visits.

8) Agree to refrain from use of vaginal device or products (for example, lubricants, creams, suppositories) throughout participation in the study. Tampons may be used except between V5 (biopsy visit) and V7 (day 14).

9) Willingness to undergo all study-related assessments and follow all study-related procedures

10) At screening and enrollment, agrees not to participate in other research studies involving drugs, medical devices, or vaginal products while enrolled in this trial

11) Participants over the age of 21(inclusive) must have documentation of a satisfactory Pap within three years prior to Enrollment consistent with Grade 0 according to the Female Genital Grading Table for Use in Microbicide Studies Addendum 1 (Dated November 2007) to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, or satisfactory evaluation with no treatment required of grade 1 or higher Pap results. If no documentation of a Pap smear can be provided, a Pap smear will be collected at the screening visit.

5.5 Exclusion Criteria

Women who meet any of the following criteria will be excluded from the study. Of note, the study is limited to premenopausal women with an intact uterus because the mucosal immune environment differs substantially between pre- and post- menopausal women. Therefore, inclusion of post-menopausal women would introduce heterogeneity into the population.

1.) Menopause (as defined as amenorrhea for one year or more without an alternative etiology)

2.) Hysterectomy

3.) Participant report of any of the following:
   a) Known adverse reaction to any of the study products (ever)
   b) Non- therapeutic injection drug use in the 12 months prior to Screening
   c) Surgical procedure involving the pelvis in the 60 days prior to enrollment (includes dilation and curettage or evacuation, and cryosurgery; does not include cervical biopsy for evaluation of an abnormal pap smear)
   d) Participation in a drug, spermicide and/or microbicide study in the 30 days prior to enrollment
   e) Currently pregnant or pregnancy within 42 days prior to enrollment
   f) Currently lactating
   g) Use of a diaphragm, NuvaRing®, or spermicide for contraception

4.) Urogenital infection or suspected infection within 7 days of enrollment including: symptomatic candidiasis, trichomonas vaginalis, and symptomatic bacterial vaginosis; or cervical infection, including *N. gonorrhoeae* (GC), *C. trachomatis* (CT), or mucopurulent cervicitis; syphilis; HSV lesions, or other sores (Note: seropositive HSV without active lesions will not be excluded); acute pelvic inflammatory disease; urinary tract infection; recent exposure to a partner with GC, CT, Trichomonas, syphilis, or NGU

5.) Antibiotic or antifungal therapy (vaginal or systemic) within 7 days of enrollment

6.) As determined by the PI, has any significant uncontrolled active or chronic cardiovascular, renal, liver, hematologic, neurologic, gastrointestinal, psychiatric, endocrine *including poorly controlled diabetes), respiratory, immunologic disorder or infectious disease

7.) Menses-like bleeding at the time of the Enrollment visit* or expected menses-like bleeding within 14 days of the Enrollment visit
(*Women who have vaginal bleeding at the scheduled Enrollment visit may return at a different date to be re-examined and possibly enrolled provided they are still within the screening window and meet all criteria.)

8.) Any condition that, in the opinion of the Investigator, would preclude provision of consent, make participation in the study unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives

6 STUDY PRODUCT

6.1 Regimen

Each participant will receive one vaginal film containing MK-2048 in either a high dose Eudragit® (12.8%) film or low dose Eudragit® (6.4%) film

6.2 Administration

After eligibility is confirmed, site staff will randomize the participant to one of two film formulations: high dose Eudragit® or low dose Eudragit®. The site staff will retrieve the film from the pharmacy.

To ensure appropriate placement, the clinician will place the film in the proximal third of the vagina using a speculum.

6.3 Study Product Formulation

Participants will be randomized to one of two formulations- high (12.8%) or low (6.4%) Eudragit® polymer. Both films contain 30mg of MK-2048. The films are 2" x2."

Calculated composition of MK-2048 30 mg extended release vaginal film (low ammonio methacrylate copolymer type B)

Component	Quantity (mg/film)	Function
MK-2048	30.0	API
Hydroxypropyl methylcellulose E5 (VIVAPHARM® HPMC E5, Hypromellose 2910)	36.7	Film forming polymer/base
Hydroxyethyl cellulose (HEC, NATRASOLTM 250L PHARM)	36.7	Film forming polymer/base
Hydroxypropyl methylcellulose (BENECEL™ K4M HPMC Type 2208	45.4	Film forming polymer/base
Hydroxypropyl cellulose (HPC, KLUCEL™ JXF PHARM)	73.4	Film forming polymer/base
Ammonio methacrylate copolymer dispersion type B (Eudragit® RS 30D)a	41.5	Extended release polymer
Polyethylene glycol 400 (PEG 400)	39.3	Plasticizer/dispersant
Propylene glycol (PG)	28.7	Plasticizer/dispersant
Purified Water[b]	11.0	Solvent
Total unit weight		

Calculated composition of MK-2048 30 mg extended release vaginal film high ammonio methacrylate copolymer type B)

Component	Quantity (mg/film)	Function
MK-2048	30.0	API
Hydroxypropyl methylcellulose E5 (VIVAPHARM® HPMC E5, Hypromellose 2910)	30.1	Film forming polymer/base
Hydroxyethyl cellulose (HEC, NATRASOLTM 250L PHARM)	30.1	Film forming polymer/base
Hydroxypropyl methylcellulose (BENECEL™ K4M HPMC Type 2208	37.2	Film forming polymer/base
Hydroxypropyl cellulose (HPC, KLUCEL™ JXF PHARM)	60.3	Film forming polymer/base
Ammonio methacrylate copolymer dispersion type B (Eudragit® RS 30D)a	68.1	Extended release polymer
Polyethylene glycol 400 (PEG 400)	32.3	Plasticizer/dispersant
Propylene glycol (PG)	23.6	Plasticizer/dispersant
Purified Water[b]	10.0	Solvent
Total unit weight	321.7	

6.4 Study Product Supply and Accountability

Study product will be manufactured and package by Benefit Coatings, Stratford, CT, in compliance with cGMP. Product release testing includes GMP Microbial Limits Testing (USP61/62). After product release, the packaged study product will be provided to the UPMC Magee-Womens Hospital Pharmacy. All study product will be available to the study staff through the Magee Pharmacy.

The Magee research pharmacist will maintain complete accountability records of all study products received for this protocol and dispensed to participants. Additional documentation will be required for study product returns, destruction (if applicable) and other related issues as outlined in instructions for DAIDS clinical trials. All unused study products must be returned to the Pharmacy after the study is completed or terminated.

The film is packaged into a foil-lined pouch and stored at room temperature of 20-25°C (68-77°F). GMP stability testing will be conducted for the clinical trial product material. Research and development level stability testing is available for the film and shows the product to remain within target specifications at all time points evaluated to date.

6.5 Study Product Dispensing

Study products will be dispensed to clinic staff only upon receipt of a written prescription signed by an authorized prescriber.

6.6 Concomitant Medications

Enrolled study participants may use concomitant medications during study participation. All concomitant medications, over-the-counter preparations, vitamins and nutritional supplements, recreational drugs, and herbal preparations will be recorded on the concomitant medications log form.

All participants will be counseled to avoid the use of spermicide and other non-study vaginal products (other than tampons during menstruation and female condoms) while participating in the study. Participants who report use of these products will be counseled regarding the use of alternative methods; however reported use of these products does not require any change in study product administration or follow-up procedures. Condoms, if provided by study staff, will not be coated with any type of spermicide.

7 STUDY PROCEDURES

This section describes visit-specific study procedures.

7.1 Pre-Screening

As part of participant outreach and recruitment strategies, study staff may pre-screen potential study participants (e.g. via telephone). During these interactions, study staff may explain the study to participants and ascertain elements of presumptive eligibility, to be confirmed at an on-site screening visit. If the participant is eligible based on a screening script her name and appointment time will be placed on the script. If she signs consent, the telephone script will then become part of her research record. If she does not sign consent, the form will be de-identified.

7.2 Visit 1: Screening Visit

Screening may take place up to 45 days prior to Enrollment. Screening procedures may occur over several visits. A combined written informed consent for screening and enrollment will be obtained before any screening procedures are initiated. For participants who do not meet the eligibility criteria, screening will be discontinued once ineligibility is determined.

The table below outlines procedure to take place at the Screening visit.

Table 1: Visit 1 Procedures

VISIT 1: SCREENING VISIT	
Component	**Procedure/Analysis**
ADMINISTRATIVE	• Written Informed Consent • Assign Study Number (PTID) • Review Eligibility • Collect Contact Information • Medical history and concomitant medications • Visit Questionnaire • HIV Pre-/Post-Test Counseling • Screening Results • Protocol counseling (including effective birth control, sexual practices restrictions and vaginal product use) • Schedule Next Study Visit, prn • Participant Reimbursement
URINE	• Pregnancy test • Urine dipstick, as clinically indicated
SALIVA	• Rapid HIV
BLOOD	• Confirmatory HIV, as clinically indicated
VAGINAL	• pH • Wet Mount, as clinically indicated • Trichomonas (NAAT) • Vaginal sample for Future Use*
CERVICAL	• GC/CT (NAAT) • Cervical sample for Future Use* • Pap smear, as indicated
CVL	• Levels of antiviral activity • Levels of glycoproteins and mucins • Levels of cytokines & innate immunity factors • Assessment of drug assay specificity for MK-2048 and Eudragit®
PHYSICAL EXAM	• Physical Exam • Vital Signs (BP) • Height & Weight
PELVIC EXAM	• Bimanual Exam, as clinically indicated • External Genital & Speculum Exam

* For participants who have consented to the collection of samples for future use and use of left-over samples for future studies.

7.3 Visit 2: Enrollment

Subjects who meet the inclusion and exclusion criteria following the Screening visit may schedule an Enrollment visit. Care will be taken to schedule the Enrollment visit at a time when the participant is not expecting to be actively bleeding or anticipating her menses within 14 days.

Before performing any enrollment procedures, study staff will confirm eligibility. The participant will undergo a pelvic examination. Pelvic specimens as described below will be collected and the study staff will assess for bleeding. If no bleeding or other exclusionary findings are evident, the participant will then be randomized to study product. The act of randomization is considered the

act of enrollment for this study. Subjects with menses like vaginal bleeding at the Enrollment visit will not be randomized/enrolled at that time. These subjects may be rescheduled if vaginal bleeding resolves before 45 days from Screening have elapsed. If vaginal bleeding does not resolve during the window period, and the subject expresses interest in enrollment for the study, she may be rescreened.

After eligibility is confirmed, the site staff will go to the Pharmacy to obtain the study product. The clinician will insert a speculum and then place the film in the proximal third of the vagina under direct visualization per the SOP. After film placement, participants will be scheduled for Visit 3. Participants who agree to optional additional PK sampling will undergo blood draw and blind vaginal swab sampling for drug levels at 1-2 time points within the first 6 hours post insertion.

Table 2: Visit 2 Procedures

VISIT 2: ENROLLMENT – DAY 0 (Day 1 of Product Use)	
Component	**Procedure/Analysis**
ADMINISTRATIVE	• Review/Confirm Eligibility • Collect/Update Contact Information • Visit Questionnaire • Update medical history and concomitant medications • Screening Results • Protocol counseling (including effective birth control, sexual practices restrictions and vaginal product use) • Assess/Document Adverse Events • Schedule Next Study Visit • Participant Reimbursement
ACCEPTABILITY	• Acceptability Questionnaire (post film insertion)
BLOOD	• Baseline MK-2048 level • Optional MK-2048 level *
URINE	• Pregnancy test • Urine dipstick, as clinically indicated
VAGINAL	• pH • Wet Mount, as clinically indicated • Gram Stain • Quantitative Vaginal Culture • Quantitative PCR for microbiota • MK-2048 level • Optional vaginal swab(s) for MK-2048 ** • Vaginal sample for Future Use***
CERVICAL	• Cervical sample for Future Use***
PHYSICAL EXAM	• Physical Exam, as clinically indicated • Vital Signs (BP)
PELVIC EXAM	• Bimanual Exam, as clinically indicated • External Genital & Speculum Exam
RANDOMIZATION	• Randomization to study product
STUDY PRODUCT	• Study Product Distribution • Clinician-inserted with speculum

*Participants may opt to have one additional blood draw at 1, 2, 3, 4, 5 or 6 hours post film insertion for MK-2048 levels.
**Participants may opt to have additional vaginal swabs collected at one or two time points (1, 2, 3, 4, 5, or 6 hours) post film insertion. The swabs will be collected by a clinician without a speculum.
***For participants who have consented to the collection of samples for future use and use of left-over samples for future studies.

7.4 Visit 3, 4, 5, 6, 7 and 8: Follow-Up Visits

Visit 3, 4, 5, 6,7 and 8 procedures are outlined in Table 4. The windows around these study visits are as follows:
Visit 3: Day 3 (2-4)
Visit 4: Day 5 (5-6)
Visit 5: Day 7 (7-8)
Visit 6: Day 10 (9-12)
Visit 7: Day 14 (13-16)
Visit 8: Day 28 (24-32)
Two cervical biopsies will be obtained at Visit 5 only. A CVL will be performed at Visits 7 and 8. Residual drug, if present, will be removed during the CVL at Visit 7. The purpose of Visit 8 is to provide a final safety assessment.

Table 3: Follow-up Visit Procedures

VISIT 3, 4, 5, 6, 7, 8 FOLLOW-UP VISITS	
Component	**Procedure/Analysis**
ADMINISTRATIVE	• Collect/Update Contact Information • Visit Questionnaire • Update medical history and concomitant medications • Protocol counseling excluding Visit 8 (including effective birth control, sexual practices restrictions and vaginal product use) • Assess/Document Adverse Events • Schedule Next Study Visit (excluding Visit 8) • Participant Reimbursement
ACCEPTABILITY	• Acceptability Questionnaire (Visit 5 ONLY; prior to biopsy)
BLOOD	• MK-2048 level
URINE	• Pregnancy test (Visit 5 & 8 and as clinically indicated) • Urine dipstick, as clinically indicated
VAGINAL	• pH • Wet Mount, prn • Trichomonas (NAAT), as clinically indicated • Gram Stain • Quantitative Vaginal Culture • Quantitative PCR for microbiota • Vaginal swab for MK-2048 level • Vaginal sample for Future Use*
RECTAL	• Rectal swab for MK 2048 (Visit 5 ONLY)
CERVICAL	• GC/CT (NAAT), as clinically indicated • Cervical biopsies- two adequate (Visit 5 ONLY) • Cervical sample for Future Use*
CVL (Visits 7 and 8 only)	• Levels of antiviral activity • • Levels of glycoproteins and mucins • Levels of cytokines & innate immunity factors • MK-2048 level • Eudragit® levels
PHYSICAL EXAM	• Physical Exam, as clinically indicated • Vital Signs (BP)

PELVIC EXAM	• Bimanual Exam, as clinically indicated • External Genital & Speculum Exam

* For participants who have consented to the collection of samples for future use and use of left-over samples for future studies.

7.5 Participants Who Become Pregnant

Urine pregnancy tests will be performed at screening, enrollment, Visit 5, Visit 8, and as clinically indicated. Participants who become pregnant will be referred for care. Should the pregnancy be diagnosed during study follow-up, pelvic exams will only be performed to evaluate a participant's reported symptom. No genital specimens will be collected in a pregnant participant.
Participants will be terminated from the study but will be contacted after their study participation to ascertain the pregnancy outcome.

Study staff will report pregnancy details to the Antiretroviral Pregnancy Registry should an enrolled participant become pregnant during follow-up.

7.6 Interim Visits

Interim visits may be performed at any time during the study (and study procedures repeated as clinically indicated), for the following or other reasons:

- For administrative reasons, e.g., a participant may have questions for study staff, or may need to re-schedule a follow-up visit.
- In response to AEs. When interim contacts or visits are completed in response to participant reports of AEs, study staff will assess the reported event clinically and provide or refer the participant to appropriate medical care.
- In the event of laboratory processing issues (i.e. inconclusive STD testing, lost or inadequate sample
- For other reasons at participant request.

Details of the interim visit will be recorded in the chart notes.

7.7 Clinical Evaluations and Procedures

Physical exams will include the following assessments:

- Vital signs:
  - Blood pressure
- Measurements of:
  - Weight (at Screening only)
  - Height (at Screening only)
- Focused physical exam (at Screening only):
  - General appearance
  - Cardiac exam
  - Respiratory exam
  - Abdomen

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

7.8 Laboratory Evaluations

Local/Clinical Laboratory
Urine pregnancy test
Rapid saliva HIV test
Wet mount if clinically indicated
Vaginal pH
Dipstick urinalysis if clinically indicated
Confirmatory HIV testing if indicated
Pap smear if indicated

MWRI Laboratories
Cervical NAAT for chlamydia, gonorrhea
Vaginal NAAT for trichomonas
Vaginal Gram stain
Quantitative vaginal cultures
Quantitative vaginal PCR for selected microbiota
Levels of cytokines and innate immunity factors from CVL
Levels of glycoproteins and mucins in CVL
Anti-viral activity from CVL
Ex vivo HIV challenge of cervical tissue

University of Colorado Lab
MK-2048 levels in CVL, cervical tissue, rectal fluid, vaginal fluid and plasma
Eudragit® levels in CVL

7.9 Specimen Collection and Processing

The site will adhere to the standards of DAIDS good clinical laboratory practice and site standard operating procedures for proper collection, processing, labeling, handling, transport, and storage of specimens. In cases where laboratory results are not available due to administrative or laboratory error, sites are permitted to re-draw and/or re-collect specimens.

7.10 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study as recommended by the CDC and NIH. All biological specimens sent to the University of Colorado lab will be transported using packaging mandated by CFR 42 Part 72. Biohazardous waste will be contained according to institutional, transportation/carrier, and all other applicable regulations.

8 ASSESSMENT OF SAFETY AND CLINICAL MANAGEMENT

8.1 Safety Monitoring

The study site investigators are responsible for continuous close safety monitoring of all study participants, and for alerting the Protocol Team if unexpected concerns arise. A sub-group of the Protocol Team, including the Protocol Chair or designee, the DAIDS Medical Officer, and the External Safety Monitor (see section 8.2) will serve as the Protocol Safety Review Team (PSRT). Close cooperation among the PSRT and the study site will be necessary to monitor participant safety and respond to occurrences of toxicity in a timely manner. Appropriate safety monitoring

will be contingent upon excellent communication between study participants and study staff, and upon cooperation among study staff, investigators, the External Safety Monitor, and the DAIDS Medical Officer. Grade 3 or higher AEs will be reported by the site to the independent safety physician, DAIDS medical officer and Merck as appropriate no later than 48 hours after identification to ensure timely evaluation of safety data.

8.2 Clinical Data Safety Review

An External Safety Monitor who is familiar with the pertinent scientific literature related to the study product will be responsible for the first review of data and safety monitoring. This physician, independent of the study sponsor, will be available to monitor data from this site. His/her minimum qualifications will include experience as a physician and experience in the conduct of clinical research. This individual will not receive salary or other support from the grant. The External (Independent) Safety Monitor model has been used successfully for other Magee-Womens Hospital Reproductive Infectious Disease studies involving investigational products. The proposed individual will meet the qualifications outlined above and have training in the importance of the objective treatment of clinical safety data.

The Data Management team for this site will generate data summaries for the Protocol Chair, the External Safety Monitor, and the DAIDS Medical Officer on at least a monthly basis. More frequent ad hoc safety reviews can be implemented during the initial enrollment and throughout the study as needed. These data summaries will include adverse event, accrual and retention data. The External Safety Monitor will evaluate adverse event data independently as well to recommend to the PSRT whether the study protocol should continue as originally designed, should be changed, or should be terminated.

Approximately once a month the PSRT will convene via email or telephone to review adverse event data. Only masked safety data will be reviewed by the PSRT. If more urgent safety matters arise, telephone calls can occur more frequently.

The IRB will be notified of any serious and unexpected adverse events according to the policies outlined in the University of Pittsburgh IRB Policy and Procedure Manual[9].

The following information will be submitted to the University of Pittsburgh IRB at the time of renewal of a research protocol, as required by the IRB guidelines:

- The frequency of monitoring during the renewal interval, including the dates of data and safety monitoring;
- A summary of any assessment performed to evaluate external factors or other relevant information that may have an impact on the safety of study volunteers or the ethics of the research study;
- A summary of the outcome of procedural reviews conducted to ensure subject privacy and research data confidentiality;
- Any conclusions regarding changes to the anticipated benefit-to-risk ratio of study participation and final recommendations related to continuing, changing, or terminating the study, with accompanying rationales as appropriate.

Furthermore, should two Grade 3 or higher related AEs be reported, enrollment will be paused and an emergency meeting of the PSRT be called to order. The PSRT will review the cumulative data and give serious consideration to discontinuing enrollment.

8.3 Adverse Events Definitions

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 groups beginning from the time of randomization.

Study participants will be provided instructions for contacting the study site to report any untoward medical occurrences they may experience, except for possible life-threatening events, for which they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation at UPMC Magee-Womens Hospital, where the study clinicians are based, and to request that a study clinician be contacted upon their arrival. All participants reporting a clinically significant untoward medical occurrence will be followed either in person or by phone until the occurrence resolves (returns to baseline) or stabilizes over a four week period.

Study site staff will document AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product.

For each study participant, AE documentation and reporting will be undertaken throughout the scheduled duration of follow-up, i.e., through Visit 8.

The PI/designee will grade the severity of each AE and the relationship of the AE to study product:

AE severity will be graded per the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 2.1, July 2017 and the Female Genital Grading Table for Use in Microbicide Studies (Appendix 1, version 1.0, November 2007) except that asymptomatic BV will not be considered an AE. AEs not included in the Female Genital Grading Table will be graded by the DAIDS AE Grading Table Version 2.1, July 2017. 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.

The relationship of all reported AEs will be assessed based on the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, dated January 2010) and the clinical judgment of the PI/designee.

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)
The study products that must be considered when AE relationships are assigned are the MK-2048 films.

The DAIDS Table for Grading Adult and Pediatric Adverse Events, the Female Genital Grading Table for Use in Microbicide Studies is available on the DAIDS Regulatory Support Center (RSC) web site:https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables

All AEs will be captured on an AE log. AEs should be reviewed at each study visit and updated as needed. For any Grade 3 or higher AEs that are continuing at a participant's study exit visit, the Protocol Chair/designee must establish a clinically appropriate follow-up plan for the AE and

review with the DAIDS Medical Officer. At a minimum, the AE must be re-assessed by study staff at least 2 weeks after the participant's study exit visit (Visit 8); additional evaluations also may take place at the discretion of the PI/designee. The same approach must be taken for any AEs deemed related to study product that are still ongoing at the study exit visit (Visit 8). For those AEs requiring re-assessment, if the AE has not resolved or stabilized at the time of re-assessment, study staff will continue to re-assess the participant at least once per month while the study is ongoing.

8.4 Reporting of Unanticipated Problems and Serious AEs to DAIDS

Relevant unanticipated problems such as study product concerns will be reported to the DAIDS MO within 1 business day. The following information about the critical event will be submitted to DAIDS MO:

A detailed description of the problem and actions the study site is taking or plans to take to address the problem, such as suspending participant enrollment, termination of research, revising study protocol or informed consent documents, increasing monitoring of the study participant, etc. Any revisions to the study protocol and informed consent must be reviewed and approved by the DAIDS MO and study site IRB prior to implementation.

Serious or continuing noncompliance and suspension/termination of IRB approval must be reported as critical events as well.

Serious Adverse events will be reported to the DAIDS MO within 24 hours of the site becoming aware of the event. As per the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use ICH definition, a Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose: -results in death,

-is life-threatening
-requires inpatient hospitalization or prolongation of existing hospitalization,
-results in persistent or significant disability/incapacity,
Or
-is a congenital anomaly/birth defect

8.5 Clinical Management of Adverse Events

By definition, an adverse event can be either new finding or symptom or a worsening of a pre-existing condition. In order to accurately capture adverse events in follow-up, a thorough baseline history will be obtained at Visit 1 and Visit 2. For example, for participants who endorse a history of headache, site staff will probe for and record details surrounding the condition such as frequency, location, duration, medication use, triggers, etc. Only by eliciting a full description will study staff be equipped to determine whether a subsequent event in follow-up is a clinically distinct event or not.

Adverse events will be elicited at each follow-up visit. Referral to appropriate care will be offered to participants as needed The study site is well equipped to manage treatment of all sexually transmitted infections and vaginitis identified during screening or during study participation except for HIV. Participants who are found to be HIV infected will be referred for care. The PI/designee should manage STI/RTI per CDC guidelines, available at http://www.cdc.gov. Observed single dose treatment should be provided whenever possible. Vaginally applied medications should not be used if possible.

8.5.1 Product Hold

In the unlikely event that a participant is intolerant of the study product immediately after placement, the site clinician will perform a pelvic exam to remove all visible product.

8.6 Social Harms Reporting

Although study sites make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could become known as HIV-infected or at "high risk" for HIV infection). For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities and/or employer.

Social harms that are judged by the study site investigator to be serious or unexpected will be reported to the DAIDS Medical Officer and the responsible site IRB at least annually or according to individual IRB requirements. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant as needed

8.7 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. The Site PI/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. Participants also may be withdrawn if the study sponsors, government or regulatory authorities, including the Office of 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.

8.8 Reporting to the FDA

The IND holder will be responsible for reporting any safety concerns to the FDA.

9 STATISTICAL CONSIDERATIONS

9.1 Review of Study Design

The primary aim of this study is to assess the safety of a single dose of a MK-2048 vaginal film. All participants will be randomized to receive a single dose of a vaginal film containing MK-2048 (either the high Eudragit® or low Eudragit® formulation). The film will be inserted by a clinician on the day of enrollment. Vaginal swabs and plasma will be collected at days 0, 3, 5, 7, 10, 14 and 28. Rectal swab and genital biopsy samples will be obtained on day 7 and cervicovaginal lavage will be collected at screening, day 14 and day 28.

9.2 Sample Size and Accrual

The primary aim of the study is to assess the local and systemic safety of a single dose of two formulations of an MK-2048 vaginal film. The primary endpoint is the proportion of participants who experience a Grade 2 or higher adverse event. The proposed total sample size is N=48 divided into 2 arms (low and high Eudragit® content assigned at a 1:1 ratio). Based on previous studies of vaginal products conducted at the study site, the accrual of 48 eligible subjects with normal reproductive tracts is expected to require the screening of approximately 90 volunteers. Since unevaluable participants will be replaced, it is anticipated that 48 women exposed to study product will be evaluable for the primary endpoint.

The sample size is based on the exact binomial probability of observing Grade 2 or higher adverse events. **Table 4** gives the upper and lower bounds for the 95% exact binomial confidence intervals of the true Grade 2 or higher adverse event rate at all possible numbers of observed events in a group of size 24 or in the full cohort of 48. Note that the results for more than 50% of events in each group are not listed, but by symmetry can be calculated as 1- the listed rate and 95% CI for the number of non-events. If none of the 48 subjects experience Grade 2 or higher adverse events, the 95% exact 2-sided upper confidence bound for the Grade 2 adverse event rate is 7.4%. In a previous study of a single dose of the high Eudragit® content placebo film, 6 grade 2 adverse events were reported in a cohort of 64 women for an event rate of 9.4%. If similar event rates are observed in the proposed study, we will be able to rule out grade 2 or higher adverse event rates greater than 22.6% in the full cohort and 32.4% in each study arm.

TABLE 4: 95% confidence intervals for the true rate at all possible observed Grade 2 or higher adverse event rates

Number of observed events	Rate (95% CI)	Number of observed events	Rate (95% CI)
0/24	0 (0, 14.2%)	0/48	0 (0, 7.4%)
1/24	4.2% (0.1%, 21.1%)	1/48	2.1% (0%, 11.1%)
2/24	8.3% (1.0%, 27.0%)	2/48	4.2% (0.5%, 14.3%)
3/24	12.5% (2.7%, 32.4%)	3/48	6.2% (1.3%, 17.2%)
4/24	16.7% (4.7%, 37.4%)	4/48	8.3% (2.3%, 20.0%)
5/24	20.8% (7.1%, 42.2%)	5/48	10.4% (3.4%, 22.6%)

6/24	25.0% (9.8%, 46.7%)	6/48	12.5% (4.7%, 25.2%)
7/24	29.2% (12.6%, 51.1%)	7/48	14.6% (6.1%, 27.8%)
8/24	33.3% (15.6%, 55.3%)	8/48	16.7% (7.5%, 30.2%)
9/24	37.5% (18.8%, 59.4%)	9/48	18.8% (8.9%, 32.6%)
10/24	41.7% (22.1%, 63.4%)	10/48	20.8% (10.5%, 35.0%)
11/24	45.8% (25.6%, 67.2%)	11/48	22.9% (12.0%, 37.3%)
12/24	50.0% (29.1%, 70.9%)	12/48	25.0% (13.6%, 39.6%)
		13/48	27.1% (15.3%, 41.8%)
		14/48	29.2% (17.0%, 44.1%)
		15/48	31.2% (18.7%, 46.3%)
		16/48	33.3% (20.4%, 48.4%)
		17/48	35.4% (22.1%, 50.5%)
		18/48	37.5% (24.0%, 52.6%)
		19/48	39.6% (25.8%, 54.7%)
		20/48	41.7% (27.6%, 56.8%)
		21/48	43.8% (29.5%, 58.8%)
		22/48	45.8% (31.4%, 60.8%)
		23/48	47.9% (33.3%, 62.8%)
		24/48	50.0% (35.2%, 64.8%)

9.3 Study Endpoints

9.3.1 Primary Endpoint

Grade 2 Adverse Events

The safety/toxicity endpoint is clinical or laboratory evidence of a Grade 2 or higher Adverse Event as defined by the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 2.1, July 2017 and the Female Genital Grading Table for Use in Microbicide Studies (Appendix 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, (Dated November 2007).

9.3.2 Secondary and Exploratory Endpoints

The secondary and exploratory endpoints of this study are as follows:

Secondary: MK-2048 pharmacokinetics

MK-2048 concentrations in plasma, tissue homogenate, and CVL rectal and vaginal swab eluents, comparing the two film formulations sampled at enrollment and post-enrollment time-points through Visit 8

Secondary: Acceptability

Reports of overall acceptability of the film and qualities of the experience with the film will be measured using a self-report survey drawing items from two sources. Overall acceptability is measured using standard language in market research methodology, each using a 5-point agreement scale followed by an open-ended explanation. Assessment of film-specific qualities of the experience is assessed by a 9-item scale drawn from previous research with participant-inserted films. Questions assess perceptions of leaking, vaginal dryness, and sensation of the film inside the vagina during and after insertion.

Exploratory: Vaginal Microenvironment

Given that there is no non-product use arm, participants will serve as their own control. Assessment of baseline microenvironment will take place during the Enrollment visit with the

exception of CVL properties. CVL will be obtained at the Screening visit so that the microenvironment can recover prior to study product administration at the enrollment visit.

Levels of lectin binding and mucins measured in CVL samples
Endogenous and exogenous glycolytic enzymes, formulated microbicide products, and microbial flora interact with mucins and may alter their structure and function. These alterations may be detected with lectins, which are sugar specific binding molecules, and can be bound to reporter molecules (such as horseradish peroxidase) in an ELISA type assay.

Nugent Score
Clinically significant changes in vaginal flora will be evaluated by a change in the Nugent score baseline (Visit 2, pre-exposure) through Visit 7. The Nugent score is graded 1 to 10 as follows:

Normal, 0 to 3
Intermediate, 4 to 6
BV, 7-10

Any shift from normal at baseline to intermediate or BV at Visit 3, 4, 5, 6, 7, or 8 or shift from intermediate or BV to normal at Visit 3, 4, 5, 6, 7, or 8 will be considered a scientifically meaningful change in vaginal microbiota.

Quantitative vaginal cultures and quantitative PCR
Meaningful changes will be defined by greater than or equal to 1 log changes in dominant members of the microbiota, including *Lactobacillus* species, *Gardnerella vaginalis*, key anaerobic microorganisms, *Enterococcus* species, *Escherichia coli*, *Staphylococcus aureus*, Group B *Streptococcus*, and *Candida* species.

Levels of anti-viral activity in CVL
In-vitro anti-HIV activity will be determined by the TZM-bl assay.

The TZM-bl assay will determine HIV-1 infection by luciferase for single round of replication. To assess the potency of the CVL, serial dilutions will be made and tested to determine the IC_{50}.

Levels of biomarkers of innate immunity
Changes in cervical cytokine expression and innate immunity factors in cervicovaginal lavage will be assessed. Changes within each group will be reported. The factors to be analyzed will be determined on the scientific evidence at the time of the analysis.

Exploratory: Pharmacokinetic (PK)/ Persistence of Eudragit® in CVL

Persistence of polymer will be determined as detected levels of polymer in vaginal fluid collected through cervicovaginal lavage.

Exploratory: Ex vivo challenge

HIV infection of cervical biopsies as measured by HIV-1 p24 replication by ELISA and confirmation either by immunohistochemistry or quantitative PCR assessment of integrated provirus in HIV-1- exposed cervical biopsies.

9.4 Blinding

All participants will receive a single dose of the MK-2048 vaginal film. They will be blinded to the formulation with respect to Eudragit® content. Evaluators of the study endpoints will also be blinded to the film formulation.

9.5 Emergency Unblinding

The Principal Investigator can proceed with unblinding for safety concerns, without prior consultation of the PSRT. Details of emergency unblinding are specified in an SOP.

9.6 Random Assignment

The randomization scheme will be generated and maintained by a member of the Data Management Center at the study site. and supplied to the Pharmacy. Women will be randomized to one of the two arms at a 1:1 ratio using a permuted block design with random block sizes of 2, 4, and 6. In order to minimize bias in group assignment, participants will be given study identification numbers. A study staff member will allot the identification numbers in sequential order as participants become eligible to enroll in the study. Numbered study film product packages will include sealed envelopes enclosing documentation of the randomized assignment to film formulation. The randomization scheme will be created for a total of 54 participants; the overage will be created to compensate for participant withdrawals or unusable products.

9.7 Data Monitoring and Analysis

9.7.1 Data Monitoring

This clinical trial will be conducted in compliance with the protocol, GCP guidelines, and applicable regulatory requirements. All research charts are maintained in locked files in a locked room. The research staff, under the direction of the primary investigator, will create and maintain electronic databases using REDCap (Research Electronic Data Capture) which is a secure, web-based application designed for clinical trial data collection that is validated for each study protocol. The databases will be backed up every night onto the University of Pittsburgh's server's back-up system. Appropriate firewall and virus scanning software are installed and updated routinely by the university support staff.

This study will utilize electronic data capture. Site staff will enter data as collected directly into a data entry system. Study data management staff will review the electronic data elements for completeness and accuracy. If there are any responses that are incomplete, unclear, or inconsistent with related data elements, the staff person will speak with the clinician in question as soon as possible to resolve the problem. If necessary, the study staff will make the appropriate change in the electronic database which tracks time and date if a change is made.

9.7.2 Primary Endpoint Analysis

The number of adverse events will be summarized by severity, body system, and relationship to study product using frequencies and percent. Individual participants will contribute once to the calculation of event rates. Differences in the prevalence of adverse events between the study arms will be assessed using Fisher's exact test.

9.7.3 Secondary and Exploratory Endpoint Analysis

Summary statistics of frequencies and percentages will be provided for categorical data. Means and standard deviations or medians and ranges will be provided for continuous data. Methods for the analyses of the secondary endpoints are described below.

Student's t- and Mann-Whitney U tests will be used, where appropriate, to evaluate differences in the following continuous variables between the study arms: MK-2048 concentrations in plasma, rectal and vaginal swab eluents, and genital tissue, levels of glycoproteins and mucins in CVL, levels of cytokines and innate immunity factors, and antiviral activity in CVL. Between group pre- to post-exposure changes in the above endpoints will also be evaluated using Student's t- and Mann-Whitney U tests, where appropriate, while paired Student's t- and Wilcoxon signed-rank tests will be used to evaluate these changes within each study arm. Mixed effects linear regression will be used to evaluate associations between days of exposure to study product and these outcomes.

Nugent score will be categorized as follows: normal (score 0-3), intermediate (score 4-6), and bacterial vaginosis (score 7-10). Differences in the categorized Nugent score pre and post exposure levels will be evaluated using Fisher's exact, while McNemar's tests will be used to evaluate pre- to post-exposure shifts between normal (score 0-3) and abnormal (score 4-10) vaginal microflora within each study arm. Generalized estimating equations will be used to evaluate differences in the marginal post exposure prevalence of bacterial vaginosis between the two film formulations.

Prevalence of microorganisms at each visit as assessed through culture and qPCR will be compared between the study arms using Fisher's exact tests, while pre- to post-exposure shifts will be evaluated within each group using McNemar's tests. Differences between the study arms in the quantity of microorganisms at each visit, as well as the change in quantity, will be evaluated using Mann-Whitney U tests. Meaningful changes will be defined by greater than or equal to 1 log changes in dominant members of the microbiota, including *Lactobacillus* species by quantitative PCR (qPCR), *Prevotella* species by qPCR, *Gardnerella vaginalis* (qPCR), *Atopobium vaginae* (qPCR) and *Megasphaera* phylotype I) qPCR). Candida species, facultative gram negative rods, staphylococci and streptococci will be evaluated by cultivation based methods. Fisher's exact tests will be used to evaluate differences in these changes between study arms. Mixed effects linear regression (for continuous outcomes) and generalized estimating equations (for binary outcomes) will be used to evaluate associations between days of exposure to study product and these outcomes.

Descriptive statistics will be performed for HIV infection of cervical biopsies as measured by HIV-1 p24 replication for each study arm and the combined cohort. Differences in HIV-1 p24 replication between study arms will be evaluated using Student's t-or Mann-Whitney U test, as appropriate.

9.7.4 Analysis Cohort

The intent-to-treat cohort (ITT) will be defined as those who were randomized. It will be used for analyses of baseline characteristics, protocol deviations and violations, and trial conduct. The evaluable cohort (Eval) is a subset of ITT participants. Evaluable participants will be defined as participants who complete the Screening and Enrollment visits and return for their day 7 visit within the window for a safety assessment. Non-evaluable participants will be replaced.

TABLE 5: Statistical Analysis Plan

Endpoint	Variable	Statistics
Primary	Adverse Events	Frequency, Percent
Secondary	MK-2048 concentration	Mean, SD, Median, Range,
	Acceptability	Frequency, Percent

Exploratory	Persistence of Eudragit® in CVL defined as any concentration above the lower limit of quantification	Mean, SD, Median, Range
	Levels of glycoproteins and mucins, in CVL	Mean, SD, Median, Range
	Antiviral activity in CVL	Mean, SD, Median, Range
	Change in vaginal flora (Nugent score)	Frequency, Percent, Median, Range
	Culture/qPCR results from vaginal organisms - prevalence	Frequency, Percent
	Culture/qPCR results from vaginal organisms - concentration	Mean, SD, Median, Range
	Change in cytokine and innate immunity factors	Mean, SD, Median, Range
	HIV infection of cervical biopsies in ex vivo challenge	Mean, SD, Median, Range

10 HUMAN SUBJECTS CONSIDERATIONS

The investigators will make efforts to minimize risks of these products to human subjects. Volunteers will take part in a thorough informed consent process throughout their participation in the study. Before beginning the study, the investigators will have obtained IRB approval. The investigators will permit audits by the NIH or any of their appointed agents.

10.1 Special Populations

Study staff will offer screening to eligible women of all ethnic and racial groups. Members of the study staff are not seeking the screening or enrollment of women in special or vulnerable populations. The following section also discusses special considerations for male partners of participants.

10.1.1 Men

Men are not included as subjects in the study because the study is testing a vaginal application of the study product. The male sexual partners of women participating in this study will not be consented or monitored for several reasons. Protocol-specified guidelines for abstinence are expected to protect male partners from exposure to the study product. In addition, based on preclinical data, no toxicity is anticipated from the study product.

10.1.2 Children

The NIH has mandated that children be included in research trials when appropriate. This study will enroll women aged 18 to 20 who are able to give informed consent. This study meets “Justifications for Exclusion” criteria for younger children as set forth by the NIH. Specifically, "the research topic to be studied is irrelevant to (young) children" and "a separate, age-specific study in (adolescent) children is warranted and preferable" at a later time.

10.1.3 Prisoners

Prisoners will not be included in this study (for screening or enrollment). Any participants incarcerated during the course of participation in the trial will not be followed during their incarceration and will be discontinued from the study. Participants who have been released from

incarceration will be permitted to return for any protocol specified follow-up or safety visits per the guidelines of the local IRB.

10.1.4 Pregnant women

Pregnancy is an exclusion criterion as the vaginal microenvironment in pregnancy differs substantially from the non-pregnant state. Including pregnant participants would make the interpretation of study results difficult. Women who become pregnant during the study period following randomization will be terminated from the study. Participants who become pregnant during the course of the study will be contacted periodically after study termination in order to determine the pregnancy outcome.

10.2 Informed Consent Process

Written informed consent will be obtained from all potential study participants prior to the initiation of any study-related procedures**. Only listed investigators who are physicians may obtain consent.** The informed consent process will give individuals all of the relevant information they need in order to decide whether to participate, or to continue participation, in this study. Potential research participants will be permitted to ask questions and to exchange information freely with the study investigators. Only listed study investigators may obtain informed consent from potential study participants. The investigators will keep research participants fully informed of any new information that could affect their willingness to continue study participation.

10.2.1 Risk/Benefit Statement

Risks

It is not expected that this trial will expose human subjects to unreasonable risk. The intervention used in this study is unlikely to cause uncomfortable side effects and is only given once. However, there may be unknown risks or side effects associated with the use of the vaginal film. Some women who used the film in other studies have reported vaginal discharge, and irritation and discomfort. Women in studies involving the MK-2048 vaginal ring reported bacterial vaginosis, vulvovaginal candidiasis, vaginal burning, vaginal erythema, vaginal pain, and vaginal itching which investigators attributed to the study product. Only the vaginal candidiasis was Grade 2; the other AEs were Grade 1.

Below is a list of potential risks of participation. There may be other risks associated with participation that are not yet known.

Procedure	Risk
Pregnancy Test	• Anxious or nervous having test or waiting for results • Denial, depression or worry with unexpected positive result
Pelvic Exam	• Discomfort with speculum
Genital and rectal specimens	• Minimal discomfort from collection of specimens

Cervical Biopsy	• Generally well tolerated but may cause pain (or pinching)
	• Cramping similar to menstrual cramps
	  ○ Cramping typically resolves within minutes after procedure
	  ○ May take ibuprofen prior to biopsy to minimize cramping
	• Vaginal spotting or bleeding for a couple days
	  ○ Typically less than a period but if heavier, instructed to contact study staff
	• Infection of biopsy area

	○ Unlikely but if foul odor or unusual discharge, instructed to contact study staff • A cut in the vagina can increase the risk of getting HIV if exposed • Risk of dislodging an IUD (if present) ○ If displaced, a new one would be inserted at no charge
Vaginal use of MK 2048 film	• Vaginal discharge, irritation, discomfort • Vaginal burning, itching, redness and/or pain • Yeast or bacterial infection • Allergic reaction
Blood draw	• Bruising, soreness, pain/discomfort, bleeding, infection at the site • lightheadedness, fainting
Participation in research, collection and storage of private health information, biospecimens and internet communication*	• Inconvenient • Breach of confidentiality
Questionnaires	• Discomfort with personal nature of questions
STD/HIV testing	• Worry or anxiety • Sadness, depression or denial with a positive test result ○ Positive gonorrhea and chlamydia test results will be reported to the Allegheny County Health Department according to the Commonwealth of Pennsylvania reporting requirements ○ May be contacted and asked questions about sexual partners

*We will make reasonable efforts to protect the privacy of information on social media. Each platform has their own privacy policies and terms of use that may change at any time. The University of Pittsburgh cannot guarantee the privacy and confidentiality of information shared on social media in this research study. Participants information may not remain private. Participants will be instructed to visit site privacy policies and update privacy settings, as necessary. Social media retains information shared across accounts for an unknown length of time and it may be shared with others including targeted advertisers.

Pregnancy, Breastfeeding and Sexual Practices

The vaginal film is not a birth control method. We do not know what effect the study drug may have on pregnancy, including the effect of the study drug on the fetuses of women who use the vaginal film when pregnant, or the babies of women who use the vaginal film when breastfeeding. Because of this, pregnant women and women who are breastfeeding may not join this study. Women who join the study must agree to avoid sexual intercourse from V2 (enrollment) until V7 (7 days after the biopsy visit) as well as 48 hours prior to study visits, use an effective method of birth control and have scheduled pregnancy tests while in the study. Effective methods of birth control include hormonal methods (like "the pill" or Depo-Provera injections), the IUD, sterilization (or "tied tubes"), abstinence (or not having sex), same sex partner, or having a partner who has had a vasectomy. Effective methods of birth control cannot include use of a diaphragm, NuvaRing, or spermicide. Participants must agree to use an effective method of birth control until the last study visit, approximately 4 weeks after enrollment.

In the unlikely event that a participant becomes pregnant during the study, study staff will refer her to available medical care and other services as needed. The study does not pay for this care.

Participants will be terminated from the study; however, since the outcome of the pregnancy is important to study staff; women will be followed through the outcome of the pregnancy.

If a participant were to become HIV infected while using the study product, the HIV virus may develop resistance to MK-2048. Developing MK-2048 resistance means that the MK-2048 may not be able to treat HIV however, MK-2048 is not currently used to treat HIV. Participants who are diagnosed with HIV in follow-up will be referred for care and exited from the study.

Benefits

As this is a Phase I study and it will be the first time these investigational films will be tested in humans, there is no expectation of potential direct benefit from the study intervention, only the potential for ancillary benefits. Information learned from this study may help in the development of vaginal products for women. Women will have exams and HIV/STD testing as part of participation in this study.

10.3 Incentives

Volunteers will not be charged for any of the study visits, study supplies or examinations. There are no costs to participants in this study. Pending IRB approval of these compensation guidelines, women will be compensated for their time and inconvenience and for their travel needs while participating in the protocol. The approved amounts of compensation for the time commitment of participants will be given out at each visit. The visits will be pro-rated and partial payment given in the event that the participant only completes a portion of the study visits. The following proposed compensation amounts were created based on common institutional practice for studies investigating vaginal products, as well as studies utilizing observation periods in the Clinical Translational Research Center.

Visit	V1	V2	V3	V4	V5	V6	V7	V8
Compensation	$30	$40	$30	$30	$100	$30	$30	$40
Incentive if all visits in window								$40
Optional procedures		*						

* V2 INCLUDES OPTIONAL SAMPLING: Two vaginal swabs collected without a speculum and one blood sample collected following film insertion.

- Optional collection times are at 1, 2, 3, 4, 5, or 6 hrs.
- Additional compensation will be provided for each sample collected ($10 per vaginal swab and $20 for blood draw plus an additional $10 for each sample collected between 4 – 6 hours).

Total compensation for all required visits = $370. Potential compensation for all visits, including the maximum compensation for optional visits = $440.

If a participant completes a visit outside of the protocol window for that visit, $10 will be deducted from the scheduled visit payment.

In addition, a parking pass, bus pass or assistance with transportation will be provided to participants as needed.

10.4 Participant Confidentiality

Members of the study staff are all trained in patient confidentiality. The log of study subject names and other protected health information is kept in a locked area. All computer information about study volunteers is kept on a computer with log-on passwords. Laboratory specimens are labeled with study numbers and date, and are delivered by study staff. In addition to the research study

staff, the NIH, study monitors, University of Pittsburgh Research Conduct and Compliance Office, the Office of Human Research Protections, and/or the University of Pittsburgh IRB may have access to participant's health information. Each member of the staff has log-on identification and password, logs off before leaving a computer screen unattended, and closes their office door when out of the office. All research records will be kept for a minimum of seven years following closure of this study (per University of Pittsburgh policy).

10.5 Communicable Disease Reporting

Study staff members will comply with all local requirements to report communicable diseases including chlamydia, gonorrhea, and HIV identified among study participants to the Allegheny County Health Department. Study investigators will include discussion of mandated reporting during the study informed consent process.

10.6 Study Discontinuation

NIAID or the University of Pittsburgh Institutional Review Board may discontinue this study at any time. Ongoing safety monitoring will track the incidence of AEs and EAEs. In the event of an abnormal number of reported AEs and/or EAEs judged to be related to study product the External Safety Monitor will contact the Principal Investigator to initiate a temporary hold on further enrollment.

11 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

11.1 Laboratory Specimens

Laboratory specimens will be handled in a manner consistent with institutional, OSHA, and GLP guidelines. Study staff members are trained in the appropriate handling of laboratory specimens. Samples such as urine that will be divided for multiple analyses will be divided according to site SOP.

TABLE 6: Laboratory Test Methods

SAMPLE	METHOD	LABORATORY
URINE	Pregnancy test Urine dipstick	Clinical Research Center CLIA #39D1031322
SALIVA	Rapid HIV	Clinical Research Center CLIA #39D1031322
BLOOD	Confirmatory HIV	UPMC Presbyterian Shadyside CP PUH CLIA# 39D0911193
	MK-2048 Level	University of Colorado (non-diagnostic laboratory)
RECTAL	MK 2048 Level	University of Colorado (non-diagnostic laboratory)
VAGINAL*	pH Wet Mount	Clinical Research Center CLIA #39D1031322
	Trichomonas (NAAT) Gram Stain Quantitative Vaginal Culture Quantitative PCR for microbiota	Magee-Womens Research Institute CLIA #39D1004688
	MK 2048 Level in CVL	University of Colorado (non-diagnostic laboratory)
	GC/CT (NAAT)	Magee-Womens Research Institute CLIA #39D1004688

CERVICAL*	Cervical biopsy tissue for ex vivo challenge	Magee-Womens Research Institute (non-diagnostic laboratory)
	Cervical biopsy tissue for MK 2048 level	University of Colorado (non-diagnostic laboratory)
	Pap smear	UPMC Magee-Womens Hospital CLIA# 39D0177794
CVL	Levels of antiviral activity	Magee-Womens Research Institute/Parikh Laboratory (non-diagnostic laboratory)
	Levels of glycoproteins and mucins	Magee-Womens Research Institute/Moncla Laboratory (non-diagnostic laboratory)
	Levels of cytokines & innate immunity factors	Magee-Womens Research Institute/Hillier Laboratory (non-diagnostic laboratory)
	MK-2048 level	University of Colorado (non-diagnostic laboratory)
	Eudragit® level	University of Colorado (non-diagnostic laboratory)

*Future Use Samples will be stored at Magee-Womens Research Institute; use and processing lab to be determined

11.1.1 Urine Samples

Study staff at the clinical site will utilize dipstick urinalysis to screen for possible urinary tract infection as clinically indicated. Urine will be tested for HCG via the Sure-Vue® test or equivalent.

11.1.2 Vaginal Samples

The assessment of vaginal flora will be based on the Nugent Scoring System for Gram Stained Vaginal Smears as well as assessment of several groups of organisms by cultivation (culture) based methods. In addition, selected microbiota will be detected using a quantitative polymerase chain reaction (qPCR) test which has been developed and validated within the laboratory. MK-2048 vaginal concentrations will be measured according to SOP.

Collection will be done as gently and carefully as possible so as to collect an adequate sample that is unlikely to disturb the mucosa or overall vaginal microenvironment or the vaginal film in situ. *Trichomonas vaginalis* will be detected using an amplified DNA assay and handled according to policies outlined in SOPs for this study.

11.1.3 Rectal Swabs

MK-2048 concentrations will be quantified according to the SOP.

11.1.4 Cervical Samples

C. trachomatis and *N. gonorrhoeae* will be detected using an amplified DNA assay and handled according to policies outlined in SOPs for this study.

Pap smears collected as part of screening will be processed and resulted by UPMC Magee-Womens Hospital cytology department.

11.1.5 Cervicovaginal Lavage Samples

MK-2048 Content in CVL

Aliquots of lavage samples will be tested for MK-2048 levels (according to SOP).

Eudragit® Content in CVL
Aliquots of lavage samples will be tested for Eudragit® levels (according to SOP)

Anti-viral Activity in CVL
Aliquots of lavage samples will be tested for anti-viral and antibacterial activity as outlined in the site SOPs.

Cervical Cytokines and Innate Factors in CVL
Cytokines (for example, IL-1β and TNFα) along with innate immune factors (for example, antiproteases) that could contribute to a proinflammatory milieu or changes in the glycomic signatures may be evaluated. The factors to be analyzed will be determined on the scientific evidence at the time of the analysis.

Glycome Studies
Aliquots of lavage samples will be tested for glycoproteins and mucins.

11.1.6 Cervical Tissue

MK-2048 Concentration in Cervical Tissue
Tissue from cervical biopsies will be processed at the University of Colorado and concentrations of MK-2048 measured.

Ex-vivo Challenge
The efficacy of study product to prevent HIV infection will be based on an *ex vivo* HIV biopsy challenge model as outlined in the site SOP.

11.2 Quality Control and Quality Assurance Procedures

Dr. Sharon Hillier, who completed a training program in clinical and public health microbiology certified by the American Board of Medical Microbiology (ABMM), directs the Infectious Disease Research Laboratory at Magee-Womens Research Institute. This laboratory is CLIA-inspected and maintains its own CLIA license. Thus, all testing done in this research laboratory is performed with the same level of quality control as required in a licensed clinical laboratory.

11.3 Specimen Storage and Possible Future Research Testing

Part of the consent form will include an explanation of future use of vaginal and cervical specimens and blood samples. Any future use or residual samples will be stored at Magee-Womens Research Institute, Microbiology Laboratory, 5th floor for an indefinite period of time. The principal investigator will assume primary responsibility for control of this area.

Any results from research done on future use or leftover specimens will not be placed in health records and will be kept confidential. The language and format employed in the consent for these purposes are an IRB-approved means commonly employed in studies performed at this and other study sites within our institution to obtain permission for use of stored samples. All primary study endpoints, protocol-specified testing, and QA/QC testing will be ascertained prior to any additional testing of stored specimens.

11.4 Biohazard Containment

Biohazardous waste will be contained according to institutional and all other applicable regulations.

12 ADMINISTRATIVE PROCEDURES

The study proposal for funding, this protocol, the informed consent document, data collection forms, and advertising flyers are all reviewed by the University of Pittsburgh Institutional Review Board prior to enrollment of participants in the study.

12.1 Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, the study site must have the protocol and the protocol consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, the site will submit all required protocol registration documents to the DAIDS 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 NOT be reviewed or approved by the DAIDS PRO, and sites will receive an Initial Registration Notification when the DAIDS PRO receives a complete registration packet. Receipt of an Initial Registration Notification indicates successful completion of the protocol registration process. The site will not receive any additional notifications from the DAIDS PRO for the initial protocol registration. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approval(s) for an amendment, the study 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 the required documents have been received. Site-specific ICF(s) WILL NOT be reviewed and approved by the DAIDS PRO and sites 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, available at http://rsc.tech-res.com/protocolregistration/

12.2 Data Coordination

Data management responsibilities will reside with the Data Management staff at Magee-Womens Hospital.

12.3 Study Monitoring

The University of Pittsburgh Education and Compliance Office for Human Subject Research (ECO-HSR) will assist with the monitoring as outlined in the study monitoring plan.

The site investigator will make study documents (e.g., consent forms, drug distribution forms, eCRFs) and pertinent hospital or clinic records readily available for inspections by the local IRB, the site monitors, the FDA, NIAID/DAIDS, the OHRP, and other local, US, or international regulatory entities for confirmation of the study data.

12.4 Protocol Compliance

All protocol amendments will be submitted to and approved by the University of Pittsburgh IRB and DAIDS prior to the implementation of an amendment.

12.5 Investigator's Records

The investigator will maintain, and store securely, complete, accurate and current study records throughout the study. Study records will not be destroyed prior to receiving approval for record destruction from the Principal Investigator and they will be maintained for a minimum of seven years following completion of the study, per the University of Pittsburgh IRB policy. Applicable records include source documents, site registration documents and reports, correspondence, informed consent forms, and notations of all contacts with the participant.

12.6 Use of Information and Publications

Publication of study results will be governed by DAIDS policies. The investigators will submit any presentation, abstract, or manuscript to DAIDS for review prior to submission.

12.7 Training Procedures

Only study staff trained in applicable study procedures and staff experienced in HIV pre-test and post-test counseling will perform these study procedures. Approved written materials consistent with the local clinical standard of care will support pre-test and post-test counseling.

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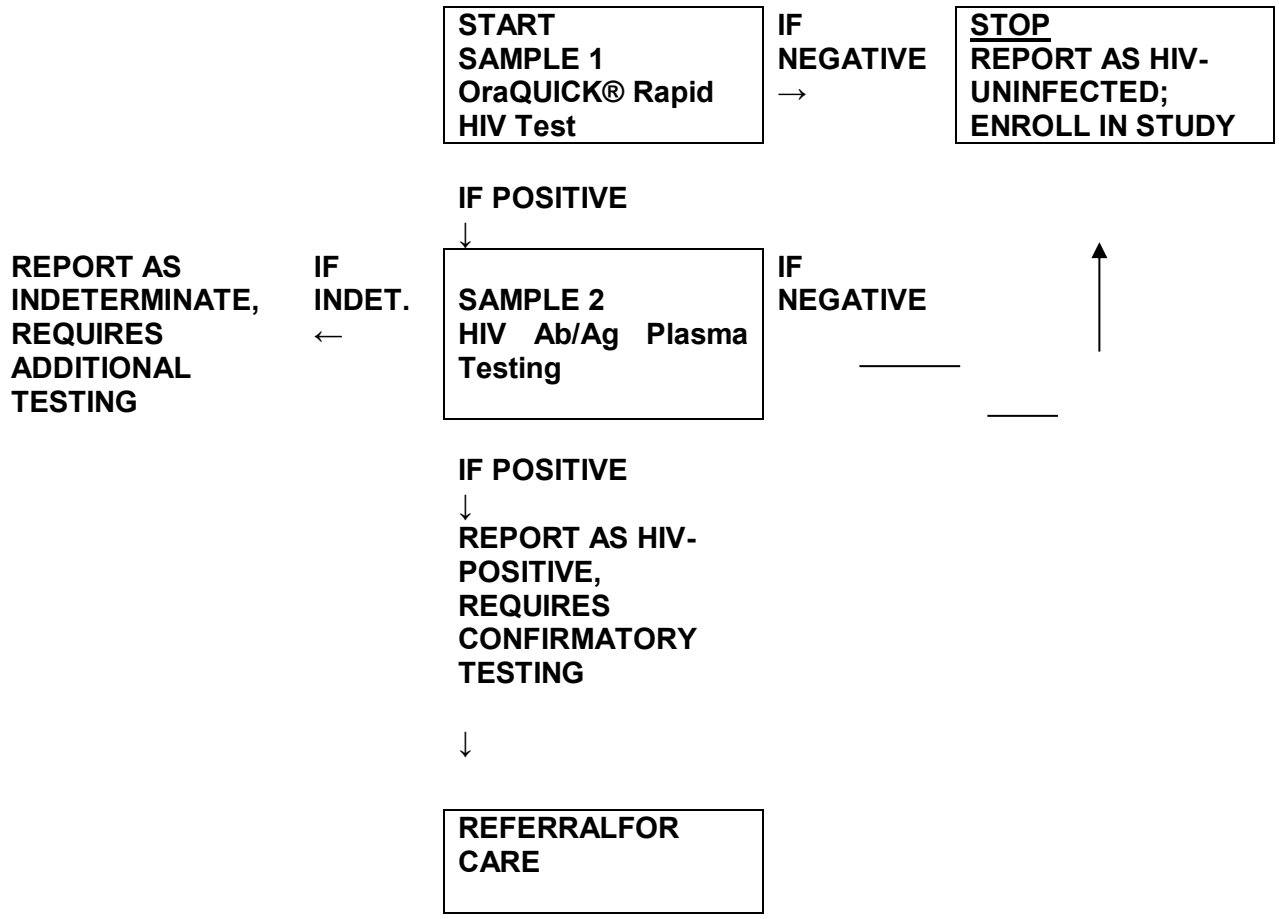
APPENDIX 1: SCHEDULE OF STUDY VISITS AND PROCEDURES

Component	Procedure/Analysis	V1: Screen	V2: Enroll	V3-7	V8
ADMINISTRATIVE	Written Informed Consent	X			
	Assign Study Number (PTID)	X			
	Review/Confirm Eligibility	X	X		
	Collect/Update Contact Information	X	X	X	X
	Medical Hx/Concomitant Meds	X	X	X	X
	Visit Questionnaire	X	X	X	X
	HIV Pre-/Post-Test Counseling	X			
	Screening Results	X	X		
	Protocol counseling	X	X	X	
	Assess/Document Adverse Events		X	X	X
	Schedule Next Study Visit	X	X	X	
	Participant Reimbursement	X	X	X	X
ACCEPTABILITY	Acceptability Questionnaire		X	V5 ONLY	
URINE	Pregnancy test	X	X	^ & V5	X
	Urine dipstick	^	^	^	^
SALIVA	Rapid HIV	X			
BLOOD	Confirmatory HIV	^			
	MK-2048 levels		X*	X	X
VAGINAL SAMPLES	pH	X	X	X	X
	Wet Mount	^	^	^	^
	Trichomonas (NAAT)	X		^	^
	Gram Stain		X	X	X
	Quantitative Vaginal Culture		X	X	X
	Quantitative PCR for microbiota		X	X	X
	Vaginal Sample for Future Use**	X	X	X	X
	MK-2048® level		X*	X	X
CERVICAL SAMPLES	GC/CT (NAAT)	X		^	^
	Cervical Sample for Future Use**	X	X	X	X
	Pap smear	^			
	Cervical Biopsies			V5 ONLY	
RECTAL SAMPLES	MK-2048 level			V5 ONLY	
CVL	Levels of antiviral activity	X		V7 ONLY	X
	Glycoproteins and mucins	X		V7 ONLY	X
	Cytokines & innate immunity factors	X		V7 ONLY	X
	Eudragit® level	X		V7 ONLY	X
	MK-2048 level	X		V7 ONLY	X
PHYSICAL EXAM	Physical Exam	X	^	^	^
	Vital Signs (BP)	X	X	X	X
	Height & Weight	X			
PELVIC EXAM	Bimanual Exam	^	^	^	^
	External Genital & Speculum Exam	X	X	X	X
STUDY PRODUCT	Study Product Randomization		X		
	Film Distribution		X		
	Film Insertion by Clinician		X		

X Required
^ PRN/as clinically indicated
* Additional OPTIONAL vaginal swabs (up to 2) and single blood sample at 1, 2, 3, 4, 5, 6hrs post film Insertion
** For participants who have consented to the collection of samples for future use and use of left-over samples for future studies.

APPENDIX II: HIV TESTING AGORITHIM



APPENDIX III: SAMPLE INFORMED CONSENT

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

STUDY TITLE: A Randomized, Double Blinded Study of the Safety and Pharmacokinetics of Two Vaginal Film Formulations Containing the Integrase Inhibitor MK2048: FAME 103

INFORMED CONSENT VERSION: Version 1.0, 12/20/2019

PRINCIPAL INVESTIGATOR: Katherine Bunge, MD
UPMC Magee-Womens Hospital, Dept of OB/GYN/RS,
300 Halket Street, PGH, PA 15213

QUESTIONS ABOUT THE STUDY: Contact the research staff at 412-641-4242 or after hours at 412-463-1337

FUNDING AGENCY: Division of AIDS, US National Institute of Allergy and Infectious Diseases, US National Institutes of Health

KEY INFORMATION

Participation is voluntary. You are being asked to take part in a research study. Research studies include only people who choose to take part. The study team members will explain the study to you and will answer any questions you might have. You should take your time to make your decision.

Summary of study. This study includes approximately 48 healthy non-pregnant women aged 18 – 45 who agree to use a single investigational (experimental) vaginal film and return for 6 follow up visits. The vaginal film, which is a thin square similar to a Listerine® breath strip, contains an investigational medication called MK2048. MK2048 is not approved by the Food and Drug Administration (FDA). This study will assess the safety and acceptability of the film.

Visits (8 in total)	Time	Key component(s) of visit
Screen	Today	Determine eligibility; includes STD/HIV testing
Enrollment	Within 45 days	Vaginal film insertion by a clinician
Required Follow-up Visits	Days 3, 5, 7, 10, 14, 28	Blood and genital* sample collection at each visit; Cervical biopsies and rectal swabs at Day 7 visit only

*Genital samples refer to vaginal, cervical and/or rectal samples

Risks. The main risks of participation include the risks of using an investigational vaginal film, blood draws and cervical biopsies. The number of study visits may be inconvenient.

Benefits. There is no direct benefit to participating, however some participants may get satisfaction taking part in a study that involves women's health.

Right to withdrawal. You may withdraw from the study at any time if you chose.

Alternatives. The alternative is not to participate in this study.

****

YOUR PARTICIPATION IS VOLUNTARY

Participation in research is a personal decision; you are under no obligation to participate. If you agree to take part, you will be asked to sign your name on this form and will be offered a copy to keep. Your regular doctor may be part of this study team. You should take your time to make a decision and discuss with others if needed.

STUDY PRODUCTS AND PURPOSE OF STUDY

Two different vaginal films will be tested in this study. Both films contain the same amount of an investigational medication called MK2048. MK2048 has been used in other studies to look at HIV prevention. The two films differ in the amount of polymer ("binding" or "delivery" agent).

Films	MK2048 dose	Size	# of participants
high polymer*	30mg	2" x 2"	24
low polymer	30mg	2" x 2"	24

*used in a previous placebo (blank, no drug) film study of 64 women and was safe and acceptable

The Food and Drug Administration (FDA) has not approved MK2048 film for use. While MK2048 has been tested in humans, including vaginal dosing via vaginal ring, this is the first time MK2048 has been tested in a film form in humans. Importantly, all the ingredients that make up the film, including the polymers have been tested in humans and have been found to be safe.

This study is testing the safety of MK2048 film when used once. Cervical biopsies, vaginal and rectal samples, and blood will be collected to see how much MK2048 gets into the genital tissue and/or bloodstream. The cervical tissue will be exposed to HIV in the lab to understand whether the film can help to prevent HIV infection when tested outside the body.

This study is not testing if MK2048 vaginal film prevents you from getting infected with HIV. Researchers do not yet know if the film will work in humans to protect against HIV. The best way to protect against getting HIV infection during sex is to use a condom every time you have sex.

Eligible women will be randomized (50/50 chance, like flipping a coin) at visit 2 (V2) to have one of two films inserted (high polymer or low polymer). You cannot choose your group. Both films are packaged identically, so neither you nor study staff will know which group you are in. All women will follow the same study visit schedule.

STUDY VISIT HIGHLIGHTS

- All research activities will take place at UPMC Magee-Womens Hospital.
- A screening visit (V1) will determine if you are eligible to participate. You are under no obligation to participate even if you are found to be eligible. Screening can occur over more than one visit, if necessary.
- If eligible and interested, you will be asked to return within 45 days to be enrolled (V2). At V2, you will have the assigned vaginal film inserted into the vagina by a research clinician.

- There are 6 follow-up visits (V3 – V8). Window periods for visits will allow some flexibility in scheduling study visits. Genital and blood samples are collected to establish your baseline and to check drug levels throughout the study.
-

This table outlines the visit schedule and study procedures.

STUDY PROCEDURES	STUDY VISIT							
	SCREEN	ENROLL	FOLLOW-UP					
	V1	V2	V3	V4	V5	V6	V7	V8
Day	-45	0	3	5	7	10	14	28
Length of Visit	1hr	1hr	30m	30m	30m	30m	30m	30m
Medical History & Medication review	x	x	x	x	x	x	x	x
Visit Questionnaire	x	x	x	x	x	x	x	x
Collect/update contact information	x	x	x	x	x	x	x	x
HIV Rapid Test	x							
HIV Confirmatory Test	^							
Review Applicable Test Results	x	x						
Protocol Counseling	x	x	x	x	x	x	x	
Urine pregnancy test	x	x			x			x
Blood draw (MK2048 level)		x	x	x	x	x	x	x
Pelvic Exam with speculum	x	x	x	x	x	x	x	x
Genital sample collection	x	x	x	x	x	x	x	x
STD testing	x							
Pap smear	^							
CVL (vaginal “wash” with saline)	x						x	x
Cervical Biopsies					x			
Rectal Swab (MK2048 level)					x			
Brief Physical Exam	x							
Height and Weight	x							
Blood pressure	x	x	x	x	x	x	x	x
MK2048 Film Insertion (by clinician)		x						
Acceptability Questionnaire		x			x			
Compensation	$30	$40	$30	$30	$100	$30	$30	$40
Incentive if all visits in window								$40
Optional procedures		*						

^ as necessary

* V2 INCLUDES OPTIONAL SAMPLING: You may choose to have up to two vaginal swabs collected without a speculum and one blood sample collected following film insertion.

- Optional collection times are at 1, 2, 3, 4, 5, or 6 hours following film insertion.
- Additional compensation will be provided for each sample collected as follows:

	1, 2, 3 hour collection	4, 5, 6 hour collection
Vaginal Swab	$10	$20

Blood Sample	$20	$30

ADDITIONAL STUDY VISIT PROCEDURE INFORMATION

- Visit Questionnaires include asking things like sexual history, vaginal product use, and vaginal symptoms
- HIV testing will be done as part of the study.
    - A saliva sample will be tested for the antibody to HIV. An antibody is a substance that blood cells make to fight infection. Exposure (contact) to the HIV virus produces antibodies.
    - Results take 20 minutes. Study staff will talk to you about the meaning of the result, how you feel, and ways to prevent HIV and other sexually transmitted infections.
    - Sometimes HIV test results are not clearly positive, but also not clearly negative. In that case, blood will need drawn and sent to the lab for further testing which could take a week.
    - If the test shows you have HIV, you cannot join the study. You will be referred for medical care and other services. Your partner(s) may have access to free HIV counseling and testing, if needed.
- You will only get the results of tests that are applicable to your clinical care, including pregnancy test, STD and HIV tests, and evaluation for vaginal complaints if performed. You will not get the results of the other tests as they are done for research purposes only.
- Protocol counseling includes visit reminders, STD/HIV and abstinence counseling, and vaginal product use.
    - You must agree to not have sex (vaginal, anal and receptive oral) from V2 – V7.
    - You must also agree to not have sex 48 hours before the other study visits.
    - You must agree not to use vaginal products during the study and no tampon use between V5 – V7.
- Blood will be drawn to test MK2048 levels. Approximately 2 teaspoons of blood will be drawn each time.
- Genital sample collection includes collecting Q-tip like swabs from the vagina, cervix and rectum. The samples will be used to look at bacteria and other markers.
- STD (sexually transmitted disease) testing will include Chlamydia, Gonorrhea and Trichomonas.
- CVL (cervicovaginal lavage) is a vaginal “wash” of the vagina with saline; the wash takes one minute.
- Cervical biopsies will be collected by a physician investigator at V5.
    - Two adequate samples will be collected, approximately 3mm each or the size of a grain of rice.
    - The biopsy sites take approximately 7 days to heal. As a reminder, do not put anything in the vagina (tampon, sex toy) and avoid any type of sex (vaginal, anal, receptive oral) for 7 days.
- Study procedures can be repeated as needed (e.g. collection or processing error, or for clinical reasons).
- Microscopic exam (looking under a microscope) of vaginal discharge, urine dipstick and bimanual exam (to feel uterus and ovaries) may be done as clinically indicated during the study.
- Interim or unscheduled visits may occur if needed (e.g. abnormal results, repeat testing, side effects).
- Study participation for the required visits end at Visit 8 (Day 28).
- If you have ongoing side effects at V8, study staff may call you until the issue stabilizes or resolves.
- In the unlikely event you become pregnant while participating in the study, the study staff will refer you for appropriate care. You will be exited from the study, but study will need to follow up with you by telephone until the outcome of your pregnancy is known.
- If you are found to have an Gonorrhea, Chlamydia or Trichomonas or a vaginal infection during the study, you may be provided with directly observed antibiotic treatment.
- The samples for this research study will be sent to Magee-Womens Research Institute (MWRI) where most of the samples will be processed. Some samples may be sent to outside investigators for processing or

analysis. Importantly and regardless of lab, all samples are sent with a unique study number. No personal identifiers (name, SSN, birthdate) will be on the samples.

RISKS AND/OR DISCOMFORTS

By participating in this study, you could have these side effects or other side effects that we do not know about.

Procedure	Risk
Pregnancy Test	• Anxious or nervous having test or waiting for results • Denial, depression or worry with unexpected positive result
Pelvic Exam	• Discomfort with speculum
Genital and rectal specimens	• Minimal discomfort from collection of specimens
Cervical Biopsy	• Generally well tolerated but may cause pain (or pinching) • Cramping similar to menstrual cramps o Cramping typically resolves within minutes after procedure o May take ibuprofen prior to biopsy to minimize cramping • Vaginal spotting or bleeding for a couple days o Typically less than a period but if heavier, contact study staff • Infection of biopsy area o Unlikely but if foul odor or unusual discharge, contact study staff • A cut in the vagina can put you at increased risk of getting HIV if exposed • If you have an IUD, there is a risk of dislodging it with performing the biopsies. If displaced, a new IUD will be inserted at no cost to you
Vaginal use of MK 2048 film	• Vaginal discharge, irritation, discomfort • Vaginal burning, itching, redness and/or pain; • Yeast or vaginal infection • Allergic reaction
Blood draw	• Bruising, soreness, pain/discomfort, bleeding, infection at the site • lightheadedness, fainting
Participation in research; collection and storage of private health information, biospecimens and internet communication*	• Inconvenient • Breach of confidentiality
Questionnaires	• Discomfort with personal nature of questions
STD/HIV testing	• Worry or anxiety • Sadness, depression or denial with a positive test result o Positive gonorrhea and chlamydia test results will be reported to ACHD according to the Commonwealth of Pennsylvania reporting requirements o May be contacted and asked questions about sexual partners

*We will make reasonable efforts to protect the privacy of information on social media. Each platform has their own privacy policies and terms of use that may change at any time. The University of Pittsburgh cannot guarantee the privacy and confidentiality of information shared on social media in this research study. Your

information may not remain private. You will be instructed to visit site privacy policies and update privacy settings, as necessary. Social media retains information shared across accounts for an unknown length of time and it may be shared with others including targeted advertisers.

Pregnancy, Breastfeeding and Sexual Practices

The vaginal film is not a birth control method. We do not know what effect the study drug may have on pregnancy, including the effect of the study drug on the fetuses of women who use the vaginal film when pregnant, or the babies of women who use the vaginal film when breastfeeding. Because of this, pregnant women and women who are breastfeeding may not join this study. Women who join the study must agree to avoid sexual intercourse from V2 (enrollment) until V7 (7 days after the biopsy visit), and 48 hours prior to each study visit, use an effective method of birth control and have scheduled pregnancy tests while in the study. Effective methods of birth control include hormonal methods (like "the pill" or Depo-Provera injections), the IUD, sterilization (or "tied tubes"), abstinence (or not having sex), same sex partner, or having a partner who has had a vasectomy. You must agree to use this method of birth control until the last study visit, approximately 4 weeks after you are enrolled.

If you do not think you can be sexually abstinent until at least 7 days after the biopsy visit, then you should not enroll in this study. In the unlikely event that 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 keep coming here for study visits as originally planned. We will change the study procedures as needed to protect your health while you are pregnant. The outcome of your pregnancy is important to study staff; you will be followed through the outcome of your pregnancy.

If you were to become HIV infected while using the study product, the HIV virus may develop resistance to MK2048. Developing MK2048 resistance means that the MK2048 may not be able to treat HIV however, MK2048 is not currently used to treat HIV.

BENEFITS

You will not directly benefit from participating in this study. You may, however, feel satisfaction knowing that information learned from this study may help in the development of vaginal products for women. You will also have exams and HIV/STD testing as part of your participation in this study.

NEW INFORMATION

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

CLINICALLY RELAVENT RESEARCH RESULTS: You will be given the results of your pregnancy, STD and HIV testing. Counseling, referrals and treatment will be discussed as applicable. There is no plan to provide you with your results for tests collected for research purposes only or results of the study in general once it is completed.

STOPPING STUDY DRUG OR BEING WITHDRAWN EARLY

The study staff may remove you from the study early without your permission if:

- The study is stopped or cancelled
- You are not able to keep appointments

- Other reasons that may prevent you from completing the study successfully or safely. This would include a newly diagnosed pregnancy or HIV. If you become pregnant, although you will be terminated from further study visits, study staff will continue to contact you through the outcome of your pregnancy.

Participants who withdraw or who are withdrawn from the study prior to completing follow up may be asked to complete a final study visit.

COSTS TO YOU
There is no cost to you or your insurance for study related visits or procedures.

REIMBURSEMENT
You will receive compensation for your time, effort, and travel expenses as detailed in the study procedure table above. If you complete all the required study visits within the designated window periods, you will receive a total of $370. If you complete all the required study visits and the optional V2 procedures, it is possible to be compensated up to $440. If you complete a visit but are seen outside of the protocol window for that visit, $10 will be deducted from the scheduled visit payment.

Parking passes or assistance with transportation will be provided as needed.

CONFIDENTIALITY
Research records will be kept and accessed by the investigators for a minimum of seven years. The computers that store any data that is collected are password and firewall protected. Study related questionnaires and specimens are identified by a unique study number to protect your confidentiality. The link to your name and study number will be kept in a separate, secure location that only the clinical research team has access to. If data from this study is shared with other (outside) investigators interested in infections, the information will be shared without personal identifiers (for example name, date of birth, SSN).

In unusual circumstances, your research records may be inspected by appropriate agencies. The University of Pittsburgh Education and Compliance Office for Human Subject Research (ECO-HSR) may review your identifiable research information (which may include your identifiable medical record information) for the purpose of monitoring the appropriate conduct of this research study. The sponsor of the study or their designee may also review your identifiable research information but only for the purposes of monitoring the conduct and data collected for the study. No information will be extracted from your medical record unless you sign a separate medical release and no information about your participation in this study will be placed in your medical record.

We will do everything possible to keep your test results confidential but this cannot be guaranteed. If others would become aware of your HIV status, it could result in discrimination which may impact your employability, insurability, or even prevent you from traveling to certain countries. If you test positive, Pennsylvania state law requires your name and results be reported to the local health department. All information will be handled in compliance with the Pennsylvania law on HIV-related confidential information.

If you test positive for gonorrhea, chlamydia, or HIV, the Commonwealth of Pennsylvania requires that your name be given to the Allegheny County Health Department. You may be contacted and asked questions about your sexual partner(s).

The investigators may use or disclose, for purposes described above, identifiable information (which may include identifiable medical information) related to your being in this study a minimum of 7 years and for as long (indefinite) as it may take to complete this study.

A description of this clinical trial will be available on http://www.clinicaltrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Your participation in this study may include whole genome sequencing. Whole genome sequencing is the mapping out of a person's unique DNA. Your genome is the unique blueprint for your body. Sometimes, because of new or inherited genetic mutations, your genes can cause a disease or increase your risk for disease. By sequencing your genome, health professionals can look at the unique variations found in your genes. Some of it matters. Some doesn't matter. Some is still unknown or uncertain.

RESEARCH-RELATED INJURY
If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation. You do not give up your legal rights by signing this form. The US National Institutes of Health (NIH) does not have a mechanism to provide compensation for research related injury.

HIPAA AUTHORIZATION: We are requesting your authorization or permission to access your protected health information for research purposes. This authorization will be valid for an indefinite period of time. We may obtain information concerning your birth control method, age, level of education, past medical and gynecologic history and results of any tests that were already done as part of your standard evaluation.

This information may be needed so that we can compare the data in your medical record to the data obtained for this study. We may use the information to identify whether you meet the conditions for participation in this study. We may also use your medical record to obtain new contact information for you while you are in the study. The information may be needed for retention purposes.

The identifiable information will be made available to members of the research team, for an indefinite period of time and may be shared with other groups, possibly including authorized representatives of the sponsor of the study, National Institutes of Health (or authorized representative that they delegate, such as representatives from monitoring or auditing companies) or authorized officials from the University of Pittsburgh Research Conduct and Compliance Office. We will protect your privacy and the confidentiality of your records, as described in this document but cannot guarantee the confidentiality of your research records, including information obtained from your medical records, once your personal information is disclosed to others outside UPMC or the University.

Your research information and data may be shared with investigators conducting other research. The shared information may be labeled by your unique study number and linked to the study data that matches the unique

study number. The link between your personal identifying information and the unique study number is maintained by the clinical research team and will not be shared with other investigators or laboratory staff.

You can always withdraw your authorization to allow the research team to review your medical records by contacting the investigator listed on the first page and making the request in writing. If you do so, you will no longer be permitted to participate in this study. Any information obtained from you up to that point will continue to be used by the research team.

YOUR RIGHTS AS A RESEARCH PARTICIPANT/VOLUNTEER

Your participation in this research study is entirely voluntary. You may want to discuss this study with your family and friends and your personal physician before agreeing to participate. If there are any words you do not understand, feel free to ask us. The investigators will be available to answer your current and future questions.

Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh, your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

CERTIFICATE OF CONFIDENTIALITY:

To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. The researchers can use this Certificate to legally refuse to disclose information that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings, for example, if there is a court subpoena. The researchers will use the Certificate to resist any demands for information that would identify you, except as explained below.

The certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally-funded projects or for the information that must be disclosed in order to meet the requirements of the US Food and Drug Administration (FDA) and other regulatory authorities.

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality will not be used to prevent disclosure to state or local authorities of child abuse and neglect, or harm to self or others.

PROBLEMS OR QUESTIONS

If you ever have any questions about the study, or if you have a research-related injury, you should contact Katherine Bunge, MD or the research staff at (412) 641-4242. If you ever have any questions about your rights as a research participant, you can contact the University of Pittsburgh IRB at 1-866-212-2668.

CONSENT FOR FUTURE USE

As part of this study, there will be samples that are collected specifically for future use and there may also be leftover samples from the main testing that could be stored for future use. These samples may be used by investigators for future research on genital tract infections, for further understanding of STDs and how the body responds to infection and/or genetic testing and may include whole genome sequencing. Whole genome sequencing is the mapping out of a person's unique DNA. Your genome is the unique blueprint for your body. Sometimes, because of new or inherited genetic mutations, your genes can cause a disease or increase your risk for disease. By sequencing your genome, health professionals can look at the unique variations found in your genes. Some of it matters. Some doesn't matter. Some is still unknown or uncertain.

The specimens would be stored at Magee Womens Research Institute by study number; your name will not be on the sample. Information linking your study number to your name will be kept in a separate, secure location in the clinical research area.

Your study samples or genetic material may lead, in the future, to new inventions or products. If the research investigators develop new products from the use of your biologic sample or genetic material, there are currently no plans to share with you any money or other rewards that may result from the development of the new product.

You would not be informed of results of the future tests since the data may not be applied to a clinical setting and may not affect clinical care. Any results from the research done on future use samples would not be put in your medical record and would be kept confidential. Samples may be given to other investigators (secondary investigators), other than Magee and UNC investigators. If samples were given to secondary investigators they would be made available without links to your personal identifying information.

There are few risks to you from future use of your specimens. Reports about research done with your specimens will not be put in your health record, but will be kept with the study records. Results from future research using your specimens may be presented in publications and meetings, but your name will not be identified.

PLEASE <u>INITIAL</u> YOUR CHOICE BELOW (chose one):

_____ I agree to the future use of my specimens as described above
initials

OR

_____ I DO NOT agree to the future use of my specimens as described above
initials

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PLEASE INITIAL WHICH, IF ANY, OPTIONAL RESEARCH PROCEDURES YOU AGREE TO BELOW:

Optional Procedure	Visit	Timepoint(s)	Initial if you agree
Up to two additional vaginal swab(s)	V2 Enrollment	1, 2, 3, 4, 5, 6 hrs after film placement	
One additional blood sample	V2 Enrollment	1, 2, 3, 4, 5, 6 hrs after film placement	

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VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research study during the course of this study, and that such future questions, concerns or complaints will be answered by a qualified member of the research team or by the principal investigator listed on the first page. I understand that I may always request that my questions, concerns or complaints be addressed by the Principal Investigator. At any time I may also contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable. By signing this form I agree to participate in this research study for the purposes described above. A copy of this consent form will be offered to me.

_____

Printed Name of Participant

_____ _____ _____

Participant Signature Date Time AM/PM

CERTIFICATION OF INFORMED CONSENT: I certify that I have explained the nature and purpose of this research to the above individual and I have discussed the potential benefits and possible risks of study participation. Any questions the individual has about this study have been answered, and we will always be available to address future questions as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

_____ _____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date