

**A Phase II, Double-Blind, Randomized, Placebo-Controlled, Multicenter Trial to
Assess the Safety and Efficacy of 5% Monolaurin Vaginal Gel Administered
Intravaginally for the Treatment of Bacterial Vaginosis**

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STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation guideline E6: Good Clinical Practice: Consolidated Guideline, the applicable regulatory requirements from US Code of Federal Regulations (CFR) (Title 45 CFR Part 46 and Title 21 CFR including Parts 50 and 56) concerning informed consent and Institutional Review Board regulations, and the NIAID Clinical Terms of Award.

All individuals responsible for the design and conduct of this study have completed Human Subjects Protection Training and are qualified to be conducting this research prior to the enrollment of any subjects. Curricula vitae for all investigators and sub-investigators participating in this trial are on file in a central facility (21 CFR 312.23 [a] [6] [iii] [b] edition).

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator:

Signed: _____

Date: _____

Name

Title

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LIST OF ABBREVIATIONS

ACOG	American College of Obstetricians and Gynecologists
AGUS	Atypical Glandular Cells of Uncertain Significance
AE	Adverse Event/Adverse Experience
ALT	Alanine Aminotransferase
ASCUS	Atypical Squamous Cells of Uncertain Significance
AST	Aspartate Aminotransferase
BV	Bacterial Vaginosis
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
cGMP	Current Good Manufacturing Practices
CIN	Cervical Intraepithelial Neoplasia
CRF	Case Report Form
CRO	Contract Research Organization
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GML	Glycerol Monolaurate
GRAS	generally recognized as safe
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HSIL	High Grade Squamous Intraepithelial Dysplasia
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IDES	Internet Data Entry System
IEC	Independent or Institutional Ethics Committee
IL	Interleukin
IND	Investigational New Drug Application
ISM	Independent Safety Monitor
IRB	Institutional Review Board

JAMA

KOH

LSIL

LOCF

Journal of the American Medical Association

Potassium Hydroxide

Low grade Squamous Intraepithelial Neoplasia

Last Observation Carried Forward

MedDRA [□]	Medical Dictionary for Regulatory Activities
MIP	Macrophage Inflammatory Protein
mITT	Modified Intent-To-Treat
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NDA	New Drug Application
NF	The National Formulary
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per-Protocol
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical and Data Coordinating Center
SIV	Simian Immunodeficiency Virus
SOP	Standard Operating Procedure
TSST	Toxic Shock Syndrome Toxin
US	United States
USP	United States Pharmacopeia

PROTOCOL SUMMARY

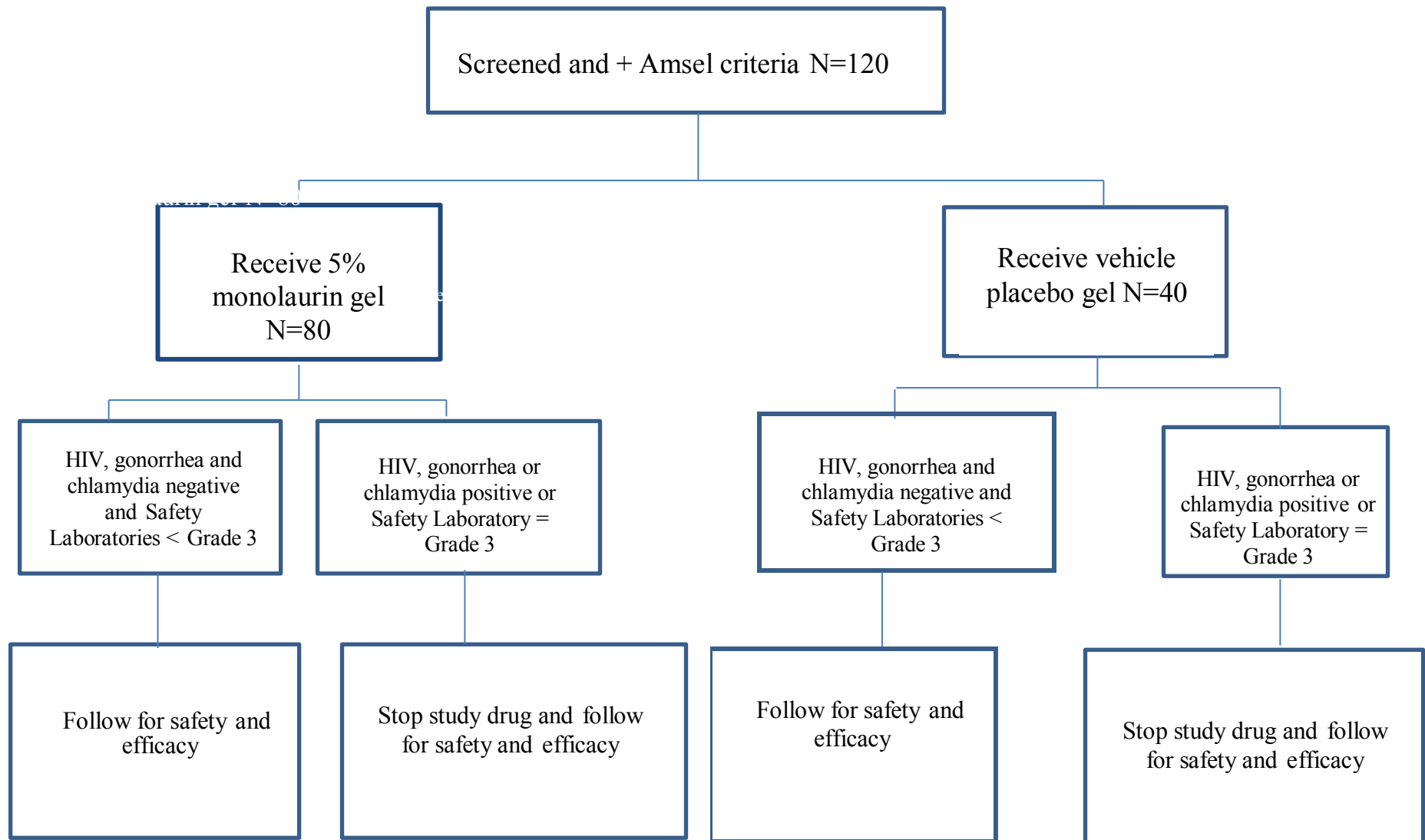
Title:	A Phase II, Double-Blind, Randomized, Placebo-Controlled, Multicenter Trial to Assess the Safety and Efficacy of 5% Monolaurin Vaginal Gel Administered Intravaginally for the Treatment of Bacterial Vaginosis
Phase:	II
Population:	120 women, 18-50 years old, with clinical evidence of bacterial vaginosis, recruited from the community around the University of Iowa Hospitals and Clinics, Cincinnati Children's Hospital Medical Center and Duke University School of Medicine.
Number of Sites:	2
Study Duration:	13 months
Subject Participation Duration:	4 weeks
Estimated Time to Complete Enrollment:	12 months
Description of Agent or Intervention:	5% Monolaurin Vaginal Gel administered intravaginally or placebo gel
Objectives:	<p><u>Primary Objectives:</u></p> <ul style="list-style-type: none">• To assess the safety and tolerability of 5% Monolaurin Vaginal Gel compared to Vehicle Placebo Gel (excipients only).• To assess the efficacy by clinical cure rate of 5% Monolaurin Vaginal Gel compared to Vehicle Placebo Gel at Visit 2. <p><u>Secondary Objectives:</u></p> <ul style="list-style-type: none">• To evaluate the therapeutic cure rate of 5% Monolaurin Vaginal Gel compared to Vehicle Placebo Gel at Visits 2 and 3.• To evaluate the clinical cure rate of 5% Monolaurin Vaginal Gel compared to Vehicle Placebo Gel at Visit 3.• To evaluate the changes in Nugent's criteria of vaginal bacterial flora at Visits 2 and 3.

Exploratory Objectives:

- To evaluate the clinical and therapeutic cure rates of 5% Monolaurin Vaginal Gel compared to Vehicle Placebo Gel for each subgroup (first episode BV and recurrent BV) at Visits 2 and 3.
- To evaluate quantitative changes in selected bacterial species at Visits 2 and 3.
- To evaluate the quantitative changes of *Candida* spp (in subjects with *Candida* spp. identified at screening) at Visits 2 and 3

Description of Study Design:

This is a Phase 2, double-blind, randomized, placebo-controlled, multi-center trial of 120 BV subjects randomized at a ratio of 2:1 to receive active test article (5% Monolaurin Vaginal Gel) or placebo (Vehicle Placebo Gel) as outpatient therapy. Each subject will self-administer intravaginal gel twice daily for 3 successive days for a total of six doses. There will be three clinic visits over 31 days.

Schematic of Study Design:**Figure 1: Schematic of Study Design**

1. KEY ROLES

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2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. Background Information

Bacterial Vaginosis

Bacterial Vaginosis (BV) is a disease of the vagina caused by bacteria. Current studies suggest that BV is caused by an imbalance of naturally occurring bacterial flora that is characterized by a paucity of hydrogen peroxide-producing lactobacilli and high concentrations of facultative anaerobic organisms such as *Gardnerella vaginalis*, *Mycoplasma hominis*, *Bacteroides* species, and others [1]. In women of childbearing age, BV is the most common cause of vaginitis accounting for 22-50% of women who have symptoms of vaginitis [2]. In a cohort of young healthy women, nearly 30% had been treated for BV [3]. In American and European studies, the prevalence often ranges from 5-36% with higher prevalence reported in women attending sexually transmitted disease clinics [4].

BV has been associated with preterm delivery, chorioamnionitis, post-abortion infection, and pelvic inflammatory disease [5-7].

Signs and Symptoms

The most common symptom of BV is an abnormal homogeneous off-white vaginal discharge (especially after sex) with an unpleasant smell. On physical exam, the discharge often coats the walls of the vagina, though there is relatively little erythema, edema or inflammation noted on the vulva, labia, or the vaginal walls [8]. Up to 50% of women with BV are asymptomatic [8].

Etiology

A healthy vagina normally contains many microorganisms; some of the common ones are *Lactobacillus crispatus* and *Lactobacillus jensenii*. *Lactobacilli*, particularly hydrogen peroxide-producing species, appear to help prevent other vaginal microorganisms from multiplying to a level where they cause symptoms. The microorganisms involved in BV are very diverse, but include *Gardnerella vaginalis*, *Mobiluncus*, *Bacteroides*, and *Mycoplasma*. A change in normal bacterial flora including the reduction of lactobacillus, which may be due to the use of antibiotics or pH imbalance, allows more resistant bacteria to gain a foothold and multiply.

Although BV can be associated with sexual activity, there is no clear evidence of sexual transmission and it is possible for sexually inactive persons to develop BV [9]. Rather, BV is a disordering of the chemical and biological balance of the normal flora. A variety of risk factors associated with BV have been identified including a history of smoking, ethnicity, intrauterine device use, and douching [4,9].

Diagnosis

The diagnosis of BV has been based clinically on Amsel and more recently Nugent scoring criteria using direct Gram stain of vaginal secretions.

Amsel criteria

In 1983, Amsel *et al.* established clinical criteria for diagnosing BV [10]. To make a diagnosis of BV, a speculum exam is performed and a swab from inside the vagina is obtained. The American College of Obstetricians and Gynecologists (ACOG) clinical guidelines state that three of the four Amsel's criteria must be positive to warrant a diagnosis of BV [2]. However, the Food and Drug

Administration (FDA) guidance to industry recommends that subjects in BV studies should meet all four Amsel criteria.

- An abnormal gray discharge.
- Vaginal pH greater than 4.5.
- A positive amine test (often called a Whiff test where a characteristic "fishy" odor develops after adding a small amount of potassium hydroxide [10% KOH] to a microscopic slide containing the vaginal discharge).
- $\geq 20\%$ of the epithelial cells seen on microscopic examination are coated with bacteria (Clue cells).

Figure 2: Light Microscopy of unstained vaginal smear showing clue cell



Nugent criteria

In 1991, Nugent *et al.* described a Gram stain scoring system of vaginal smears to diagnose BV [11]. The Nugent score is calculated by assessing for the presence of large Gram-positive rods (*Lactobacillus* morphotypes), small Gram-variable rods (*G. vaginalis* morphotypes), and curved Gram-variable rods (*Mobiluncus spp.* morphotypes). The Nugent score can range from 0 to 10. A score of 7 to 10 is consistent with BV while 4-6 is considered intermediate and 0-3 is negative for BV. Compared to the Amsel criteria, the Nugent score allows for assessment of alteration in vaginal flora as a continuum rather than a dichotomy.

In the research setting, the Nugent Score is considered the gold standard method for diagnosis of BV [12]). Scoring is as follows:

Table 1: Laboratory examination of vaginal smears and the determination of the Nugent Score

N Score = The sum of the scores for each bacterial morphotype listed below. (Note: Number of Organisms seen/high power field as determined by averaging a representative field by two independent evaluators)						
Lactobacilli	SCORE	<i>Gardnerella, Bacteroides</i>	SCORE	Curved gram-negative bacilli	SCORE	Sum= *N SCORE
≥ 30	0	0	0	0	0	0
<30-5	1	<1	1	<1	1	3
<5-1	2	1 to <5	2	1 to <5	1	5
<1	3	5 to <30	3	5 to <30	2	8
0	4	≥ 30	4	≥ 30	2	10

Current Treatments

The Centers for Disease Control and Prevention (CDC) recommends metronidazole 500 mg orally twice daily for 7 days, intravaginal metronidazole gel 0.75% once daily for 5 days or clindamycin cream 2% intravaginally once daily for 7 days as first line therapy for BV [9].

Alternative regimens using oral tinidazole, oral clindamycin or clindamycin ovules are also provided. However, up to 30% of women will have recurrent BV within 3 months of therapy [13]. Frequent emergence of vaginal candidiasis results from antibiotic suppression of vaginal flora, and *Candida species* are frequent commensal organisms in vaginal flora. Given BV's high recurrence rates and the risks for antimicrobial resistance, additional treatment options are desirable.

2.1.1. Monolaurin (GML)

Monolaurin (GML)

Monolaurin, also commonly referred to as glycerol monolaurate (GML), is a monoglyceride surfactant. It is an ester formed from glycerol and lauric acid. Laboratory studies have shown that GML has antibacterial, antifungal and antiviral properties.

GML Mechanism of Action

Several published studies indicate that monolaurin's primary mechanism of action is the insertion of its fatty acid 12-carbon chain into the microorganism's plasma membrane thus interfering with plasma membrane signal transduction and transcription [14-19].

In addition to its direct effects on pathogens, monolaurin stabilizes animal and human cell membranes to interfere with the damaging effects of bacterial exotoxins, endotoxins, and hypotonic solutions, including reduction of chemokines (interleukin [IL]-8, macrophage inflammatory protein [MIP]-3 α) that cause vaginal inflammation [15,19,20]. This property is advantageous in the prevention of infection transmission [20]. Typically, agents that cause vaginal inflammation promote transmission of sexually transmitted infections such as human immunodeficiency virus (HIV) [21-24].

A study at the University of Minnesota demonstrated that GML's action occurs at the

(Schlievert unpublished data).

Monolaurin's mechanism of action of blocking signals at the pathogen's plasma membrane provides an advantage over other antibiotics with regard to the development of resistance. The cell walls of bacteria have 10-20 two-component membrane signaling systems that can all be targeted by GML [14, 25]. This high number of targets suggests that it may be more difficult for bacteria to develop resistance to monolaurin. Two published studies demonstrate that bacteria with long-term exposure to sub-inhibitory concentrations of GML remain sensitive to its bactericidal properties. Petschow *et al.* investigated the susceptibility to metronidazole, tetracycline, and monoglycerides (including monolaurin) to three test strains of *Helicobacter pylori*. The frequency of resistance of variants to either metronidazole or tetracycline when tested at five times the minimum inhibitory concentration was 10^{-5} to 10^{-7} , whereas no resistant variants of *Helicobacter pylori* were found for any of the monoglycerides tested [26]. Schlievert *et al.* did not observe any increase in resistance to GML's antimicrobial and anti-exotoxin effects with yearlong passage of *Staphylococcus aureus* MN8 on sub-growth-inhibitory concentrations of GML [25].

The antimicrobial activity of monolaurin was examined *in vitro* against ~40 pathogenic vaginal organisms, including bacteria, yeasts, protozoa, and viruses [25]. Bacterial organisms studied included *Gardnerella vaginalis*, *Bacteroides fragilis*, *Mycoplasma hominis*, various *Streptococcus* spp., *Neisseria gonorrhoeae*, *Escherichia coli*, and others. GML was effective in inhibiting the growth of all potentially pathogenic *Candida* species (11 strains of *Candida albicans* were tested *in vitro* as well as one strain of each of four other major *Candida* species) providing for the potential to treat pathogenic fungal infections in the vagina. Since *Candida* is also controlled by monolaurin, fungal overgrowth secondary to antibiotic therapy should not occur. Additionally, lactobacilli, as normal vaginal microflora, are not significantly affected by monolaurin suggesting GML may preserve normal bacterial species during treatment while inhibiting overgrowth of other pathogens such as yeast species. For the microorganisms relevant to the proposed indication, the highest minimum microbicidal concentration of monolaurin was 500 µg/mL, which is 100-fold lower than the concentration of monolaurin in the proposed drug product (50 mg/mL).

Additionally, Li *et al.* demonstrated that 5% Monolaurin Vaginal Gel prevents transmission and infection of simian immunodeficiency virus (SIV) in 12 rhesus macaque monkeys [20]. Peterson *et al.* showed that GML (250 µg/0.25 mL), when co-administered vaginally to rabbits with toxic shock syndrome toxin-1 (TSST-1), completely prevented TSST-1-induced Toxic Shock Syndrome after 24 hours [15].

In addition to its broad and appropriate antimicrobial activity, monolaurin has been shown *in vitro* to reduce the production of inflammatory cytokines by human vaginal epithelial cells and human T and B cells in response to superantigen and antigen stimulation, and to protect mammalian cells from exotoxin. *In vivo* studies in rhesus monkeys and humans have shown that monolaurin, but not vehicle control, significantly reduces cytokine production.

In summary, monolaurin has been shown to have important antimicrobial effects on pathogens found vaginally in women. In addition, monolaurin also inhibits the production of exotoxins by susceptible microorganisms and stabilizes eukaryotic cells to inhibit microbe-induced inflammation. The compound does not affect lactobacilli, the normal flora of the human vagina. Finally, the breadth of antimicrobial activity suggests that the adverse event (AE) of vaginal microbial overgrowth of other microorganisms is highly unlikely.

Pharmacokinetics (PK)

There have been no formal PK studies of Monolaurin Vaginal Gel in animals or humans to date. However it is believed that monolaurin systemic absorption will be minimal, and any monolaurin absorbed across the vaginal wall would be quickly metabolized by plasma lipases to lauric acid and glycerol, or re-esterified to a triglyceride, all common compounds found in dietary fats and lipids [27]. There is no evidence that the presence of monoglycerides of food fats has any deleterious effect on cells or tissues [28]. Additionally, glyceryl monostearate, a 21 carbon monoglyceride, is approved for use as an excipient in vaginal cream at concentrations up to 17% (e.g., PREMARIN® Vaginal Cream).

Phase 2 Clinical Studies using Monolaurin Vaginal Gel

The following two clinical studies were performed at the University of Minnesota, and were designed as single-center, double-blind device clinical studies using Monolaurin Vaginal Gels containing up to 5% monolaurin.

- **Device Clinical Pilot Study (Protocol 2005-024) – Status: Completed**

A single-center, randomized, double-blinded, device clinical pilot study of Monolaurin Vaginal Gel enrolled 36 women [29]. The objective was to determine the effects of an intravaginal gel containing GML on vaginal *Candida albicans*, *Gardnerella vaginalis*, and *Lactobacillus spp.* and to determine GML persistence vaginally.

The key endpoints in the study were differences in colony counts of *Candida albicans*, *Gardnerella vaginalis*, and *Lactobacillus spp.* between vaginal swabs taken before (at Visit 1) and after use of the intravaginal gel at Visit 2, approximately 12 hours after the last administration; and differences in GML amounts vaginally between swabs taken before and after use of GML-containing gel.

The study included non-pregnant women, 18-50 years of age who suspected they might have a vaginal infection. Women were administered 5 mL of gel containing 0%, 0.5% or 5% of GML every 12 hours for 2 consecutive days for a total of four doses. Vaginal swabs were collected before and immediately after the first gel administration and 12 hours after the final gel administration for the following tests: vaginal pH, wet mount and KOH test, Gram staining, yeast and bacterial cultures (*Candida albicans*, *Gardnerella vaginalis*, and *Lactobacillus spp.*) and GML measurement (Gas Chromatograph-Mass Spectroscopy assay). Subjects were randomly assigned to receive one of the three study products (control, low or high concentration of GML) and were provided three more applications/doses of the appropriate study product formulation for self-administration. At about 12 hours after the final gel administration, vaginal swabs were again collected and the same tests performed.

A total of 36 women were analyzed in this study. The numbers of subjects receiving gels were as follows: control, N=14; 0.5% monolaurin, N=13; and 5% monolaurin, N=9.

Results compared the prevalence of the three study microorganisms, comparing Visit 1 to Visit

Because of the small numbers of study participants, data for GML (0.5% and 5%) were evaluated individually but also pooled because of sample size for determination of significant differences between visits.

The prevalence of *Lactobacillus* between Visits 1 and 2 for all treatment groups was not significantly different, providing evidence that the gels with or without GML have no effect on lactobacilli.

Women who received the 0.5% GML gels showed significant reductions in *Candida* colony-forming units (CFU) between Visits 1 and 2 ($P=0.001$) but not for the 5% GML ($P=0.15$) due to sample size (6 at Visit 1, 2 at Visit 2). When GML treatment groups (0.5% and 5% GML) were combined in the analysis, significant reductions of both *Candida* counts between Visits 1 and 2 were found ($P=0.015$). The reduction of *Candida* in the GML gel-treated women who showed reductions between Visits 1 and 2 was nearly to the minimum level of detection in the analyses (10^2 CFU).

A control gel effect was found for women with *Gardnerella vaginalis*. Significant reductions were seen in *Gardnerella vaginalis* counts between Visits 1 and 2 for women who received the control ($P=0.006$). Women who received the 0.5% and 5% gels did not individually show significant reductions between Visits 1 and 2, but when GML treatment groups (0.5% and 5% GML) were

combined in the analysis, the counts of *Gardnerella vaginalis* were found to be significantly lower between Visits 1 and 2 ($P=0.015$).

GML was detected vaginally in the women immediately after initial application of the GML gels (data not shown); GML was not detected ($<0.7 \mu\text{g/mL}$) in women who received control gels.

GML was undetectable in women during the second visit, regardless of gel treatment, indicating that GML did not persist in vaginal secretions for the 12 hours between treatments. No AEs were reported by any of the women.

- **Device Clinical Safety Study of 5% GML Applied Vaginally – Status: Analysis and Final Report in Preparation**

In this single-center, double-blind, parallel design, randomized study subjects (HIV negative, healthy, non-pregnant women) receive once [REDACTED] vaginal 5% GML or vehicle placebo gel for [REDACTED]

The primary objective of the study is to determine the safety of [REDACTED] of vaginal GML [REDACTED] by HIV negative, healthy women for [REDACTED] [REDACTED]. A secondary objective is to determine the tolerability and acceptability of a 5% GML vaginal gel.

The primary endpoint of the study is to assess the effect of vaginal GML on [REDACTED]
[REDACTED]
[REDACTED]

The secondary endpoints are as follows:

- 1) To assess the effect of GML on [REDACTED]
[REDACTED]
- 2) To determine the effect of vaginal GML on the [REDACTED] as determined by [REDACTED]
[REDACTED]
- 3) To determine the acceptability and tolerability of vaginal GML based on [REDACTED]
[REDACTED]

The protocol specifies enrollment of [REDACTED] women ages 18-45, [REDACTED] [REDACTED]. At the time of enrollment, women are randomized to either the treatment or placebo group. The treatment group receive a [REDACTED] vaginal applicator containing either [REDACTED]
[REDACTED] or [REDACTED]
[REDACTED]

For both groups, vaginal gels are administered [REDACTED]
[REDACTED]

Subjects return to clinic every [REDACTED]
[REDACTED] At Visits [REDACTED]
[REDACTED]
[REDACTED]

The primary endpoint is based on [REDACTED] A subject is declared [REDACTED]
[REDACTED]

[REDACTED] A subject is required to [REDACTED]
[REDACTED] The secondary endpoints are based on [REDACTED]
[REDACTED]
[REDACTED] by the subjects.

This study is currently in the process of being reported and results may be used to power definitive pivotal studies to contribute to a New Drug Application (NDA) filing.

Initially, 7 women were enrolled and completed the study. These women consistently reported that

[REDACTED]
[REDACTED] This
was deemed [REDACTED] by study participants, and thus [REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED] The use of these [REDACTED]
[REDACTED] The
study has recently been completed, and the data are presently being analyzed. [REDACTED]
[REDACTED] by any of the women.

Hereafter, the gels referred to in this protocol will be identified as 5% Monolaurin Vaginal Gel, and Vehicle Placebo Gel, which has the same formulation except not containing 5% GML.

2.2. Rationale

Extensive *in vitro* data demonstrates the antimicrobial activity of monolaurin on the spectrum of microorganisms associated with BV, while having a favorable effect on eukaryotic membrane stability by modification of inflammatory response.

New therapies for BV are needed and 5% Monolaurin Vaginal Gel has the potential to eradicate flora associated with BV while maintaining some of the normal flora that is considered protective for vaginal health, and with the added potential of preventing the emergence of vaginal candidiasis.

This study is designed to evaluate the safety and provide early efficacy in treatment of women with BV.

2.3. Potential Risks and Benefits

2.3.1. Potential Risks

There are no known safety pharmacology studies of GML's effects on the cardiovascular and respiratory systems. However, GML has low systemic absorption making these toxicities less likely.[27]

The initial nonhuman primate study exposing rhesus macaque monkeys to 5% GML gel (N=9), or vehicle (N=3) for 6 months provided evidence that prolonged gel exposure does not induce inflammation, supported by colposcopic biopsy data that showed that normal mucosal integrity was maintained. GML reduced the early inflammatory production of interleukin 8 by vaginal epithelial cells, but the quantitative aerobic microflora did not differ between the 5% gel and the control gel, with no suppression of lactobacilli even with such prolonged daily exposure [30].

An initial study in 36 women exposed to gels containing 0.5% (N=13), 5% (N=9), or 0% (N=14) GML every 12 hours for 2 days reported no AEs, and no negative effects on lactobacilli, while reducing *Candida* and *Gardnerella vaginalis* [29].

The only minor side effect noted was vaginal leakage.

There may be other side effects from GML, even serious ones that are not yet known. There are no known risks of GML to pregnant women, fetuses, or infants of breastfeeding women. The risks are thought to be minimal due to GML's low systemic absorption. However, the use of GML in pregnancy or breastfeeding may involve risks to the subject, fetus, or nursing infant that are currently unforeseeable. This study will exclude pregnant and breastfeeding women and ensure that women of childbearing potential take precautions to avoid pregnancy during the study.

2.3.2. Known Potential Benefits

Participation will contribute to the knowledge about the safety and early effects of the treatment on symptoms of BV and the changes in vaginal flora. Subjects will receive a gynecological physical exam and routine laboratory tests to assess their health status.

Society may benefit from the potential development of a new treatment for BV.

3. OBJECTIVES

3.1. Study Objectives

Primary Objectives:

- To assess the safety and tolerability of 5% Monolaurin Vaginal Gel compared to Vehicle Placebo Gel (excipients only).
- To assess the efficacy by clinical cure rate of 5% Monolaurin Vaginal Gel compared to Vehicle Placebo Gel at Visit 2.

Secondary Objectives:

- To evaluate the therapeutic cure rate of 5% Monolaurin Vaginal Gel compared to Vehicle Placebo Gel at Visits 2 and 3.
- To evaluate the clinical cure rate of 5% Monolaurin Vaginal Gel compared to Vehicle Placebo Gel at Visit 3.
- To evaluate the changes in Nugent's criteria of vaginal bacterial flora at Visits 2 and 3.

Exploratory Objectives:

- To evaluate the clinical and therapeutic cure rates of 5% Monolaurin Vaginal Gel compared to Vehicle Placebo Gel for each subgroup (first episode BV and recurrent BV) at Visits 2 and 3.
- To evaluate quantitative changes in selected bacterial species at Visits 2 and 3.
- To evaluate the quantitative changes of *Candida* spp (in subjects with *Candida* spp. identified at screening) at Visits 2 and 3.

3.2. Study Outcome Measures

3.2.1. Safety Outcomes

All safety outcomes will be assessed in the safety analysis population of all subjects who received one or more doses of study product. Outcomes will be reported by treatment arm.

- Primary Safety Outcome –
 - Number of subjects reporting solicited urogenital AEs following the first dose of the study product through Visit 2 (Day 8-15).
 - Number of subjects reporting serious adverse events (SAEs) considered product-related following the first dose of the study product through Visit 3 (Day 22-31)
- Secondary Safety Outcomes –
 - Number of subjects experiencing non-laboratory non-solicited AEs following the first dose of the study product through Visit 3 (Day 22-31).

- Number of subjects experiencing laboratory AEs following the first dose of the study product through Visit 2 (Day 8-15).

3.2.2. Efficacy Outcomes

3.2.2.1. Primary and Secondary Efficacy Outcomes

- Primary Efficacy Outcome – Proportion of subjects with clinical cure in each study arm at Visit 2 (Day 8-15).
- Secondary Efficacy Outcomes –
 - Proportion of subjects with therapeutic cure in each study arm at Visit 2 (Day 8-15) and at Visit 3 (Day 22-31).
 - Proportion of subjects with Nugent score of 3 or less (negative for BV) and 4-6 (intermediate) in each study arm at Visit 2 (Day 8-15) and at Visit 3 (Day 22-31).
 - Proportion of subjects with clinical cure in each study arm at Visit 3 (Day 22-31).

3.2.2.2. Exploratory Efficacy Outcomes

- Proportion of subjects with clinical cure in each study arm by subgroup (first time or recurrent infection) by Visit 2 (Day 8-15) and by Visit 3 (Day 22-31).
- Proportion of subjects with therapeutic cure in each study arm by subgroup (first time or recurrent infection) by Visit 2 (Day 8-15) and by Visit 3 (Day 22-31).
- Mean count of *Lactobacillus spp.*, *Gardnerella spp.*, and *Mobiluncus spp.*, respectively, in each study arm at baseline, Visit 2 (Day 8-15) and at Visit 3 (Day 22-31).
- Mean fungal colony count in vaginal secretions in each study arm at baseline, at Visit 2 (Day 8-15) and at Visit 3 (Day 22-31).

4. STUDY DESIGN

This is a Phase 2, double-blind, randomized, placebo-controlled, multi-center trial enrolling 120 subjects with BV who will be randomized at a ratio of 2:1 to receive active test article (5% Monolaurin Vaginal Gel) or placebo (Vehicle Placebo Gel). There will be three clinic visits over 31 days.

The study will seek women 18-50 years old with signs and symptoms of BV. Subjects may be identified during a routine clinic visit, referred by their care provider, or may be recruited directly to the research clinic. For women recruited following a clinic visit, the study team may review the Amsel criteria with the nonstudy clinician, but the expectation is that these tests will be repeated by study personnel to document each component of the Amsel testing after the subject has signed an informed consent.

Subjects will be educated about the study and will be asked to sign an informed consent prior to any evaluation.

Eligibility will be reviewed and if subjects qualify for participation, demographics, a medical history, including number of episodes of BV in the past year, concomitant medications review, and a physical exam including a gynecological exam will be performed. Women of childbearing potential will undergo a urine pregnancy test. Vaginal swabs will be taken to perform a KOH test, vaginal pH, and to review a wet prep. Subjects must have evidence of BV using the Amsel criteria (subjects must have any three of the four Amsel criteria: presence of discharge, greater than or equal to 20% clue cells on wet prep, positive “whiff test” on KOH prep, vaginal pH of greater than 4.5). Vaginal swabs for Gram stain of vaginal smear, and assessment for *Candida spp.*, selected bacterial species, *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* will be collected. Those with clinical evidence of active genital ulcer disease or evidence of trichomonas on wet prep or OSOM[®] test will be excluded from the study. Blood samples will be collected for safety assessments (HIV, WBC count, hemoglobin, platelets, neutrophil count, creatinine, aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin, random glucose). Subjects who continue to qualify for participation will be randomized at a ratio of 2:1 to receive 5% Monolaurin Vaginal Gel or Vehicle Placebo Gel administered vaginally morning and night for 6 consecutive doses (3 days). All subjects will be provided a 5-day memory aid to assess for local solicited events with instruction on use prior to discharge. The investigational drug (active test article or placebo) will be supplied in a kit containing sufficient prefilled vaginal applicators for six consecutive doses over 3 days. Subjects will be instructed on the proper use of the applicators and the first dose will be self-administered with observation by study personnel in the clinic. Subjects will be instructed to refrain from sexual intercourse including receptive oral sex or insertion of substances or objects intravaginally between Visit 1 and Visit 2 and 48 hours prior to Visit 3. Subjects who experience a new or worsening solicited reactogenicity symptom that is grade 2 or higher will be asked to contact the study site to confirm the severity. A determination will be made whether to continue or discontinue the study product by a licensed study clinician. No investigational or commercial agents or therapies other than test article may be administered with the intent to treat the subject for BV. For all treatment groups, each subject will be evaluated in the clinic at screening and at Visit 2 (Day 8 [Window Days 8-15]) and Visit 3 (Day 28 [Window Days 22-31]) following study drug administration.

Visit 2 (Day 8 [Window Days 8-15]): At this visit, the study team will review the memory aid, the intercurrent medical history with assessment of AEs and SAEs, changes to the concomitant medications, a gynecological exam and a targeted physical exam based on symptoms will be performed, and vaginal pH and vaginal swabs for Gram stain, saline wet mount, KOH 10% wet mount and culture will be obtained. If signs and symptoms of vaginitis fail to resolve, the study clinician will refer the subjects to their clinician for standard treatment for BV or other etiologies of vaginitis at the clinician's discretion and the subject will be classified as a clinical failure at Visit 2 and/or 3. Follow-up hematology and chemistry labs (WBC count, hemoglobin, platelets, neutrophil count, creatinine, AST, ALT, total bilirubin, random glucose) for safety evaluation will be performed.

Visit 3 (Day 28 [Window Days 22-31]) will include a review of the intercurrent medical history, concomitant medications, AEs and SAEs, and a gynecological exam and a targeted physical exam based on symptoms. Vaginal pH and vaginal swabs for Gram stain, saline wet mount, KOH 10% wet mount and culture will be performed.

Subjects who have positive HIV, *Chlamydia*, or *Neisseria gonorrhoeae*, or a grade 3 laboratory results from Visit 1 will be contacted within 24 hours of receipt of laboratory results and will be asked to follow up with their provider. These subjects will be asked to discontinue the study therapy if the test result returns during the study product administration period of the study.

These subjects will continue in efficacy and safety follow up.

At visits 1, 2 and 3, an extra vaginal swab will be collected for future use, if subject consents to future use of specimens.

4.1. Substudies (if applicable)

None

5. STUDY ENROLLMENT AND WITHDRAWAL

5.1. Subject Inclusion Criteria

It is the intent of this study to enroll women who are generally healthy with evidence of BV on gynecological evaluation. Subjects may have asymptomatic BV, but must meet any three of the four Amsel criteria for BV as defined in section 7.1. Inclusion criteria must be assessed by a clinician licensed to make medical diagnoses. Subjects must meet all of the following inclusion criteria to participate in this study:

1. Non-pregnant, non-breastfeeding females between the ages of 18 and 50 years, inclusive.
2. Women of childbearing potential* must agree to practice reliable contraception** for the 28-day period before enrollment through 30 days following treatment.

*(not surgically sterile via tubal ligation, bilateral oophorectomy or hysterectomy, or who have not been postmenopausal for ≥ 1 year)

** Acceptable birth control methods for the purposes of this study may include, but are not limited to, abstinence from intercourse with a male partner, monogamous relationship with vasectomized partner, barrier methods to include condoms and diaphragms, intrauterine devices, and licensed hormonal methods. NuvaRing[®] contraceptive use will be prohibited from this study since the device can alter vaginal secretions.

3. Presenting with signs of BV (as per Amsel Criteria). Subjects must meet any three of the four criteria for enrollment*
*Presence of discharge, greater than or equal to 20% clue cells on wet prep, positive “whiff test” on KOH prep, vaginal pH of greater than 4.5.
4. Not currently menstruating or expected to in the next 4 days.
5. Able to understand and comply with planned study procedures.
6. Willing to abstain from sexual intercourse, insertion of tampons, douches, or other intravaginal medications or objects between Visit 1 and Visit 2 and 48 hours prior to Visit 3.
7. Provide written informed consent before initiation of any study procedures and be available for all study visits.
8. No known history of HIV.

5.2. Subject Exclusion Criteria

Subjects who meet any of the exclusion criteria at Visit 1 will be excluded from study participation. Exclusion criteria must be assessed by a clinician licensed to make medical diagnoses. Subjects will not be able to participate if they have any of the following:

1. Signs or symptoms of vaginal/cervical/pelvic infection on screening or clinical diagnosis of vaginal/cervical/pelvic infection in the past 14 days.
*(including but not limited to yeast vulvovaginitis, chlamydia, gonorrhea, trichomonas, genital ulcer disease, pelvic inflammatory disease). Self-treatment for presumed yeast vaginitis is not an exclusion if treatment was discontinued 7 days or greater prior to enrollment.
2. Treatment for BV within the past 14 days.
3. Cervical or vaginal high grade squamous intraepithelial dysplasia (HSIL), atypical glandular cells of uncertain significance (AGUS) or cervical intraepithelial neoplasia grade 2 (CIN2) or higher*
*Atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesion (LSIL) or cervical intraepithelial neoplasia grade 1 (CIN1) are acceptable. Individuals with a history of atypical glandular cells of uncertain significance (AGUS), HSIL or CIN2 and who have received subsequent evaluation and/or treatment with follow up normal PAP smear are eligible. Patient report will be accepted.
4. History of undiagnosed vaginal bleeding.
5. Use of a systemic, vaginal, or perineal antibiotic within 7 days prior to enrollment in this study.
6. Use of an immunosuppressive or immunomodulatory drug* for two or more consecutive weeks within 6 months prior to enrollment
*such as >0.5 mg/kg/day or ≥ 20 mg total dose/day of prednisone orally or >800 μ g of inhaled beclomethasone (nasal and non-genital topical steroids are allowed).
7. History of allergic reactions attributed to compounds of similar chemical or biologic composition to Monolaurin Vaginal Gel.
8. Uncontrolled concurrent illness*. Subjects with a history of organ or marrow transplant are excluded.
*Including, but not limited to, ongoing or active infection, active liver, kidney or autoimmune diseases (a history of thyroid disease will be permitted as long as the thyroid disease is now stable), symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
9. Acute illness within 3 days before receipt of study product (per investigator's discretion).
10. Pregnant women and women who are planning to become pregnant within 30 days after the final study dose, or women who are breastfeeding.
11. Immunosuppression as a result of an underlying illness or treatment or use of anticancer chemotherapy or radiation therapy (cytotoxic) within the preceding 36 months.
12. Active neoplastic disease* or a history of any hematologic malignancy. Active neoplastic disease is defined as neoplastic disease or treatment for neoplastic disease within the past 5 years.

*(excluding non-melanoma skin cancer)

13. Received an experimental agent* within 30 days before receipt of study product or expect to receive an experimental agent during the 1 month study period.

*(vaccine, drug, biologic, device, blood product, or medication)

14. Any condition that would place the subject at an unacceptable risk of injury, render her unable to meet the requirements of the protocol, or that may interfere with successful completion of the study.
15. A history of alcohol or drug abuse* during the previous 1 year that in the opinion of the site investigator would interfere with study procedures

*For example, daily excessive alcohol use or frequent binge drinking as determined by the investigator, or daily marijuana use.

5.3. Treatment Assignment Procedures

Subjects, study site and laboratory personnel will be masked to the treatment assignments of study subjects.

5.3.1. Randomization Procedures

Subjects will be randomly assigned to active drug or placebo in a 2:1 ratio. Subjects will be stratified by clinical site.

The list of randomized treatment assignments will be prepared by statisticians at the Emmes Corporation and included in the enrollment module of the Emmes Corporation's Internet Data Entry System (IDES). IDES will assign each volunteer a treatment code from the list after demographic and eligibility data have been entered into the system. A designated individual at each site will be provided with a treatment key, which links the treatment code to the actual treatment assignment, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the IDES User's Guide. Manual back-up randomization procedures are provided in the Manual of Procedures (MOP) for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable.

5.3.2. Masking Procedures

The applicators of vaginal gels will be selected by the unmasked Site Research Pharmacist or designated unmasked study personnel and will be distributed to masked study personnel with no labels that identify the product or applicators to the site as Monolaurin Vaginal Gel or Vehicle Placebo Gel.

The volunteers, the study personnel who perform study assessments after administration, data entry personnel at the sites, and laboratory personnel will be masked to treatment assignment. The Data and Safety Monitoring Board (DSMB) may receive data in aggregate and presented by treatment group. The DSMB may be unmasked to individual study treatment assignments, as needed, to adequately assess safety issues. Refer to the MOP for unmasking procedures.

5.3.3. Reasons for Withdrawal

Any enrolled subject may withdraw or be withdrawn from the study for the following reasons:

- The subject withdraws consent
- The study is terminated
- For any reason that, in the opinion of the investigator, precludes the subject's participation in the study
- The subject no longer meets inclusion/exclusion criteria
- The subject develops an SAE that warrants withdrawal
- Other safety-related reasons at the discretion of the Principal Investigator (PI), DMID Medical Monitor, DSMB, or the subject

Any subject who has received at least one dose of study drug will be continued in efficacy and safety follow-up, if the subject agrees.

Subjects who test positive for chlamydia, gonorrhea, HIV, or are incidentally found to have a PAP result consistent with AGUS or HSIL, or have a Grade 3 laboratory abnormality will be discontinued from receiving study product if the result is available within the 3 days of study drug administration. They will be continued in follow up for safety and efficacy if they have received one dose of study drug. (PAP smears are not part of the study protocol, but may have been done outside of the study protocol by the subject's provider. Patient report of PAP smear abnormalities may be accepted as a criterion for discontinuation of study product).

If a subject develops a grade 3 laboratory abnormality while still receiving study product, they will be contacted and asked to discontinue study product and will be continued in safety and efficacy follow up. If any subject experiences a severe AE related to the study product, further doses will be discontinued. Additionally, subjects who experience a new or worsening solicited reactogenicity symptom that is grade 2 or higher will be asked to contact the study site to confirm the severity. A determination will be made whether to continue or discontinue the study product by a licensed study clinician. A subject who withdraws voluntarily from or has been discontinued from receiving further treatment will be encouraged to permit continued follow-up of AEs and to follow scheduled visits. If the subject agrees, study procedures (e.g., blood sampling for safety and vaginal sampling) may be continued.

Subjects may withdraw from participation in the study at any time. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs). Refer to Section 7.4 for procedures to be followed if a subject withdraws from the study.

5.3.4. Handling of Withdrawals

In the case of subjects who fail to appear for a follow-up assessment, extensive efforts (i.e., documented phone calls and certified mail) should be made to locate or recall them, or at least to determine their health status. These efforts should be documented in the subjects' records and their health status documented in the appropriate CRF. If a subject is withdrawn prior to completion of the study, the subject will not be replaced.

5.3.5. Termination of Study

The NIAID/DMID has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- Incidence or severity of AEs indicating a potential health hazard
- Data recording is inaccurate or incomplete
- The Investigator has not been adhering to the protocol or applicable regulatory guidelines in conducting the study

6. STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1. Study Product Description

Monolaurin (GML)

Monolaurin, also commonly referred to as GML, is a monoglyceride surfactant. It is an ester formed from glycerol and lauric acid. Monolaurin Vaginal Gel is a clear, colorless, non-sterile glycol-based gel for vaginal administration.

FDA recognizes monoglycerides, such as monolaurin, as direct food substances that are generally recognized as safe (GRAS) (21 CFR 184.1505). Monolaurin is also considered safe as an ingredient in cosmetics and tampons (e.g., o.b.[®] optiBalance). As stated, monolaurin is commercially available as a food grade substance from several suppliers. HLS will use [REDACTED]

[REDACTED] for the initial Investigational New Drug Application (IND) phase 2 clinical trials.

This formulation promotes rapid drug distribution, as well as adequate coverage, throughout the vaginal cavity. The acidic pH keeps monolaurin in its non-ionized form in the vagina.

Monolaurin, which is practically insoluble in water, is highly soluble in this formulation.

Vehicle Placebo Gel

The Vehicle Placebo Gel utilizes the same vehicle formulation as used in the Monolaurin Vaginal Gel.

6.1.1. Acquisition

5% Monolaurin Vaginal Gel and Vehicle Placebo Gel will be provided by Hennepin Life Sciences. Upon request by DMID, the investigational product will be transferred to the following address:

DMID-Clinical Agents Repository
Contract Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD, 20876
Tel: (240) 477-1350
Fax: (240) 477-1360
Email: DMID.Car@ThermoFisher.com

Study product will be shipped to the investigational sites upon request and approval by DMID.

6.1.2. Formulation, Packaging, and Labeling

The vaginal gel applicators will be packaged as a kit sufficient for twice daily administration for 3 consecutive days (six applicators). Each vaginal gel dispensing kit will be individually labeled with the protocol number, dosing instructions, recommended storage conditions, the name and address of the manufacturer, the kit number, Investigational Use Statement ("Caution: New Drug –

Limited by Federal [USA] Law to Investigational Use”) and that the agent should be kept out of reach of children.

6.1.2.1. 5% Monolaurin Vaginal Gel

Monolaurin Vaginal Gel is a clear and colorless non-sterile glycol-based gel for vaginal administration. Monolaurin Vaginal Gel is comprised of 5% monolaurin and the following excipients: [REDACTED]

Active test article will be comprised of 5% monolaurin prepared as pre-filled vaginal gel applicators of identical likeness to placebo. The components of the applicator are biocompatible and latex-free.

6.1.2.2. Vehicle Placebo Gel

The Vehicle Placebo Gel is a clear to opaque, colorless to light gray, non-sterile glycol-based gel for vaginal administration and contains the following: [REDACTED]

[REDACTED] The Vehicle Placebo Gel contains the same excipients as the active product.

The Vehicle Placebo Gel will be comprised of the identical vehicle contained in the active test article prepared as pre-filled vaginal gel applicators of identical likeness to active test article. The components of the applicator are biocompatible and latex-free.

6.1.3. Product Storage and Stability

Vaginal applicators containing active test article or placebo will be stored at room temperature (15-30°C.) with transient spikes up to 40°C permitted if they last less than 24 hours.

6.2. Dosage, Preparation and Administration of Study Intervention/Investigational Product

Preparation

No formal preparation is required for the study product. The unmasked Research Pharmacist or designated unmasked study personnel maintaining the study product accountability will select the appropriate kit based on the randomization scheme and will label the kit with the subject's name, subject ID number, treatment number, and any other hospital required identifying information.

Administration

Study personnel will provide both verbal and written instructions for proper storage and administration. The first dose will be administered by the subject while the subject is still in clinic. The remaining 5 doses will be administered twice daily (morning and evening) with at least 8 hours between doses by the subject at home. If the subject received her first dose after 3 pm, she will be instructed to administer the next dose the following morning.

Dose of Study Agents/Interventions

Active test article will be composed of 5 mL of 5% Monolaurin Vaginal Gel prepared as pre-filled vaginal gel applicators of identical likeness to placebo. Vehicle Placebo Gel will contain 5 mL of the vehicle used in the active test article.

6.3. Modification of Study Intervention/Investigational Product for a Participant

If a subject is found to have a positive test for HIV, chlamydia or gonorrhea or Grade 3 laboratory abnormality from Visit 1, she will be notified of the results, withdrawn from receiving further study product, but will be followed for safety and efficacy. All subjects who have received even a single dose of study drug will be followed for safety and efficacy.

If any subject experiences a severe AE related to the study product, further doses will be discontinued.

If the subject misses a dose, she should note the missed dose on the memory aid, save the unused applicator, and resume dosing at the next recommended scheduled dose.

6.4. Accountability Procedures for the Study Intervention/Investigational Product(s)

The unmasked Research Pharmacist will be responsible for maintaining complete records and documentation of study product receipt, accountability, dispensation, storage conditions, and final disposition of the study product. All study products, whether administered or not, must be documented on the appropriate study product accountability record or dispensing log.

Study personnel will document on the CRF the time of the application of the first study dose. The first dose applicator may be discarded. Subjects will be instructed to return the kit (containing used and/or unused vaginal applicators) to study personnel at the clinic for Visit 2. The used kit and applicators will be placed in a sealed plastic bag for transport to the study site. Once the study personnel have verified the test article accountability, the kits and used applicators may be discarded in an appropriate medical waste container.

Upon completion or termination of the study and after the final monitoring visit, any remaining unused study product will either be returned or destroyed appropriately at the clinical site as per Sponsor requirements and instructions.

6.5. Assessment of Subject Compliance with Study Intervention/Investigational Product

Study personnel will count the number of used/unused applicators contained in each returned subject kit and record this information in the test article accountability section of the subject's CRF.

6.6. Concomitant Medications/Treatments

Administration of any medications, therapies, or vaccines will be documented in the subject's source documentation and reported on the subject's data acquisition screen. Concomitant medications will include all medications, vitamins, and supplements taken within the 30 days before enrollment and through Visit 3 (Day 28) or early termination, whichever occurs first.

Each subject will be instructed to not use any systemic, vaginal, or perineal antibiotic, antifungal, immunosuppressant, or anti-inflammatory medication except oral nonsteroidal anti-inflammatory medication during study participation from study Day 1 through Visit 3 unless absolutely necessary.

Treatment with any prescription medication(s), over-the-counter preparations and non-prescription medication(s) intended to treat the subject for vaginosis will be used only if subject is considered a clinical failure. In this case, she will be referred to her provider for standard of care treatment. All treatment medications must be documented on the concomitant medications CRF.

7. STUDY SCHEDULE

7.1. Screening and Enrollment (Visit 1, Day 1)

Subjects should be scheduled for visits when they are not menstruating and instructed to avoid inserting any substance into the genital tract for 48 hours before examination at Visit 1, when possible. Subjects may present after having clinical evaluation by a non-study clinician or may present to the research clinic directly.

The following procedures will be performed:

- Obtain signed informed consent. This should be performed prior to conducting any of the assessments listed below.
- Review inclusion/exclusion criteria and collect demographic information.
- Obtain medical history and concomitant medications (prescription and over-the-counter drugs, supplements, and vitamins taken 30 days prior to enrollment will be reviewed and documented).
- Review of contraceptive methods and recent menstrual history.
- Review sexual history.
- Review subject report of history of previous episodes of BV and if noted, the number of episodes of BV in the previous 12 months and treatments given.
- Assessment of current symptoms of BV
- Perform physical exam.
- Perform urine pregnancy test for female subject of childbearing potential prior to pelvic exam. Must be negative prior to receipt of study drug.
- Gynecologic exam for evaluation of the cervix, vagina, and vulva:
 - Assessment for genital tract infections and lesions. If evidence of active lesions consistent with herpes simplex virus infection or other sexually transmitted infection (e.g., syphilis), subject will be excluded and may be referred to her provider for further management.
 - Document vaginal discharge (quantity, color, odor, and consistency).
 - Document objective signs of inflammation (edema, erythema, excoriation). Inspection and appearance of the cervix, vagina, and vulva, and document presence or absence of objective signs of inflammation (edema, erythema, excoriation).
 - Document vaginal secretion pH testing using pH paper 4-6.
 - Saline “wet mount” for microscopic examination (performed by licensed clinician or laboratory staff certified or trained to read vaginal wet mounts):
 - Document the presence of and percentage of cells that are “clue cells.”

- If motile flagellated protozoa are seen, the subject will be excluded from participating in the trial and referred to her provider to be treated using established standards.
- KOH 10% “wet mount” for microscopic examination (performed by licensed clinician or laboratory staff certified or trained to read vaginal KOH preparations):
 - Document presence or absence of fishy odor on “whiff test”: fishy (amine) odor released by the addition of a drop of 10% KOH to the vaginal discharge.
 - Document presence of pseudohyphae or budding spores.

The subject will be enrolled based on demonstration of any three of the four following Amsel criteria:

- Off-white (milky or gray), thin, homogenous discharge with minimal or absent pruritus or inflammation of the vulva or vagina.
- $\geq 20\%$ “Clue” cells of total epithelial cells on microscopic examination of saline wet mount.
- Vaginal secretion pH >4.5 .
- Positive “whiff test”.

*****If the subject does not meet clinical criteria (based on Amsel criteria) for BV, she will not have further vaginal sampling performed and will not proceed to randomization and will not receive study drug.**

If subject demonstrates evidence of BV:

For those subjects who have evidence of BV based on the above criteria and continue to meet inclusion/exclusion criteria, samples will be collected in the following order:

- Vaginal swabs for the following:
 - Gram stain of vaginal smear (local laboratory preparation and shipment to the University of Iowa Clinical Pathology Laboratory).
 - Culture or molecular testing for the following will be taken:
 - OSOM® rapid Trichomonas testing. OSOM testing is required if the wet prep shows no evidence of motile flagellated protozoa. If the wet prep was positive for motile protozoa, then the diagnosis of trichomonas has already been confirmed and OSOM testing is not required. If positive for Trichomonas, the subject will be excluded from participating in the trial and referred to her provider to be treated using established standards.
 - Bacteria and Candida species (to be sent to the Central Laboratory).
 - Chlamydia trachomatis - testing should be performed as per local standards (to be sent to the Local Clinical Pathology Laboratory). If study subject has had Chlamydia trachomatis testing performed within the past 5 days

and it was negative or is still pending, this data may be used for screening. Vaginal swab testing is the preferred method for testing in this study, but if urine testing has been done within the past 5 days, this is acceptable. If Chlamydia testing is positive, the subject will be contacted by telephone and letter and referred to her provider for further management.

- Neisseria gonorrhoeae - testing should be performed as per local standards (to be sent to the Local Clinical Pathology Laboratory). If study subject has had Neisseria gonorrhoeae testing performed within the past 5 days and it was negative or is still pending, this data may be used for screening. Vaginal swab testing is the preferred method for testing in this study, but if urine testing has been done within the past 5 days, this is acceptable. If gonorrhea testing is positive, the subject will be contacted by telephone and letter and referred to her provider for further management.
- Future Use: If the subject consents to future use, one Copan ESwab in liquid amies will be collected for future use (to be transported on cold packs or ice to the research laboratory).
- Obtain approximately 15 cc of blood to be sent to the local Clinical Pathology Laboratory for WBC count, hemoglobin, platelet, neutrophil count, creatinine, AST, ALT, total bilirubin, random glucose, and HIV testing. If HIV testing has been performed within the past 5 days and was negative or is still pending, this test can be used for screening.
- Once all samples have been obtained, the subject will be enrolled in IDES and randomly assigned to a treatment group.
- Subjects will be counseled on proper administration and storage of the vaginal gel.
- Subjects will self-administer the first dose of the investigational vaginal gel or vehicle placebo gel with observation by study personnel while in the clinic using the first applicator in the assigned study kit.
- Subjects will receive 5 further doses (vaginal applicators with instructions) for self-administration twice-daily (morning and evening) with at least 8 hours between doses.
- Subjects will be instructed to return used applicators to the disposal bag provided in the kit to return to the clinic at Visit 2 (between Study Days 8-15).
- Review for AEs/SAEs
- Provide counseling to refrain from penile-vaginal or receptive oral sexual intercourse or insertion of any substance (other than study product), products, or objects between Visit 1 and Visit 2 and 48 hours prior to Visit 3.
- Provide memory aid and instruct subject on how to complete the memory aid for 5 days from the first day of therapy.
- Counsel subjects on avoidance of pregnancy (for all female subjects of childbearing potential).

***If a subject's laboratory results show a positive gonorrhea, chlamydia, HIV test or grade 3 abnormality, study personnel will contact the subject to inform her of the results and instruct**

her to discontinue study treatment. Telephone contact is the preferred method of contact, though initial contacts may include other forms of communication with follow-up discussions provided by telephone or in person.

7.2. Visit 2 (Day 8 [Window: Days 8-15])

All subjects will return for Visit 2 on Day 8 (Window: Days 8-15) at which time the following assessments will be performed.

- Review changes in medical history:
 - Review symptoms of BV since Visit 1 and treatments for BV other than investigational drug.
 - Review all concomitant medications since Visit 1.
- Review for sexual intercourse, insertion of intravaginal substances, or objects since Visit 1.
- Review for AEs and SAEs.
- Review Memory Aid and collect applicator kit.
- Perform targeted general physical exam based on symptoms.
- Perform gynecologic exam of cervix, vagina, and vulva:
 - Assessment of vaginal discharge (quantity, color, odor, and consistency).
 - Record presence or absence of objective signs of inflammation of cervix, vagina and vulva (edema, erythema, excoriation) and presence or absence of lesions.
- Collect vaginal secretions for pH testing using pH paper 4-6.
- Perform KOH 10% wet mount on vaginal (must be performed by a licensed clinician or trained laboratory personnel) and record “whiff test”: fishy (amine) odor released by the addition of a drop of 10% KOH to the vaginal discharge.
 - Evaluate for pseudohyphae or budding spores (must be performed by a licensed clinician or trained laboratory personnel).
- Perform saline wet mount (must be performed by a licensed clinician or trained laboratory personnel).
 - Evaluate and record presence and percent of ‘Clue’ cells in vaginal secretions.
 - Record for presence of flagellated protozoa (if positive, refer to provider for further therapy).
- Collect vaginal swabs for:
 - Gram stain of vaginal smear.
 - Swabs for culture of *Gardnerella spp.*, *Mobilluncus spp.*, *Lactobacilli spp.*, *Candida spp.*
 - Future Use: If the subject consents to future use, one Copan ESwab in liquid amies will be collected for future use (to be transported on cold packs or ice to the research laboratory).

- Obtain approximately 10 cc of blood for:
 - WBC count, hemoglobin, platelet, neutrophil count.
 - Creatinine, ALT, AST, total bilirubin, random glucose.
- Counsel subjects to refrain from penile-vaginal or receptive oral sexual intercourse or insertion of any substance, products, or objects into vagina for 48 hours prior to Visit 3.
- Counsel subjects on avoidance of pregnancy (for all female subjects of childbearing potential).
- Subjects will be counseled to come in for an unscheduled visit if their vaginitis symptoms recur/worsen between Visit 2 and 3.
- Document Investigator assessment of clinical cure.

If the signs and symptoms of BV fail to resolve and further treatment is required (in the Investigator's opinion), the subject will be referred to her provider for standard treatment. Therapy will be recorded on the appropriate source document and electronic CRF (eCRF) pages and the subject will be followed through Visit 3 for safety. At Visit 3, these subjects will be classified as a clinical failure.

7.3. Visit 3, Final Study Visit (Day 28 [Window: Days 22-31])

All subjects will return for Visit 3 on Day 28 (Window: Days 22-31) at which time the following assessments will be performed:

- Review changes in medical history.
- Review all concomitant medications since Visit 2.
- Review of BV symptoms and treatments for BV other than investigational drug.
- Review history of sexual intercourse, insertion of intravaginal substances or objects within the past 48 hours.
- Review Memory aid if solicited events continued at last visit and collect applicator kit, if not done at Visit 2.
- Review AEs/SAEs: If AEs or SAEs continue at Visit 3, the study team will follow until resolution or stabilization of the event. Laboratory abnormalities that were stable at Visit 2 (i.e., were abnormal at Visit 1 prior to receipt of study drug and continued to be abnormal but no worse at Visit 2) do not need to be repeated and are considered stabilized. Laboratory abnormalities that occurred between Visit 1 and Visit 2 or worsened at Visit 2 should be repeated until the test has stabilized or resolved.
- Perform targeted general physical exam based on symptoms.
- Gynecologic exam of cervix, vagina, and vulva:
 - Assessment of vaginal discharge (quantity, color, odor, and consistency)
 - Assess cervix, vagina, and vulva for presence or absence of objective signs of inflammation (edema, erythema, excoriation) and/or genital lesions.
- Collect vaginal secretions for pH testing using pH paper 4-6.

- Perform KOH 10% wet mount on vaginal secretions (performed by licensed clinician or trained laboratory personnel)
 - Record “whiff test”: fishy (amine) odor released by the addition of a drop of 10% KOH to the vaginal discharge.
 - Evaluate for pseudohyphae or budding spores (performed by a licensed clinician or trained laboratory personnel).
- Perform saline wet mount (performed by a licensed clinician or trained laboratory personnel).
 - Evaluate and record presence and percent of “clue cells” in vaginal secretions.
 - Record for presence of flagellated protozoa (if positive, refer to provider for further therapy).
- Collect vaginal swabs for:
 - Gram stain of vaginal smear
 - Swabs for culture of *Gardnerella spp.*, *Mobiluncus spp.*, *Lactobacilli spp.*, and *Candida spp.*
- Future Use: If the subject consents to future use, one Copan ESwab in liquid amies will be collected for future use (to be transported on cold packs or ice to the research laboratory). Document Investigator assessment of clinical cure.

7.4. Early Termination Visit

For subjects who are withdrawn from the study early, follow-up assessments will be completed according to the protocol schedule, if possible, including review of concomitant medications, history of sexual intercourse, insertion of intravaginal substances or objects and BV symptoms, gynecological exam, targeted physical exam if indicated based on symptoms, review of clinical cure, blood for WBC count, hemoglobin, platelet, neutrophil count, creatinine, AST, ALT, total bilirubin, random glucose), vaginal sampling (wet mount, KOH preparation, and vaginal swabs for Gram stain, culture of *Gardnerella*, *Mobiluncus*, *Lactobacillus* and *Candida spp.*), return of applicator kit (if applicable) and reason for termination or withdrawal. If subject declines to return for an in-person visit, the study personnel will seek permission to contact the subject by telephone 22-31 days from introduction of treatment to assess for AEs and SAEs. If subject continues to have signs and symptoms of BV or other genital infections, she will be referred to her provider for further management.

7.5. Unscheduled Visit

Unscheduled visits may occur at any time during the study. The procedures below are to be performed at any unscheduled visit. See the MOP for instructions for documentation and data reporting.

- Obtain interim medical history and solicit information on vaginal symptoms.
- Perform a targeted physical exam if indicated based on symptoms.
- Perform gynecological exam and collect appropriate samples (e.g., appropriate clinical samples for genital infectious pathogens).

- Review concomitant medications.
- Review of memory aid, if applicable.
- Review of AEs/SAEs, if applicable.

8. STUDY PROCEDURES/EVALUATIONS

8.1. Clinical Evaluations

Medical History: Will be obtained by interview of the subjects prior to randomization. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited.

Concomitant Medications: All current medications and medications taken in the 30 days before enrollment (prescription and over-the-counter drugs, supplements and vitamins) will be included through Visit 3 (Day 28) after enrollment. Assessment of eligibility also will include a review of prohibited medications (per the exclusion criteria).

Physical Examination: This examination will be conducted at Visit 1 and will assess general appearance the following areas/symptoms: skin, inguinal lymph nodes, head, eyes, ears, nose, throat, neck, respiratory, cardiovascular, abdomen, extremities, musculoskeletal, and neurological.

Gynecological Examination: This examination will be conducted at each visit and will assess for presence of discharge, appearance of the cervix, vulva, and vaginal mucosa, and presence of specific lesions. Bimanual exam is not required, but may be performed at the discretion of the licensed clinician.

Targeted Physical Examination: This may be conducted at any study visit based on interim medical history.

Solicited Events Assessments: Study personnel will take a brief history of subjects for assessment of AEs. This will include genital signs and symptoms of odor, pain, tenderness, discharge, swelling, itching, and dryness.

8.2. Laboratory Evaluations

8.2.1. Clinical Laboratory Evaluations

Clinical laboratory evaluations will be performed by the local Clinical Pathology Laboratory. Specific tests to be performed are described below:

- HIV testing will be performed as per local standards (performed at Visit 1 only). It is recommended that an antibody or antibody/antigen test be used for screening. If antibody or antibody/antigen testing results in an indeterminate test, HIV PCR may be used to further evaluate the HIV status of the subject.
- Hematology includes: WBC count, hemoglobin, platelet, neutrophil count.
- Clinical chemistry includes: creatinine, AST (SGOT), ALT (SGPT), and total bilirubin, and glucose (random).
- Urine Pregnancy test: Urine pregnancy test in females of childbearing potential will

be performed in the research clinic, local clinical care clinic or Local Pathology Laboratory. Result must be negative within 8 hours before first administration of study product.

- Sexually transmitted infection testing: Testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Samples will be obtained and processed as per local clinic guidelines and will be sent to the site Clinical Pathology Laboratory. Either culture or molecular testing for *Neisseria gonorrhoeae* and chlamydia is acceptable. Trichomonas testing using wet preps may be performed in the clinic as per local clinic guidelines. The OSOM® rapid Trichomonas testing will be performed on all subjects who do not have Trichomonas identified on wet prep at screening.

One or more of the laboratory parameters may be repeated at any time during the study as determined by the Investigator, if indicated by an AE. Grade 3 abnormal laboratory values should be repeated within 10 days if possible and followed up as clinically relevant.

8.2.2. Special Assays or Procedures

Vaginal Swabs (Please refer to MOP for details on vaginal swab collection)

The study coordinator or investigator will label the tubes with a unique identification number and store the vaginal swabs inside the supplied transport tubes in an insulated cooler with cold packs or placed in the refrigerator (2-8°C) within 1 hour of collection. The swabs will be packaged in accordance with the federal shipping guidelines for diagnostic biological material. A reconciliation system will be used to facilitate tracking of vaginal swab specimens. For subjects enrolled at the Cincinnati Children's Hospital Medical Center and Duke University School of Medicine vaginal swabs will be shipped within 24 hours of collection by overnight delivery on Monday through Thursday using cold packs in a Styrofoam container to the designated central laboratory. For subjects enrolled at the University of Iowa, specimens will be transported by hand to the central laboratory Monday through Friday. If transported within 1 hour of collection, the samples may be transported at room temperature. If the time exceeds 1 hour after collection, the samples should be transported in an insulated cooler with cold packs.

If the subject consents to future use, a vaginal swab will be collected for future use. A Copan ESwab in liquid amies will be used to swab the vaginal walls. The study coordinator or investigator will label the tubes with a unique identification number. Once collected the swab will be broken off in the transport tube that contains the liquid amies solution and will be transported on cold packs or ice to the research lab within 1 hour of collection. In the research laboratory, the tube should be vortexed for 5 minutes and then using sterile procedures with sterile pipets, aliquot the liquid amies solution into three cryovials that have been labeled with global trace bar codes.

Gram stain Assay (University of Iowa)

Slides will be fixed using the SOP for using CytoPrep fixation (see the MOP) at the local site from vaginal swabs collected from every subject at Visits 1, 2 and 3 for central scoring. The fixed slide will be sent to the University of Iowa VTEU team where they will be transported to the University of Iowa Clinical Pathology Laboratory for staining and analysis. The smear will be evaluated for bacterial morphotypes and interpreted in accordance with Nugent's criteria

(relative populations of the morphotypes *Lactobacillus*, *Gardnerella*, *Bacteroides*, *Peptostreptococcus*, and *Mobiluncus spp.* [11]) using the standardized 0-10 point scoring system to interpret the Gram stain results as follows:

- 0-3 classified as normal/no BV detected
- 4-6 classified as intermediate
- 7-10 indicative of BV

Each stained slide will be read by two independent observers who will be masked to the other's results. Discordant results (results that differ in the absolute Nugent score) will be re-reviewed by the initial two readers to reach a consensus. If a consensus cannot be reached, the slide will be reviewed by a third observer to resolve the discrepancy. A single Nugent score value will be recorded.

The same Gram stained slides used to assess the presence of bacterial morphotypes will also be evaluated for the presence of yeast by using the 100x objective. A categorical score will be assigned to each specimen slide, after the reader viewed and examined the entire area of each stained slide to determine the presence or absence of yeast cells showing blastoconidia (cell buds).

8.2.3. Specimen Preparation, Handling, and Shipping

Instructions for specimen preparation, handling, storage, and shipping are included in the MOP.

8.2.3.1. Instructions for Specimen Preparation, Handling, and Storage

After collection, study required vaginal swab tubes will be placed at 2-8°C within 1 hour of collection and stored at 2-8°C until shipped. If subjects have consented to future use, the future use vaginal swabs will be transported on cold packs or ice to the research laboratory where they will be vortexed for five minutes and then aliquoted into three cryovials that will then be frozen at -70 C or colder.

8.2.3.2. Specimen Shipment

Prior to transport, vaginal swab tubes containing specimens (which have been stored at 2-8°C) will be placed in a small plastic bag with a handful of cotton balls, and then placed in a Styrofoam™ container and cardboard box with a disposable ice pack. They will be shipped to the University of Iowa by overnight delivery, by placing the package in a drop box or calling for pickup by FedEx. Specimens will be shipped daily Monday through Thursday.

For those who have consented to future use, the future use specimens (cryovials containing aliquots of the liquid amies solution obtained from the vaginal swabs), will be sent on dry ice to the Fisher repository.

“All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations (49 CFR Part 173: Shippers, General Requirements for Shipments and Packaging

and under the US Department of Transportation- Pipeline and Hazardous Materials Safety Administration (PHMSA) and according to individual carrier guidelines, as applicable.”

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9. ASSESSMENT OF SAFETY

9.1. Specification of Safety Parameters

Investigators are required to report promptly to the sponsor any AE that may reasonably be regarded as caused by or probably caused by the drug. If the AE is alarming, the investigator shall report the adverse effect immediately (§ 312.64(b)). Safety will be assessed by incidence and severity of solicited and non-solicited AEs and SAEs. A DSMB will be convened by DMID to review safety information from study participants.

Sponsors are specifically required to notify all participating investigators and FDA in a written IND safety report of any AE associated with the use of the drug that is both serious and unexpected, and any finding from tests in laboratory animals that suggests a significant risk for human subjects (§ 312.32(c)(1)(i)(A),(B)). More generally, sponsors are required to keep each participating investigator informed of new observations discovered by or reported to the sponsor on the drug particularly with respect to AEs and safe use (§ 312.55(b)).

Also, Investigators are required to report promptly to the IRB all unanticipated problems involving risks to human subjects or others including AEs that should be considered unanticipated problems (§§ 56.108(b)(1), 312.53(c)(1)(vii), and 312.66).

9.2. Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1. Adverse Events

AE monitoring and reporting is a routine part of every clinical trial. All clinical site staff and the Sponsor's employees share in the responsibility for reporting AEs. All clinical site staff and the Sponsor's employees who learn about or are notified of a SAE must collect and promptly report (24 hours) data according to the regulations. Failure to report AEs according to the timeframes and definitions as outlined in the regulations can result in regulatory actions.

Adverse Event: ICH E6 defines an AE as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for "serious adverse events" should be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or are considered stable.

Any medical condition that is present at the time that the subject is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product.

Severity of Event: All AEs will be assessed by the clinician using a protocol defined grading system. For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity.

- Mild: events require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity. AEs characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products: The clinician's assessment of an AE's relationship to test article (study drug) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Related – There is a reasonable possibility that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

9.2.2. Solicited Events

Solicited events assessments will be captured on a memory aid starting on Day 1, the first day of therapy and continuing for 5 days.

The subject will record the presence and intensity of vulvo-vaginal solicited events on the memory aid.

Table 2: Solicited events & grading

	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe
Vaginal odor	Mild unpleasant odor	Moderate unpleasant odor	Severe, unpleasant odor
Vaginal pain	Pain causing no or minimal interference with usual social and functional activities	Pain causing greater than minimal interference with usual social and functional activities or the need for non-narcotic medication	Pain causing inability to perform usual social and functional activities or the need for narcotic medication
Vaginal tenderness	Mild tenderness	Moderate tenderness	Severe tenderness
Vulvar/vaginal itching	Itching causing no interference with usual social and functional activities	Itching causing interference with usual social and functional activities	Itching causing inability to perform usual social and functional activities
Vaginal dryness	Dryness not interfering with sexual function, social, and functional activities	Dryness causing greater than minimal interference with usual sexual, social and functional activities	NA
Vaginal discharge by participant's report	Mild increase in participant's usual amount of discharge, regardless of color or quantity; no sanitary protection required	Moderate to profuse increase in discharge requiring pad use or other hygienic intervention	NA
Vulvar inflammation (Redness/Swelling)	Mild swelling, or redness; no interference with usual, social and functional activities	Moderate swelling, or redness; interference with usual, social and functional activities	Severe swelling or redness, or presence of mucosal ulceration; inability to perform usual, social and functional activities

Any symptoms still present on Day 5 will continue to be followed by subject memory aid notations until symptom resolution. Subjects will also be asked to record any medications taken and any emergency room or physician visits (other than routine check-ups).

Severity of solicited events symptoms are graded according to the table above. For symptoms not specifically mentioned will be graded using the following scale:

- Grade 1: Mild (does not interfere with activity)
- Grade 2: Moderate (interferes with activity)
- Grade 3: Severe (prevents daily activity)

9.2.3. Serious Adverse Events

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening AE*,
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

*Life-threatening AE. An AE is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE, had it occurred in a more severe form, might have caused death.

All SAEs will be:

- Reviewed and evaluated by a study clinician
- Recorded on the appropriate Adverse Event CRF
- Followed through resolution by a study clinician
- A study clinician will determine whether the SAE is considered related to study treatment.

9.2.4. Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Collection of specific safety laboratory data is outlined in the study visit schedule. Toxicity tables are based on the FDA toxicity tables developed for normal healthy adult volunteers.

Laboratory values that are abnormal based on the toxicity tables will be reported on the appropriate CRF.

In the event a subject experiences an AE or vulvar or vaginal reaction that is still present at the end of the subject’s participation in the study, the subject must be followed until resolution of the event or until the event or abnormality stabilizes to the investigator’s and/or sponsor’s satisfaction. Follow-up procedures, evaluations, and outcomes will be recorded on the subject’s CRFs.

9.3. Reporting Procedures

9.3.1. Adverse Events

AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Solicited AEs will be collected from the time of the first product administration through Day 5. Non-solicited AEs and SAEs will be collected from the time of first product administration throughout the entire study period. Information to be collected includes event description, date of onset, investigator assessment of severity, investigator assessment of relationship to study product, date of resolution of the event, seriousness, and outcome. The intensity of non-serious AEs will be assessed by a licensed study clinician (i.e., medical doctor, nurse, nurse practitioner, physician's assistant). The causality of non-serious AEs will be assessed by a licensed clinician able to make diagnoses listed on the Form FDA 1572. All AEs occurring during the AE reporting period of the study will be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or until considered stable.

Any medical condition that is present at the time of enrollment will be considered as pre-existing at Baseline and will not be reported as an AE. If the severity or frequency of any pre-existing medical condition increases during the study period, then it will be recorded as an AE.

AEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

9.3.1.1. Serious Adverse Events

Information to be collected includes event description, date of onset, investigator assessment of severity, investigator assessment of relationship to study product, date of resolution of the event, seriousness, and outcome. The intensity of SAEs will be assessed by a licensed study physician. The causality of SAEs will be assessed by a licensed physician able to make diagnoses listed on the Form FDA 1572.

Serious adverse events will be recorded on the appropriate Adverse Event CRF and will be reported to the site's Independent Safety Monitor. Events will be reviewed and followed to resolution by a study physician. SAEs will be collected on each subject until her last study visit.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

**DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com**

Additionally, materials must be submitted to the Statistical and Data Coordinating Center (SDCC), Emmes Corporation, through the IDES system. Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The DMID Medical Monitor and Clinical Protocol Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.

9.3.2. Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the AE. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow-up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3. Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported in IDES. No further treatment will be administered to pregnant subjects, but all study-mandated blood samples will be obtained and the subject will continue in follow-up for safety events (see Section 7.6). In addition, pregnant subjects will no longer undergo pelvic exams or swabs collection. Pregnancies will be followed to pregnancy outcome pending the subject's permission.

9.4. Type and Duration of Follow-up of Subjects after Adverse Events

AEs will be followed until resolved or subject is considered stable.

9.5. Halting Rules

Study enrollment and dosing will be halted and an ad hoc DSMB review will be performed if any of the following occur at any time during the study:

Study Halting Events:

1. One or more subject experiences a treatment-related SAE.

2. One or more subject experiences vulvar and/or vaginal ulceration, abscess, or necrosis associated with study product administration.
3. Two or more subjects experience a treatment-related severe (Grade 3) unsolicited adverse event.
4. Three or more subjects who received at least one treatment dose experience the same severe (Grade 3) solicited AE as evaluated by a licensed clinician.
5. Three or more subjects who received at least one treatment dose experience a severe (Grade 3) study related laboratory abnormality in the same laboratory parameter.
6. An overall pattern of symptomatic, clinical, or laboratory events that the DMID, Medical Monitor, or DSMB consider associated with study product and that may collectively represent a serious potential concern for safety.

9.6. Safety Oversight (ISM plus DSMB)

9.6.1. Data and Safety Monitoring Board

Because this is a large Phase II Randomized Clinical Trial, a DSMB will be created to review this study. The DSMB will be composed of three to four members and may include at least one physician involved in research, one biostatistician and potentially an expert in the field of microbiology. This committee will be charged with reviewing the protocols with respect to ethical and safety standards and making recommendations if necessary. During the studies, the Committee will meet (either in person, via teleconference or web-based meetings) at least annually to review safety issues and to monitor center performance in execution of the protocol.

Prior to each meeting, a formal, detailed report will be generated with emphasis placed on safety issues.

A formal DSMB review will occur after 30 subjects have received at least one dose of study drug and have completed Visit 2. The DSMB will either recommend continued enrollment of the remaining subjects or discontinuation of enrollment. Though the timing of the DSMB review will be expedited, enrollment may continue while awaiting the DSMB review. In addition, a computerized system will be used to acquire any data regarding halting criteria throughout the study.

Additionally, a single fully blinded interim analysis is planned to reassess the adequacy of the sample size based on subjects meeting eligibility for the Modified Intent-to-Treat (mITT) cohort (see also Section 11). After eighty subjects have been enrolled and have available test results for Nugent score, HIV, chlamydia and gonorrhea, the fraction of subjects who are enrolled to date who are eligible for the mITT cohort will be estimated. Based on this calculation, the enrollment target may be increased from 120 subjects to a maximum of 150 as required to achieve the target of 90 subjects eligible for the mITT cohort. The study sponsor and investigators will have access to the data shared in fully blinded reports issued to the DSMB.

If any of the halting rules are met, the study will not proceed with the remaining enrollment without a review by and recommendation from the DSMB to proceed. Upon completion of this review and receipt of the advice of the DSMB, DMID will determine if study entry or study dosing should be interrupted or if study entry and study dosing may continue according to the protocol.

The study is masked. The DSMB has the option to request unmasking to make determinations regarding study outcome.

9.6.2. Independent Safety Monitor (ISM)

Safety oversight for this study will include an ISM in close proximity to each of the clinical research sites. The ISM will receive and evaluate SAEs blinded and provide an independent written summary to the DMID Clinical Project Manager and DMID Medical Monitor. The ISM may be asked by the DMID Medical Monitor to review additional safety events (i.e. significantly increased frequency of non-serious adverse events).

10. CLINICAL MONITORING

10.1. Site Monitoring Plan

Site monitoring will be conducted to ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP) guidelines and applicable regulatory requirements, and that the study is conducted in accordance with the protocol and sponsor's standard operating procedures. The DMID or its designee will conduct site-monitoring visits as specified in the monitoring plan.

Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, CRFs, informed consent forms (ICFs), medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.

11. STATISTICAL CONSIDERATIONS

11.1. Introduction

This is a Phase 2, double-blind, randomized, placebo-controlled, multi-center trial comparing 5% Monolaurin Vaginal Gel to Vehicle Placebo Gel as outpatient therapy. A total of 120 eligible subjects with BV will be enrolled. Treatment assignments will be randomly allocated in a 2:1 ratio of test article to placebo, with the randomization stratified by clinical site. Each subject will receive intravaginal gel twice daily for three successive days for a total of 6 doses. There will be 3 clinic visits over 31 days.

11.2. Data Analysis Populations

The Per-Protocol (PP) efficacy analysis population includes all randomized subjects who met all inclusion/exclusion criteria, did not have any significant protocol deviations, complied with the assigned study treatment, returned to the study site for the Test of Cure visit within the specified window (Visit 2 Study Day 8 relative to the first day of treatment [Window: Days 8-15]), or discontinued study product early due to lack of treatment effect or received vaginosis or systemic antimicrobial therapy other than study drug during the study. Subjects with Visit 1 test results including a grade 3 laboratory abnormality, a positive Visit 1 HIV, *Chlamydia*, or *Neisseria gonorrhoeae* test, a PAP result consistent with HSIL, AGUS, or CIN2 or greater, or a Visit 1 Nugent score < 4 will be excluded from the PP population.

A subject is compliant with study treatment if she uses at least 5 of 6 doses of study product, as assessed by the study coordinator's count of used / unused applicators in each returned subject kit. If a kit is not returned, compliance will be assessed by the subject's self-report on the memory aid. Significant protocol deviations include sexual intercourse including receptive oral sex or insertion of substances or objects intravaginally between Visit 1 and Visit 2.

The Modified Intent-to-Treat (mITT) efficacy analysis population includes all randomized subjects who met all inclusion/exclusion criteria excluding those who have a Nugent score < 4 at Visit 1 or are positive for other concomitant vulvovaginal infections at baseline which may interfere with the efficacy assessment. The mITT population will be used as the primary analysis population for the test of 5% Monolaurin Vaginal Gel to Vehicle Placebo Gel.

The Evaluable efficacy analysis population includes all subjects in the mITT population who received study treatment and returned for at least one post-baseline visit.

The Intent-to-Treat (ITT) efficacy analysis population includes all randomized subjects.

The safety population includes all randomized subjects who received at least one dose of study product.

11.3. Study Objectives and Endpoints

The primary study objectives are to compare 5% Monolaurin Vaginal Gel with vehicle placebo gel (excipients only) with respect to 1) safety and tolerability, and 2) efficacy by clinical cure at Visit 2. The primary safety outcome measures are the number of subjects reporting solicited urogenital AEs following the first dose of the study product through Visit 2 (Days 8-15) and the number of

subjects reporting SAEs considered product related following the first dose of study product through Visit 3 (Day 22-31). The primary efficacy outcome measure is the proportion of subjects with clinical cure in each study arm at Visit 2 (Days 8-15).

A clinical cure is defined by normal Amsel criteria. Normal Amsel criteria are defined as: normal physiological vaginal discharge, whiff test negative for any amine “fishy” odor, saline wet mount less than 20% for clue cells, and vaginal pH is ≤ 4.5 .

A clinical failure is defined by a) one or more abnormal Amsel criteria, b) early discontinuation of study therapy due to lack of treatment effect, c) use of any vaginosis therapy other than study product during the study, or d) in the investigators opinion, requires additional treatment for vaginosis.

Subjects who do not meet the criteria for either the clinical cure or clinical failure definitions are not evaluable for clinical cure.

A secondary efficacy outcome measure is the proportion of subjects with therapeutic cure in each study arm at Visit 2 (Day 8-15) and at Visit 3 (Day 22-31). Therapeutic cure is defined as both a clinical cure AND a bacteriological cure (Nugent score 0-3). Subjects who were clinical failures, or have a Nugent score >3 are therapeutic failures. Subjects who are not evaluable for clinical cure, or do not have a Nugent score result are not evaluable for therapeutic cure.

Table 3: Bacterial Vaginosis: Determination of Therapeutic Cure

If the clinical cure outcome is...	and the Nugent (bacteriological) score result is...	then the overall therapeutic cure outcome is...
Cure	0-3	Cure
Cure	>3	Failure
Cure	Not Evaluable	Not Evaluable
Failure	0-3	Failure
Failure	>3	Failure
Failure	Not Evaluable	Failure
Not Evaluable	0-3	Not Evaluable
Not Evaluable	>3	Failure
Not Evaluable	Not Evaluable	Not Evaluable

Subjects who are non-evaluable for clinical cure at the Test of Cure visit are excluded from the PP population, but included in the mITT, ITT, and Evaluable populations as treatment failures. Last Observation Carried Forward will be used as a sensitivity analysis. Non-evaluable therapeutic cures will be handled similarly.

11.4. Study Hypotheses

There are three planned formal tests of hypotheses which compare 5% Monolaurin Vaginal gel with Vehicle Placebo Gel (excipients only), with respect to the primary safety and efficacy outcomes. The first hypothesis test compares the proportion of subjects in each study arm who experience solicited urogenital AEs through Visit 2 (Day 8-15 study window). The second

hypothesis test compares the proportion of subjects in each study arm who experience product related SAEs following the first dose of the study product through Visit 3 (Day 22-31). The third hypothesis test compares the proportion of subjects who achieve clinical cure at Visit 2 (Day 8-15). The null hypothesis for each comparison is that there is no difference in proportions between study arms, with a two-sided alternative which considers the possibility of a difference in either direction. Each test will be conducted using a Fisher's exact test at the 5% two-sided level of significance level without adjustment for multiplicity. The two safety hypotheses will be tested in the safety data analysis population. The primary efficacy analysis will be conducted in the mITT efficacy analysis population, and repeated as a secondary analysis in the PP efficacy analysis population, Evaluable efficacy analysis population and Intent-to-Treat efficacy analysis population.

11.5. Sample Size Considerations

The statistical information goal for the study is 90 subjects eligible for the primary efficacy analysis of clinical cure in the mITT efficacy population. This is an ad-hoc sample size determined by logistical and feasibility considerations, as there is insufficient pilot data upon which to base more formal sample size calculations.

Subjects who have a Nugent score <4 at Visit 1 or are positive for other concomitant vulvovaginal infections at baseline which may interfere with the efficacy assessment will be excluded from the mITT population.

Based on observed rates of these outcomes at the participating clinics, it is anticipated that a total of 120 subjects will be enrolled to reach this target. However, enrollment will be continued past 120 subjects to a maximum of 150, if necessary to reach the informational goal of 90 eligible subjects.

The table below shows the statistical power conferred by this sample size for a two-sided level 5% Fisher's exact test comparing binomial proportions, under different assumed proportions.

Table 4: Power to compare binomial proportions using a two-sided level 0.05 Fisher's Exact Test with a sample size of N = 90 (60 subjects in the 5% Monolaurin Vaginal Gel arm, 30 subjects in the Vehicle Placebo Gel arm) Conditions in which Power is $\geq .80$ are highlighted

Vehicle Placebo Gel (N=30)	5% Monolaurin Vaginal Gel (N=60)											
		.05	.10	.20	.30	.40	.50	.60	.70	.80	.90	.95
	.05	.02	.04	.38	.82	.97	>.99	>.99	>.99	>.99	>.99	>.99
	.10	.13	.03	.15	.52	.85	.98	>.99	>.99	>.99	>.99	>.99
	.20	.53	.22	.03	.12	.42	.78	.96	>.99	>.99	>.99	>.99
	.30	.86	.61	.15	.03	.12	.41	.76	.95	>.99	>.99	>.99
	.40	.98	.89	.47	.13	.04	.13	.41	.76	.96	>.99	>.99
	.50	>.99	.98	.79	.42	.13	.04	.13	.42	.79	.98	>.99
	.60	>.99	>.99	.96	.76	.41	.13	.04	.13	.47	.89	.98
	.70	>.99	>.99	>.99	.95	.76	.41	.12	.03	.15	.61	.86
	.80	>.99	>.99	>.99	>.99	.96	.78	.42	.12	.03	.22	.53
	.90	>.99	>.99	>.99	>.99	>.99	.98	.85	.52	.15	.03	.13
	.95	>.99	>.99	>.99	>.99	>.99	>.99	.97	.82	.38	.04	.02

With respect to the safety objectives, for a sample size of 60 subjects in the treatment arm with no observed serious adverse events related to study product, the 95% confidence interval for the estimated proportion is (.00,.06) (Clopper-Pearson interval).

In actuality, the safety analysis cohort will be larger than the mITT cohort, and the confidence interval will be more precise than this.

11.6. Planned Interim Analyses

The DSMB will meet and review safety data and enrollment data when 30 subjects have completed Visit 2 and at least annually as outlined in the DSMB charter. The study will be monitored to determine if any of the safety halting rules described in Section 9.5 are met. Additionally, a single fully blinded interim analysis is planned to reassess the adequacy of the sample size based on subjects meeting eligibility for the mITT cohort. After eighty subjects have been enrolled and have available test results for Nugent score, HIV, chlamydia and

gonorrhea, the fraction of subjects who are enrolled to date who are eligible for the mITT cohort will be estimated. Based on this calculation, the enrollment target may be increased from 120 subjects to a maximum of 150 as required to achieve the target of 90 subjects eligible for the mITT population. The study sponsor and investigators will have access to the data shared in fully blinded reports issued to the DSMB.

11.7. Final Analysis Plan

The primary and secondary analyses are briefly described in this section. Further details of the analyses as well as the analysis plan for the exploratory analyses will be described in a separate statistical analysis plan document.

11.7.1. Safety Analyses

11.7.1.1. Overview of Safety Analyses

Solicited Adverse Event Data

Descriptive techniques will be used to summarize the solicited adverse events data. Local and systemic solicited events will be tabulated, with symptom type crossed by severity grade.

Tables will be prepared for each treatment arm, and for the combined safety analysis cohort. The data in each table will also be depicted graphically, as a bar-chart of symptom incidence, and provided as listings of events. In the tables, figures and listings, color-coding using a heat chart (yellow, orange, red) will be used to highlight severity (mild, moderate, severe).

The solicited AE outcome data consist of multiple symptoms types, with ordinal severity grades, taken repeatedly over a 5-day reporting period following first administration of study product. To facilitate analysis and interpretation, the data will be simplified in different ways in different analyses. For example, individual symptoms will be used to form the composite endpoint “any local symptom”. The ordinal severity grades will be dichotomized, *e.g.*, none or mild versus moderate or severe. The grade will be summarized by the maximum severity over the five day reporting period so as to obtain a single observation per subject.

Unsolicited Adverse Event Data

Unsolicited AEs will be coded by MedDRA[®] for preferred term and system organ class. The proportion of subjects and exact 95% confidence intervals of AEs in aggregate, as well as by MedDRA[®] categories, will be computed. The number of SAEs is likely to be small in this study and will be reported by a detailed listing showing the type, MedDRA[®] coding, relevant dates (treatment dosing dates and AE onset and resolution dates), severity, relatedness, and outcome for each event.

Clinical Safety Laboratories

Results of safety laboratories assessed prior to and after administration of study product will be summarized by treatment group and grade. Changes in laboratory parameters pre- and post-

treatment will also be examined. Standard summary statistics, including means, standard deviations and 95% confidence intervals, will be computed. Additionally, graded results will either be analyzed as a binary variable (normal, abnormal), or a multinomial variable (Grade 0, Grade 1, Grade 2, Grade 3) using either logistic regression or ordinal logistic regression respectively to make treatment group comparisons.

11.7.1.2. Analyses of Primary and Secondary Safety Outcomes

All safety outcomes will be assessed in the safety analysis population of all randomized subjects who received one or more doses of study product. Outcomes will be reported overall and by treatment arm.

- Primary Safety Outcome –
 - Number of subjects reporting solicited urogenital AEs following the first dose of the study product through Visit 2 (Day 8-15)
 - Number of subjects reporting serious adverse events (SAEs) considered product-related following the first dose of the study product through Visit 3 (Day 22-31)
- Secondary Safety Outcomes –
 - Number of subjects experiencing non-laboratory non-solicited AEs following the first dose of the study product through Visit 3 (Day 22-31)
 - Number of subjects experiencing laboratory AEs following the first dose of the study product through Visit 2 (Day 8-15).

Two tests of hypotheses will be conducted in the safety analysis population. Each test will be conducted using a Fisher's exact test at the 5% two-sided level of significance level without adjustment for multiplicity. The point estimate for the arm-specific proportions and difference in proportions as well as corresponding two-sided level 95% confidence intervals will be reported. As described in Section 11.7.1.1, additional descriptive analyses of solicited adverse event data, unsolicited adverse event data, and clinical safety laboratory values will be performed.

11.7.2. Efficacy Analyses

11.7.2.1. Overview of Efficacy Analyses

11.7.2.1.1. Analyses of Primary and Secondary Efficacy Outcomes

- Primary Efficacy Outcome – the proportion of subjects with clinical cure in each study arm at Visit 2 (Day 8-15).

A test of hypothesis for the primary efficacy outcome will be conducted in the mITT efficacy analysis population, and repeated as a secondary analysis in the PP efficacy analysis population, Evaluable efficacy analysis population, and Intent-to-Treat efficacy analysis population. Fisher's exact test at the 5% two-sided level of significance level will be used without adjustment for multiplicity. The point estimates for the arm-specific proportions and difference in proportions as well as corresponding 95% confidence intervals will be reported.

- Secondary Efficacy Outcomes –
 - Proportion of subjects with therapeutic cure in each study arm at Visit 2 (Day 8-15) and at Visit 3 (Day 22-31).
 - Proportion of subjects with clinical cure in each study arm by Visit 3 (Day 22-31)
 - Proportion of subjects with Nugent score of 3 or less (negative for BV) and 4-6 (intermediate) in each study arm at Visit 2 (Day 8-15) and at Visit 3 (Day 22-31)

The analyses of the secondary efficacy outcomes will be conducted in the mITT efficacy analysis population and repeated in the PP efficacy analysis population, Evaluable efficacy analysis population and Intent-to-Treat efficacy analysis population. The point estimates for the arm-specific proportions and difference in proportions as well as corresponding 95% confidence intervals will be reported.

Additional descriptive analyses of the primary and secondary efficacy outcomes will be performed including tabular and graphical displays of the data, reporting outcomes overall and separately by treatment arm.

11.7.2.1.2. Analyses of Exploratory Efficacy Outcomes

- Proportion of subjects with clinical cure in each study arm by subgroup (first time or recurrent infection) by Visit 2 (Day 8-15) and by Visit 3 (Day 22-31).
- Proportion of subjects with therapeutic cure in each study arm by subgroup (first time or recurrent infection) by Visit 2 (Day 8-15) and by Visit 3 (Day 22-31).
- Mean count of *Lactobacillus spp.*, *Gardnerella spp.*, and *Mobiluncus spp.*, respectively, in each study arm at baseline, at Visit 2 (Day 8-15) and at Visit 3 (Day 22-31).
- Mean fungal colony count in vaginal secretions in each study arm at baseline, at Visit 2 (Day 8-15) and at Visit 3 (Day 22-31).

Analyses of the exploratory efficacy outcome measures will be detailed in the separate statistical analysis plan document.

12. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance (QA) reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, X-rays, Gram stain slides, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. Data collection forms will be derived from the eCRFs and be provided by the SDCC. The authority to collect this information is under 42 U.S. Code 285f.

The site may provide certified copies of the medical record or other source documents for monitoring. Medical history and inclusion/exclusion evaluations are by patient report and do not require review of the medical records prior to enrollment.

13. QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the investigational site is responsible for conducting routine QA and quality control (QC) activities to internally monitor study progress and protocol compliance. The site PI will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site PI will ensure all study personnel are appropriately trained and applicable documentation is maintained on site.

DMID-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, ICH/GCP guidelines, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

The SDCC will implement QC procedures beginning with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

14. ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1. Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997). The investigator's Institution will hold a current Federalwide Assurance (FWA) issued by Office for Human Research Protections (OHRP) for federally funded research.

14.2. Institutional Review Board

Prior to enrollment of subjects into this trial, the approved protocol and the ICF will be reviewed and approved by the appropriate IRB. The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial and a copy will be provided to DMID. Notification of the IRB's composition, or the IRB's FWA number, will be provided to DMID. Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the investigator for submission to the IRB.

14.3. Informed Consent Process

All subjects must sign an ICF that complies with the requirements of both 21 CFR 50 and 45 CFR 46.

Prior to participation in the trial, subjects will receive a comprehensive explanation of the proposed treatment, including the nature and risks of the trial, alternate therapies, any known AEs, the investigational status of the components, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their vaginal swab specimens and serum samples. Subjects will be allowed sufficient time to consider participation in the trial, after having the nature and risks of the trial explained to them. The consent form must not include any exculpatory statements.

DMID will provide the investigator, in writing, any new information that significantly affects risk related to a subject's receipt of the investigational product. This new information will be communicated by the investigator to subjects who consent to participate in the trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented, if necessary.

14.4. Exclusion of Women, Minorities, and Children (Special Populations)

This study will be inclusive of all healthy non-pregnant women, non-breastfeeding who meet the inclusion/exclusion criteria, regardless of religion, or ethnic background. Only individuals who

are 18 to 50 years old, inclusive, will be included at this time. Pregnant and breastfeeding women and children are excluded for safety reasons. Prisoners are excluded due to the rigorous study schedule that would not be feasible in an incarcerated population. Should the outcome of this study be deemed acceptable, additional trials may be initiated in other populations.

14.5. Subject Confidentiality

Subjects will have code numbers and will not be identified by name. Subject confidentiality is strictly held in trust by the participating investigators, their staff, the sponsor(s), and their agents. This confidentiality extends to genetic and biological sample tests, in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. Documented evidence that shows that a potential investigator is aware of and agrees to the confidential nature of the information related to the study must be obtained by means of a confidentiality agreement. No information concerning the trial or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All information provided by the sponsor and all data and information generated by the site as part of the study (other than a subject's medical records) will be kept confidential by the investigator and other site staff. This information and data will not be used by the investigator or other site personnel for any purpose other than conducting the study. These restrictions do not apply to: (1) information that becomes publicly available through no fault of the investigator or site staff; (2) information that it is necessary to disclose in confidence to an IRB solely for the evaluation of the study; (3) information that it is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results that may be published as described in Section 16. If a written contract for the conduct of the study that includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

The study monitor, applicable regulatory authorities, such as the FDA, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the site principal investigator. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this trial. The participating sites will permit access to such records.

14.6. Study Discontinuation

If the study is discontinued, enrolled subjects will continue to be followed for safety assessments. Subjects will be instructed to discontinue use of study product. If signs or symptoms of BV or other genital infections persist either during the study or at the last visit, the subject will be referred to her provider for further management.

If the study drug is discontinued prior to completion of the 3-day course of therapy, subjects will continue to be evaluated for symptoms of ongoing BV or other genital infections and will be referred for further therapy if signs and symptoms persist.

Subjects will be compensated for the participation in this study. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

14.7. Future Use of Stored Specimens

Subjects will be asked for permission to allow the study team to obtain extra vaginal swabs and to keep extra swab specimen for possible use in future research studies, such as testing for viruses or bacteria. Samples will be stored at a central clinical storage facility. Samples may be shared with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product. Each sample will be encoded (labeled) *only* with a barcode and a unique tracking number to protect subject's confidentiality.

There are no benefits to subjects in the collection, storage, and subsequent research use of specimens. Reports about future research done with a subject's samples will NOT be kept in their health records. Subjects can decide if they want their samples to be used for future research or have their samples destroyed at the end of the study. A subject's decision can be changed at any time prior to the end of the study by notifying the study doctors or nurses in writing. However, if a subject consents to future use and some of their blood or swab specimens have already been used for research purposes, the information from that research may still be used.

14.8. Information Provided to Subjects at Study Completion

There is increasing discussion in the research community in support of disclosure of individual research results. Several ethical principles have been invoked, including respect for persons who have the right to access information about their own health regardless of the utility of those results, if they wish to do so. Surveys suggest that many research participants value and desire the return of research results. Arguments against disclosure include: i) individual research results are often of experimental nature and difficult to translate into useful clinical applications;

ii) some results have the potential to provoke unnecessary distress and anxiety; and iii) expectations of disclosure may set a false inducement for participation.

In this protocol, we intend to provide each participant with a lay summary of overall study results. In addition, participants may choose to receive their individual results (*e.g.*, their treatment assignment or individual endpoint data, as appropriate) and will be informed about any risks and benefits of disclosure. Offering participants the option to decline and explaining the clinical significance of individual treatment is intended to minimize the negligible risks associated with disclosure. Interested subjects will be asked to provide contact information for receipt of the information. Results will be provided in an IRB-approved written document after the Clinical Study Report has been finalized.

15. DATA HANDLING AND RECORD KEEPING

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRFs and provided by the SDCC to the sites to record and maintain data for each subject enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent ink is required to ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF should be consistent with the data collection form/source documents or the discrepancies should be documented.

The sponsor and/or its designee will provide guidance to investigators on making corrections to the data collection forms and eCRFs.

15.1. Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. AEs must be graded, assessed for severity and causality, and reviewed by the site PI or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The Emmes Corporation will serve as the SDCC for this study, and will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2. Data Capture Methods

Clinical data (including AEs, concomitant medications, and solicited events data) will be entered into a 21 CFR Part 11-compliant IDES provided by The Emmes Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms/source documents.

15.3. Types of Data

Data for this study will include safety, laboratory and outcome measures (e.g., solicited events, clinical cure, and therapeutic cure).

15.4. Timing/Reports

Safety data will be reviewed by the DSMB per the DSMB charter for this study. The DSMB will make a recommendation at that time as to the advisability of proceeding with further enrollment or use of study product. Interim statistical reports may be generated as deemed necessary and appropriate by DMID.

15.5. Study Records Retention

Records and documents pertaining to the conduct of this study, including data collection forms, source documents, consent forms, laboratory test results, and medication inventory records, must be retained by the investigator for at least 2 years following submission of a Biologics License Application or until DMID authorizes transfer or destruction of study records. No study records will be destroyed without prior authorization from NIAID.

15.6. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported via The Emmes Corporation's IDES.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as in the subject's chart. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

16. PUBLICATION POLICY

Following completion of the study, the investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of DMID to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before subject enrollment. For trials that began enrollment prior to this date, the ICMJE member journals will require registration by 13 September 2005, before considering the results of the trial for publication.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), would be exempt from this policy.

17. LITERATURE REFERENCES

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SUPPLEMENTS/APPENDICES

APPENDIX A: SCHEDULE OF EVENTS

Table 5: Schedule of Events

	Visit 1	Visit 2	Visit 3	Early Termination	Unscheduled
	Day 1	8 (Window Days 8- 15)	28 (Window Days 22- 31)		
Informed Consent	X				
Inclusion/Exclusion	X				
Demographics	X				
Medical History ¹	X				
Concomitant Medication	X	X	X	X	X
Intercurrent Medical History ²		X	X	X	X
Physical Exam	X				
Targeted Physical Exam		{X}	{X}	{X}	
Urine Pregnancy Test	X				
Gynecological Exam ³	X	X	X	X	X [†]
Vaginal Swabs for Nugent Score, bacterial/fungal cultures, trichomonas testing and appropriate samples for gonorrhea, chlamydia testing ⁴	X	X	X	X	X [†]
Vaginal Swab for Future Use (if subject consents to future use sample collection)	X	X	X	X	
Randomization	X				
Study Drug Administration	X				
Distribute Memory Aid and Study Drug Kit	X				
Counsel on intravaginal products, objects, sexual intercourse, and pregnancy avoidance	X	X			
Safety Labs	X	X	X [^]	X	
Telephone Contact to Stop Study Treatment	{X}				
Review Vaginal Symptomology	X	X	X	X	X
Review for insertion of intravaginal products, objects, and sexual intercourse		X	X	X	
Review Memory Aid and Collect Study Drug Kit		X	X ⁺	X	
Assessment of Clinical Cure		X	X	X	
Assessment of Therapeutic Cure		X	X	X	
AE/SAE	X	X	X	X*	X

¹ Include menstrual, contraceptive, sexual history and history of previous episodes of vaginitis.

² Include use of intravaginal products or objects, sexual intercourse, treatment for vaginitis (other than the study product).

³ Evaluate cervix, vagina, and vulva. Perform Vaginal pH, Saline Wet Mount, KOH Wet Mount, "Whiff Test".

- ⁴ Collect vaginal swabs for study specific tests (gram stain, cultures for bacterial flora and yeast) and trichomonas OSOM testing (if saline wet mount is negative, Visit 1 only) and institution specific samples for culture or molecular testing for *Chlamydia*, *Neisseria gonorrhoeae*. (Visit 1 only). Samples for gonorrhea and chlamydia testing will be consistent with standard of care at the institution).
- ⁵ Provide instructions on proper use of study drug applicator and observe first dose. Remaining 5 doses will be administered by the subject at home on Day 2 and Day 3.
- ⁶ Safety Laboratories Include: White blood cell count, hemoglobin, platelet count, absolute neutrophil count, creatinine, ALT, AST, total bilirubin, random glucose, HIV test (Visit 1 only).
- { } Targeted physical examination if indicated based on review of symptoms.
- [] Subjects will be called regarding grade 3 laboratory abnormalities within 24 hours of receipt of results and told to discontinue study product. Subjects with positive HIV, chlamydia or gonorrhea tests will be contacted as soon as possible after results are available and referred to their primary care provider for appropriate treatment.
- ⁺ Memory Aid will be reviewed *only* if solicited events continued at Visit 2. Study Drug Kit returned *only* if not returned at Visit 2.
- ^{*} If subject declines to return for an in-person visit, the study personnel will ask to contact the subject by telephone to assess for AE/SAEs on Day 22-31.
- [†] Evaluate cervix, vagina, and vulva. Collect appropriate clinical samples for genital infectious pathogens.
- [#] Assess for vaginal discharge (quantity, color, odor, and consistency) and record presence or absence of objective signs of inflammation of cervix, vagina, and vulva (edema, erythema, excoriation).
- [^] Laboratory abnormalities that occurred between Visit 1 and Visit 2 or worsened at Visit 2 should be repeated until resolved or has stabilized. Further testing beyond Visit 3 is at the discretion of the PI or study investigator.

APPENDIX B: Toxicity Table

Table 6: Toxicity Table

HEMATOLOGY	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Hemoglobin (Female) - g/dL	11.0-11.5	9.5-10.9	<9.5
WBC Increase - cell/mm ³	11,001-15,000	15,001-20,000	>20,000
WBC Decrease - cell/mm ³	2,500-3,500	1,500-2,499	<1500
Platelets Decreased - cell/mm ³	120,000-130,000	100,000-119,999	<100,000
Neutrophils Decrease - cell/mm ³	1,500-1,799	1,000-1,499	<1000
CHEMISTRIES			
Glucose – Hypoglycemia mg/dL	60-64	55-59	<55
Glucose – Hyperglycemia Random – mg/dL	140-159	160-200	>200
Creatinine – mg/dL	>ULN-1.7	1.8-2.0	>2.0
AST U/L	44-105	106-175	>175
ALT U/L	34-105	106-175	>175
Bilirubin (serum total) mg/dL	1.3-2.0	2.1-2.5	> 2.5

APPENDIX C: Female Genital Grading Table for Physical Exam Findings

Table 7: Female Genital Grading Table for Physical Exam Findings

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe
Vulvar Edema	Mild, non-pitting edema	Moderate, 1-2+ pitting edema	3+ pitting edema, severe enough to require urinary drainage, or weeping edema +/- skin breakdown
Vulvar Erythema	Erythema covering less than 50% of vulvar surface	Erythema covering 50% or more of vulvar surface	NA
Vulvar Lesions	Blisters, ulcerations, pustules- no treatment indicated	Blisters, ulcerations or pustules, with treatment indicated	Severe epithelial disruption with hospitalization indicated
Vaginal Edema	Mild-moderate engorgement	Loss of rugae and friability	NA
Vaginal Erythema	Erythema covering less than 50% of vaginal surface	Erythema covering 50% or more of the vaginal surface	NA
Vaginal Discharge	Mild increase in amount	Moderate - significant increase in amount with pooling in vagina on examination	NA
Vaginal Abrasions or lacerations	Superficial disruptions and disruptions extending through the mucosa with minimal impact on life	Large disruptions extending through the mucosa or large superficial disruptions, hospitalization not indicated	Large disruptions extending throughout the mucosa or large superficial disruptions, hospitalization indicated
Cervical Edema and Friability	Edema without friability	Friable cervix	NA
Cervical Erythema	Erythema covering less than 50% of cervix	Erythema covering 50% or more of cervix	NA
Cervical Lesions	Blisters, ulcerations, or pustules, no treatment indicated	Blisters, ulcerations or pustules with treatment indicated	NA