Lactobacillus Probiotic for Prevention of Recurrent UTI

Short Title: Probiotic Phase 3 Study

Investigational Product:	LACTIN-V
Protocol No.:	UW/LB-002
Original Protocol:	June 9, 2005
Current Protocol:	February 22, 2015
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INVESTIGATOR'S SIGNATURE PAGE

I have read the attached protocol entitled, “Lactobacillus Probiotic for Prevention of Recurrent UTI” dated 1/14/15, and agree to conduct the study according to the provisions described therein.

I agree to comply with the International Conference on Harmonization Guideline on Good Clinical Practice and applicable FDA regulations set forth in 21 CFR Parts 50, 54 and 312.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior consent of Ann Stapleton, M.D.

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[Investigator Name] Date

GLOSSARY OF TERMS

Abbreviation	Definition
AE	adverse event
ATP	according-to-protocol
BV	bacterial vaginosis
C. trachomatis	*Chlamydia trachomatis*
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFU	colony forming units
CMH	Cochran-Mantel-Haenszel
CRA	Clinical Research Associate
CRF	case report form
CTV-05	the LACTIN-V strain of *Lactobacillus crispatus*
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
Eval	evaluable
FDA	Food and Drug Administration
g	gram
GC	gonorrhea
GCP	good clinical practices
H_2O_2	hydrogen peroxide
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICF	informed consent form
ID	identification
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	intent-to-treat

IUD	intrauterine device
KOH	potassium hydroxide
L. crispatus	*Lactobacillus crispatus*
L. jensenii	*Lactobacillus jensenii*
LMP	last menstrual period
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligrams
MMWR	*Morbidity and Mortality Weekly Report*
N. gonorrhoeae	*Neisseria gonorrhoeae*
NIH	National Institutes of Health
OTC	over-the-counter
Pap smear	Papanicolaou smear
PCR	polymerase chain reaction
rep-PCR	repetitive sequence polymerase chain reaction
rUTI	recurrent urinary tract infection
SAE	serious adverse event
SD	standard deviation
spp.	species
Subject ID	subject identification number
STD	sexually transmitted disease
T. vaginalis	*Trichomonas vaginalis*
UTI	urinary tract infection
WHO	World Health Organization

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1 STUDY SITE INFORMATION AND PERSONNEL

1.1 Site Names and Locations

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2 BACKGROUND AND RATIONALE

2.1 Background information on Recurrent Urinary Tract Infection (rUTI)

Urinary tract infection (UTI) is a very common outpatient diagnosis among healthy young women, resulting in considerable morbidity and health care costs. In 1997, UTI accounted for approximately 11 million doctor visits. Approximately 33% of these women experience two or more UTIs per year. A recent population survey of women in the United States found that the lifetime risk of UTI was over 60% and that the estimated societal cost of UTIs exceeds 25 billion dollars over 20 years (1). To date, the only effective method of reducing the high risk of UTI in pre-menopausal women with a history of recurrent urinary tract infection (RUTI) is antimicrobial prophylaxis, a strategy that is becoming less effective as antimicrobial resistance amongst uropathogens increases (2). In addition, antibiotic resistance, even among community-acquired UTIs, is making treatment of these infections more problematic (3).

Thus, novel, safe and effective non-antimicrobial prevention strategies are urgently needed. One such approach, for which there is strong evidence at the mechanistic level, is use of an H_2O_2-producing *Lactobacillus* to restore the normal vaginal microbiota (4). If effective in reducing *E. coli* colonization and UTI, this strategy would reduce the costs and morbidity associated with urinary tract infections, including time lost from work, health care costs, antimicrobial use, and the ultimate development of antimicrobial resistance.

Many studies have confirmed that the critical event preceding a UTI is colonization of the vaginal introitus with gut microbiota, most commonly *E. coli* (5, 6). Women with recurrent UTI are more likely to be vaginally colonized with *E. coli* or other *Enterobacteriaceae* than women without recurrent UTI, and demonstrate prolonged periods of persistent colonization. Recent studies have verified these findings and have demonstrated a coincident loss of the normally predominant vaginal lactobacilli [10, 11, 12]. In a study in women of reproductive age, 15% of 302 women who had vaginal colonization with *L. crispatus* or *L. jensenii*, both H_2O_2 producers, were colonized with *E. coli* as compared with 27% of women who did not have these lactobacilli species present ($p = 0.01$) (7). This and other data suggest that H_2O_2-producing lactobacilli may have an important role in the maintenance of the normal vaginal ecology and in the prevention of vaginal colonization with *E. coli* and thus *E. coli*-induced UTI.

Mechanistic studies also support the ability of H_2O_2-producing lactobacilli to exclude *E. coli* vaginal colonization. Thus, Osset and colleagues showed that such lactobacilli are capable of competitive exclusion, blocking adherence of *E coli* to vaginal epithelial cells (8). In addition, *L. crispatus* inhibits the growth of *E. coli* in both liquid and solid media through the action of H_2O_2. Finally, recent studies at the University of Washington have

shown that *L. crispatus* adheres extremely well to vaginal epithelial cells from women with recurrent UTI, suggesting it will colonize such women's vaginal epithelium (9).

2.2 History of product development

2.2.1 Pre-clinical research

In 1987, research conducted by Giorgi et al. suggested that the most common *Lactobacillus* in the human vagina is not *Lactobacillus acidophilus*, but rather *Lactobacillus crispatus*, *Lactobacillus jensenii* and *Lactobacillus gasseri* (10). Additional studies performed in the laboratories of Sharon Hillier, PhD at the University of Pittsburgh confirmed that *Lactobacillus crispatus i*s the predominant H_2O_2-producing *Lactobacillus* found in the vagina of a healthy woman (7).

LACTIN-V, the product under investigation in this Phase 3 study, contains *Lactobacillus crispatus* CTV-05 bacteria preserved by spray drying with a simple drying medium. The product is prepared as a powder in an applicator for intravaginal use. *Lactobacillus crispatus* is a naturally occurring organism isolated from the vagina of a healthy woman in 1993. Using repetitive sequence polymerase chain reaction (rep-PCR) DNA fingerprinting, investigators distinguished *L. crispatus* CTV-05 from other *Lactobacillus* strains derived from the human vagina. Additionally, it was demonstrated that this strain produces high levels of lactic acid and H_2O_2 (11).

Osel, Inc. plans to develop LACTIN-V for the following indications:

1. To reduce the recurrence rate of urinary tract infection in women with recurrent uncomplicated UTI (RUTI), including acute, uncomplicated cystitis and pyelonephritis.

2. To reduce the recurrence rate of bacterial vaginosis (BV) in women with recurrent BV.

2.2.2 Clinical research

2.2.2.1 Lactin-Vaginal

The first product containing *L. crispatus* CTV-05, Lactin-Vaginal, was under development by The Medicines Company and subject to an IND sponsored by the NIH (BB-IND 6603). In previous clinical studies (three non-IND and two IND) conducted between 1991 and 2000, a total of 304 women received Lactin-Vaginal and were considered evaluable. Although the overall results of the Phase II/III study with Lactin-Vaginal indicated no significant improvement in the cure rate for BV when Lactin-Vaginal was administered with a single 2 gram dose of metronidazole, the

subset of subjects who received Lactin-Vaginal and were colonized with *Lactobacillus crispatus* CTV-05 had a significantly higher clinical cure rate at 30 days than those who were not colonized.

In this Phase II/III study, Lactin-Vaginal was shown to be safe. Adverse events that were reported in the phase II/III study of Lactin-Vaginal (Protocol No. 99-033) were equally distributed between the Lactin-Vaginal and placebo control substance groups, and thus were unlikely related to the *L. crispatus* CTV-05, with the exception of increased vaginal discharge while using the capsules. All 355 women received a single 2 gram oral dose of metronidazole and then were randomized to either Lactin-Vaginal or a control vaginal capsule. Adverse events reported in at least 10% of subjects in this study were abdominal pain, nausea, upper respiratory infections, urinary tract infections, bacterial vaginitis, yeast vaginitis, back pain, urinary frequency, genital pruritus, intermenstrual bleeding, menorrhagia and vaginal discharge. Other less commonly occurring AEs reported included dysuria, urinary urgency, nocturia, dysmenorrhea, vaginal burning and/or pain, genital burning, genital erythema, genital edema, genital lesions, vaginal odor, diarrhea, constipation, dyspareunia, and skin rash. The following Serious Adverse Events were reported in subjects who received Lactin-Vaginal, but all were considered unrelated to treatment: a spontaneous abortion which occurred 69 days after randomization; a ruptured ectopic pregnancy which occurred 79 days after randomization; and a gall bladder removal 92 days after randomization.

Osel, Inc. has acquired the commercialization rights for this product and also the manufacturing facilities used to produce Lactin-Vaginal. Osel, Inc. planned to develop the product for the following indications:

1. To reduce the recurrence rate of BV.
2. To reduce the recurrence rate of urinary tract infections in women with recurrent uncomplicated UTI (RUTI)

2.2.2.2 LACTIN-V

Two rounds of important modifications have been made to the original Lactin-Vaginal product by The Medicines Company. The new product developed by Osel, Inc. was called LACTIN-V.

The first formulation of LACTIN-V (Formulation 1) incorporated the following changes into the manufacturing process and was used in the trials LV 001 – LV004:

- Preparation of a new Master Cell Bank;
- Reformulation to reduce the quantity of fermentable sugars in the final study product; this reformulation has resulted in a more potent study product;

- Replacement of the fermentation medium with an animal by-product-free medium;
- Changes in the fermentation process leading to the harvest of bacteria in a "log growth phase" versus "stationary growth phase";
- Increase in the number of viable bacteria per capsule;
- Reduction in the size of gelatin capsules
- Reduction in the volume of the capsule

LACTIN-V (Formulation 1) contains *Lactobacillus crispatus* CTV-05 bacteria preserved as a dry powder. Using repetitive sequence polymerase chain reaction (rep-PCR) DNA fingerprinting, investigators distinguished *L. crispatus* CTV-05 from other *Lactobacillus* strains derived from the human vagina. Additionally, it was demonstrated that this strain produces high levels of lactic acid and H_2O_2 (11).

LACTIN-V (Formulation 1) has been tested in several phase I and phase II studies:

LACTIN-V Study LV-001

Osel, Inc. completed an initial Phase I study of LACTIN-V (Formulation 1) in healthy pre-menopausal women (Study No. LV-001) under BB-IND 11363. In LV-001, 60 women were randomized (1:1:1:1) between four groups: low dose LACTIN-V at 5 x 10^6 cfu/capsule; high dose LACTIN-V at 5 x 10^8 cfu/capsule; control capsule; and untreated. Commencing at Visit 1 (Day 1), all capsule recipients inserted one capsule daily for five consecutive days and returned to the clinic for two follow-up visits (Visit 2 between Days 6–8 and Visit 3 between Days 26–34). Safety was evaluated by symptoms, pelvic examinations, colposcopy and urinalyses. Colonization and shifts in other vaginal microbiota were assessed at Day 7 (+/-1) and at Day 30 (+/-4). Colonization data indicates that approximately twice as many subjects in the LACTIN-V 5 x 10^8 cfu/capsule dose arm (approximately 40% colonization rate) versus the LACTIN-V 5 x 10^6 cfu/capsule dose cohort (approximately 20% colonization rate) were successfully colonized with *L. crispatus* strain CTV-05 at Day 30.

No serious adverse events were reported in Protocol No. LV-001, and no subject left the protocol due to an adverse event. Long-term safety was assessed by a telephone follow-up at Month 6. Most AEs were mild to moderate. No single severe AE was experienced by more than one subject. Three subjects each experienced a single severe, treatment-related AE: abdominal pain, vaginal candidiasis, vaginal burning sensation, vaginal hemorrhage and vaginal irritation. There were no notable, unexpected AEs, no SAEs and no deaths. No subject left the study due to an AE. The most frequent (≥10%) non-gynecologic AEs reported regardless of causality were abdominal pain NOS (25.0%), headache NOS (23.3%) and diarrhea NOS (10.0%). The most frequent (≥10%) gynecologic AEs reported were vaginal discharge (35.0%), genital pruritis female

(26.7%), vaginal hemorrhage and vaginal odor (15.0%, each), and vaginal burning sensation and vaginal candidiasis (11.7%, each). Forty (88.9%) of subjects participating in this study experienced an AE that was considered by the Investigator to be possibly or probably related to study treatment (LACTIN-V or control capsule). The most common (≥10%) non-gynecologic AEs reported as possibly or probably related to study treatment were abdominal pain NOS (22.2%), headache NOS (17.8%) and diarrhea NOS and vaginal candidiasis (11.1%, each). The most common (≥10%) gynecologic AEs that were reported by the Investigator as possibly or probably related to study treatment were vaginal discharge (46.7%), genital pruritis female (31.1%), vaginal odour (20.0%), vaginal hemorrhage (17.8%) and vaginal burning sensation (15.6%).

Results obtained indicate that LACTIN-V is safe and well tolerated when administered to healthy subjects once a day for five days at either 5×10^6 cfu/capsule or 5×10^8 cfu/capsule. The safety and vaginal microbiota changes in the placebo control group demonstrated that it was a valid comparison group for use in future studies, instead of using an untreated group.

LACTIN-V Study LV-002

Osel, Inc. conducted a second Phase I study, Protocol No. LV-002, in a cohort of healthy pre-menopausal women with a history of uncomplicated recurrent UTI. The design of this study was similar to Protocol No. LV-001 with the exception that there were 30 women randomized (1:1) to receive either LACTIN-V Formulation 1 (5×10^8 cfu/capsule) or the control capsule once daily for five days. Safety was evaluated by symptoms, urinalyses and pelvic examination. Colonization was assessed at Day 7 (+/-1) and at Day 30 (+/-4), in addition to shifts in other vaginal microbiota. Long-term safety was assessed by a telephone follow-up at Month 6. In general, the study product was well tolerated.

A high rate of positive *L. crispatus* strain CTV-05 assays at study baseline in the Evaluable Cohort (33.3% and 7.7% in the LACTIN-V and the control capsule groups, respectively) may have been due to a lack of specificity of the assay. The CTV-05 rep-PCR assay, which was still under development at the time, may have lacked sufficient specificity in this study to distinguish between endogenous, H_2O_2-producing *L. crispatus* and the CTV-05 (LACTIN-V) strain of *L. crispatus*. The CTV-05 colonization rates observed in subjects receiving LACTIN-V in this study (40.0% at Visit 2 and Visit 3) were lower than anticipated, possibly due to the high rate of endogenous lactobacilli at baseline in this population of healthy pre-menopausal women. All study subjects in the Evaluable Cohort were vaginally colonized with H_2O_2-producing lactobacilli at study entry, and they remained colonized throughout the study. It was anticipated that in women with disrupted vaginal microbiota and reduced lactobacilli following antibiotic treatment of recurrent BV or RUTI, the CTV-05 colonization rates would be higher.

In Protocol No. LV-002, LACTIN-V appeared to be safe and well tolerated when administered intravaginally for five consecutive days at a dose of 5×10^8 cfu/capsule.

All AEs were mild to moderate; no severe or severe, related AEs were reported. There were no notable, unexpected AEs, no SAEs and no deaths. No subject left the study due to an AE. Fourteen subjects in the LACTIN-Vgroup reported a total of 37 events. In the control group, 14 subjects reported a total of 41 events. Overall, the most frequent (≥10%) non-gynecologic AEs reported regardless of causality were headache NOS (43.3%), abdominal pain NOS (23.3%) and back pain (13.3%). The most frequent (≥10%) gynecologic AEs reported were vaginal discharge (43.3%), female genital pruritis (13.3%), vaginal candidiasis (13.3%) and dysmenorrhea (10.0%). Twenty-two (73.3%) of the 30 subjects participating in this study experienced an AE that was considered by the Investigator to be possibly or probably related to study treatment (10 subjects in the LACTIN-V group, 12 subjects in the control group). The most common (≥10%) AEs that were reported by the Investigator as possibly or probably related to study treatment were vaginal discharge (43.3%), female genital pruritis (13.3%) and vaginal candidiasis (13.3%). Six subjects in the LACTIN-V group reported a total of six events that were considered to be possibly related to study treatment, five subjects reported six events that were probably related, and one subject reported an event that was considered by the Investigator to be related to study treatment (mild vaginal discharge). In the control group, eight subjects reported a total of eleven events that were considered to be possibly related to study drug, six subjects reported six events that were probably related, and one subject reported an event that was considered to be related to study treatment (moderate vaginal discharge). None of the AEs reported were considered to be severe. Five subjects in the LACTIN-V group reported eight events that were considered to be moderate. All of these events were considered unrelated to study treatment, with the exception of a single occurrence of vaginal candidiasis, which was considered to be possibly related. Five subjects in the control group reported a total of eight moderate AEs. Long-term safety was assessed by a telephone follow-up at Month 6. The study was published in 2007 (12)

LACTIN-V Study LV-003

In 2005-2006, Osel Inc. conducted a Phase II, randomized, double-blind, placebo-controlled, multi-center study to assess safety, colonization rate and efficacy of preventing recurrence of bacterial vaginosis (BV). The study was conducted in 149 women with a history of recurrent BV who had recently been treated with a course of intravaginal metronidazole or clindamycin. Following completion of the prescribed course of antibiotic, each subject was randomized (1:1) at Visit 2 (Day 1) to receive either one LACTIN-V (Formulation 1) capsule at a dose of 5×10^8 colony forming units (cfu) per capsule daily for five days followed by one capsule per week for 10 weeks or the placebo capsule on the same schedule. Of those subjects who had *Lactobacillus* culture results, the percentage of subjects who were colonized with the *L. crispatus*, CTV-05 strain was significantly greater in the LACTIN-V group compared to the placebo group for both the modified intent-to-treat (MITT) and according-to-

protocol (ATP) cohorts [41.7% vs. 6.2%, respectively, in the MITT cohort (p=0.0000) and 59.3% vs. 12.1%, respectively, in the ATP cohort (p=0.0023)]. Although not reaching levels of statistical significance, the rate and the incidence of reported symptomatic BV episodes was lower in the LACTIN-V group compared to the placebo group (in both the MITT and ATP cohorts). In addition, the time to first recurrence of symptomatic BV was longer in LACTIN-V subjects compared to placebo subjects in the MITT cohort (118.7 days) in the LACTIN-V group and 98.7 days in the placebo group. Although overall encouraging, the provided data could not fully answer the research question of efficacy of prevention of BV recurrence due to low sample size and a low colonization rate of 41.7% for the MITT cohort and 59.3% for the ATP cohort.

For Protocol No. LV-003, 111 of 149 subjects (74.5%) reported at least one AE (76.7% of LACTIN-V subjects and 72.4% of placebo control substance subjects). Overall, the most common AEs reported were vaginal discharge (38.9%), vaginitis bacterial NOS (24.2%), genital pruritis female (23.5%) and vaginal odour (22.1%). Adverse events were mostly mild (67.8%) or moderate (28.2%) in severity, and the majority of subjects had AEs that were assessed as possibly or probably related to study product (56.4%). No safety signal was observed for the LACTIN-V group in regard to AEs, with the exception of a higher rate (≥5% difference between treatment groups) of nausea, diarrhea, dysuria, vaginal discharge and vaginal odour. One subject experienced three simultaneous SAEs (hemiparesis, blurred vision in right eye and headache); however, as this subject was in the placebo control substance group, the SAEs were unrelated to LACTIN-V. Two subjects in the LACTIN-V group discontinued study treatment due to AEs; one subject became pregnant and the other reported nausea and diarrhea, which required treatment. No deaths were reported during this study.

LACTIN-V study LV-004

A conducted by Osel Inc. was planned to involve 100 women with a history of recurrent, uncomplicated UTIs who had recently been treated for UTI with a course of antibiotics. Only subjects treated with amoxicillin/clavulanate (Augmentin), a quinolone or nitrofurantoin would have been eligible for enrollment. Subjects were to be randomized to one of two groups (50 subjects per group) to either receive one capsule of LACTIN-V Formulation 1 (at 5 x 10^8 cfu/capsule) or one placebo daily for five days followed by one capsule per week for 10 weeks. In addition to safety, this study was to evaluate the preliminary efficacy of LACTIN-V in reducing the incidence of symptomatic, probable and asymptomatic UTI. Subjects were to be queried at each clinic visit for urinary symptoms, including dysuria, frequency, urgency, hematuria, flank pain or suprapubic pain. This study was to also evaluate vaginal colonization with lactobacilli, *Lactobacillus crispatus*, strain CTV-05 and *E. coli* following administration of LACTIN-V.

The trial was halted due to logistical difficulties after only four subjects had been enrolled at three of the six study sites. Due to an enrollment rate that was much slower than expected, even after the addition of two more study sites, Study No. LV-004 was closed to further recruitment much in advance of reaching the target enrollment number of 100. Among the four subjects that completed the study and were being followed for long-term safety, a total of six AEs were reported during the study in two subjects in the LACTIN-V group. All of the AEs were considered by the Investigator to be of moderate severity and unrelated to study treatment. There were no severe or severe related AEs. There were no notable, unexpected adverse events, no serious adverse events and no deaths.

LACTIN V study UW/LB-001

Following the problems with low enrollment described above for LV-004, a study with similar study design using LACTIN-V for prevention of recurrent UTI was undertaken at the University of Washington. In addition to safety, this study was to evaluate the preliminary efficacy of LACTIN-V in reducing the incidence of symptomatic, probable and asymptomatic UTI. In the study UW/LB-001, 100 young healthy women with a history of recurrent UTI received antimicrobials for acute UTI and then were randomized to one of two groups (50 subjects per group) to either receive one capsule of LACTIN-V Formulation 1 (at 5×10^8 cfu/capsule) or one placebo daily for five days followed by one capsule per week for 10 weeks. Participants were followed up at 1 week and 10 weeks after intervention and for UTIs; urine samples for culture and vaginal swabs for real-time quantitative 16S ribosomal RNA gene polymerase chain reaction for *L. crispatus* were collected. This study was to also evaluate vaginal colonization with lactobacilli, *Lactobacillus crispatus*, and strain CTV-05 following administration of LACTIN-V.

Recurrent UTI occurred in 15% (7/48) of women receiving Lactin-V compared with 27% (13/48) of women receiving placebo (relative risk [RR], .5; 95% confidence interval, .2–1.2). High-level vaginal colonization with *L. crispatus* (**>**10^6 16S RNA gene copies per swab) throughout follow-up was associated with a significant reduction in recurrent UTI only for Lactin-V (RR for Lactin-V, .07; RR for placebo, 1.1; $P < .01$).

Adverse effects were reported by 56% of participants who received Lactin-V and by 50% of participants who received placebo; the most common adverse effects included vaginal discharge or itching or moderate abdominal discomfort. One participant in the placebo group discontinued treatment because of adverse effects. The incidence of BV or candidal vaginitis was low in both treatment groups (0%–5%), and there was no significant difference in rates of pyuria between the 2 groups (rate at visit 3 among women in the Lactin-V group, 13%; rate at visit 3 among women in the placebo group, 22%; rate at visit 4 among women in the Lactin-V group, 32%; rate at visit 4 among women in the placebo group, 33%). No serious AEs or episodes of pyelonephritis were reported in either group. This study was published in 2011 and concluded that Lactin-V after treatment for cystitis is associated with a reduction in recurrent UTI (13).

LACTIN-V study LV-005

As a result of the non-optimal colonization rate using LACTIN-V (Formulation 1) delivered in capsules, as seen in Protocols No. LV-003 and LV-004, it was recognized that the gelatin capsule used in the previous LACTIN-V dosage form dissolved slowly in the vagina, likely contributing to sub-optimal colonization of the bacteria. To circumvent this problem and improve the colonization rates of *L. crispatus* CTV-05, a new round of study product and protocol improvements incorporated the following measures:

- Elimination of the gelatin capsule
- Development of a pre-filled vaginal applicator for administration of the LACTIN-V powder formulation directly into the vagina without a capsule (Formulation 1 in applicator)
- Increasing the amount of *L. crispatus* CTV-05 per dose (i.e., per applicator) from 5 x 10^8 (150 mg) to 2 x 10^9 cfu/dose (600 mg)
- Elimination of the lag time between completion of antibiotic treatment and initiation of LACTIN-V dosing

In late 2007 Osel commenced a small phase I dose-ranging clinical study (LV-005) to assess the safety, tolerability and acceptability of LACTIN -V (Formulation 1) powder delivered with pre-filled vaginal applicators in healthy pre-menopausal women. Study Protocol No. LV-005 also assessed higher doses than used in the prior LV-001 to LV-004 studies; increasing the amount of *L. crispatus* CTV-05 per dose (i.e., per applicator) from 5 x 10^8 (150 mg) to 1 x 10^9 (300 mg) and 2 x 10^9 cfu/dose (600mg). Twelve healthy volunteers were enrolled in 3 blocks of 4 (5 x 10^8, 1 x 10^9, and 2 x 10^9 cfu/dose). Each block was randomized in a 3:1 ratio of active product to placebo. Participants used study product for 5 consecutive days, returned for follow-up on days 7 and 14, and had phone interviews on days 2 and 35.

All 12 subjects took 5 doses and completed study follow-up. No SAEs were reported for Study Protocol No. LV-005. All 12 participants took 5 doses and completed study follow-up. Overall, 45 adverse events (AEs) occurred, of which 31 (69%) were genitourinary (GU) AEs. GU AEs appeared evenly distributed between the 3 treatment blocks and between LACTIN-V and placebo arms. The most common GU AEs were vaginal discharge in 5 subjects (42%), abdominal pain in 4 subjects (33%), metrorrhagia in 4 subjects (33%), vulvovaginitis in 4 subjects (33%), vaginal candidiasis in 3 subjects (25%), and vaginal odor in 3 subjects (25%). Forty-one (91%) AEs were mild (grade 1) in severity. All 4 moderate AEs (grade 2) were unrelated to product use. No grade 3 or 4 AEs or serious adverse events (SAE) occurred. Laboratory parameters and colposcopy findings were within normal limits or clinically insignificant. The product was well tolerated and accepted. The study concluded that all 3 dosage levels of LACTIN-V appeared to be safe and acceptable in healthy volunteers. This study was published in 2009 (14).

LACTIN-V study LV-006

Following the phase I clinical study LV-005, Osel, Inc. completed a phase IIa clinical study to further assess the colonization efficiency of *L. crispatus* CTV-05 in otherwise healthy pre-menopausal women diagnosed with BV, administered immediately after completion of a standard antibiotic treatment with metronidazole (MetroGel), using the pre-filled vaginal applicator. In addition, safety, tolerability and acceptability of LACTIN-V (Formulation 1) were assessed.

In this Phase IIa study, twenty-four women with BV were randomized in a 3:1 ratio of active product to placebo. Participants used LACTIN-V at 2 x 10^9 colony-forming units (cfu)/dose or placebo for 5 initial consecutive days, followed by a weekly application over 2 weeks. They returned for follow-up on Days 10 and 28. Sixty-one percent of the 18 women randomized to the LACTIN-V group were colonized with *L. crispatus* CTV-05 at Day 10 or Day 28. Among LACTIN-V users with complete adherence to the study regimen, 78% were colonized at Day 10 or Day 28. Of the 120 adverse events (AEs) that occurred, 108 (90%) and 12 (10%) were of mild and moderate severity, respectively. AEs were evenly distributed between the LACTIN-V and placebo group. Of the total AEs, 93 (78%) were genitourinary in origin. The most common genitourinary AEs included vaginal discharge (46%), abdominal pain (46%), dysuria (21%), pollakiuria (21%), vaginal odor (21%), and genital pruritus (17%). No grade 3 or 4 AEs or serious AEs occurred and no deep epithelial disruption was seen during colposcopic evaluation. The product was well tolerated and accepted. The applicator and dosage modifications resulted in a more potent LACTIN-V product (Formulation 1 in applicator) and an improved clinical protocol, leading to higher colonization and thus greater efficacy to prevent recurrent BV (15).

In early clinical studies conducted by The Medicines Company between 1991 and 2000, a total of 304 women received Lactin-Vaginal and were considered evaluable. Although the overall results of the phase II/III study with Lactin- Vaginal indicated no significant improvement in the cure rate for BV when the study product was administered with a single 2 gram dose of metronidazole, the subset of subjects colonized with *L. crispatus* CTV-05 had a better clinical outcome. In this study, Lactin-Vaginal was demonstrated to be safe, with no category of adverse events occurring at a statistically higher frequency in the Lactin-Vaginal group than among controls. Later studies conducted by Osel, Inc. with the product LACTIN-V (Formulation 1 in capsule) at 5 x 10^6 or 5 x 10^8 cfu/capsule (Protocol No. LV-001 through LV-004) included more than 200 women (half of them being exposed to LACTIN-V) and provided excellent data on the safety of the study product.

2.3 Proposed next step

The goal of the proposed studies is to test the preliminary efficacy of LACTIN-V in recurrent UTI, as well as to confirm previous safety data. The pre-filled vaginal applicator delivery system will be employed for LACTIN-V as a powder formulation of *Lactobacillus crispatus* CTV-05 provided at a dose of 600 mg (containing 2 x 10^9 CFU of *L. crispatus* CTV-05 per dose), as in published trials using LACTIN-V for BV (15, 16).

3 STUDY PURPOSE, OBJECTIVES AND ENDPOINTS

3.1 Purpose

The purpose of this study is to evaluate the efficacy and safety of *Lactobacillus crispatus* strain CTV-05 (LACTIN-V) in pre-menopausal women with uncomplicated recurrent urinary tract infection (RUTI). The purpose, objectives, endpoints, and design of this study will be similar to the completed Phase 2 study of the safety and preliminary efficacy of LACTIN-V (13).

3.2 Study Objectives

3.2.1 Primary Objective

The primary objective of this study is to evaluate the efficacy of LACTIN-V, compared to placebo, in pre-menopausal women with recurrent uncomplicated UTI.

1. To evaluate the number (percent) of subjects in each treatment group with at least one recurrence of symptomatic UTI.
2. To evaluate the efficacy of LACTIN-V, compared to placebo, in reducing the incidence of symptomatic UTI.
3. To evaluate the time to recurrence, in subjects receiving LACTIN-V as compared to placebo, of symptomatic UTI

3.2.2 Secondary Objectives

1. To evaluate vaginal colonization with total *Lactobacillus* (endogenous and exogenous) in subjects receiving LACTIN-V versus placebo.
2. To evaluate vaginal colonization with *Lactobacillus crispatus* strain CTV-05 (the LACTIN-V strain of *Lactobacillus*).
3. To evaluate vaginal colonization with *E. coli* in subjects receiving LACTIN-V versus placebo.
4. To evaluate shifts in vaginal microbiota in subjects receiving LACTIN-V versus placebo, as assessed by Nugent score of vaginal gram stains at all visits.

5. To evaluate shifts in the composition of vaginal microbiota in subjects receiving LACTIN-V versus placebo, as assessed by qualitative and/or quantitative DNA amplification of stored vaginal fluid specimens from all visits.
6. To evaluate the safety of LACTIN-V over four months.

3.3 Case Definitions

3.3.1 Symptomatic UTI

Symptomatic UTI is defined as an episode with one or more UTI symptoms (dysuria, urgency, frequency) prompting evaluation and treatment, with a positive urine culture. A positive urine culture is defined as ≥ 10^2/ml of an uropathogen in these patients.

3.3.2 Probable UTI

Probable UTI is defined as an episode with one or more UTI symptoms (dysuria, urgency, frequency) prompting evaluation and treatment. This can occur in the absence of a MSU culture.

3.3.3 Recurrent UTI

In this study, for the purpose of eligibility, the diagnosis of recurrent UTI is defined as one or more symptomatic, treated UTIs in the prior 12 months.

3.4 Study Endpoints

3.4.1 Primary Endpoint

The primary endpoint of this study is to demonstrate the efficacy of *Lactobacillus crispatus* strain CTV-05 (LACTIN-V) in pre-menopausal women with RUTI, when administered vaginally at a dose of 2 x 10^9 CFU once per day for five consecutive days followed by once per week for 8 weeks, for preventing RUTI.

3.4.2 Secondary Endpoints

To evaluate changes in the vaginal environment and determine the extent of colonization of CTV-05 over the course of the study and to assess AE's reported by subjects or thru physical exam by investigators.

4 RATIONALE

4.1 Overview

The goal of this Phase 3 study is to obtain preliminary data on LACTIN-V's ability to reduce the UTI recurrence rate, decrease UTI incidence and/or increase time to first UTI recurrence in pre-menopausal women who have a history of recurrent uncomplicated UTI, and to continue to evaluate the safety of LACTIN-V. LACTIN-V will be administered at a dose of 5 x 10^9 CFU of *L. crispatus* strain CTV-05/powder applicator once daily for five consecutive days then once per week for the following 10 weeks. Subjects will be followed off LACTIN-V or placebo for an additional 10 weeks following this dosing regimen for recurrent UTI, vaginal microbiota measures, and AEs.

4.2 Rationale for Dose and Regimen

LACTIN-V is available in a dose level of 5 x 10^9 CFU/applicator for this Phase 3 study in the rUTI population, which is the same dose studied in a Phase 1 Study and a Phase 2a BV study (Protocols LV-005 and LV-006, respectively). No serious adverse events have been reported.

Preliminary data from Protocol LV-001 have shown that subjects receiving LACTIN-V at the 5 x 10^8 CFU/capsule dose level had a 40% colonization rate (6/15 subjects) with the *L. crispatus* strain CTV-05, as compared to a 20% colonization rate (3/15 subjects) in the 5 x 10^6 CFU/capsule treatment group (Osel Inc., personal communication). At a dose of 2 x 10^9 CFU/applicator (Protocol LV-006), 61% of women randomized to the LACTIN-V group were colonized with *L. crispatus* CTV-05 at Day 10 or Day 28. Among LACTIN-V users with complete adherence to the study regimen, 78% were colonized at Day 10 or Day 28.

The dosing regimen selected for this study was based on the dosing regimen in the Phase 1 and 2a studies with LACTIN-V, in which subjects inserted one capsule daily for five consecutive days, followed by one applicator weekly for 2 weeks. In this Phase 3 study, as in Protocol UW/LB-001, subjects will receive one intravaginal applicator daily for five consecutive days (Days 1–5), followed by one applicator weekly for 10 weeks (starting on Day 8) for a total of 15 applicators over 11 weeks. Weekly dosing has been added for 10 weeks following the initial five-day course so that *L. crispatus* strain CTV-05 colonization can be maintained throughout the study period.

4.3 Rationale for Study Design

The study will enroll 276 pre-menopausal women currently with a symptomatic UTI who report at least one other symptomatic UTI episode treated within the past 12 months. Subjects will be randomized to receive either LACTIN-V (5 x 10^9 CFU/applicator) or a placebo applicator in a 1:1 ratio. The Randomization Visit (Visit 1) must occur within 3–

10 days of completing antibiotics since the vaginal microbiota is thought to be maximally disrupted at this time, thus providing L*. crispatus* CTV-05 the best chance of successful colonization.

It is not anticipated that the entry UTI episode will necessarily have fully resolved at the time of randomization (Visit 1), as mild symptoms may still be present, and the dipstick and urinalysis may still be positive or equivocal several days after completing antibiotics. However, the urine culture is anticipated to be negative at Visit 1, following completion of antibiotics; therefore, subjects with a positive urine culture at this visit will be considered an antibiotic treatment failure.

All subjects will return to clinic at 2, 4, 8, 12, and 16 weeks to assess efficacy and safety. Colonization with *L. crispatus* CTV-05 and other lactobacilli, as well as *E. coli*, will be assessed at all visits. Nugent scores will be determined at baseline and all subsequent visits to evaluate vaginal microbiota pre- and post-treatment.

4.4 Rationale for Study Endpoints

4.4.1 Efficacy

Preliminary efficacy will be assessed by evaluating subjects for Symptomatic UTI. Subjects will also be queried at each visit for any UTI symptoms occurring since the prior visit. In addition, subjects will be instructed to return to clinic for an additional visit if they develop symptoms of UTI during the four-month study period.

4.4.2 Safety

Safety will be evaluated using the following safety measures:

1. Subjects will be queried about adverse events at all visits.
2. Subjects will be given a Study Diary to record symptoms.
3. Genital examinations at all visits. Complete pelvic exams at visit one and six or if clinically indicated.

4.4.3 *Lactobacillus* Colonization

Vaginal colonization with lactobacilli will be assessed by vaginal culture. This culture information will be both specific to *L. crispatus* CTV-05 and non-specific (total vaginal lactobacilli, including endogenous and exogenous lactobacilli).

Total *Lactobacillus* colonization and H_2O_2 production will be assessed using culture. Colonization specifically with *L. crispatus* CTV-05 will be assessed using a DNA fingerprinting technique known as rep-PCR (11), a repetitive element sequence-based PCR, which will be used to identify the specific strain of *L. crispatus* (CTV-05) by

amplifying its unique pattern of DNA fragments. This technique, which makes use of repetitive sequences dispersed throughout a bacterial genome for the direct amplification of genomic DNA, generates bacterial species- or strain-specific fingerprint patterns. Rep-PCR can be used to distinguish probiotic *L. crispatus* strain CTV-05 from other *L. crispatus* species. The *Lactobacillus* cultures and CTV-05 rep-PCR will be performed in the lab of Ann Stapleton, M.D. at the University of Washington. The rep-PCR data collected will be used to assess the correlation between colonization and clinical endpoints.

Vaginal fluid specimens will be stored from each visit for analysis of shifts in the composition of vaginal microbiota by qualitative and/or quantitative DNA amplification methods. DNA amplification will be performed in the lab of Ann Stapleton M.D. at the University of Washington.

4.4.4 *E. coli* Colonization

Vaginal colonization with *E. coli* will be assessed by vaginal culture at all visits. The *E. coli* cultures will be performed in the lab of Ann Stapleton, M.D. at the University of Washington.

5 STUDY DESIGN

5.1 Description of the Study

This is a Phase 3, randomized, double-blind, placebo-controlled study. Safety will be assessed using self-reported symptoms, pelvic examinations and vaginal cultures. The study will be conducted in 276 women with UTI symptoms and a history UTI in the past 12 months. The duration of the antibiotic course prior to study entry is anticipated to be 3–5 days¸ however the length of treatment will be decided by the treating provider.

At the Screening Visit (Visit 0), subjects will be evaluated for eligibility with a medical history, physical examination, genital examination and vaginal swabs for specimen collection.

If found eligible based on the results of the Screening Visit (v0), the subject will be prescribed antibiotics and scheduled to return for the randomization visit in 3-10 days after antibiotic completion. At Visit 1 subject will be randomized to receive either LACTIN-V at a dose of 5 x 10^9 CFU/applicator daily for five days followed by one applicator per week for 8 weeks or the placebo applicator on the same schedule. The LACTIN-V applicator will contain active *Lactobacillus* (strain CTV-05) in maltodextrin and a preservation matrix and the placebo capsule will contain maltodextrin and preservation matrix only. LACTIN-V is supplied as a powder formulation in a pre-filled, single-use applicator. The preservation matrix contains xylitol, trehalose, and sodium ascorbate. LACTIN-V placebo applicators contain 200 mg of the powder formulation without *L. crispatus* CTV-05.

Visit 0 (Screening Visit) will occur at the time of the UTI diagnosis. Visit 1 should occur within 3–10 days of completing antibiotic. Visit 2 will occur at Week 3, Visit 3 at Week 6, and visits will be monthly thereafter for 3 months.

At Visit 1, each subject who continues to meet all inclusion and exclusion criteria will be randomized and will receive a box containing 15 applicators. Two extra applicators will be kept at the study site in case a lost or damaged applicator needs to be replaced. The first study applicator will be inserted under supervision in clinic at this visit. The remaining 14 applicators will be inserted daily at bedtime on Days 2–5, and then weekly. Women are to abstain from sexual intercourse and tampon use for 24 hours after each applicator is used. If the subject's partner uses condoms, they will be instructed to use non-spermicidal condoms to avoid killing the *L. crispatus*. The use of other intravaginal products including douching is prohibited throughout the four-month study period. Subjects must agree not to self-medicate with antibiotics or antifungals during the four-month study period. However, subjects may be treated for any on-study vaginal or other infection if a health care provider deems such treatment necessary. Subjects who receive antibiotic or antifungal treatment after commencing the study should continue to insert the study applicator according to their allocated schedule.

Subjects will be provided with a Study Diary to use to record applicator use information during the study. The Study Diary will also be used by subjects to record any symptoms. Subjects will be instructed on how to use these memory aids to remind them to insert the applicator on the designated day and to record the date and time when each applicator was inserted.

Self-reported symptoms will be evaluated at all study visits. Subjects will be queried about the following symptoms:

Nausea or vomiting
Abdominal Discomfort
Diarrhea
Constipation
Rash
Abnormal Vaginal Discharge/Bleeding
Abnormal Vaginal Odor
Genital itching or burning
External genital irritation/swelling
Painful Urination
Urinary Frequency
Urinary Urgency
Blood in urine

Headache Other:_____

Subjects will be instructed to call the study clinic if they have any unexpected symptoms at any time during the study.

Pelvic examinations will be performed at all visits to evaluate the external genitalia; cervix and vaginal mucosa for signs of irritation at Visits 1 and 6 and if clinically indicated.

In addition to safety, this study will evaluate the efficacy of LACTIN-V in reducing the incidence of Symptomatic UTI. Subjects will be queried at each clinic visit for urinary symptoms, including dysuria, frequency, urgency, hematuria, flank pain or suprapubic pain. Urinary dipstick, urinalysis and urine culture will be performed at each clinic visit. Subjects will be asked to return to clinic as soon as possible if they develop symptoms of UTI during the four-month study period.

5.2 Reduction of Bias

Measures proposed to limit bias include making the LACTIN-V and placebo applicators similar in appearance. Both study personnel and the subject will be completely blinded to the random treatment assignment.

6 STUDY MATERIALS AND METHODS

6.1 Study Materials

6.1.1 LACTIN-V and Placebo Applicators

Each LACTIN-V applicator contains 200 mg of LACTIN-V powder with 2 x 109 CFU of *L*. *crispatus* CTV-05. The LACTIN-V powder formulation also contains maltodextrin, xylitol, trehalose, and sodium ascorbate. LACTIN-V placebo applicators contain 200 mg of the powder formulation without *L*. *crispatus* CTV-05.

6.1.2 LACTIN-V

6.1.2.1 Background

The Medicines Company originally licensed world-wide rights to a proprietary organism, *Lactobacillus crispatus* strain CTV-05, which had undergone clinical development as an adjunctive treatment for vaginal infections (Lactin-Vaginal). Acquired in 1999 from a small biotech company, GyneLogix, Inc., in Louisville, Colorado, the product had been developed in conjunction with the NIH through completion of a Phase 2 study. The

Medicines Company elected a strategic direction focusing on acute care products sold to hospitals and this led to a decision to out-license rights to continued development of this product to Osel, Inc.

6.1.2.2 The Product

LACTIN-V contains *Lactobacillus crispatus* CTV-05 bacteria preserved by lyophilization in a simple freeze drying medium. CTV-05 is a strain of *Lactobacillus crispatus*, a gram-positive rod isolated from the vagina of a healthy woman. *Lactobacillus crispatus* is found naturally in the vaginas of healthy women and is commonly found as a component of the natural human intestinal microbiota. It is a facultative anaerobe, homofermentor of lactic acid, fastidious in its growth, and capable of H_2O_2 production. Unlike most commercially available strains of *Lactobacillus*, CTV-05 adheres well to epithelial cells and is thus able to colonize the vaginal epithelium. The powder is loaded into an applicator for vaginal administration. The filled applicator is packaged under nitrogen with desiccant in a Mylar foil pouch. The manufacturing and formulation of this organism are carried out in a proprietary process.

6.1.2.3 Manufacturing and Packaging Information

LACTIN-V contains *Lactobacillus crispatus* CTV-05 bacteria preserved by spray drying with a simple drying medium. *Lactobacillus crispatus* is a naturally occurring organism isolated from the vagina of a healthy woman in 1993. The final product appears as an off-white to light yellow powder, which is delivered as a vaginal applicator. The applicators pre-filled and individually packaged.

6.2 Study Methods

6.2.1 Subject Selection

6.2.1.1 Recruitment

The volunteers for this study will be recruited from Hall Health Primary Care Center at the University of Washington (Seattle, WA), as well as thru posted flyers and UW Daily Ads. The study will follow Health Insurance Portability and Accountability Act (HIPAA) guidelines where applicable.

6.2.1.2 Eligibility

6.2.1.2.1 Inclusion Criteria

1. Pre-menopausal women aged 18–45 years of age at date of the Screening Visit.

2. Meets criteria for current symptomatic acute uncomplicated cystitis, defined as one or more UTI symptoms (dysuria, urgency, frequency, suprapubic pain), and pyuria (>10 wbc) on hemocytometer count, confirmed with a positive urine culture with greater than 10^2/ml of an uropathogen.
3. Completion of all screening procedures. Screening will take place at the visit at which acute uncomplicated cystitis (UTI) diagnosis is made.
4. History of one Symptomatic UTI episode treated within the past 12 months.
5. Agree to return for the Randomization Visit (Visit 1) within 3–10 days of completing antibiotic treatment for UTI.
6. Regular menstrual cycles (21–35 days) or amenorrheic due to birth control method. Women using IUD(C) must be 2 months post insertion of IUD.
7. Subject is willing to insert vaginal applicators.
8. Capable of providing informed consent.
9. Able to read and understand English.
10. Agree to abstain from self-medication with antibiotics for UTI symptoms.
11. Agree to abstain from antibiotic prophylaxis for recurrent UTI.
12. Agree to abstain from the use of any other intra-vaginal product (i.e., contraceptive creams, gels, foams, sponges, lubricants, douches, etc.).
13. Agree to abstain from sexual intercourse for 24 hours after each applicator is inserted.
14. Agree to not use tampons for 24 hours after each applicator is inserted.
15. Agree to use an adequate method of birth control throughout study to avoid pregnancy. Acceptable methods include bilateral tubal-ligation, male partner with a vasectomy, steroidal contraceptive (oral, patch, injectable, implantable), IUD, condoms (to be provided), or abstinence. If condoms are used, they should be non-spermicidal condoms provided by the site.

6.2.1.2.2 Exclusion Criteria

1. Complicated cystitis or pyelonephritis (uncomplicated or complicated)
2. Vaginal or cervical infection, currently or within the past 30 days, with bacterial vaginosis (treated, symptomatic infection), yeast, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* or genital Herpes simplex.

3. Symptomatic BV and/or yeast at Visit 1 (women who have Asymptomatic BV at Visit 1 may participate in the study).
4. High risk for sexually transmitted diseases and/or HIV, including: diagnosis of *N. gonorrhoeae*, *C. trachomatis* or *T. vaginalis* on two or more occasions during the previous six months;
5. History of chronic vaginal, urinary or pelvic symptoms not attributable to UTI.
6. Pregnancy or within two months of last pregnancy (all subjects must have a negative urine pregnancy test prior to randomization).
7. Lactation.
8. Antibiotic therapy (vaginal or systemic) less than three days prior to Randomization Visit (Visit 1).
9. Antifungal therapy (vaginal or systemic) less than thirty days prior to the Randomization Visit.
10. Antibiotics planned within four months (including prophylactic antibiotics).
11. Investigational drug use within 30 days or current participation in any urogential studies.
12. Menopause.
13. Any significant disease or acute illness that in the Investigator's assessment could complicate the evaluation.
14. Known HIV infection.
15. Immunosuppressive drug within 60 days.
16. Known allergy to any component of LACTIN-V or the placebo capsule.
17. Unavailable for follow-up visits.
18. Have any social or medical condition that, in the opinion of the Investigator, would preclude provision of informed consent, make participation in the study unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.

Subjects who develop the following exclusion criteria while on study will discontinue study treatment, but continue to be followed for safety and efficacy endpoints whenever possible.

- Pregnancy
- Lactation
- HIV infection
- Treatment with immunosuppressive drug
- Allergy to LACTIN-V or placebo capsule

Subjects who develop any other exclusion criteria while on study will be managed at the discretion of the Investigator, in consultation with the Medical Monitor.

6.2.1.3 Screening Failures and Subject Replacement

Volunteers with the following diagnoses at Visit 1 will be considered screening failures and will be treated or referred for appropriate follow-up. All screening failures will be replaced.

1. Complicated cystitis or uncomplicated or complicated pyelonephritis.
2. Abnormal findings on pelvic examination that would interfere with the evaluation of safety.
    a. Unable to visualize cervix.
    b. Clinically significant inflammation, erosions and/or petechiae of external genitalia, vagina or cervix on visual examination.
    c. Clinically significant tenderness on bimanual examination.
3. Evidence of a sexually transmitted disease.
4. Symptomatic BV or yeast.

6.2.1.4 Withdrawal of Randomized Subjects

Study subjects who have been randomized may be withdrawn from receiving further investigational product for the following reasons:

1. Allergy to the study product.
2. Adverse event.
3. Unable or unwilling to use LACTIN-V applicators for the duration of the study.
4. Failure to follow protocol requirements that would significantly affect study outcomes.
5. Pregnancy.
6. Medical reasons unrelated to study products. This determination will be at the discretion of the Investigator, but might include major new diagnoses such as a malignancy requiring chemotherapy.
7. Personal reasons (voluntary request or withdrawal of consent).
8. Physician decides that it is not in the best interest of the subject to continue participation.
9. Physician or sponsor decides, for whatever reason, to discontinue the study.

For randomized subjects who are withdrawn from receiving further product, the site will make every effort to continue to follow all subjects for efficacy through duration of study.

6.2.1.5 Discontinuation and Subject Replacement

The study subject may choose to leave the study at any time. Reasons for leaving the study will be recorded. Study subjects who enroll in the study but who do not return after three attempts to contact them will be considered study "lost to follow-up".

Once a subject is randomized, she will not be replaced. Withdrawals and drop-outs will not be replaced.

6.2.2 Blinding and Unblinding of Treatment Assignment

6.2.2.1 Blinding

The most important factor for the success of a blinded study is to maintain the blind throughout the entire course of the trial. In this study, the treatment assignment to LACTIN-V and the placebo applicator will be maintained by Pacita Roberts, M.S., the study statistician. Treatment assignment will be blinded to all other study staff and subjects.

The study product will be prepackaged in accordance with a previously generated randomization schedule. It will be supplied to the study site labeled only with the Protocol Number and Subject Number. As study product is shipped to the sites, a list of Subject Numbers that corresponds to the study product will be enclosed.

A block size of four has been chosen to minimize the potential imbalance between LACTIN-V and placebo. Within each block, subjects will be randomized in a 1:1 ratio to receive either LACTIN-V or placebo. Subjects will be randomized on site in sequential order based on the list of Subject Numbers provided to the site. Lists will not have any treatment assignment associated with the Subject Number.

Applicators will be individually packaged. No one involved in the conduct of the trial will be aware of the specific treatment allocated to any particular subject. Only authorized persons, including the study statistician, will have access to treatment assignments in case of necessary unblinding (see below). The LACTIN-V applicator cannot be distinguished from the placebo applicator (appearance or smell) either before or during administration.

6.2.2.2 Unblinding

Breaking the blind on an individual basis should be considered only when knowledge of the treatment assignment is considered essential by the subject's physician for her care. In the event of an emergency situation for which knowledge of the treatment assignment is necessary for clinical management, the site should notify the Medical Monitor or Clinical Program Manager. Any unintentional unblinding should be reported immediately to Ann Stapleton, M.D. documented during the trial, and reported and explained at the end of the trial in a study report, irrespective of the reason for its occurrence.

6.2.2.3 Unblinding Procedure

Tamper-proof envelopes containing treatment group assignment for each Subject Number will be available to Pacita Roberts, M.S. The tamper-proof envelopes will be stored in a secured location for emergency unblinding purposes only. If the Investigator determines that a subject has experienced a significant adverse event that may be related to the study product and requires unblinding in order to make patient care decisions, he or she should notify the Medical Monitor and Clinical Program Manager immediately. A Request for Emergency Unblinding Form, with the Subject Number, date, time, person making the request, description of the serious adverse event, onset date, and the reasons for unblinding, will be faxed to the Medical Monitor and Clinical Program Manager for review. If unblinding is approved, an authorized person at the University of Washington will open the tamper-proof envelope for the individual subject and promptly report the treatment assignment to the Investigator. In the event that the Medical Monitor or Clinical Program Manager cannot be reached quickly, the Investigator will be provided with a list of representatives who may be contacted to request emergency unblinding. An emergency unblinding log will be maintained by University of Washington.

6.3 General Procedures

6.3.1 Informed Consent

Women who attend a study clinic (or referring clinic) for urinary symptoms and are diagnosed with acute, uncomplicated UTI (including acute uncomplicated cystitis) will be advised that a study is underway. They will be asked if they would like to hear more about the research. If they are interested, written informed consent will be obtained from each study subject prior to commencing any procedure or assessment associated with the Screening Visit (Visit 0). Study subjects will receive a copy of the signed and dated Informed Consent Form (ICF). If the ICF is altered during the study, subjects will be re-consented with the IRB-approved revised ICF, and a copy of the new signed consent form will be provided to the subject.

6.3.2 Confidentiality of Records

Informed consent forms, contact information, and other data collection instruments containing personal identifiers will be securely stored separately in locked offices located at the project site. Only limited study site staff will have access to these files. Only a study identification number will identify the subject in research records. All data will be encoded and entered into a computer using the Subject ID.

6.3.3 Visit 0

Visit 0 is the Screening Visit. This visit is conducted at the time of the UTI diagnosis.

- Visit 0 will occur on the day that the UTI is diagnosed.

Each woman who agrees to be screened will be asked to sign the informed consent form written in English. The screening process will be explained to interested women and written informed consent will be obtained from each woman who agrees to participate. The subject will be asked to provide either a contact phone number or email address, which will be recorded on a subject contact information form. This will be used to remind subjects of follow-up visits.

Women who give informed consent to enrollment will undergo the following procedures and assessments at Visit 0:

1. Detailed structured interview to obtain medical and sexual history. This will include assessment of STD and HIV risk.
2. Review eligibility criteria.
3. Vital signs and weight.
4. Clean catch urine dipstick performed in clinic, with urinalysis, culture and susceptibility testing sent to research laboratory.
5. The subject will then undergo a physical examination and focused genital examination by a study physician or nurse..
6. Pelvic examination, to be conducted in the following order:
    a. Examination of the external genitalia
    b. Vaginal swab for pH, wet mount (clue cells and trichomonas), KOH and amine test (whiff test) performed in clinic.
    c. Swab of vaginal fluid to be stored for assessment of vaginal microbiota by DNA amplification.

Women diagnosed with any symptomatic vaginitis at Visit 0 will be treated and excluded from the study.

6.3.4 Visit 1

Visit 1 is the Randomization Visit. This visit will be scheduled if urine culture confirms the presence of a uropathogen in quantity greater than or equal to 10^2/ml.

- Visit 1 must occur within 3–10 days of completing the antibiotic, and after the subjects' anticipated menses.

It is possible that the entry UTI episode will not be fully resolved at the time of randomization (Visit 1), as mild symptoms may still be present, and the dipstick and urinalysis may still be slightly positive or equivocal several days after completing antibiotics.

The following procedures and assessments will be performed at Visit 1:

1. Eligibility confirmed.
2. All baseline symptoms to be recorded on the CRF.
3. Urine pregnancy test performed in clinic.
4. Clean catch urinalysis and culture.
5. Vital signs.
6. Pelvic examination, to be conducted in the following order:
    a. Examination of the external genitalia
    b. Speculum insertion and examination of vagina, fornices and cervix with naked eye. Only water must be used to lubricate the speculum at all study visits. Other lubricants could kill *L. crispatus*.
    c. Vaginal swab for pH, wet mount (clue cells), KOH and amine test (whiff test) performed in clinic.
    d. Vaginal swab for gram stain Nugent score.
    e. Vaginal swabs for culture (total lactobacilli and *E. coli*) and rep-PCR.
    f. Swab of vaginal fluid to be stored for assessment of vaginal microbiota by DNA amplification.
    g. Bimanual examination
7. Subject randomized.
8. First investigational applicator inserted under supervision.
9. Instructions provided to subject regarding:

a. Study Diary
b. Dates for initial 5 day applicator use and weekly applicator use will be marked on the Study Calendar, and staff will review with the subject how to record. If subject experiences unanticipated menses she may defer scheduled applicator use and will then record the day of actual use.

6.3.5 Visits 2 thru 5

The following procedures and assessments will be performed at follow-up Visits 2 thru 5 (unless otherwise specified):

1. All reported AEs to be recorded on the AE CRF.
2. Clean catch urine dipstick performed in clinic.
3. Urinalysis and urine culture.
4. Urine pregnancy test performed in clinic only if clinically indicated.
5. Focused physical examination, if indicated for assessment of symptoms and adverse events.
6. Pelvic examination to be conducted in the following order:
   a. Examination of the external genitalia.
   b. Vaginal swab for pH, wet mount (clue cells), KOH and amine test (whiff test) performed in clinic.
   c. Vaginal swabs for culture (total lactobacilli and *E. coli*) and rep-PCR sent to laboratory
   d. Vaginal swab for gram stain Nugent score sent to laboratory (Visit 5 only).
   e. Swab of vaginal fluid to be stored for assessment of vaginal microbiota by DNA amplification.
7. All diagnoses of UTI are to be recorded on the UTI CRF.

Subjects diagnosed with Symptomatic UTI during study participation are to be treated as clinically indicated and should continue to use study applicators as scheduled.

6.3.6 Additional Unscheduled Visits

Subjects will be instructed to return to the site if they experience symptoms of UTI during the four-month study period, or to report an adverse event that requires further evaluation.

The following list of procedures and assessments for the Unscheduled Visits is a guide only. The components of the history and physical examination conducted at this visit will be determined by the Investigator or his/her designee. All history and physical data from this visit should be recorded on the Symptomatic Visit CRF:

1. Focused physical examination, if indicated for assessment of symptoms.
2. Pelvic examination, if indicated for assessment of symptoms. A pelvic examination should be performed for all vaginitis symptoms and should be conducted as follows:
    a. Examination of the external genitalia.
    b. Speculum insertion and examination of vagina, fornices and cervix with naked eye. Only water must be used to lubricate the speculum at all study visits. Other lubricants could kill *L. crispatus*.
    c. Vaginal swab for pH, wet mount, KOH, clue cells and amine test (whiff test) performed in clinic.
    d. Swab of vaginal fluid to be stored for assessment of vaginal microbiota by DNA amplification.
3. Clean catch urine collection for dipstick, urinalysis, and/or urine culture with susceptibility testing if indicated. Urine testing should be conducted for all urinary symptoms while on study.
4. All diagnoses of UTI are to be recorded on the UTI CRF. Subjects diagnosed with UTI during study participation are to be treated as clinically indicated and should continue to receive study treatment.

If the subject has a new symptom (or increase in the severity of an existing symptom), the AE CRF and if necessary, the SAE CRF, should be completed.

6.3.7 UTI Episodes While On Study

Subjects will be instructed to return to the study site if they have persistent or new urinary symptoms. If a UTI is diagnosed by a non-study physician, medical records for that visit will be obtained in order to document the recurrence of UTI.

All antibiotics used to treat UTI should be recorded on the Concomitant Medications Log.

6.4 Clinical Procedures/Evaluations

6.4.1 Pelvic Examinations

Each subject will be examined in the dorsal lithotomy position in designated examination rooms. Only water must be used to lubricate the speculum at all study visits. Other lubricants could kill *L. crispatus*.

6.4.2 Investigational Product Instructions

At Visit 1 (Randomization Visit), each study subject will be counseled on how to use the applicators. Written instructions for administration, storage and handling will be provided to each study subject (Appendix B), along with a Study Diary to record applicator use times and symptoms throughout the study. Each subject will be instructed to use the applicator on scheduled days, and weekly insertion dates will be marked on the Study Diary, which the subject will take home.

Instructions will be given regarding what to do in the event that the subject forgets to use an applicator on the prescribed day (see Section 6.6).

Subjects will be instructed to defer insertion of applicators on days of heavy menstrual flow.

Information will be reviewed with the study subject at each of the follow-up visits, and study personnel will address any questions or concerns that may arise.

Subjects will be asked to return both used and unused applicators.

6.5 Early Discontinuations

If a study subject should discontinue the study prior to Visit 6, all end of study procedures and CRFs should be completed.

6.6 Missed Applicators

During the initial five-day treatment period, if it is already within eight hours of her next dose, she should skip the missed applicator. The subject should record the missed dose on the Study Diary. After Day 5, if a subject misses an applicator, she should be instructed to use the missed applicator as soon as possible (but no more than four days from the scheduled date) and the date should be recorded on the Study Diary.

6.7 Lost Applicators

Extra applicators will be provided to each subject together with the required 13 applicators. If a subject loses or damages a study applicator, she may take one of the extras provided, as long as the total number of applicators used does not exceed 13. Lost or damaged applicators should be documented on the Study Diary.

6.8 Antibiotic Use While On Study

All subjects who receive antibiotics during the four-month study period for UTI or any other infection may remain on study and continue study treatment. All antibiotic use should be reported on the Concomitant Medication Log CRF.

6.9 Laboratory Procedures

6.9.1 Specimen Collection and Processing

- ***Wet Mount Slides (All Visits)***: Wet mount slides will be evaluated for signs of BV (clue cells), trichomonas and/or candida (yeast). If vaginal symptoms develop during the study, additional wet mounts will be done as indicated. These tests will be done in the clinical laboratory of the UTI Research Unit at the Hall Health Primary Care Center.
- ***Urine Dipstick Testing (All Visits)***: A clean catch urine sample will be tested on site for the presence of red blood cells, white blood cells, nitrite, leukocyte esterase and protein.
- ***Urine Pregnancy Test (Visits 1 and 6):*** A urine sample will be tested on site to confirm the absence of pregnancy.

6.9.2 Laboratory Testing

All testing will be performed in the laboratory of Ann E. Stapleton, M.D. at the University of Washington.

- ***Urine Hemocytometer (All Visits)***: Will be performed during the study visit at Hall Health. Urine samples will be assessed for red blood cells and white blood cells.
- ***Urine Dipstick (All Visits)*** Will be performed during the study visit at Hall Health. Urine samples will be assessed for LE, nitrite and blood.
- ***Urine Culture (All Visits)*****:** Cultures of midstream, voided clean catch urine samples will be performed in the lab of Ann E. Stapleton, M.D. at the University of Washington.
- ***Culture of vaginal specimens for Gram stain (All Visits)*****:** A sterile swab will be used to collect fluid from the vaginal walls. Gram stain and cultures will be done in the lab of Ann E. Stapleton, M.D. at the University of Washington.
- ***Lactobacillus culture and rep-PCR for Lactobacillus CTV-05 (All Visits)*****:** A sterile swab will be used to collect fluid from the vaginal walls. Specimens will be analyzed by culture and rep-PCR in the lab of Ann E. Stapleton, M.D. at the University of Washington.

- ***E. coli culture (All Visits):*** A sterile swab will be used to collect fluid from the vaginal wall. Specimens will be analyzed by culture in the lab of Ann E. Stapleton, M.D. at the University of Washington.
- ***Qualitative and/or quantitative DNA amplification of vaginal microbiota:*** A sterile swab will be used to collect fluid from the vaginal walls at each visit and stored. DNA amplification will be performed in the lab of Ann E. Stapleton, M.D. at the University of Washington.

6.9.3 Compliance Monitoring

The study subject will be questioned at Visits 2-6 regarding her use of the applicators and any symptoms she experienced during or following applicator use.

7 MANAGEMENT OF ADVERSE AND OTHER EVENTS

7.1 Adverse Events

An adverse event (AE) is any untoward and/or unintended sign, including an abnormal laboratory finding, symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. Pre-existing events or conditions that increase in frequency or severity during or as a consequence of use of a drug in human clinical trials will also be considered adverse events. Any medical condition or clinically significant laboratory abnormality with an onset date before the first date of study product administration is considered to be a pre-existing condition and should be documented as such.

In this protocol, pregnancy will be considered to be an AE, and will be followed for outcome.

7.1.1 Adverse Event Recording

Adverse events may include illnesses, discomfort, pain, or any medical problem such as cold symptoms or other complaints. They may be clinically significant laboratory abnormalities, or pre-existing conditions which change in severity or nature during the course of the protocol. All AEs, whether serious or non-serious and whether or not related to the study product, must be recorded on the Adverse Event (AE) page of the case report form. Study subjects will be instructed to call or return to the study clinic if they have any unexpected symptoms.

7.1.2 Adverse Event Grading

Each AE should be graded for severity using the following scale:

- Mild: the subject was aware of the AE, but was still able to do all activities; no or minimal medical intervention/therapy required.
- Moderate: the subject had to discontinue some activities due to the AE; no or minimal medical intervention/therapy required.
- Severe: the subject was incapacitated by the AE and unable to perform normal activities; significant medical intervention/therapy required, hospitalization possible.
- Potentially life-threatening: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

7.1.3 Assessment of Causality

Each AE should be assessed for its causal relationship to the study product. The Investigator is responsible for making an assessment of whether or not it is reasonable to suspect a causal relationship between an AE or SAE and the administration of the investigational product. The causality assessment will be reviewed by Ann Stapleton, MD and the Medical Monitor. The assessment of causality will only be reclassified by the Investigator. "Associated with the use of the drug" means: "There is a reasonable possibility that the experience may have been caused by the drug."

In assessing causality the following should be considered:

- Alternative etiology - Is the AE due to the underlying disease being treated or is the AE a new diagnosis?
- Known relationship – Has the AE been observed before with this investigational product or class?
- Temporal relationship – What was the time of onset and resolution in relationship to the receipt of the investigational product?
- Concomitant medication – Is the AE a known side effect of another medication?
- Re-challenge – Does re-challenge result in recurrence of the AE?

For each AE, an assessment of the relatedness to the test agent should be made using the following scale:

- Not Related: Onset of the AE has no reasonable temporal relationship to administration of the investigational product, a causal relationship to administration

of the investigational product is biologically implausible or the event is attributed to an alternative etiology.

- Unlikely Related: Onset of the AE has a reasonable temporal relationship to study product administration and although a causal relationship is unlikely, it is biologically plausible.
- Possibly Related: Onset of the AE has a strong temporal relationship to administration of the investigational product, cannot be explained by the subject's clinical state or other factors, and a causal relationship is biologically plausible.
- Probably or Definitely Related: Onset of the AE shows a distinct temporal relationship to administration of the investigational product that cannot be explained by the subject's clinical state or other factors, or the AE occurs on rechallenge, or the AE is a known reaction to the product or chemical group, or can be predicted by the product's pharmacology.

7.2 Serious Adverse Events

A serious adverse event (SAE) is any adverse event occurring at any dose that meets the following criteria:

- results in death
- is life-threatening
- requires hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- the event was another, important medical event that may not have met the above criteria but based upon appropriate medical judgment, it jeopardized the subject or required medical or surgical intervention to prevent one of the outcomes listed above

Any AE that is serious (whether expected or unexpected) must be reported by facsimile within 24 hours of the Investigator or site staff becoming aware of the event to Ann E. Stapleton, M.D., and the University of Washington Institutional Review Board. Sites will use Adverse Event Reporting Forms when submitting SAE reports.

It is the responsibility of the Investigator to report all SAEs to the sponsor, to provide the most complete report possible, and to assess each SAE for its relationship to the study product. Each SAE occurring in this study must be followed to resolution.

The study will be suspended or stopped if two or more product-related SAEs are reported.

7.3 Unexpected Adverse Events

An unexpected adverse event is any AE having a specificity or severity not consistent with the current Investigator Brochure.

7.4 Serious Adverse Event Reporting

All serious adverse events (SAEs) must be reported to Ann E. Stapleton, M.D. and the Medical Monitor as follows:

- Any AE that is serious (whether expected or unexpected) must be reported by facsimile or email to Ann E. Stapleton, M.D. and the University of Washington Institutional Review Board using the University of Washington Event Reporting Forms within 24 hours of the Investigator or site staff becoming aware of the event.
- All SAE reports should include the Investigator's assessment of the relationship of the event to the study product.

  Any subject with an SAE, whether or not considered related to the study product or procedures, will be discontinued from further study treatment.

7.5 Regulatory Reporting of Serious Adverse Events

In the event of a fatal or life-threatening SAE, Ann E. Stapleton, MD will notify the FDA by telephone or facsimile within seven calendar days of receipt of the report. Ann Stapleton, M.D. will follow all 7-day alert reports with a written IND safety report within 15 calendar days of receipt of the case.

Serious adverse event cases that concern non-fatal, non-life-threatening events which are unexpected and at least possibly related to the study product will be submitted in writing to the FDA as 15-day IND safety reports within 15 calendar days of receipt.

Ann Stapleton, MD will require Investigators to obtain follow-up information on all SAEs.

7.6 Unblinding of Study Treatment Assignment

If during the course of the blinded portion of this study a medical emergency requires knowledge of a subject's treatment assignment, the study blind may be broken by authorized persons for that specific subject.

7.7 Pregnancy

If a woman becomes pregnant while on study, this should be reported immediately to the Investigator and the Medical Monitor. All pregnancies will be recorded as an AE and will be followed for outcome.

8 GENERAL ADMINISTRATIVE PROCEDURES

8.1 Protocol Modifications

Protocol modifications will be avoided to the extent possible. Potential modifications include (but are not limited to) refinement of counseling strategies and recruitment techniques, revision of questionnaires and other subject-related documents, and refinement of intervention. Any protocol modification must be approved by Ann Stapleton, M.D. prior to implementation. Protocol amendments will be submitted to the IRB as indicated, depending upon the nature of the modification.

8.2 Clinical Monitoring

There will be no independent data monitoring committee for this Phase 3 study.

The Medical Monitor and Principal Investigator will regularly review the safety data throughout the course of the study.

8.3 Individual Stopping Rules

Any subject with an SAE, whether or not considered related to the study product or procedures, will be discontinued from further study treatment.

8.4 Study Suspension or Termination

As stated above, any SAE must be reported by the Investigator or designee to Ann Stapleton, M.D. and the Medical Monitor. The study will be suspended if two or more SAEs considered “Possibly” or “Probably” related to the product are reported in any study of LACTIN-V. The study will only be resumed if the Medical Monitor and the FDA agree to do so. Study suspension or termination will be reported to the University of Washington IRB.

8.5 Roles and Responsibilities of the Study Site

The study site has the following responsibilities:

- Complete the site University of Washington IRB application.

- Complete the following prior to study start: signature(s) on any applicable clinical trial agreements, FDA forms, financial disclosures, debarment certifications and protocol signature page; CVs and medical licenses (if applicable) of all Investigators; and, if applicable, recruitment materials and laboratory documentation.
- Conduct the study under procedures defined by GCP (Good Clinical Practices).
- Report all new or updated SAE information promptly to Ann Stapleton, M.D. and the University of Washington's IRB within 24 hours of first knowledge.
- Report any instances of unintentional unblinding immediately to Ann Stapleton, M.D.
- Submit all significant protocol deviations promptly to University of Washington's IRB
- Resolve all queries regarding the data that arise in the course of the study..
- Provide clinical laboratory services where required by the protocol.
- Obtain annual approval from University of Washington's IRB.
- Complete monthly update and submit to Ann Stapleton, M.D. in report form on the first business day of each month during the study.
- Submit all Expedited Safety Reports and Investigator Letters to University of Washington's IRB within three business days of receipt.

8.6 Protocol Deviations

All significant protocol deviations that the Investigator or site staff believes are of major importance (for example, incorrect randomizations, subject enrolled but not eligible) should be reported to Ann Stapleton, M.D. as soon as possible. Significant protocol deviations may include the following:

- Deviations from the Inclusion/Exclusion criteria that may affect subject safety.
- Deviations (omission or delay) of safety monitoring procedures.
- Deviations in the administration of study product.
- Deviations in obtaining informed consent.

Significant protocol deviations will be reported to the University of Washington IRB, and will also be recorded on the CRF using the Significant Protocol Deviation page. These forms will be completed and faxed to the IRB.

All protocol deviations occurring at each site that are not recorded on these forms will be listed in the Protocol Deviation Log, which will be routinely reviewed by the principal investigator.

9 DATA MANAGEMENT

9.1 Data Collection and Entry

Case Report Forms (CRFs) will be completed at the study site with only the subject identification (Subject ID), which consists of a 2-digit site number and a 4-digit randomization number (the Subject Number). CRF's will be forwarded to the University of Washington for entry onto datasets using Teleform scanning software (Cardiff Software, Inc.). These datasets will be transferred electronically to the study statistician, who will manage and analyze the data. At several points during the project, the data will be examined for out-of-range values, missing data and logical inconsistencies, and adjustments will be made as needed. Final analysis files will be created at the conclusion of data collection and clean-up. Once the last subject has completed Visit 6 and data are cleaned, all analyses will be performed on the data sets through Visit 6. Data will be managed and analyzed using a combination of local and centralized computing resources at the University of Washington. SAS will be used for data management.

10 STATISTICAL CONSIDERATIONS

10.1 Primary Endpoint

The primary endpoint of this Phase 2 study is to evaluate the efficacy of *Lactobacillus crispatus* strain CTV-05 (LACTIN-V) in pre-menopausal women who have a history of recurrent uncomplicated UTI when the product is administered vaginally at a dose of 2 x 10^9 CFU/capsule once per day for five consecutive days followed by once per week for 10 weeks. The main analysis of interest will be the comparison of UTI during the follow-up period, looking at:

1. UTI incidence over the 16-week study period in the LACTIN-V treatment group (Group A) as compared to the placebo group (Group B) of:
    a. Symptomatic or Probable UTI
    b. Symptomatic UTI
2. Time to recurrence, as measured from date of the Randomization Visit (Day 1) to the date of first UTI diagnosis post-randomization, in the LACTIN-V group (Group A) compared to the placebo group (Group B) of:
    a. Symptomatic or Probable UTI
    b. Symptomatic UTI
3. Comparison of the number (%) of subjects over the 16-week study period with at least one:
    a. Symptomatic or Probable UTI
    b. Symptomatic UTI

10.2 Secondary Endpoints

The secondary endpoints of this study are parameters of vaginal environment and colonization and adverse events due to the study product.

1. Vaginal colonization with total lactobacilli (as assessed by vaginal culture) and *Lactobacillus crispatus* strain CTV-05 (as assessed by rep-PCR) at Visits 1 through 6 in the LACTIN-V group (Group A) as compared to the placebo group (Group B).
2. Vaginal colonization with *E. coli*, in the LACTIN-V group (Group A) as compared to the placebo group (Group B), as assessed by the presence of *E. coli* on vaginal culture at Visits 1 through 6.
3. Shifts in the vaginal microbiota of individual subjects, as assessed by Nugent score of vaginal gram stains at Visits 1 through 6, in the LACTIN-V group (Group A) as compared to the placebo group (Group B).
4. Shifts in the composition of vaginal microbiota, as assessed by qualitative and/or quantitative DNA amplification of stored vaginal specimens from all visits.
5. Presence and/or quantitation of H_2O_2, lactic acid, and cytokine profiles at Visits 1 through 6, in the LACTIN-V group (Group A) as compared to the placebo group (Group B).
6. Safety and tolerability will be evaluated by the number (%) of subjects with any treatment emergent adverse event, reported symptoms and/or abnormal pelvic examination results over the four-month study period.

10.3 Measurement

There are six scheduled study visits during which clinical assessment and measurements will be made in this study:

Activity	Schedule
Visit 0	Screening
Visit 1	Randomization (Day 3-10 post antibiotics)
Visit 2	Week 3
Visit 3	Week 6
Visit 4	Week 10
Visit 5	Week 14
Visit 6	Week 18

Safety will be assessed at each visit using self-reported adverse events, symptoms and pelvic examination findings. Pelvic examinations at each visit will be used to evaluate subjects for signs of irritation involving the external genitalia, cervix or vagina.

The presence of a Symptomatic UTI episode will be assessed at each visit by the investigator using the case definitions in Section 3.3. Subjects will also be queried at each study visit regarding any Probable UTI episodes, which may have occurred since the prior visit. The number of days from the date of the Randomization Visit to the date when a recurrence of UTI is first diagnosed will be used to calculate the time to UTI recurrence. A new on-study UTI (Symptomatic or Probable UTI) can only be diagnosed after the urine culture at Visit 1, or at another interim study visit after confirming resolution of the prior UTI.

Vaginal microbiota will be assessed using the Nugent Scoring System for Gram Stained Vaginal Smears at Visits 1 through 6. Vaginal cultures of total lactobacilli and *E. coli* will be evaluated at Visits 1 through 6. Colonization with the exogenous *Lactobacillus crispatus* strain CTV-05 will be evaluated by the presence or absence of CTV-05 by rep-PCR at Visits 1 through 6. Vaginal fluid specimens from each visit will be stored for assessment of shifts in the composition of vaginal microbiota using qualitative and/or quantitative DNA amplification methods. Presence and/or quantity of H_2O_2, lactic acid, and cytokines will be assessed.

10.4 Statistical Power and Sample Size Justification

Based on previous studies in this population, we estimate that 40% of women with a history of rUTI and recent treatment for a UTI will have a recurrence within the 4 month follow-up period, based on data from a recently completed rUTI study at this site (17). We plan to enroll 250 women to allow for >80% power to detect a 50% reduction in UTI incidence, also factoring in some drop-out, non-compliance and loss-to-followup, plus a cushion in case of a recurrence rate of less than 40%.

10.5 Study Instruments

Responses to safety questions, results of clinical examinations, and results of laboratory testing will be recorded on source documents for visits and laboratory evaluations.

The study will continue enrolling subjects until all subjects are enrolled and have completed Visit 6, or until there is any finding that warrants change in study procedures, as explained previously (see Section 7.2 and 7.4).

10.6 Randomization

Subjects will be randomized in a double-blind fashion. The randomization assignments will be produced using a computerized blocked randomization process, assigning equal numbers of subjects to either intervention or placebo. The randomization assignments, along with the sequentially assigned identifying number (ID#), will be given to the Hall Health pharmacy, where the probiotic/placebo will be packaged into identical envelopes

marked with the ID# prior to distribution to clinic personnel. Subjects who have successfully completed the Screening Visit and the Visit 2 pre-randomization assessments will be randomized sequentially as they become eligible for the study.

10.7 Cohorts for Analyses

The intent-to-treat (ITT) cohort is defined as subjects who are randomized in the study.

The modified intent-to-treat (MITT) cohort is defined as subjects who are randomized and have received at least one dose of study product. It is a subset of the ITT cohort. It will be used for analyses of primary and secondary endpoints, baseline characteristics, trial conduct, and significant protocol deviations.

The evaluable (Eval) cohort is defined as the subset of the MITT cohort who had at least one follow-up visit assessment post-baseline (Visit 2 or later) and are not an antibiotic treatment failure at Visit 1 (defined as a requiring treatment for UTI symptoms at Visit 1). Subjects with any significant protocol deviations may be included in this cohort.

The according-to-protocol (ATP) cohort is the subset of Eval with subjects who meet all protocol specifications. Those subjects who developed exclusion criteria while on study will be excluded from this cohort.

Both Eval and ATP cohorts will be used to analyze the primary endpoints.

10.8 Analysis Methods

Summary statistics of frequencies, percentages, and 95% confidence intervals will be provided for categorical data. Means, standard deviations (SD), medians, and ranges (minimum, maximum) will be provided for continuous data. Chi-square test (Cochran-Mantel-Haenszel [CMH] methods), Fisher's exact test, or McNemar's test will be used for categorical variable comparisons and a t-test or rank-sum test will be used for continuous variable comparisons. Specifically, the following table lists the key variables to be analyzed by endpoint.

Table 1 Analysis Variables and Methods

Endpoint	Variable	Type	Statistics	Method
Primary	Percent of subjects with any Symptomatic/Probable UTI	Categorical	Frequency, Percent	95% confidence intervals
	Incidence of Symptomatic/Probable UTI	Categorical	Frequency, Percent	CMH
	Time to recurrence of first Symptomatic UTI	Numeric	Mean time to recurrence	Survival analysis, log rank test
Secondary	Change in vaginal microbiota (Nugent score)	Categorical	Frequency, Percent	Shift table (from Visit 1 to Visit 6), CMH
	Total *Lactobacillus* vaginal colonization	Categorical	Frequency, Percent	95% confidence interval at Visits 1-6
	Colonization with CTV-05	Categorical	Frequency, Percent	95% confidence interval at Visits 1-6

Endpoint	Variable	Type	Statistics	Method
	Total *E. coli* vaginal colonization	Categorical	Frequency, Percent	95% confidence interval at Visits 1-6
	Change in vaginal microbiota (DNA Amplification)	Categorical	Frequency, Percent	CMH 95% confidence interval
	H2O2, lactic acid, cytokines	Numeric	Medians, Percentiles	Wilcoxon rank-sum test
	Adverse Events, Serious Adverse Events, Symptoms, and signs of irritation on pelvic examination	Categorical	Frequency, Percent	Fisher's exact test may be used for selected AE preferred terms, symptoms or findings

10.9 Analysis of Primary Endpoint

10.9.1 Recurrence Rate of UTI

The number (%) of subjects with any Symptomatic/Probable UTI (defined in Section 3.3) episode over the four-month study period will be assessed, as well as the number (%) of subjects with Symptomatic UTI. The number (%) in each treatment group will be presented. 95% confidence intervals will be calculated to evaluate any difference between the treatment groups.

10.9.2 Incidence of Symptomatic UTI episodes

The number of Symptomatic/Probable UTI (defined in Section 3.3) episodes per subject in each treatment group will be summarized by treatment group, as well as the number Symptomatic UTI episodes. Number and percent of subjects with 0, 1, 2, …, n episodes will be displayed by treatment group. P-value will be calculated by CMH methods to evaluate any difference between the treatment groups.

10.9.3 Time to Recurrence of the first Symptomatic/Probable UTI episode

The time (number of days) to the first recurrence of Symptomatic/Probable UTI, as well as the time to first Symptomatic UTI, will be calculated for each subject, as measured from the date of the Randomization Visit (Visit 1) to the date of first Symptomatic/Probable UTI diagnosis post-randomization. Kaplan-Meier life-table

analysis will be used to describe the survival rates and the logrank test will be used to compare the two treatment groups.

10.10 Analysis of Secondary Endpoints

10.10.1 Shifts in Vaginal Microbiota, as Assessed by Nugent score

Significant changes in vaginal microbiota will be evaluated by the Nugent score with shift tables from Visit 1 (Day 1) to Visit 6. The Nugent score is graded as 0 to 10 and categorized as follows:

1 Normal, if between 0 and 3;
2 Intermediate, if between 4 and 6;
3 Bacterial vaginosis (BV), if between 7 and 10.

See Appendix A, Nugent Scoring System for Gram Stained Vaginal Smears, for complete Nugent scoring criteria.

The following shift table will be used to summarize the change in Nugent score from Visit 1 (Day 1) to Visit 6 (or final score).

Table 2 Nugent Score Shift Table

	Final Nugent Score	**0–3**	**4–6**	**≥ 7**	**Total**
Baseline Nugent Score	**0–3**				
	4–6				
	≥ 7				

Changes within each group and between groups will be reported.

10.10.2 Vaginal Colonization with Lactobacilli

Total lactobacilli (based on culture) will be scored as 1+, 2+, 3+, or 4+.

Colonization with *L. crispatus* strain CTV-05 (based on rep-PCR) will be scored as present or absent (frequency). Number and percent of subjects with positive CTV-05 in each treatment group will be presented. 95% confidence intervals will be calculated to evaluate any difference between the treatment groups.

The differences of these scores between Visit 1 (Day 1, Baseline) and Visits 3 and 6 for each subject will be evaluated within each group and between treatment groups.

10.10.3 *E. coli* Vaginal Colonization

The number (%) subjects with *E. coli* colonization in each treatment group will be summarized by treatment group and visit. 95% confidence intervals will be calculated to evaluate any difference between the treatment groups at a given visit.

10.10.4 Change in Vaginal Microbiota, as assessed by DNA amplification.

The number (%) of specific *Lactobacillus spp*. and uropathogens will be summarized by treatment group and visit. 95% confidence intervals will be calculated to evaluate any difference between the treatment groups at a given visit.

10.10 5 Safety and Tolerability.

The safety endpoints include specific solicited adverse events (AEs), all other reported AEs, serious adverse events (SAEs) and signs of irritation on pelvic examination. The adverse events emerging during the study, their severity and relation to study product will be recorded. Each event will be coded with system organ class and preferred term based on the Medical Dictionary for Regulatory Activities (MedDRA). Number (%) of subjects with AEs will be summarized by system organ class, preferred terms, and treatment group. Severity of AEs and relationship to study product will also be summarized by system organ class, preferred terms, and treatment group. SAEs and reasons for premature termination from study will be summarized by treatment group in frequency and percent. In addition, signs of irritation of the external genitalia, vagina or cervix from pelvic examination, along with their severity and relation to study product will be summarized by treatment group. Furthermore, symptoms and physical findings will be summarized.

10.10.6 Subject Characteristics and Disposition

The subjects' age, weight, ethnic background, and other demographic characteristics will be summarized by treatment group. All subjects who discontinue the study will be identified and reasons for their discontinuation will be recorded.

10.10.7 Assessment of Protocol Compliance

Every attempt will be made to select subjects who are eligible and will comply with the protocol. Noncompliant subjects may be discontinued from the study. Protocol deviations will be listed and summarized by treatment arm.

11 RISKS AND DISCOMFORTS TO STUDY SUBJECTS

11.1 LACTIN-V and Placebo Applicator Users

11.1.1 Risks

The potential risks of LACTIN-V are currently unknown. Pelvic examinations and analyses of vaginal discharge and vaginal microbiota were included in the Phase 1 studies designed to assess LACTIN-V's safety and colonization. The initial Phase 1 safety study of LACTIN-V in healthy women, Protocol LV-001, included colposcopy to evaluate for inflammation or other lesions of the vaginal, cervical or vulvar epithelium. There were no significant differences noted in these safety parameters between treated subjects and those who remained untreated or received the placebo capsule (Osel, Inc., personal communication).

Risks from the administration of LACTIN-V are low since colonization with this type of organism is strongly associated with improved vaginal health. No systemic risks are anticipated, since this is an organism that is applied topically, and thus not absorbed. These risks will be explained in the written informed consent form. Pregnant women, fetuses, prisoners, children, persons with or at risk of STD or women undergoing in vitro fertilization will not be included in this study.

Potential risks to subjects participating in this study include genital or vaginal irritation or symptoms. In the initial Phase 1 study of LACTIN-V (LV-001), the following adverse events were experienced in (at least two) more LACTIN-V treated subjects when the treated groups were compared to the placebo and untreated groups: vaginal discharge, abdominal pain, genital pruritis, vaginal odor, vaginal burning sensation, vaginal candidiasis and diarrhea (Osel, Inc., personal communication). There is also the risk of an allergic reaction to the latex in the condoms provided to subjects who are unaware of their latex allergy and who use these condoms.

11.1.2 Benefits

Research subjects, whether they receive LACTIN-V or placebo, will have the benefit of an office visit and urine culture, often omitted for this condition. Subjects will also have the benefit of having a pap smear. In addition, subjects participating in this clinical trial may contribute to the body of knowledge in the field of preventing recurrent urinary tract infections.

11.1.3 Procedures for Minimizing Potential Risks

Allergic Reactions (including anaphylaxis): A study subject with a known allergy to components of the capsule will be excluded from enrollment. If an allergy or sensitivity

occurs during the course of the study, the study subject will be advised to immediately discontinue product use and seek medical attention. This event will be recorded on an Adverse Event (AE) Form.

Allergies to Latex: Subjects with known allergy to latex will be provided with non-latex condoms.

11.1.4 Compare Potential Benefits with Potential Risks

The potential benefits to women with a history of recurrent UTI will depend on the outcome of the study. The subjects in this study will have the benefit of a pap smear, urine culture, and several pelvic examinations. These procedures may detect underlying diseases sooner.

11.1.5 Costs and Compensation

The subjects will not be charged for any of the study visits, study supplies or examinations. Subjects will be reimbursed for study participation to cover inconvenience, travel, parking and time off work. The visits will be prorated and partial payment given in the event that the subject only completes a portion of the study. The subject will be responsible for any medical care related to pregnancy that occurs during study participation. If a subject is diagnosed with a recurrence of UTI, vaginal candidiasis, BV, STD or other infection during this study, the subject will be responsible for the cost of any treatment not provided by the study site or covered by their insurance.

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STUDY PROCEDURES FLOW CHART

	Visit 0 Screening Visit	**Visit 1 Randomize 3-10 days Post abx treatment**	**Visit 2 Week 3**	**Visit 3 Week 6**	**Visit 4 Week 10**	**Visit 5 Week 14**	**Visit 6 Week 18**
Study Treatment (LACTIN-V or placebo)		1 applicator qd x 5d then q week x 10 weeks					
Informed consent	X						
Clean catch urine, dipstick, hemacytometer	X	X	X	X	X	X	X
Clean catch Urine culture	X	X	X	X	X	X	X
Urine pregnancy test		X					X
Eligibility and medical history	X	X					
Concomitant Medications	X	X	X	X	X	X	X
Vital signs	X						
Physical Exam	X						
Pelvic examination		X					X
Vaginal swab for pH, KOH (yeast) and wet mount (clue cells, trichomonas, amine test)	X	X	X	X	X	X	X
Vaginal swab for Gram stain	X	X	X	X	X	X	X
Vaginal swab for *Lactobacillus* and *E. coli*	X	X	X	X	X	X	X

	Visit 0 Screening Visit	**Visit 1 Randomize 3-10 days Post abx treatment**	**Visit 2 Week 3**	**Visit 3 Week 6**	**Visit 4 Week 10**	**Visit 5 Week 14**	**Visit 6 Week 18**
Culture and CTV-05 rep-PCR							
Vaginal swab stored for DNA amplification of vaginal microbiota[3]	X	X	X	X	X	X	X
Dispense Study Diary		X	X	X	X	X	X
Review Study Diary			X	X	X	X	X
Dispense study medication; subject inserts 1st applicator		X					
Dispense condoms		X					
Safety assessment			X	X	X	X	X

APPENDICES

Appendix A Nugent Scoring System for Gram Stained Vaginal Smears

Appendix B Instructions

Appendix C Diary

Appendix A

Nugent Scoring System for Gram Stained Vaginal Smears

The Nugent scoring system uses a 0- to 4-point scale for the evaluation of the vaginal microbiota and is based on the weighted sum of the following three bacterial morphotype scores calculated from slide exam under oil immersion (1000X):

- *Lactobacillus*: large Gram-positive rods
- *Gardnerella*/*Bacteroides* spp.: small Gram-variable *coccobacilli*/small Gram-positive rods
- *Mobiluncus* spp.: thin, curved Gram-variable rods

The criterion for bacterial vaginosis according to Nugent's criteria is a total score of 7 or more with a score of 4 to 6 being considered intermediate and a score of 0 to 3 being considered normal.

Nugent Scoring System for Gram's Stained Vaginal Smears[1]			
SCORE[2]	*Lactobacillus* morphotypes	*Gardnerella*/*Bacteroides* spp. morphotypes	Curved Gram-variable rods
0	4+[3]	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

1 Source: Draft Guidance for Industry: Bacterial Vaginosis – Developing Antimicrobial Drugs for Treatment (July 1998)

2 Morphotypes are scored as the average number seen per oil immersion field (minimum of 10–20 fields should be examined). Each morphotype is then given a score form the left hand column. The TOTAL SCORE is calculated by adding up the individual morphotype scores = *Lactobacillus* + *Gardnerella*/*Bacteroides* + curved Gram-negative rods.

3 QUANTIFICATION SCALE: 0 = no morphotypes seen; 1+ = < 1 morphotype per field; 2+ = 1 to 4 morphotypes; 3+ = 5 to 30 morphotypes; 4+ = > 30 morphotypes per field.

Appendix B

Study Diary Instructions

The "Diary" on the other side will help you to record when you insert your study applicators. Please mark the daily boxes to record any activity or symptoms.

When to Insert the Study Applicator

You will use the first applicator in the clinic on Day 1. All other applicators will be used at bedtime.

Starting tomorrow evening, you will insert an applicator at bedtime. Continue using one applicator a day at bedtime. For the first week, you will be inserting an applicator daily for 5 days. In the following weeks, it will be done once a week. The study staff will mark your diary for the recommended SCHEDULED DATE.

If you forget to insert the applicator please insert it as soon as you remember.

Recording When You Insert the Study Applicator: Use the Diary on the Other Side

Write the date and time in the Diary box for each applicator that you insert (Dose 1, Dose 2, etc.). If you insert your applicator after midnight, record the actual date and time of insertion.

Recording Activity

Check the box for each day on which an activity occurred.

Recording Symptoms

Write **1=Mild, 2=Moderate or 3=Severe** in the box for each symptom you have during the first 7 days of the study.

To Insert A An Applicator

Wash your hands thoroughly with warm water and soap.

Open **one** applicator package. Do not use anything sharp, like scissors, which could damage the applicator.

Holding the applicator between your thumb and fingers, insert into the opening of the vagina (like a tampon), and push plunger to dispense the contents.

Storage and General Instructions

Store the applicators in their box at room temperature until use.

Please avoid having sexual intercourse for 24 hours after use or use the condoms provided by the study.

Do not use another vaginal product, such as contraceptive creams, gels, foams, sponges, lubricants, douches, etc. during this study unless your doctor prescribes it.

Do not use tampons for 24 hours after applicator use.

Do not insert the applicator if you are having heavy bleeding from your period.

If you lose or damage an applicator, use another instead. If you somehow lose or damage more than 2 applicators or the whole box, please call the study clinic immediately for further instructions.

Place used applicators back in their original pouches and return at clinic visits, and return any unused applicators at the end of the study.

Appendix C

Study Diary: see attached