Randomized Controlled Trial of Treatment of Male Partners of Women With BV

Study Protocol & Statistical Analysis Plan

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RANDOMIZED CONTROLLED TRIAL OF TREATMENT OF MALE PARTNERS OF WOMEN WITH BV

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Principal Investigator/Protocol Chair: Jane R Schwebke, MD

NIAID Medical Monitor: Mohamed Elsafy

Draft or Version Number: 3.0

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Statement of Compliance

This trial will be conducted in compliance with the protocol, International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP) and the applicable regulatory requirements.

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46 and 21 CFR including parts 50 and 56 concerning informed consent and IRB regulations, if under IND, 21 CFR 312).

- Completion of Human Subjects Protection Training

SIGNATURE PAGE 1

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

Principal Investigator (UAB): Jane R. Schwebke, M.D.
Signed: _____ Date: 11-9-18
Name: Jane R. Schwebke, M.D.
Title: Professor of Medicine

SIGNATURE PAGE 2

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Protocol Chair (UAB): Jane R. Schwebke, M.D.
Signed: _____ Date: 11-9-18
Name: Jane R. Schwebke, M.D.
Title: Professor of Medicine

Principal Investigator (UAB): Jane R. Schwebke, M.D.
Signed: _____ Date: 11-9-18
Name: Jane R. Schwebke, M.D.
Title: Professor of Medicine

Site Investigator (Wayne State): Jack Sobel, M.D.
Signed: _____ Date: _____
Name: Jack Sobel, M.D.
Title: Professor of Medicine/Infectious Diseases

APPENDICES

AE	Adverse Event/Adverse Experience
BID	Twice a day
BV	Bacterial vaginosis
CFR	Code of Federal Regulations
UAMS	Center for International Data Evaluation and Analysis
CT	*Chlamydia trachomatis*
DCF	Data collection form
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GC	*Neisseria gonorrhoeae*
GCP	Good Clinical Practice
Gm	Grams
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
MedDRA ©	Medical Dictionary for Regulatory Activities
Mg	Milligram
MIC	Minimal Inhibitory Concentration
ML	Milliliter
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NAATS	Nucleic Acid Amplification Testing
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health

OCC	Operations Coordinating Center for the STI CTG
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH
OHRP	Office for Human Research Protections
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PO	By mouth
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
STD	Sexually Transmitted Disease
UAB	University of Alabama at Birmingham

Full Title: RANDOMIZED CONTROLLED TRIAL OF TREATMENT OF MALE PARTNERS OF WOMEN WITH BV

Short Title : Treatment of male partners to prevent recurrent BV

Phase: III

Principal Investigator: Jane R. Schwebke, MD

Population: 368 heterosexual couples, ages 18 or greater in good general health; females have history of recurrent bacterial vaginosis (BV) attending STD and women health clinics in Birmingham, AL and Detroit, MI

Number of Sites: Two: University of Alabama at Birmingham; Wayne State University

Study Duration: 5 years

Subject Duration: 16 weeks (females); 3 weeks (males)

Description of Agent or Intervention:

For the treatment of BV in the female: metronidazole 500 mg PO BID for 7 days

For the treatment of the male sexual partner:

- Metronidazole 500 mg PO BID for 7 days versus placebo capsules PO BID for 7 days

Objectives:

Primary: To determine if the treatment of male partners of women with recurrent BV significantly decreases the recurrence rate of BV in the female.

Secondary: To determine concordance rates of the biotypes/strains of *Gardnerella vaginalis* a cultivatable organism highly associated with BV in sexual couples.

Secondary: To archive genital specimens from sexual couples to be used in the future to determine the prevalence and concordance of novel organisms in women with BV and their sexual partners using state-of –the art molecular techniques and to explore potential behavioral and demographic factors associated with these organisms.

Outcome Measures

Since cures in these women may be short-lived and missed between visits we will define our outcome measure as recurrence/persistence of BV.

Primary Outcome Measure

Follow-up visits:

Recurrence/Persistence of BV

- Positive 3 - 4 Amsel criteria (vaginal pH $\geq$ 4.7, clue cells, positive whiff test; or Nugent score 7-10

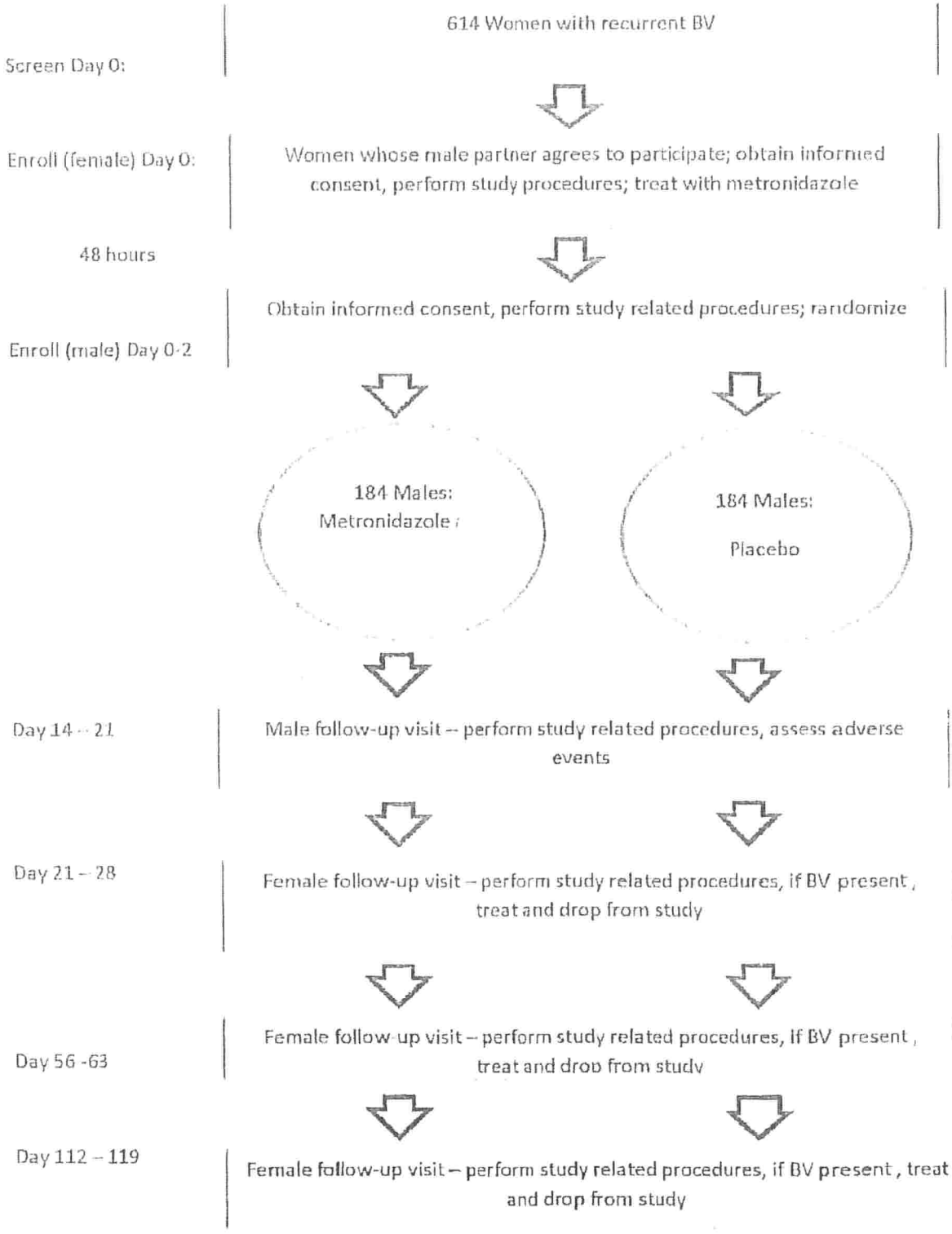
No Recurrence/Persistence: Presence of 0 -2 Amsel criteria; AND Nugent score 0-6.

Unevaluable: Subject did not return for the visit or the subject could not be evaluated for any reason.

Secondary Outcome Measure

Time to recurrence of BV.

DESCRIPTION OF STUDY DESIGN:



KEY ROLES

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1. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

BV is the most prevalent vaginal infection, present in 20-25% of women in the general population and in 40-50% of women attending STD clinics [1,2]. BV has been associated with upper genital tract complications such as preterm labor and delivery and PID [3]. Data strongly suggest that BV facilitates acquisition/transmission of STDs including HIV [4-8]. BV is characterized by changes in the vaginal flora from a lactobacillus - predominance to a marked decrease in lactobacilli, particularly those which produce hydrogen peroxide [9-11]. Large numbers of anaerobes and facultative anaerobes occur, some of which may be found in women without BV in small amounts. The pathogenesis of BV is poorly understood. Epidemiologic correlates of BV include a history of prior STDs, number of sexual partners, a new sexual partner preceding symptom onset, and douching [1,12-15].

Symptomatic BV is characterized by vaginal discharge and/or odor. However, half of all women with BV are asymptomatic [1]. The diagnosis of BV is complicated by the fact that no single pathogen has been implicated. The two most commonly used diagnostic methods are Amsel and Nugent criteria. Amsel criteria are based on the composite findings of vaginal pH, volatile amines in the vaginal fluid, "clue" cells (squamous epithelial cells covered by bacteria, and homogenous vaginal discharge. The Nugent criteria is based on the quantity of morphotypes on Gram stain - 1) large, gram positive rods which represent lactobacilli, 2) small, gram variable rods which represent *Bacteroides/Gardnerella* and 3) curved rods which are *Mobiluncus*. A composite score indicates the presence or absence of BV [16]. This criterion is useful in that it describes what appear to be progressive changes in the flora and assigns them a numerical number of 0-10 with 0 as optimal flora, 4-6 an intermediate flora with increases in *G vaginalis*, and 10 representing the most dramatic of changes.

Sexual transmission of BV has been frequently suggested and the association of BV with sexual activity is strong. Prospective studies show that sex with a new partner is the most significant risk factor for incident BV [17,18]. In their original report on *Haemophilus vaginalis* (now *Gardnerella vaginalis*), Gardner et al believed the disease was sexually transmitted based on high recurrence rates and the isolation of *H. vaginalis* from over 90% of the male partners [19]. *G vaginalis* and anaerobes have been isolated from the semen and genital tract of males [20,21]. The presence of BV related microorganisms in the male genital tract suggests a possible reservoir and supports the theory of sexual transmission of BV. BV in sexually inexperienced females has been documented in only one study which relied on sexual histories provided by adolescent girls [22]. More recently, among college students, the presence of BV was confirmed only amongst those who had experienced some type of sexual activity, mainly penile-vaginal intercourse [23].

In addition, several recent studies have suggested that BV may be an STD. Bradshaw et al. sought to identify risk factors associated with recurrence of BV over a 12 month period following oral metronidazole therapy. At one month follow up, 23% of women had a recurrence of BV; by 12 months 58% of women had recurrent BV and 69% had recurrence of abnormal vaginal flora. Factors associated with recurrence were prior history of BV, lack of hormonal contraceptive use, having a regular sex partner throughout the study and having a female sex partner. Having a new sex partner during the study period was not associated with recurrence. Women with a regular sex partner were more likely to have used condoms <50% of the time than those with new sex partners, although condom use was not associated with recurrence in this cohort. The authors suggested that women treated for BV are reinfected by their regular sex partner but not by new partners, who would be less likely to carry the causative organisms for BV. In our own study, we examined risk factors for incident BV in 96 women seeking care at our STD clinic in Birmingham. Incident BV was significantly associated with exposure to a new sexual partner. Women who developed BV were significantly more likely to have not used a condom with their occasional partner. Among women who developed BV, their male partners reported significantly more sexual partners in the previous 30 days vs. those women not developing BV. Regarding condom use, another recent treatment study for BV showed that women who had unprotected sex during the 30-day follow-up period were significantly more likely to develop recurrent BV than those women who either abstained or used condoms. In a treatment study we recently completed, unprotected sex during the course of the study was significantly associated with treatment failure. Most recently, Ness et al showed that consistent condom use was associated with a decrease in the risk for BV with the strongest association being in women with lactobacillus predominant normal flora as opposed to intermediate flora. Perhaps most compelling is the recent work by Swidsinski et al which has demonstrated *Gardnerella* biofilms in the urine of male partners of women with BV. All partners of women with BV were either positive for *Gardnerella* biofilm or their samples had insufficient epithelial cells for analysis. All women with BV had male partners without evidence of *Gardnerella* biofilm in the urine[24]. Additionally they present a chain of infection among sexual partners infected with the identical strains of *Gardnerella*[24].

Microbial Pathogenesis of BV

G. vaginalis is universally associated with BV but has not been proven to be the pathogen. Although published data indicate that it is present in "normal" women the definition of normal used in that study was not sufficiently strict and included women with intermediate Nugent scores [25]. Many women have intermediate vaginal flora not quite consistent with the definition of BV yet these women cannot be described as having optimal vaginal health. We have shown that many women exhibit transient increases in bacterial morphotypes consistent with *G vaginalis* on vaginal smears without ever fulfilling Amsel criteria and in the past were considered to be "normal". These women have a distinctly different vaginal flora pattern than women with a stable lactobacillus-predominant flora throughout the month [26]. Our work has

indicated that women with a strict definition of consistently normal vaginal flora do not have *G. vaginalis* present using the most sensitive of techniques available (unpublished data). The vagina provides an especially rich culture medium, the presence of glycogen being an in vivo growth requirement [27]. In males, *G vaginalis* has been repeatedly recovered from the urethra and from seminal fluid [19,21,28-30].

Gardner et al isolated *Gardnerella* from the urethras of 86% of sexual partners of women with BV [19]. Pheiffer demonstrated concordance among 79% of couples [31]. Biotyping of *G. vaginalis* has yielded compelling results supporting the sexual transmission of this bacterium. Piot et al found that biotypes isolated from women with BV and from the urethras of their sexual partners were identical 90% of the time [32]. Briselden and Hillier found that lipase producing strains were associated with BV and that while not statistically significant there was an association between acquisition of a new biotype and new sex partners [33]. Further evidence of the pathogenicity of *G vaginalis* can be found from analysis of cytokine levels in the vaginal fluid of women with normal, intermediate, and BV flora. Elevated cytokine levels are associated primarily with increased concentrations of *G vaginalis* even among those women with intermediate vaginal flora patterns [34]. Recent studies have suggested that BV is a biofilm community primarily composed of *G vaginalis* at its onset and gradually becoming more complex in terms of additional species. This could account for the observations of clinicians that women who suffer repeated episodes of BV are often more difficult to cure of their disease. A recent study examining vaginal biopsies demonstrated by fluorescence in-situ hybridization the presence of an adherent vaginal biofilm that predominantly hybridized with *G vaginalis*. The proportion of all other bacterial groups detected in the biofilm, including *Atopobium*, was significantly lower [35]. Sloughing of this biofilm leads to the clue cell which is characteristic of BV. Newly published in vitro work has shown that *G. vaginalis* is more virulent than other BV-associated anaerobes and that the latter may be opportunists that colonize after *G. vaginalis* has initiated the infection[36]. Additionally, a recent study by Swidsinski et al using male and female urine specimens showed that *Gardnerella* is present in dispersed and a cohesive (biofilm) forms and that in sexual partners concordance of the cohesive forms was 100% leading the authors to conclude that *Gardnerella* biofilm is sexually transmitted[24].

Metronidazole or clindamycin are the original and currently recommended therapies. Initial cure rates are approximately 50-80% and recurrence rates of 50-70% have been shown[37]. In a study of suppressive therapy for recurrent BV, Sobel et al showed that among the women with a history of recurrent BV assigned to placebo suppression, 50% had developed recurrent BV by 12 weeks[38]. Although the epidemiology of BV strongly suggests sexual transmission, treatment of the sexual partner is currently not recommended. This is based on studies of the treatment of male partners of women with recurrent BV[39-41]. These studies, which randomized the male to receive single dose metronidazole versus placebo, showed no benefit in reducing recurrence rates in the female. However, compliance with medication on the part of these asymptomatic males is questionable. Medication was delivered by the female and no measures of compliance were undertaken. Additionally, short-course single-dose therapy for females with BV has a cure rate of only 50%, thus the males may also have been inadequately treated. In addition, it is often difficult to differentiate between recurrence and persistence since cures may be short-lived.

1.1.1 Summary of Relevant Clinical Studies

Overview of Male Partner Treatment Trials in Bacterial Vaginosis

Few studies have examined the effect of partner treatment on BV, and the results of these studies are controversial, owing to the small numbers of patients studied and methodological flaws. A review of the literature (Medline and EMBASE) shows that there are six randomized, controlled trials of

treatment of sexual partners of women with BV, the most recent published in 1997 [39-44]. In four of these trials, partners were treated with oral metronidazole; in the remaining two trials partners were treated with either oral clindamycin or oral tinidazole. The overall conclusion from these trials is that partner treatment of BV does not appear to confer a benefit in terms of improved cure rates or reduction in recurrence. However, four of the trials have either very small treatment groups, very large dropout rates or both (Mengel, Moi, Swedberg, Colli). As mentioned above, several of the trials utilized single dose metronidazole or tinidazole to treat the male. Treatment of women with BV with single dose metronidazole has been shown to be ineffective [45]. In addition, the trial by Mengel did demonstrate a benefit in partner treatment when looking at Gram stain criteria, further adding to the controversy of the conclusions from these trials. A recent systematic review of these studies by Mehta concluded that each of the trials had significant flaws including randomization flaws, inadequate dosing of medication, lack of adherence measures and limited power [46].

1.1.2 Description of the Study Agents

Women will be treated with the standard dose of oral metronidazole at 500 mg PO BID for 7 days. Male partners will be randomized to the same dose of oral metronidazole versus placebo capsules.

1.2 Rationale

Although new data has added considerable credence to the hypothesis that BV is indeed sexually transmitted, we continue to accept data on the efficacy of treatment of the male sexual partner in preventing recurrent BV that is outdated and not rigorous in its methodology. A recent systematic review of the published partner treatment studies strongly supports that in light of the inadequacies of previous studies, new studies are needed on order to answer this important question [46]. The combination of the hypothesis that *Gardnerella* is pivotal in the pathogenesis of BV combined with the growing acceptance in the scientific community that BV is an STD begs for the reevaluation of male treatment.

Current management of women with BV is clearly inadequate as demonstrated by initial cure and recurrence rates. If indeed BV is sexually transmitted then clearly treatment of males will be necessary to achieve control of this infection. Control and decreased prevalence of BV is needed to decrease the burden of public health complications associated with this infection.

Although the optimal antibiotic for use in this study could be debated, we have chosen metronidazole as there is the most experience with this drug in BV. It should be noted that it is the hydroxy metabolite of metronidazole that is active against *Gardnerella* [47].

1.3 Potential Risks and Benefits

1.3.1 Potential Risks

The major side effects associated with metronidazole are gastrointestinal. Metallic taste, nausea, vomiting, abdominal cramping, and diarrhea are possible. Metronidazole may rarely cause peripheral neuropathy, headache, discoloration of the tongue, skin rash, decreased blood counts, confusion, seizures, and a disulfiram type of reaction in conjunction with alcohol. However, these are rare events.Metronidazole interacts with lithium, Coumadin, Dilantin, or Antabuse and therefore subjects requiring these medication are excluded from participation.

1.3.2 Known Potential Benefits

Metronidazole is licensed by the FDA for the treatment of anaerobic gynecologic infections and has been in use for many years for the treatment of BV with an excellent safety profile. Treatment of the male partner may have no direct benefit for the male partner or may result in decreased rates of recurrent BV in his female partner.

This study may improve understanding of the microbiology and sexual transmissibility of BV by determining the prevalence and concordance of *Gardnerella vaginalis*. In addition, future investigation with stored specimens may provide insight into the role of unculturable agents in the sexual transmission of BV.

2. STUDY OBJECTIVES

The specific aims of this study are to:

- 1. To conduct a RCT of the treatment of male partners of women with recurrent BV (the return of positive 3 - 4 Amsel criteria (vaginal pH $\geq$ 4.7, clue cells, positive whiff test, homogenous discharge); or Nugent score 7-10) with a 7 day dosing regimen of metronidazole versus placebo alone for the male to determine if treatment of the male partner results in lower recurrence rates in the female index case. The expected outcome is that treatment of the male partner will reduce the likelihood of recurrence of BV in the female index case.

  2. To determine concordance rates of *Gardnerella vaginalis*, a cultivatable organism which is highly associated with BV, in sexual couples and to examine concordance of *G vaginalis* biotypes and strain patterns.

  3. To archive genital specimens from sexual couples to be used in the future to determine the prevalence and concordance of novel organisms in women with BV and their sexual partners using state-of the-art molecular techniques and to explore potential

behavioral and demographic factors associated with these organisms

3. STUDY DESIGN

3.1 Description of the Study Design

This study will be performed as a phase III randomized, double-blinded trial to evaluate the efficacy of 1) metronidazole 500 mg PO BID for 7 days versus 2) placebo capsules alone for treatment of the male sexual partner of women with recurrent BV. The primary outcome is the rates of recurrent BV between these two groups. Although this is a phase III study we will carefully monitor potential toxicity in the males since it is currently not standard of care to treat males for this indication.

Women and men in this study will be recruited from STD or women's clinics in Birmingham, AL and Detroit, MI.

Women with symptomatic BV defined by Amsel criteria [1] and no evidence of STD will be invited to participate. Inclusion criteria are that the woman be at least 18 years of age (19 in Alabama), heterosexual, have symptoms of vaginal odor and/or discharge, meet the modified clinical (Amsel) criteria for BV (all must have a vaginal pH of $\geq$4.7, a positive whiff test, and clue cells to be eligible), have an enrollment Nugent score of 7 or greater, and a history of recurrent BV, defined as 2 or more episodes of BV in the previous year. She must have a regular current sex partner who would be willing to participate.

Females will be administered questionnaires containing detailed questions regarding sexual activity including new sex partners since the prior visit. A urine pregnancy test will be performed. A speculum examination will be conducted and findings noted. The vaginal pH will be noted (colorpHast Indicator Strips, EM Science, Gibbstown NJ). Vaginal fluid will be placed into a small amount of saline for microscopy and "whiff" test, and into TVPouch media for culture of *T vaginalis*. A Gram stained smear of the vaginal fluid will be made. Additional vaginal swab specimens will be collected for *G vaginalis* culture (including biotyping and PFGE if also isolated from male partner) and archive. Nucleic acid amplification testing for *N gonorrhoeae* and *C trachomatis* will be performed. A urine specimen will be obtained to determine the presence of *Gardnerella* biofilm using the method of Carnoy solution fixation and FISH as described by Swidsinski et al[24]. The subject will be carefully instructed as to how to take her medication (metronidazole 500 mg twice a day for 7 days) . She will be asked to keep a diary of any side effects, symptoms, douching, other medication and sexual activity and asked to avoid the latter three behaviors if at all possible or to at minimum to use a condom with intercourse for the first two weeks of the study. She will be counseled to abstain from alcohol for the duration of the treatment.

At the female enrollment visit an appointment will be made for the male partner to enroll in the study within 72 hours (we find that virtually all of our patients and their partners have cell phones or pagers). Women who cannot contact their partners at the time of their enrollment visit and confirm an appointment will not be enrolled into the study. Women will be asked to remind their male partner twice a day to take their medication.

Follow-up visits for females will be conducted at day 21, 8 and 16 weeks. At each follow-up visit, a questionnaire will be administered, diary reviewed, pelvic examination conducted and specimens (Gram stain, Amsel criteria, *G vaginalis* culture and detection of novel organisms) obtained as done at the enrollment visit. Participants will be asked to return the medication packages, a standard way of assessing adherence to the regimen. Cure will be ascertained using the clinical criteria for BV of Amsel [1] as well as the Nugent scoring system [16]. The persistence or disappearance of specific organisms will be analyzed in relationship to these standard definitions of cure. Women who fail initial therapy or have recurrence of symptomatic BV during the course of the study will be re-treated with 7 days of metronidazole and dropped from the study at that time. Any woman found to have a positive screening test for gonorrhea or chlamydia will be treated appropriately and instructed to notify her partner of the need to be treated. Women with intercurrent vaginal yeast infections, which may occur, will be treated with oral fluconazole and continued in the study. Women with a positive culture for trichomonas will be dropped from the study as their male partners will require treatment with metronidazole.

Subjects will receive an incentive for each study visit, in accordance with local IRB recommendations. This could be a check or gift certificate or a similar incentive.

Women whose male partner failed to enroll into the study within 72 hrs will be so notified and dropped from the study at the first follow-up visit.

Male Study Procedures

Males referred by their female sexual partner will be seen within 72 hours of enrollment of the female. Males will be consented and asked behavioral and historical questions using a gender appropriate questionnaire, with special emphasis on number of current sexual partners. A couple verification screening tool will be utilized to be certain they are current sexual partners[22]. The confidentiality of their answers will be emphasized. They will be examined and two external swabs from the coronal sulcus and a 10 ml first void urine specimen will be obtained. These specimens will also be used for *G vaginalis* culture and archived for possible use in detection of novel organisms. The urine will also be used for NAATS for *N gonorrhoeae* and *C trachomatis* and for the determining the presence or absence of *Gardnerella* biofilm[24]. They will then be randomized to one of two treatment arms:

1. Metronidazole 500 mg PO twice a day for 7 days .
2. Placebo capsules PO twice a day for 7 days.

Both arms will also contain an instruction sheet on metronidazole.

Packaging and encapsulation of the study drugs are described in section 5.

Subjects will be instructed on dosing and potential side effects. They will be asked to refrain from sexual activity or at least unprotected sex until they have finished the medication. They will be counseled to abstain from alcohol during the treatment. They will remain in the study. They will be given a diary to record dosing, symptoms, and sexual activity if it occurs.

If subjects test positive for gonorrhea or chlamydia they will be notified and treated appropriately and instructed to inform their partner of the need for treatment. If their female partner has a positive culture for trichomonas, they will be treated with metronidazole and dropped from the study.

Males will be asked to return to the clinic for a follow-up visit at day 21-28. The examination and collection of specimens for *G vaginalis* and archive will be repeated. The diary will be reviewed and clarified especially with regards to medication dosing, side effects, and intercurrent sexual activity – if the latter has occurred the research nurse will verify if it occurred with the index patient or with another partner. Pill containers will also be collected.

Subjects will receive an incentive for each study visit, in accordance with local IRB recommendations. This could be a check or gift certificate or a similar incentive.

Randomization

After the study participant has signed an informed consent form to participate in the study, and has been screened and determined to be eligible for the study, the participant will be randomized. The study will use a stratified, blocked randomization scheme. Stratification will be by clinical center. The blocked randomization is planned to ensure balanced groups within strata.

All arms will be blinded. At the time of randomization, each study participant will be assigned a unique study medication kit number. Participants who are randomized but withdraw prior to receiving study medication will be replaced.

The University of Arkansas Medical Center, the data coordinating center, will be responsible for creating the randomization procedure; allocation concealment will involve opaque envelopes.

All participants and clinical investigators participating in this trial will be masked with respect to treatment assignment. Unmasking is discouraged and will only be considered if justified on a case-by-case basis as assessed by the protocol chair. Requests to unmask a participant should be made in writing by the site investigator to the protocol chair with a letter that includes the study ID number, date of randomization, and brief description of the rationale for unmasking. Electronic requests are preferred and accepted. In the case of an emergent safety issue that could be life threatening or a serious safety issue, the Protocol Chair will contact the DMID Medical Monitor. This streamlined procedure would be used when deemed necessary to protect the subject's health.

The protocol chair must communicate the decision to the site investigator with a written copy of the decision whether unmasking is necessary. A copy of the decision should be forwarded to the data management center. If unmasking is deemed necessary, the data management center will provide the site investigator with the treatment identification. Neither the data management

center nor the site investigator should involve the site pharmacist in this process. If a participant is to be unmasked, the site investigator should communicate the information directly to the subject and his local physician. Documentation of the unmasking procedures, including who was unmasked and when, should be submitted in a timely fashion to the Protocol Chair and the data management center.

3.2 STUDY ENDPOINTS

The study endpoint is recurrent BV defined by clinical criteria (positive 3-4 Amsel criteria, vaginal pH≥4.7, clue cells, positive whiff test, homogenous discharge) or microbiological criteria (Nugent score 7-10).

4. STUDY POPULATION

4.1 Description of the Study Population

Subjects will be recruited at the public health STD clinics and women's clinics located in Birmingham, AL and Detroit, MI. If a subject presenting for routine care is diagnosed with recurrent BV (history of 2 or more episodes in the past 12 months), she will be approached about potential participation in the study. The study will screen 614 women to enroll 368 couples. Study retention will be enhanced by the use of reminder telephone calls and texts and subject reimbursements. Advertisement in local papers and possibly internet will also be used.

4.1.1 Subject Inclusion Criteria

Females meeting all of the following criteria will be considered eligible for enrollment in this study:

1. At least 18 years of age
2. Heterosexual with a regular partner.
3. History of 2 or more episodes of BV in the previous 12 months.
4. Symptoms of BV including vaginal discharge and/or odor.
5. Positive Amsel criteria for BV including vaginal pH >4.5, positive whiff test, presence of clue cells and/or Nugent score 7-10
6. Willingness to provide informed consent.
7. Willingness to abstain from sexual intercourse or use condoms during the study.
8. Willingness to abstain from alcohol for the first week of the study.

Males meeting all of the following criteria will be considered eligible for enrollment in the study:

1. At least 18 years of age .
2. Sexual partner to a female who meets study eligibility.
3. Willingness to provide informed consent.
4. Willingness to abstain from sexual intercourse or use condoms during the study.
5. Willingness to abstain from alcohol for the first week of the study.

4.1.2 Subject Exclusion Criteria

Subjects meeting any of the following criteria at baseline will be excluded from study participation:

1. Allergy to metronidazole and other nitroimidazole derivatives
2. Failure of the male partner to keep his appointment to be seen within 72 hours.
3. Pregnant or breast feeding (females).
4. HIV or other chronic disease which in the opinion of the investigator would interfere with the ability to participate in this study.
5. Subject requires concurrent lithium, coumadin, dilantin, or antabuse.
6. Presence of trichomonas on wet prep of vaginal fluid (females).
7. History of convulsive seizures, peripheral neuropathy, or blood dyscrasias.

4.2 Strategies for Recruitment and Retention

We anticipate that recruitment of women with recurrent BV will be achievable due to the women's strong desire to rid themselves of this recurrent infection. Recurrent BV is a common problem in each of the participating clinics. We will also enhance recruitment with the aid of flyers posted on campuses and advertisements in local papers/online for the study. The study will also be registered with clinical trials.org. Study retention will be enhanced by the use of reminder telephone calls and texts and subject reimbursements.

5. STUDY AGENT/INTERVENTIONS

5.1. Study Product Acquisition

All drugs to be used in this study are FDA-approved for use in the US for anaerobic gynecological infections. Metronidazole 500 mg generic tabs and placebo will be purchased in bulk from the UAB research pharmacy. The UAB pharmacy will package the metronidazole (active or placebo) into individual doses. This includes encapsulation, packaging, and blinding and labeling according to the randomization schedule for each site. The UAB pharmacy will send the packaged study product to the sites.

5.2 Formulation, Packaging and Labeling

Each active tablet of metronidazole will be placed in a gelatin capsule with lactose filler. Each placebo capsule will be filled with lactose only and be identical in appearance to the capsule with the active ingredient. The lactose filler is Lactose U.S.P. and the small amount should not be a problem even for persons who are lactose-intolerant. They may experience some mild bloating. The gelatin capsules are Swedish orange capsules from Capsugel.

The active and placebo tablets or capsules will be placed into amber prescription vials and labeled with a DRUG number and allocated according to the randomization scheme generated by UAMS. Examples of the labels are shown below.

Metronidazole/placebo:

DRUG #9999 Date_____
Take 1 tablet twice a day with water
Metronidazole/placebo 500mg # 14
Dr Jane Schwebke 205-934-3411
Investigational Use Only

Vials will be placed into plastic bags with a sheet of instruction for metronidazole in each arm.

5.3 Product Storage and Stability

All study medications are stable at room temperature.

5.4 Preparation, Administration and Dosage of Study Intervention/ Investigational Product

The antibiotics to be used in this study are licensed by the FDA for anaerobic gynecological infections. Metronidazole is recommended by the CDC for the treatment of BV.

Each participant will receive a single vial of medication. Section 5.2 explains the packaging and labeling. Section 4 explains the design and study arms. After randomization of the participant, the study clinician will obtain the next available DRUG number and vial of medication for that

DRUG number. The patient will be given instructions on how and when to take the medication and to bring the bottle to the clinic at the first follow-up visit (visit #2).

Dosage will be as follows:

Metronidazole for females – 500 mg tablets, 1 tablet twice a day for 7 days.

Metronidazole/placebo for males – 500 mg tablets (active or placebo), 1 tablet twice a day for 7 days.

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

UAB will ship packaged study medications to the investigational sites. At each site, study product will be kept in a locked storage cabinet at the Clinic and administered by the study personnel. A drug accountability log should be filled out for all study medications at the sites. Unused product will be returned to UAB at the end of the study.

5.6 Assessment of Subject Compliance with Study Intervention/ Investigational Product

Subjects will be asked to bring the study medication bottles to the study personnel at their follow-up visit. Study personnel will review adherence to the study medication schedule with the subjects, including the subject's study medication diary, and record this on the appropriate data collection form.

5.7 Concomitant Medications and Procedures

Drugs not allowed while on study include lithium, Coumadin, Dilantin and antabuse.

Subjects who test positive for *Neisseria gonorrhoeae* or *Chlamydia trachomatis* will be notified as soon as possible and treated according to local guidelines as well as their sexual partners.

Females who develop vaginal candidiasis will be treated with oral fluconazole and continued in the study.

Subjects with a positive culture for trichomonas will have their sexual partners treated with 2 gm single dose metronidazole. The female will be retreated with metronidazole if she has had unprotected sex. The couple will be discontinued from the study.

Subjects with persistent BV according to the clinical failure criteria, at the follow-up visits or at the end of the study will be treated with a second course of oral metronidazole.

6. STUDY PROCEDURES/EVALUATIONS

6.1 Clinical Evaluations

- Medical history to include general health, sexual history, drug use, STD history, current symptoms. To be obtained by research clinician or assistant using standardized questionnaire at enrollment and follow-up visits.
- Medications history to include prescription medications taken within the past 30 days; if on lithium, coumadin, Dilantin,or antabuse, subject is ineligible for the study.
- Physical exam to be targeted to genitals. To be performed at enrollment and each follow-up visit including unscheduled visits for adverse events.
- Counseling procedures regarding abstinence or use of condoms and avoidance of alcohol during the treatment phase of the study for alcohol and ideally for the course of the study for sex.
- Study medication bottles and the medication diary will be reviewed for adherence.

6.2 Laboratory Evaluations

6.2.1 Clinical and Research Laboratory Evaluations and Specimen Collection

Specimens for evaluation in females include:

1. Amsel criteria at time of enrollment consisting of vaginal pH, vaginal wet prep for presence of clue cells and whiff test to be repeated at each visit.
2. Vaginal wet prep to look for motile trichomonads.
3. Pregnancy test.
4. Gram stained smear of vaginal fluid for Nugent criteria to be repeated at each follow-up visit (collect duplicate slides).
5. Vaginal swab for *T. vaginalis* culture obtained at initial visit.
6. Endocervical swab for NAATS for gonorrhea and chlamydia to be obtained at initial visit.
7. Vaginal swab for *G. vaginalis* culture and archive obtained at each visit.
8. Urine specimen for detection of biofilm obtained at each visit.

Specimens for evaluation in males include:

1. Swab of urethral meatus for G. vaginalis culture and archive at each visit

2. Swab of the coronal sulcus for *G. vaginalis* culture and archive at each visit.

3. 10 ml first void urine specimen for NAATS testing for gonorrhea and chlamydia (visit 1 only) and *Gardnerella* culture and biofilm at each visit.

6.2.2 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations, 42 CFR Part 72.

6.2.3 Specimen Preparation, Handling and Shipping

The Principal Investigator will be responsible for complying with all regulations for shipment of potentially hazardous materials. This includes appropriate training for study personnel as well as ensuring that protocol collaborators are in compliance. The following websites are resources for shipping regulations.

- *Department of Transportation. 49CFR171-180. Hazardous Materials Regulations. http://www.hazmat.dot.gov/rules.htm*
- *Public Health Service 42CFR72. Interstate Shipment of Etiologic Agents. 42CFR Part 72. Federal Register, Vol. 45, No. 141-Monday, July 21, 1980. http://www.cdc.gov/od/ohs/biosfty/shipregs.html*
- *Dangerous Goods Regulations. International Air Transport Association (IATA). http://www.iata.org*
- *Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens. World Health Organization, 1997. http://www.who.int/emc/biosafety.html*
- *United States Postal Service. 39CFR111. Mailability of Etiologic Agents. Codified in the Domestic Mail Manual 124.38: Etiologic Agent Preparations. DMM Issue 55, January 10, 2000. http://www.access.gpo.gov/*
- *Occupational Health and Safety Administration (OSHA). 29CFR1910.1030. Occupational Exposure to Blood borne Pathogens. http://www.osha.gov*

6.2.4 Instructions for Specimen Storage

Each site will query participants at the time of consent concerning their permission to use their specimen's in future related studies. If the participant declines such use, their specimens will be destroyed at the end of the study.

6.2.5 Specimen Shipment Preparation, Handling and Storage

Please refer to the Manual of Operations for detailed information on specimen collection, testing, interpretation, storage and shipping.

Vaginal Gram stains will be interpreted on-site and then sent to UAB for review.

At each clinical site, specimens will be processed for the assay for *N. gonorrhoeae* and *C. trachomatis* and tested per the manufacturer's instructions using the licensed GEN-PROBE TMA assay (Aptima Combo II, Gen-Probe, San Diego, CA).

Culture for *T. vaginalis* will be performed at the local site using InPouch TV (BioMed Diagnostics, White City, OR)

Gardnerella culture will be performed at the local site and isolates frozen and stored for future shipment to the UAB laboratory for processing for biotyping.

Urine for *Gardnerella* biofilm and specimens for archive will be sent to UAB.

7. SUBSTUDIES

If a substudy is added to this ongoing parent study at a later time, a protocol amendment is required.

Definition: A substudy asks a separate research question from the parent protocol and does not overlap with the parent protocol's objectives, but uses all or a subset of study participants or specimens.

A concept sheet for a proposed study should be approved by the NIAID Project Officer/Program Officer prior to development of a full protocol for the substudy. Once the concept for a substudy is approved by the Program Office, a decision will be made by NIAID, in conjunction with the Investigator as to whether the concept is appropriate as a substudy or should be a stand-alone study. The concept sheet should include a description of the substudy and its objectives and its impact on the parent study.

8. STUDY SCHEDULE

Schedule of visits for females:

Visit 1 – Enrollment

Visit 2 – Day 21-28

Visit 3 – Day 56-63

Visit 4 - Day 112-119

Schedule of visits for males:

Visit 1 – Enrollment (within 72 hours of female partner)

Visit 2 – Day 21-28

Reminder calls will be made prior to the next scheduled visit.

8.1 Screening

Subjects may be enrolled in via two different pathways. One potential pathway applies to subjects attending the STD or Women's Clinic for a routine visit with complaints consistent with symptomatic BV. These women will have a vaginal wet prep, pH, and whiff test performed as part of their routine evaluation. Subjects diagnosed with symptomatic BV as a result of vaginal pH >4.5, presence of clue cells on wet mount microscopy of the vaginal fluid, and positive "whiff" test will be referred to the study recruiter or clinician for determination of level of interest in participating in the study, the informed consent process, and further screening. Potential subjects must meet all inclusion and exclusion criteria to be eligible for participation in the study. Screening and enrollment should occur at the same clinic visit. The subject must verify that her sexual partner is willing to participate in the study and present for enrollment within 72 hours. If the subject agrees to participate, the study recruiter/clinician will conduct the informed consent process.

The other potential pathway refers to women with vaginal symptoms who may respond to an advertisement for study participation. For these women the process will be the same as described above except that they will be consented for the study prior to examination and specimen collection.

8.2 Enrollment/Baseline (Visit #1)

Female

At enrollment, the research clinician will confirm/clarify subject's symptoms with respect to BV as well as any known drug allergies, and will review the initial wet prep result to confirm eligibility. A study questionnaire will be administered. An examination will be conducted to obtain additional study specific specimens including vaginal swabs for Gram stain, culture for *T. vaginalis* and *G. vaginalis*, and archive. Subjects will be asked to provide a first fraction urine specimen for pregnancy testing and NAAT (nucleic acid amplification testing) for gonorrhea and chlamydia and examination for biofilm.

After confirmation of a negative pregnancy test, subjects will be assigned the next consecutive number in the randomization scheme which will link with their partner's study number. They will be provided with metronidazole 500 mg tablets to be taken twice a day for 7 days. Subjects will be counseled regarding abstaining from alcohol until they finish their medication and sexual intercourse, or using condoms, ideally for the course of the study. Condoms will be provided and subjects reimbursed for study visit according to local procedures.

All study specimens will be processed as described in the Manual of Operations labeled with the date and study number and sent to the local site's research laboratory for further processing/handling.

Male

Male subjects will be consented and administered study specific questionnaires. A genital examination will be performed and two external swabs will be obtained from the coronal sulcus for *G. vaginalis* culture and archive. A first fraction urine specimen will be obtained for NAATS testing for gonorrhea and chlamydia as well as examination for biofilm and *G. vaginalis* culture. Men will be randomized to oral metronidazole versus placebo tablets alone. They will be counseled to avoid alcohol until they finish their medication and to avoid unprotected sex during the course of the study.

All study specimens will be processed as previously described in 7.2, labeled with the date and study number and sent to the local site's research laboratory for further processing/handling.

8.3 Male Follow-up (Visit #2, Day 21 to 28)

Male subjects will be asked to return to the Clinic one week after completion of therapy (Day 21-28). Study medication bottles and the medication diary will be reviewed for adherence. Subjects will be queried regarding interim sexual activity and symptoms, and any possible side effects of the study medication will be recorded. A genital examination will be performed. Coronal sulcus specimens will be obtained for *G. vaginalis* culture and archive. First fraction urine will be obtained for examination for biofilm and *G. vaginalis* culture. If there was a positive screening test for STD from enrollment he will be treated according to local guidelines and instructed to inform his partner of the need for treatment. Subjects will be reimbursed for study visit according to local procedures.

8.4 Female Follow-up (Visit #2, Day 21-28; Visit #3, Day 56-63, Visit #4, Day 112-119)

Subjects will be asked to return to the Clinic at the days listed above n. Study medication bottles and the medication diary will be reviewed for adherence. Subjects will be queried regarding interim sexual activity, symptoms, and any possible side effects of the medication. A pelvic

examination will be conducted to obtain specimens for vaginal Gram stain, Amsel criteria, *G. vaginalis* culture, and archive. If there was a positive screening test for STD from enrollment she will be treated according to local guidelines and instructed to inform her partner of the need for treatment. Intercurrent vaginal yeast infections may be treated with single dose oral fluconazole. If her trichomonas culture is positive, her partner will require treatment with metronidazole and the couple will be discontinued from the study. Women whose male partner failed to enroll in the study within 72 hours will be so notified and dropped from the study at this visit. Subjects will be reimbursed for study visit according to local procedures.
Subjects will be evaluated for clinical outcome according to the following definitions:

Recurrence/Persistence of BV:

- Positive Amsel criteria (vaginal pH $\geq$ 4.7, clue cells, positive whiff test; or Nugent score 7-10.

No Recurrence/Persistence of BV:

- Presence of 0- 2 Amsel criteria and Nugent score 0-6

Unevaluable:

- Subject did not return for the visit or the subject could not be evaluated for any reason.

Subjects meeting the criteria for recurrence/persistence will be added to the cumulative failures for the study. Women who fail treatment will be re-treated with oral metronidazole and dropped from the study.

8.5 Early Termination Visit

Potential reasons for early termination would include voluntary withdrawal or a positive culture for trichomonas or as a sexual partner to a subject with a positive culture for trichomonas. Subjects will **not** be terminated for failure to take study medications as directed (intent-to-treat analysis).

Procedures at the early termination visit will include review of study medication adherence, review of possible side effects, administration of follow-up questionnaire, targeted physical examination, and collection of specimens as outlined in male and female follow-up visits. Specimen handling is described in section 6. If subjects have the signs or symptoms of BV as defined for clinical failure, they will be treated according to local guidelines for recurrent or persistent BV.

8.6 Unscheduled Visits

An unscheduled visit would most likely occur for treatment for a positive test for gonorrhea and/or chlamydia or as a sexual partner to a subject with a positive test. These visits will consist of counseling about the STD an administration of treatment for that STD. Some sites may elect

to inform the subject of the need for treatment and refer that subject to a specific STD Clinic for the necessary treatment.

9. ASSESSMENT OF SAFETY

The primary safety concern of this study is gastrointestinal tolerability of the antibiotic regimen.

9.1 Specification of Safety Parameters

Gastrointestinal side effects to be assessed include nausea, vomiting, abdominal pain, and diarrhea. These will be graded on a scale of 1 to 5 utilizing the NCI Common Toxicity Criteria Table (version 3.0) as shown in the table below.

Adverse event	Grade	Description
Nausea	1	Loss of appetite without alteration in eating habits
	2	Oral intake decreased without significant weight loss, dehydration or malnutrition; intravenous (IV) fluids indicated < 24 hours
	3	Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or total parenteral nutrition (TPN) indicated >= 24 hours
	4	Life-threatening consequences
	5	Death
Vomiting	1	1 episode in 24 hours
	2	2 - 5 episodes in 24 hours; IV fluids indicated < 24 hours
	3	>= 6 episodes in 24 hours; IV fluids, or TPN indicated >= 24 hours
	4	Life-threatening consequences
	5	Death
Abdominal pain	1	Mild pain not interfering with function
	2	Moderate pain; pain or analgesics interfering with function, but not interfering with activities of daily living (ADL)
	3	Severe pain; pain or analgesics severely interfering with ADL
	4	Disabling
	5	Death
Diarrhea	1	Increase of < 4 stools per day over baseline; mild increase in ostomy output compared to baseline
	2	Increase of 4 - 6 stools per day over baseline; IV fluids indicated < 24 hours; moderate increase in ostomy output compared to baseline; not interfering with ADL
	3	Increase of >= 7 stools per day over baseline; incontinence; IV fluids >= 24 hours; hospitalization; severe increase in ostomy output compared to baseline; interfering with ADL
	4	Life-threatening consequences (e.g., hemodynamic collapse)

	5	Death

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

Any adverse events reported at the follow-up visits will be recorded.

9.3 Adverse Events, Serious Adverse Events

At the follow-up visits, any gastrointestinal side effects reported will be recorded, as will other adverse events if volunteered by the subject. Adverse events will be recorded on the appropriate DCF and summarized by the data center. Serious adverse events will be immediately reported to DMID and FDA MedWatch.

9.4 Adverse Events

ICH E6 Good Clinical Practice Guidelines defines an Adverse Event (AE) as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an adverse event may come to the attention of study personnel during study visits and interviews or by a study recipient presenting for medical care. All adverse events must be graded for intensity and relationship to study product. All Adverse Events including local and systemic reactions not meeting the criteria for "serious adverse events" should be captured on the appropriate data collection form. Information to be collected includes event description, time of onset, investigator assessment of severity, relationship to study product, and time of resolution/stabilization of the event. All adverse events occurring while in study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution. Any medical condition that is present at the time that the subject is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study it should be recorded as an AE. AEs and SAEs will be reported if they occur at any time during the subject's participation in the study.
However, since the study product is a licensed drug and it is unlikely that an AE or SAE occurring beyond day 28 would be related to the study product, we will only assess safety for study medication at visits 1 and 2. Safety assessments for study procedures will continue throughout the study.

Intensity of Event: The following guidelines will be used to quantify intensity.

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

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

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

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

9.5 Serious Adverse Events

A Serious Adverse Event (SAE) is defined as an AE meeting one of the following conditions:

- Death during the period of protocol defined surveillance
- Life Threatening Event (defined as a subject at immediate risk of death at the time of the event)
- An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity.

Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

9.6 Reporting Procedures

The Investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study product.

This protocol follows the NIAID guidelines for reporting of adverse events. All adverse events that meet the expedited reporting requirements of NIAID are reported to DMID and FDA

MedWatch. These events will be referred to as serious adverse events (SAEs). The study sites will report the serious adverse events as outlined in the Study Manual. It is the responsibility of each site to provide adverse event information to their local IRB as required by their IRB.

The data center will provide a listing of adverse events to the protocol chair for review on a monthly basis. They will compile these events in a secure tabular format and forward to the Protocol Specialist for posting on the study website within the NIAID. The Protocol Team will have access to the specific study website. For blinded studies, the serious adverse events will be reviewed and tabulated without treatment assignment.

Accrual summaries for this trial will also be posted to the study website. The progress of the study will be reviewed independently by the Protocol Chair. For this phase III trial, stopping the trial for toxicity or efficacy is based on meeting criteria stated in the protocol, and the protocol team will determine whether these criteria have been met.

All serious adverse events will be:

- recorded on the appropriate serious adverse event data collection form
- followed through resolution by a study physician
- reviewed by a study physician.

Any AE considered serious by the Principal Investigator or Subinvestigator or which meets the aforementioned criteria must be submitted on an SAE form to DMID and FDA MedWatch.

The study clinician will complete a Serious Adverse Event Form for all SAEs, whether related or unrelated. The form will be sent by fax to DMID within 24 hours of site awareness of the event. Other supporting documentation of the event may be requested by DMID and should be provided as soon as possible.

DMID Pharmacovigilance Group
Clinical Reserch Operations and Management Support (CROMS)
6500 Rock Springs Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 SAE Fax Number: 1-800275-7619
SAE Email: PVG@dmidcroms.com

9.7 Reporting of Pregnancy

Pregnant women are not eligible to participate in the study. Women are counseled regarding prevention of pregnancy and encouraged to make every effort to avoid pregnancy during study participation. This is not due to safety issues but rather due to the potential confounding effects of hormones in pregnancy on the vaginal flora. If a study participant becomes pregnant during

study participation, she will be continued in the study and the data center will be notified. Metronidazole is not contraindicated during pregnancy and since the dose is for 7 days it is likely that the subject would have finished the medication prior to learning she is pregnant. The basic information about the pregnancy will be recorded on the "Pregnancy" case report form. If there are complications during the pregnancy, the complications will be recorded as adverse events in the usual way. The participant is asked to report outcome of the pregnancy. If there is a congenital anomaly in the infant, this will be recorded as a serious adverse event (SAE) in the data forms for the mother (i.e., the study participant).

9.8 Procedures to be followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

All positive results for gonorrhea, chlamydia, and trichomonas will be reported within 24 hours of test completion to the appropriate study personnel by the research laboratory. In the event of a positive test for gonorrhea, chlamydia, or trichomonas study personnel will contact the subject to arrange for treatment. Couples requiring treatment for trichomonas will be dropped from the study. Test results will be reported by name to the local health department as required by law.

9.9 Type and Duration of the Follow-up of Subjects after Adverse Events

All SAEs and AEs will be followed until resolved, stabilized, or the subject is lost to follow up, or the subject completes the study. This will be accomplished by interviews at follow-up visits for mild adverse events or telephone contact by study personnel for more severe adverse events.

9.10 Halting Rules for the Protocol

The PI will request a safety review by the Data and Safety Monitoring Board (DSMB) if there is one unexpected SAE that is attributable to the study drug or if there are 3 or more subjects with study product related severe (grade 3) AEs. If any of the halting rules are met, the study will not continue with the remaining enrollments or study treatments without a review by and recommendations from the DSMB to proceed. DMID retains the authority to suspend additional enrollment and administration of study product during the entire study, as applicable. See section 10.2 for description of the DSMB.

9.11 Stopping Rules for an Individual Participant

A study participant will be discontinued from further Study Agents/Procedures if continued participation in the study would not be in the best interest of the participant. They will be discontinued if they develop a severe (grade 3) AE or a SAE related to the study product. Subjects may voluntarily withdraw their consent for further study participation at any time and for any reason, without penalty.

9.12 Premature Withdrawal of a Participant

Participants will be withdrawn from the study if they require treatment for a positive culture for trichomonas. At that visit all laboratory assessments will be performed as would be performed at a follow-up visit.
Voluntary withdrawal from the study is always an option for participants.

9.13 Replacement of a Participant who Discontinues Study Treatment

These subjects will not be replaced.

10. CLINICAL MONITORING STRUCTURE

10.1 Site Monitoring Plan

All study data forms will be sent to the study PI for review. In conjunction with the protocol data managerqueries will be generated as needed. The protocol data manager will perform remote data entry into the study database. Range checks will be placed on each field to eliminate entry of out-of-range values. Edit check programs will be run periodically on the database to identify and resolve inconsistencies between forms or data collected at different points in time. UAMS staff members will interact with UAB site staff to resolve any data problems.

A study monitor within UAB but external to the UAB STD Program will visit the study sites to evaluate compliance with regulatory issues and to review data by checking source documents.

The monitor will send these monitoring reports to the study site, the Investigator of Record and the Protocol Specialist. In the event that major violations are identified, sites are asked to provide a plan to correct deficiencies within 30 days. If needed, a repeat site visit is conducted. In the event that a site does not correct deficiencies in a pre-determined time frame, the Investigator of Record has the option of taking action against the site. Possible actions include, but are not limited to, suspending enrollment of new subjects until deficiencies are corrected; recommending a decrease in funding to the site; and requiring specific training for site investigators or staff members.

10.2 Safety Monitoring Plan

Safety reviews of this protocol will be undertaken by a Data and Safety Monitoring Board (DSMB). The DSMB will consist of several independent experts (at least 3) that make recommendations to DMID and the study investigators.

An Organizational DSMB teleconference must be held before subject enrollment can begin. The DSMB will meet at the beginning of the study, at routine timeframes during the study and at the end of the study to review and provide recommendations regarding the safety and efficacy endpoints. They will meet at least annually. The DSMB will meet to assess safety and compliance and to review accumulating safety data as well as the overall progress of the study. The DSMB will assess any events that trigger the halting rules or as needed to provide a recommendation to DMID.

The DSMB will operate under the rules of a DMID-approved charter that will be reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will share its recommendations with the PI and DMID.

Meetings of the DSMB will be arranged and documented by DMID.

11. STATISTICAL CONSIDERATIONS

11.1 Overview and Study Objectives

This randomized placebo controlled trial will be conducted as a Phase III study of the efficacy of the treatment of male partners of women with recurrent BV to prevent future recurrences. We hypothesize that BV recurrence rates will be significantly reduced in women whose male partners receive treatment with metronidazole.

11.1.1 Primary Objectives

To compare the recurrence rates of BV between women whose male partners received metronidazole versus placebo.

To evaluate the safety and tolerability of metronidazole in the treatment of male partners of women with recurrent BV.

11.1.2 Secondary Outcome Measures

To determine concordance rates of *Gardnerella vaginalis*, a cultivatable organism which is highly associated with BV, in sexual couples and to examine concordance of *G vaginalis* biotypes and strain patterns.

To archive genital specimens from sexual couples to be used in the future to determine the prevalence and concordance of novel organisms in women with BV and their sexual partners using state-of the-art molecular techniques and to explore potential behavioral and demographic factors associated with these organisms.

11.2 Study Hypotheses

We hypothesize that treatment of male partners of women with BV will reduce the BV recurrence rate among these women, and that there will be a high level of concordance between sexual partners with respect to *G. vaginalis* biotypes.

11.3 Study Population

Women age 18 or greater with a history of recurrent BV whose male partners agree to participate in this randomized trial of male treatment with metronidazole versus placebo.

11.4 Description of the Analysis

Analysis Populations

The primary analysis population will be the "intent-to-treat" population which will include all couples who are randomized and for whom the male partners are provided with the study medication. A secondary analysis of efficacy will be performed on the "per-protocol" population which is comprised of all enrollees whose male partners complete the protocol defined treatment.

Statistical Analysis Plan

The primary endpoint is recurrence at 16 weeks. Fisher's exact test will be used to compare the two treatment arms with respect to the proportion of women who have had a recurrence on or before the 16 week follow-up visit.

Time to recurrence will be assessed using the Kaplan-Meier method and the two treatment arms will be compared with respect to time to recurrent using the log-rank test.

The impact of medication adherence and sexual activities with a new partner (for women and men) will be assessed using the logistic regression model for recurrence rate and the proportional hazards model for the time to recurrence. To address the role of potential confounding factors such as circumcision of the male partner, condom use, vaginal douching, additional sexual partners, and other behavioral measures, logistic regression model and proportional hazard model will be used to assess the difference in BV recurrent rate and time to recurrence, respectively, between intervention arms after adjusting for those factors.

The proportion of couples that are concordant for *G. vaginalis* biotype will be estimated using the binomial proportion and its 95% confidence interval. If the lower bound of the 95% confidence interval for concordance is greater than 50%, then we will conclude that there is a correlation between *G. vaginalis* biotypes between partners. A similar analysis will assess concordance between couples with respect to strains based on biotypes and biochemical identification. As an exploratory analysis we will evaluate concordance between partners with respect to specific biotypes and strains. McNemar's chi-square test will be used to assess the concordance between partners with respect to specific biotypes and strains. Because this aim is exploratory, there are no plans to adjust the significance level for multiple testing.

Randomization and Stratification

Blocked randomization will be used with stratification for clinical site.

11.5 Measures to Minimize Bias

The primary outcome measure is BV recurrence rate measured as the proportion of study participants who do not meet the criteria for clinical cure. Participants who do not complete the course of therapy or who do not return for the cure visit or who meet the criteria for BV recurrence will be considered as BV recurrences. Since this is a superiority study, designating unevaluable or lost participants as recurrences is consistent with intent-to-treat analysis. This approach avoids overestimating efficacy differences between treatment groups that might apply only to a compliant subset of study participants. It is also consistent with ICH guidelines [49].

11.6 Appropriate Methods and Timing for Analyzing Outcome Measures

Female participants will be evaluated for BV recurrence at each of the 3 follow-up visits. If the study participant meets the criteria for BV recurrence (section 8.3) at any of the 3 visits, she will be considered a BV recurrence and will discontinue study participation.

11.7 Sample Size Considerations

Sample Size Estimation

The primary objective of this randomized, placebo-controlled clinical trial is to determine if the recurrence rate for women with a history of recurrent BV is reduced if their male partners are treated with a 7 day dosing regimen of metronidazole.. Based on Sobel et al[48] the recurrence rate for women with BV is 59% after 16 weeks. To detect a reduction in the recurrence rate to 40% at the two-sided 0.05 significance level with power of 0.90 will require 154 couples per arm. To allow for a potential 16% drop-out rate, 368 couples will be enrolled. It is estimated that 40% of the male partners will not keep appointments. Thus, 614 women will need to be screened to enroll 368 couples.

11.8 Participant Enrollment and Follow-Up

There will be two sites, each enrolling equal numbers of subjects. We will plan for a 3-month start-up time for the sites, followed by a maximum of 51 months of enrollment and follow-up, and will then use the final 6 months for data queries, close-out of sites and analysis of results.

11.9 Safety Review

No interim analysis is planned. Reports of adverse events will be monitored by the protocol chair on a regular basis from each site. The DSMB will meet at least annually. If there is a high rate of a particular severe adverse event, this will be further investigated and may lead to stopping the study depending on the circumstances.

11.10 Final Analysis Plan

See Statistical Analysis Plan.

12. QUALITY CONTROL AND QUALITY ASSURANCE

Each site will be provided with a copy of the study Manual of Operations as well as the Quality Management Plan for quality management procedures. Data will be evaluated for compliance with protocol and accuracy in relation to source documents. The study will be conducted in accordance with procedures identified in the protocol. A site initiation visit will be conducted for each site with review of the protocol and study procedures for all involved staff at that site.

Procedures in the study manual must be used at all clinical and laboratory sites. Regular monitoring will be performed according to GCP/ICH.

The investigational site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The Principal Investigator will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The Principal Investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site. The Data Coordinating Center (DCC) will implement quality control procedures beginning with the data

entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

13. ETHICS/ PROTECTION OF HUMAN SUBJECTS

13.1 Institutional Review Board/Ethics Committee

The study will be conducted in full conformity with the Declaration of Helsinki, or with the International Conference for Harmonization Good Clinical Practice (ICH-GCP) regulations and guidelines, whichever affords the greater protection to the subject.

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate ethics review committee or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are placed into use. In both the United States and in other countries, only institutions holding a current U. S. Federal-Wide Assurance issued by the Office for Human Research Protections (OHRP) may participate.

13.2 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects. Consent forms describing in detail the study interventions/products, study procedures and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product. The consent form for the study will include a section for the participant to either agree or disagree to have specimens stored for possible future studies related to BV. The consent forms will be IRB approved and the subject will be asked to read the document, or have the document read to him, and to review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to any procedures being done specifically for the study. The subjects will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the trial. Any participant who consented to future testing of frozen specimens may withdraw consent at any later time. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.2.1 Informed Consent/Assent Process (in Case of a Minor)

Minors will not be enrolled in this study.

13.3 Exclusion of Women, Minorities, and Children (Special Populations)

Women and minorities are included in this study. Children less than 18 years of age will not be enrolled due to the probable difficulty in obtaining parental consent for this group.

13.4 Participant Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the sponsor.

The study monitor, or other authorized representatives of the sponsor, and the FDA may inspect all documents and records required to be maintained by the Investigator, including but not limited to medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

13.5 Study Discontinuation

In the event that the study is discontinued, subjects currently taking study drug will be notified to stop immediately and depending on the individual circumstances will be offered treatment with standard regimens.

13.6 Data Management Responsibilities

The following is a summary of data management activities.

The DCC will format the CRFs based on the content developed by the Protocol Chair. Final approval for the CRFs will be obtained from the Protocol Chair, Data Manager and Study Statistician. All study subjects will be assigned a unique participant identifier that will be required on all CRFs, study collection instruments, and files used in analysis.

The DCC will design a study-specific 21 CFR Part 11 compliant clinical database. Error specifications will be written in consultation with the statistician to check for accuracy, completeness, and consistency on the CRFs. The error specifications will be run regularly and will produce 'discrepancies' that will be sent to the Data Manager to resolve with the sites.

Data will be entered and verified through double data entry. Illegible, out-of-range, or other data that cannot be entered will be flagged and sent to study staff for clarification.

The DCC will provide reports on study progress. Adverse event coding will be done by the Protocol Chair.

Data freezes will be requested by the statistician for all interim and final reports. Thorough cleaning and closure of subject data will be performed at study close-out. Prior to closure, all participant data will be complete or accounted for.

14. DATA CAPTURE METHODS

All data will be recorded on CRFs created by the DCC. Each CRF must be initialed and dated by the study staff member who completed it.

The source documents include signed informed consent forms, laboratory reports, and patient records. These documents should be maintained in the participant's file, and should be available for review during monitoring visits.

Completed CRFs will be emailed encrypted to Dr. Lee to be entered into the database. The original CRFs will be retained at the site in the participant's clinic study file. The original and sent copies must match in every detail. Therefore, no changes may be made on any copy after the CRFs have been sent . Before the CRFs are sent, any change to the data should be dated and initialed by the person making the change. Use of opaque correction fluid is not permitted. Changes made to data after the CRFs are sent must be documented.

All CRFs should be stored securely. File cabinets should be locked, maintained, and viewed by study staff only. No names or personal identifying information will be contained on the CRFs. Access to all CRFs will be restricted to authorized personnel.

CRFs that have been sent will be filed in participant file folders, ordered by participant number, in a file cabinet or drawer designated for the study. These CRFs are stored in a locked file room at UAB.

All data will be stored in UAMS's 21 CFR Part 11 compliant clinical database.
Error specifications will be developed to check data for accuracy and completeness. All discrepancies with the error specifications will be posted for the sites using an Internet-based system, which allows for electronic communication between site staff and DCC staff. DCC staff will update the database based on the investigator's or designee's written response. Investigators or designees must keep copies of all discrepancies stapled to the CRF in the participant's study file.

14.1 Types of Data

Data for this study includes safety, laboratory, and outcome measures as per section 8 (Study Procedures/Evaluations).

14.2 Timing/Reports

The DCC will generate reports that provide information helpful for operational decisions. The contents and frequency of these reports will be based on the study team's requests. Ad hoc reports will also be generated as needs arise. The contents will be based on requests from the study team.

CDM will produce data freezes for the study based on requests from the Protocol Chair or Biostatistician. Data will be uploaded to a secure web-portal and access to download those data will be granted via login and password to individuals listed in the request for a data freeze.

14.3 Study Records Retention

All paper CRFs and source documents will be retained after completion of the trial for a minimum of five years. Records may not be destroyed without written permission from NIAID/DMID. The investigator may withdraw from the responsibility to maintain records and transfer custody of the records to another person who will accept responsibility for them. Notice of transfer must be given to the sponsor preferably before, but no more than ten days after, the transfer.

14.4 Protocol Deviations

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

These practices are consistent with Good Clinical Practice:

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

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled Protocol-required activity. The sites will report protocol deviations by fax to Investigator of Record. The BV Study Manual will provide information on specific deviations and how to report deviations. Additional deviations must be promptly reported to the Protocol Chair for determining if it meets the criteria for an actual protocol deviation.

All deviations from the Protocol must be addressed in study subject source documents. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI and study staff are responsible for knowing and adhering to their IRB requirements.

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Appendix A

Procedures - Female		Screening/ Enrollment	Day 21-28	Day 56-63	Day 112-119	Premature Discontinuation
Signed Consent Form		X				
Assessment of Eligibility Criteria		X				
Review of Medical History		X				
Completion of Study Questionnaire		X	X	X	X	X
Review of Concomitant Medications		X	X	X	X	X
Randomization & Administration of Study Medication		X				
Physical Exam	Symptom-Directed	X	X	X	X	X
Assessment of medication –related Adverse Events			X			
Assessment of study procedure-related adverse events			X	X	X	X
Laboratory	Amsel Criteria	X	X	X	X	X
	Vaginal wet prep for motile trichomonads	X	X	X	X	X
	Urine Pregnancy test	X				
	Vaginal Gram stain for Nugent score	X	X	X	X	X
	Vaginal swab for *T. vaginalis* culture	X				

Procedures - Female		Screening/ Enrollment	Day 21-28	Day 56-63	Day 112-119	Premature Discontinuation
	Endocervical swab for NAATS for gonorrhea and chlamydia	X				
	Vaginal swab for *G. vaginalis* culture and archive	X	X	X	X	X
	Urine specimen for detection of biofilm	X	X	X	X	X
Other Procedures	Review study medication adherence		X			

(X) – As indicated/appropriate.
At baseline, all procedures should be done before study intervention.

Procedures - Male		Screening/ Enrollment	Day 21-28	Premature Discontinuation
Signed Consent Form		X		
Assessment of Eligibility Criteria		X		
Review of Medical History		X		
Completion of Study Questionnaire		X	X	X
Review of Concomitant Medications		X	X	X
Randomization & Administration of Study Medication		X		
Physical Exam	Symptom-Directed	X	X	X
Assessment of Medication-related Adverse Events			X	
Assessment of Study Procedure-related Adverse Events			X	X
	Swabs of coronal sulcus and meatus for G. vaginalis culture and archive	X	X	
	10 ml first fraction urine for NAATS GC/CT and Gardnerella biofilm and culture	X		

Procedures - Male		Screening/ Enrollment	Day 21-28	Premature Discontinuation
Other Procedures	Review study medication adherence	X	X	

(X) – As indicated/appropriate.
At baseline, all procedures should be done before study intervention.

Appendix B: Site Roster

City, state	Site name	Site Investigator
Birmingham, AL	University of Alabama at Birmingham	Jane R. Schwebke, MD
Detroit, MI	Wayne State University	Jack Sobel, MD

Randomized Clinical Trial of Treatment of Male Partners of Women with BV

Statistical Analysis Plan
Draft Version

Prepared by:

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I. Study Objectives

Primary Study Objective: to determine if the treatment of male partners of women with recurrent BV significantly decreases the recurrence rate of BV in the female.

Secondary Study Objective: to determine the concordance rates of the biotypes/strains of *Gardnerella vaginalis* a cultivatable organism highly associated with BV in sexual couples; and to archive genital specimens from sexual couples to be used in the future to determine the prevalence and concordance of novel organisms in women with BV and their sexual partners using state-of–the art molecular techniques and to explore potential behavioral and demographic factors associated with these organisms

II. Analysis Populations

The primary analysis population will be the "intent-to-treat" (ITT) population which will include all couples who are randomized and for whom the male partners are provided with the study medication. A secondary analysis of efficacy will be performed on the "per-protocol" (PP) population which is comprised of all enrollees whose male partners complete the protocol defined treatment.

III. Blind Review of the Data

A blinded review is planned at the end of the study to determine:

- Exclusion of data or participants from analysis populations
- Definition, identification and timing of outcome measures (BV recurrence in women)
- Definition of and methods for dealing with protocol deviations

IV. Analysis of Baseline Participant Characteristics

Analysis Populations: ITT and PP as defined in section II.

Randomization: The frequency and percentages of male participants randomized on the study will be tabulated by treatment arm and by clinic.

Age of participant: Summary statistics (N, mean, median, and standard deviation, minimum and maximum) will be generated for each treatment arm and the two treatment arms combined for male partners and female partners. The t-test for independent samples will be used to compare the two treatment arms with respect to age for each gender. Two-way analyses of variance will be used to compare the two treatment arms across clinics with respect to age for each gender.

Ethnicity (Hispanic, Latina or of Spanish Descent): The frequency and proportion of participants that fall into each of the ethnicity categories (yes, no) will be generated for the each treatment arm and for both treatment arms combined for male and female partners. Fisher's exact test will be used compare the two treatment arms with respect to ethnicity for male and female partners. The Cochran-Mantel-Haenszel test will be used to compare the two treatment groups with respect to ethnicity across clinics for male and female partners

Race: The six racial categories (Black or African American, White, Hawaiian or other Pacific Islander, Asian, Native American or American Indian, or Other) are dichotomous variables. A derived racial category variable will be created that assigns a single racial category to each participant. Participants that check only one of the six racial categories will be assigned to that racial category, and those that check multiple racial categories will be assigned to a category entitled "more than one race." The frequency and proportion of participants on the 7 racial categories will be generated for the each of the treatment groups. It is anticipated that these categories may be further condensed into three categories: Black or African American, White, and Other (which is comprised of the other 4 racial categories). Pearson's chi-square analyses will be used to compare the two treatment arms with respect to the distribution of race based on these three categories for each gender. The Cochran-Mantel-Haenszel test will be used to compare the two treatment groups with respect to racial category across clinics for each gender.

Marital Status: Pearson's chi-square test will be used to compare the two treatment arms with respect to marital status for male and female partners. The Cochran-Mantel-Haenszel test will be used to compare the two treatment arms with respect to marital status across clinics for each gender.

Educational Level: The last grade completed variable will be categorized into the following educational levels:

- ≤ 8 = 8^{th} grade or less
- 9-11= some high school
- 12 = completed high school (or GED)
- 13-15 = some postsecondary school education
- 16= completed college
- >16 = post-college education

The frequency and proportion of participants that fall into each of the educational levels will be generated for each treatment arm and for both treatment arms combined for male and female partners. Pearson's chi-square tests will be used to compare the two treatment arms with respect to distribution of educational level. The Cochran-Mantel-Haenszel test will be used to compare the two treatment groups with respect to educational level across clinics for male and female partners.

Sexual History: The t-test for independent samples will be used to compare treatment groups with respect to the age at sexual debut, days since last sexual activity, and number of partners (total, occasional, regular, new) over various time periods (previous 30 days, previous 3 months, lifetime) for male and female partners. Pearson's chi-square test will be used to compare the two treatment groups with respect to the proportions of participants whose most recent partner was a new partner, frequency of condom use in the last 3 months and reported use of a condom during the last sexual encounter for male and female partners.

History of Sexually Transmitted Diseases (STDs): Pearson's chi-square test will be used to compare the two treatment arms with respect to the number of times participants have previously had the following STDs: gonorrhea, chlamydia, MPC/cervicitis, trichomonas, syphilis and BV, and whether they had a history of genital warts or herpes, for female and male partners.

Symptoms: Fisher's exact test will be used to compare the two treatment groups with respect to the proportions of participants who report the following symptoms for male and female

partners: drip or discharge, urinary frequency, burning on urination, genital soreness/swelling/irritation, genital itching, abdominal pain, genital ulcers/lesions/sores, odor/small and skin rash.

V. Analysis of Participant Follow-up

A. Analysis Population: ITT

B. Presentation of Participant Flow

The following information will be presented for each treatment arm, and for each clinic within each treatment arm: number of individuals screened for study participation; the number of enrolled participants and the percentages of screenees that enrolled.

For male partners, the proportions of enrollees who completed treatment and who attended the day 14-21 visit will be summarized for each treatment arm and the two treatment arms will be compared using Fisher's exact test. Female partners are to attend three follow-up visits (day 21-28, day 56-63, and day 112-119); however, if BV present is at a visit, the participant will be withdrawn from the clinical trial.

C. Presentation of Protocol Violations

Summary tables for treatment arm and clinic will be used to present the number and percentage of protocol violations for female and male partners. A line listing of protocol violations will be included as an appendix to the report.

D. Presentation of Discontinuations

For each treatment and clinic, the number and percentage of male study participants who discontinue prior to completing treatment will be summarized. The Cochran-Mantel-Haenszel chi-square test will be used to compare the two treatment arms with respect to the proportions of male participants who discontinued treatment prematurely.

For each treatment and clinic, the number and percentage of female study participants who discontinued prior to being assessed for BV recurrence will be summarized. The Cochran-Mantel-Haenszel chi-square test will be used to compare the two treatment arms with respect to the proportions of female participants who discontinued study participation prematurely.

VI. Primary and Secondary Objectives

The primary study objective is to determine if the treatment of male partners of women with recurrent BV significantly decreases the recurrence rate of BV in the female. Female participants will be classified as having had a recurrence of BV if they met the protocol definition of recurrence at any of the follow-up visits. Fisher's exact test will be used to compare the two treatment arms with respect to the proportion of female participants who experience a recurrence of BV. For female participants who experience a BV recurrence, the time to the first occurrence of BV recurrence will be defined as the time from the date of randomization of the male partner to the date of the first follow-up visit in which BV was present. For female participants who do not experience a BV recurrence, their censored time to first occurrence of BV recurrence is defined as the time from the data of randomization of the male partner to the date of the last follow-up visit in which criteria for BV were not met. For both treatment arms, the Kaplan-Meier estimates of the cumulative proportion of participants without BV will be estimated and displayed graphically. The log-rank test will be used to compare the two treatment arms with respect to the time to first BV recurrence.

The secondary objectives are to determine the concordance rates of the biotypes/strains of *Gardnerella vaginalis,* a cultivatable organism highly associated with BV in sexual couples, and to archive samples. The Kappa statistic will be used to assess the agreement between male and female sexual partners with respect to the biotypes/strains of *Gardnerella vaginalis*.

Graphs/Plots: Graphical summaries for cumulative number of patients with BV recurrence against time for each treatment arm. Kaplan-Meier curves will be generated for time to first BV recurrence for each treatment arm.

Missing Values: There are no plans to impute data for analysis.

Subgroup Analyses: Subgroup analyses by race/ethnicity will be performed for the primary and secondary outcomes.

VII. Analysis of Safety Data

Adverse Events at the Event Level: For each type of adverse event, the number of occurrences will be tabulated by treatment arm and gender based on severity grade, whether or not the adverse event is classified as serious, whether or not the adverse event was treated, and the outcome of the adverse event.

Adverse Events at the Event Level Associated with Study Product: For each type of adverse event, the number of occurrences associated with study product will be tabulated by treatment arm and gender based on severity grade, whether or not the adverse event is classified as serious, whether or not the adverse event was treated, and the outcome of the adverse event.

Serious Adverse Events at the Event Level: For each type of serious adverse event, the number of occurrences will be tabulated by treatment arm and gender based on severity grade, whether or not it was treated, and the outcome of the serious adverse event.

Serious Adverse Events Associated with Study Product at the Event Level: For each type of serious adverse event, the number of occurrences associated with study product will be tabulated by treatment arm and gender only based on severity grade, whether or not it was treated, and the outcome of the serious adverse event.

Adverse Events at the Participant Level: For each type of adverse event, each participant will be categorized by the episode of the event with the highest severity. The number of patients who experience each adverse event will be tabulated by treatment arm and gender based on severity grade, whether or not the adverse event is classified as serious, whether or not the adverse event was treated, and the outcome of the adverse event.

Adverse Events at the Participant Level Associated with Study Product: For each type of adverse event associated with study product, each participant will be categorized by the episode of the event with the highest severity. The number of patients who experience each adverse event associated with study product will be tabulated by treatment arm and gender

based on severity grade, whether or not the adverse event is classified as serious, whether or not the adverse event was treated, and the outcome of the adverse event.

Serious Adverse Events at the Participant Level: For each type of serious adverse event, each participant will be categorized by the episode of the event with the highest severity. The number of patients who experience each serious adverse event will be tabulated by treatment arm and gender based on severity grade, whether or not the serious adverse event was treated, and the outcome of the serious adverse event.

Serious Adverse Events at the Participant Level Associated with Study Product: For each type of serious adverse event associated with study product, each participant will be categorized by the episode of the event with the highest severity. The number of patients who experience each serious adverse event will be tabulated by treatment arm and gender based on severity grade, whether or not it was treated, and the outcome of the serious adverse event.