Statistical Analysis Plan

Treating male partners of women with BV to reduce recurrence: randomised controlled trial

Version: *1*

Dated: *5th October 2023*

Protocol number/Alfred HREC number: *506/18*

Trial Registration: *ACTRN12619000196145*

Melbourne Sexual Health Centre, Alfred Health

Central Clinical School, Monash University

1. PROJECT SUMMARY

Full Title	Treating male partners of women with BV to reduce recurrence: a randomised controlled trial
Short Title	Step Up Trial
Protocol Version	Version 7.0, 14/09/2020
HREC	Alfred Hospital Ethics Committee: number 506/18
Main Study Site	Melbourne Sexual Health Centre 580 Swanston Street, Carlton, VIC 3053
Study Participants	Women with BV and their male partners
Planned Sample Size	342 couples
Interim Analysis	150 randomised couples
Chief Investigator	Professor Catriona Bradshaw
Coordinating Principal Investigator	Dr Lenka Vodstrcil
Trial Steering Committee	Prof Catriona Bradshaw, Dr Lenka Vodstrcil, Prof Christopher Fairley, Prof Kathy Petoumenos, Prof Matthew Law and Prof Deborah Bateson
Investigators	Alfred Health, Melbourne Sexual Health Centre Prof Catriona Bradshaw; Prof Christopher Fairley; Prof Marcus Chen Monash University, Central Clinical School Dr Lenka Vodstrcil, Dr Jade Bilardi, Dr Erica Plummer, Prof Eric Chow University of Melbourne Prof Jane Hocking Royal Women's Hospital Prof Suzanne Garland; Dr Gerald Murray; Dr Jennifer Danielewski University of New South Wales, Kirby Institute Prof Kathy Petoumenos; Prof Basil Donovan; Prof Matthew Law; Prof John Kaldor University of Sydney Prof Deborah Bateson Family planning sites (NSW, SHINE, Sexual Health Victoria) Dr Kathleen McNamee; Dr Sally Sweeney; Dr Clare Boerma; Dr Amy Moten
Funding	NHMRC Project Grant (APP1138165) NHMRC Investigator Grant (to CSB; GNT1173361)

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3. ABBREVIATIONS

AE	Adverse event
BV	Bacterial Vaginosis
BVAB	BV associated bacteria
CONSORT	Consolidated Standards of Reporting Trials
DSMB	Data Safety Monitoring Board
GCP	Good Clinical Practice
IUD	Intrauterine device
HREC	Human Research Ethics Committee
mITT	modified intention-to-treat
MSHC	Melbourne Sexual Health Centre
MTZ	Metronidazole
NS	Nugent Score
PPA	per protocol analysis
PID	Pelvic inflammatory disease
RCT	Randomised controlled trial
SAE	Serious adverse event
STI	Sexually transmitted infection

4. INTRODUCTION

Bacterial vaginosis (BV) is the most common vaginal syndrome in women, affecting 30% of women globally(1). BV is not caused by a single agent, but is characterised by marked disruption of the vaginal microbiota with high loads of anaerobic BV-associated bacteria (BVAB) and depletion of key *Lactobacillus* species(2). BV increases a woman's risk of acquiring HIV and STIs, transmitting HIV, preterm delivery, low birth weight, and miscarriage(3-6). It has a profound impact on self-esteem, sexual relationships, and quality of life(7). Currently recommended antibiotics cure 80% of women within 1 month(8), but over 50% of women experience recurrence within 3–6 months(9, 10). BV imposes a heavy burden on the healthcare system, as recurrences result in multiple presentations to clinical services, and its obstetric and reproductive sequelae have very significant implications for healthcare expenditure(6, 11).

In the last 20 years, there have been no advances in BV cure. Our research suggests a clear reason for this – we have only been treating women. There is now overwhelming epidemiological and microbiological evidence to indicate that sexual transmission is involved in BV acquisition and recurrence: **1)** BV has the epidemiological characteristics of an STI, it is associated with inconsistent condom use, higher numbers of partners, and early age of sexual debut(12); **2)** Re-exposure to a regular male partner and lack of condom use are associated with BV recurrence(9, 13); **3)** Male partners of women with BV have BVAB in their urethral and penile microbiota more often than male partners of women without BV(14-16). **4)** Male circumcision reduces the abundance of BVAB in men and the development of BV in their female partners(17). These data strongly suggest that male carriage of BVAB and their reintroduction to treated women is playing a key role in BV recurrence.

RESEARCH AIMS & HYPOTHESIS: We hypothesise that male carriage and sexual transmission of BVAB plays a key role in the high rates of BV recurrence in treated women, and that concurrent oral and topical antibiotic therapy of male partners will clear these species from male partners, reduce the risk of reinfection and BV recurrence in women, and be cost-effective.

APPROACH: This a pragmatic, open-label, RCT involving couples, where the female index has BV diagnosed at large sexual health and family planning services in Australia.

The purpose of this analysis plan is to:
- ensure that the analysis is appropriate to the aims and design of the trial;
- reduce bias that may arise from knowledge of results as the analysis is conducted; and
- facilitate transparency and enable others to perform or reproduce the analysis.

The researchers and biostatisticians involved in developing the statistical analysis plan have had no access to data and will not analyse the data until the database is locked. The statisticians preparing the interim statistical results were blinded to allocation.

5. STUDY METHODS

5.1 TRIAL DESIGN

Step Up is a multicentre open-label randomised controlled trial of female treatment alone (standard of care) *vs* female and male partner treatment for prevention of BV recurrence(18). All women will receive first-line therapy. The trial is implemented in accordance with Good Clinical Practice (GCP guidelines) and will be reported according to the CONSORT statement. The trial takes place at 5 sites in Australia (Vic, NSW, SA), was approved by Human Research Ethics Committees governing each trial

site (Multi-site clinical trial, with reviewing HREC: Alfred Hospital Ethics committee 506/18), and is registered on the Australia and New Zealand Clinical Trial Registry (ACTRN12619000196145).

5.2 STUDY POPULATION

Any woman with BV diagnosed at baseline (3-4 Amsel's criteria, including pH >4.5, Amine+, vaginal discharge, Clue cells >20%[1], NS≥4[1]) and with a male RSP >8 weeks will be assessed for eligibility at her visit. Her male RSP will be contacted, and if eligible, offered enrolment within a week of the female being enrolled.

The combination of at least 3 Amsel's criteria and a NS between 4 and 10 will be used to diagnose BV in this trial.
The Amsel's criteria (19) include:

i) presence of a characteristic homogenous vaginal discharge;
ii) elevated vaginal pH >4.5;
iii) positive amine test (noticeable fishy odour);
iv) ≥20% Clue cells on microscopy.

The Nugent method scores bacterial morphotypes on vaginal Gram stain (20):

i) a NS of 0–3 represents a lactobacillus dominant "healthy" vaginal microbiota,
ii) a NS of 4–6 an intermediate state with few lactobacilli and increased anaerobes,
iii) a NS of 7–10 is classified as Nugent-BV.

Combining a NS of 4–10 with at least 3 Amsel's criteria optimises the clinical diagnosis of symptomatic BV.

Recruitment of women at sites without onsite laboratory facilities: As some women are recruited from sites where microscopy is not available, women at these sites will be eligible for enrolment if they have the following three Amsel's criteria:

i) a vaginal pH >4.5 *and*
ii) a noticeable fishy odour on examination *and*
iii) typical BV vaginal discharge on examination.

Note that the presence of a fishy odour noted by the clinician during examination will suffice for a positive amine test and the formal addition of potassium hydroxide to secretions is not required.

[1]A slide of vaginal secretions will be collected from women recruited without microscopy onsite and sent to MSHC for Nugent score and Clue cell reporting. Women with a NS<4 will be deemed a screening failure if the male has not been randomised and the couple a protocol violation if the male has enrolled and already been randomised. If either occurs, any specimens will be discarded appropriately. Non-evaluable participants will be replaced.

5.3 INCLUSION CRITERIA

Females will be eligible for inclusion if they fulfil the following criteria:

- Aged 18 years to pre-menopausal (defined as still menstruating within the last 12 months); *and*
- have symptomatic BV defined as ≥3 Amsel's criteria and a Nugent score (NS) of 4–10; *and*
- have a regular male partner for the prior 8 weeks who is willing and eligible to participate; *and*
- are treated with a first line recommended antimicrobial therapy for BV (including the following 3 options: 7 days of oral metronidazole 400mg bd is the preferred option but if not tolerated or is

contraindicated 7 days of topical vaginal clindamycin cream or 5 days of intravaginal metro gel is acceptable); *and*
- have sufficient English language proficiency to understand the study requirements; *and*
- provide written informed consent; *and*
- are willing and able to comply with protocol requirements including clinic visits at 4 and 12 weeks ; *and*
- do not meet any of the exclusion criteria listed below.

Males nominated as the regular partner of a woman fulfilling inclusion criteria will be *eligible* if they:

- are aged 18 years or above; *and*
- enrol within a week of their partner with confirmed BV; *and*
- have sufficient English language proficiency to understand the study requirements; *and*
- provide written informed consent; *and*
- are able to comply with protocol requirements; *and*
- do not meet any of the exclusion criteria or contraindications listed for metronidazole.

5.4 EXCLUSION CRITERIA

Participants will be deemed ineligible for the study if they meet any of the following exclusion criteria:

- are a current sex worker; *or*
- have concurrent sexual partners; *or*
- are known to be HIV positive

Additionally, females will be excluded if they:

- are being currently treated for pelvic inflammatory disease with 14 days of metronidazole +/- ceftriaxone; *or*
- are not being treated with first line recommended antimicrobial therapies for BV at baseline or
- are pregnant or breast feeding; *or*
- have a hypersensitivity or a contraindication to all first line drugs used to treat BV: nitroimidazoles and clindamycin (note they can be enrolled if they are able to take one of these agents)

Additionally, males will be excluded if they:

- have a hypersensitivity or a contraindication to either nitroimidazoles or clindamycin (they cannot be enrolled if they are allergic to even just one of these drugs); *or*
- have significant fungal balanitis requiring a topical antifungal cream (antibiotics are likely to worsen candida balanitis).

5.5 INTERVENTION

All female participants will receive first line recommended treatment, which is oral metronidazole (MTZ) 400 mg twice daily for 7 days, or if contraindicated, either alternate first line therapy: 7-day regimen of topical vaginal 2% clindamycin or 5 days of vaginal metronidazole gel (21).

Male partners randomised to the Intervention Group will receive oral MTZ 400mg twice daily and topical 2% clindamycin cream to be applied to the glans penis and upper shaft (under the foreskin if uncircumcised) twice daily for 7 days.

5.6 COMPARATOR/CONTROL

As topical placebos are highly likely to affect the penile microbiota and therefore influence the efficacy estimates, males randomised to the *Control Group* will not receive a placebo. The control for this trial is therefore current standard of care: treatment of the female with first line antibiotic therapy (no male treatment). This trial will therefore be an open-label randomised controlled trial.

5.7 FOLLOW UP

Women and their male partner will be followed for up to 12 weeks (12 weeks - no BV recurrence and <12 weeks if BV recurrence occurs prior to this point). Women will have a combination of home and clinic follow up and males will be able to complete all follow up at home.

6. RANDOMISATION AND SEQUENCE GENERATION, ALLOCATION CONCEALMENT AND BLINDING

A computer-generated randomisation sequence in a block of 6 and 1:1 ratio with stratification by intrauterine device (IUD)-use, circumcision status and recruitment site will be created by an independent biostatistician.

As this is an open-label randomised controlled trial, both the researchers and participants will know which group they were in. All laboratory staff performing Nugent scoring, Clue cell reporting and amine testing will be blinded to randomisation group for all clinic visits with onsite laboratories. The molecular biologists who performed Nugent scoring and noted Clue cells on slides collected at home and sent back via the post will be blinded to allocation group.

7. OUTCOME MEASURES AND DEFINITIONS

7.1 PRIMARY OBJECTIVE

To determine whether the *combined antibiotic treatment of male partners* of women receiving therapy for BV significantly reduces the risk of BV recurrence compared to *no male treatment*, in the 12 weeks after randomisation.

7.2 STUDY OUTCOMES AND DEFINITIONS

Primary efficacy endpoint:

BV recurrence defined as **≥3 Amsel's criteria & Nugent score (NS)=4–10 within 12 weeks** [a]

The Primary Analysis will be a modified intention-to-treat (mITT) analysis. Women will be analysed as randomised and will be eligible to be included in the mITT if they:

- returned for ≥1 clinical visit
- took one or more doses of antimicrobial therapy
- are part of the evaluable population (excluding people deemed to be non-evaluable or protocol violations as defined below)

Secondary efficacy endpoints:

1. BV recurrence defined as NS≥7 within 12 weeks [b]
2. BV recurrence defined as ≥3 Amsel's criteria within 4 weeks [a]
3. BV recurrence defined as NS≥7 within 4 weeks [b]
4. Recurrence of a microbiota dominated by BV-associated bacteria within 12 weeks [b]

5. Recurrence of a microbiota dominated by BV-associated bacteria within 4 weeks [b]

[a] requires onsite visit for this endpoint

[b] able to reach this endpoint on self-collected home swabs

Other measured outcomes:

1. Adherence to antibiotic therapy and predictors of adherence in men
2. Acceptability of antibiotic therapy to men
3. Adverse events in men (safety analysis)

Non-Evaluable subjects:

Subjects who enrol (and the male partner is randomised) but are then rendered non-evaluable include:

1. Female participants with a baseline Nugent score <4 following randomisation – this will occur in sites without onsite Nugent scoring facilities when slides are sent away for scoring, i.e. family planning or sites reporting Clue cells but no Nugent score;
2. Female participants recalled within the first week of enrolment for treatment of PID requiring 14 days of metronidazole +/- ceftriaxone.

Protocol violation for primary mITT and PPA (per protocol analysis; may still be eligible for some of the other analyses):

1. Female does not return for clinical assessment (i.e. doesn't come back for any follow up visits);
2. Female formally withdraws at any point during the study and provides no data at all or states previous collected data cannot be used;
3. Male formally withdraws prior to day 8 endpoint (NB. If he withdraws after providing day 8 data on adherence and his partner continues, her data could still be used in all analyses and his data could contribute to safety analyses);
4. Male partner does not provide a signed PICF;
5. Female or male partner (if in treatment group) report that they took no antibiotic therapy.

Protocol deviation for specific analyses:

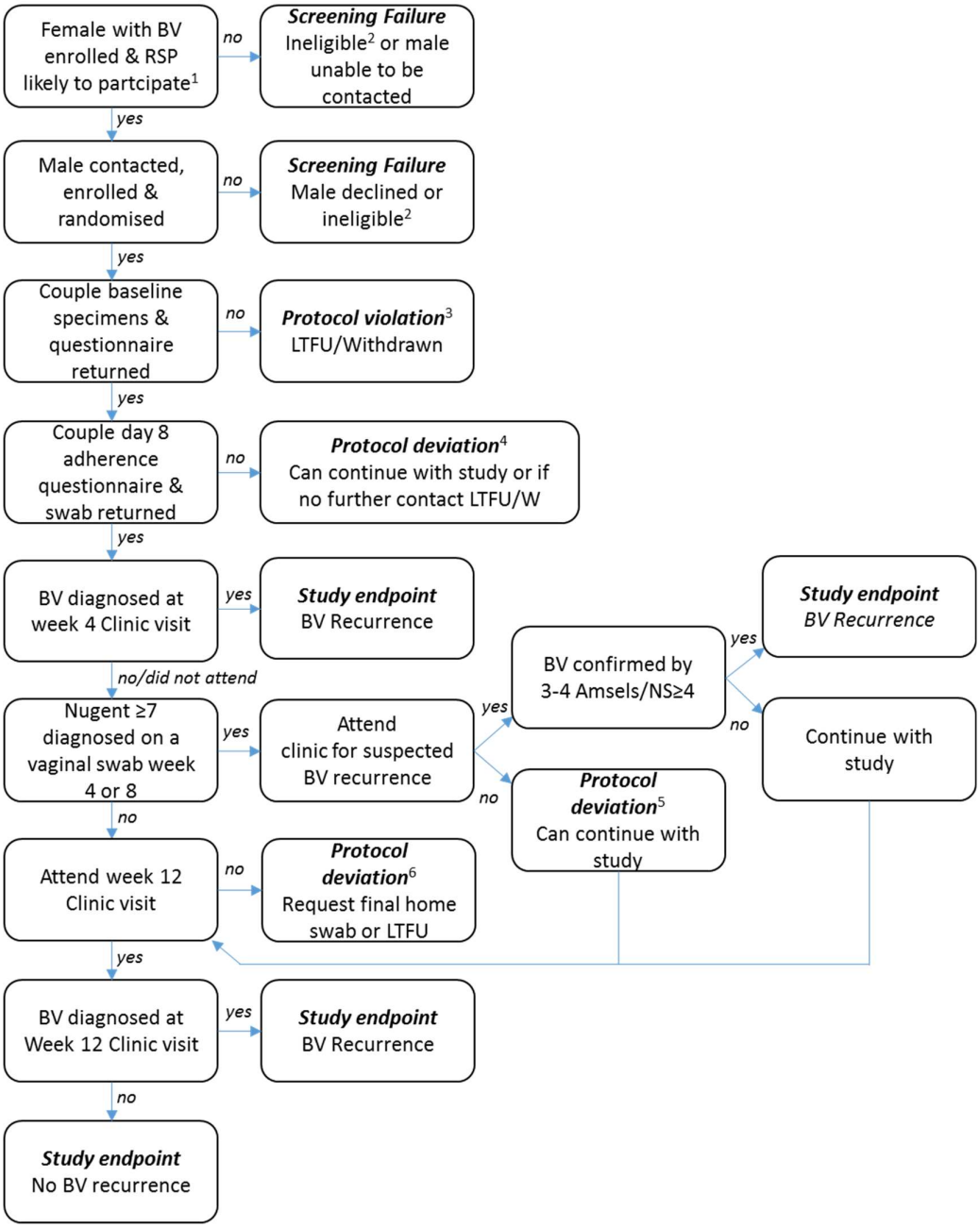
1. *Safety analysis:* if a male participant does not return their day 8 questionnaire they will not be eligible for the safety analysis which measures adverse effects
2. *Analyses for secondary outcomes:* If a female participant does not attend any clinic visits but returns ≥1 swab, she will be eligible for the Nugent and microbiota secondary endpoints

Specific Comments:

1. If a female attends at week 4 but not week 12, she is still eligible for the primary endpoint analysis, which is recurrence within 12 weeks but will contribute less person time to this analysis than a participant who stays in until week 12.
2. *Adherence:* For the mITT analysis (primary analysis) participants need to take one or more doses of antimicrobials (including men if randomised to the intervention group). For the *per protocol* analysis participants need to take ≥10 doses of oral metronidazole or topical clindamycin (in males) and ≥5 doses of intravaginal clindamycin or intravaginal metronidazole gel in females if these alternative first line regimens are prescribed.

8. ALGORITHM FOR DEFINITIONS

The study involves enrolment of the woman with BV prior to recruitment of her male partner who is then randomised once consent is obtained. Participants are then followed for up to 12 weeks as per the algorithm below:



[1]been together ≥8 weeks, indicated/confirmed on phone that RSP likely to participate; [2]refer to eligibility criteria; [3]*protocol violation:* may be ineligible for modified ITT (mITT, primary analysis) or per protocol analysis (PPA); [4]eligible for mITT and PPA if week 4 and/or 12 provided, not eligible for safety analysis; [5]if female participant never attends clinic visits but returns ≥1 swab, eligible for secondary endpoints; [6]if female attends at week 4 but not week 12, eligible for mITT; if no visits but return a swab, eligible for secondary endpoints; if no visits or swabs, *protocol violation* (see above).

9. DATA COLLECTION

9.1 AT RECRUITMENT

Participants will complete a paper-based questionnaire at recruitment covering demographics, sexual behaviours, contraceptive use, and information regarding prior BV (females only). This includes: number of lifetime partners; types of sexual activity (oral, penile-vaginal, anal); use of contraceptives (including any hormonal contraceptive, IUD, condom use if applicable); details about vaginal douching (female only) and smoking practices.

Clinic data collected at recruitment from women includes Amsel's criteria (discharge, odour, vaginal pH, presence of Clue cells), Nugent score, presence of yeasts, and any additional STIs detected.

9.2 FOLLOW UP: DAY 8 (POST-TREATMENT) AND WEEKS 4, 8 AND 12

Participants will be instructed to complete paper-based questionnaires the day after treatment completion (all women, and all couples randomised to treatment) or the day after the female partner completes treatment (all male controls). Paper-based questionnaires will also be completed at each monthly follow up point (weeks 4, 8 and 12). The questionnaires at weeks 4, 8 and 12 are identical, and if the participant reaches study endpoint prior to week 12, this constitutes their "endpoint" questionnaire. If a participant attends for an interim review visit, they will also be instructed to complete the "follow up" questionnaire.

The follow up questionnaires collect data (where applicable) detailing sexual behaviours (e.g. frequency & types of sex, condom use, change in partnerships, new partnerships etc), contraceptive use, menstrual dates, genital symptoms, and any interim treatments. The day 8 questionnaire also collects data on adherence and any experienced adverse effects, as below.

At all clinic visits (week 4, 12 or interim review visit), additional data collected from women includs Amsel's criteria, Nugent score, presence of yeasts and additional STIs detected.

9.3 ADVERSE EVENTS REPORTING & DRUG ADHERENCE MONITORING

In the day 8 questionnaire, participants are asked to report all adverse events (AEs), adherence, and reasons for non-adherence.

Participants are also instructed to contact the study team if they experience any unexpected AEs. In addition, data on serious AEs (SAEs) that is recorded by participants in the Day 8 questionnaire will be relayed to the chief investigator.

An SAE is defined as any untoward medical occurrence that:

- results in death;
- is life-threatening;
- requires inpatient hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability/incapacity;
- or consists of a congenital anomaly or birth defect.

Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

All adverse events and adherence are based on self-report.

If reported, a SAE occurring to a participant will then to be reported to the Alfred Hospital Ethics Committee in a timely manner, using the Alfred SAE Report Form: (https://www.alfredhealth.org.au/contents/resources/research/SAE-report-form.docx). N.B. There

were no related SAE reported in the prior pilots of male partner treatment (Alfred Human Ethics Number 264/15).

9.4 DATA SAFETY AND MONITORING BOARD

An independent Data Safety and Monitoring board was formed at the time of protocol publication, and comprises two medical practitioners with knowledge of sexual health and an independent statistician. The DSMB members were invited to review the DSMB Terms of Reference when they joined.

10. STATISTICS AND ANALYSES

Some anticipated statistical analyses (including original sample size calculations) were included in the published protocol (18).

As outlined in the published study protocol, this detailed statistical analysis plan has been written close to final data lock, but before any analyses have been performed, and defines all analyses to be performed in greater detail.

10.1 SAMPLE SIZE [as published (18)]

BV recurrence rates after recommended therapy are high. Sobel *et al* reported 50% recurrence within 3 months, and our group 52% recurrence within 6 months (9, 10). We have chosen *12 weeks of follow up, as we believe this will be the optimal duration over which to measure the direct effect of treatment of the male partner on BV recurrence.* Importantly this duration of follow-up is considered appropriate as it balances sufficient time for BV recurrence to occur but minimizes the risk of introduction of new untreated partners into the "treated partnership". To obtain an estimate of the efficacy that we could expect from male partner treatment, we used the reduction in risk reported from consistent condom use (50%) (9, 13) and male circumcision (40–60%) (17). Prior to commencing the trial we assumed a 12-week BV recurrence rate in the control group of 40%, and conservatively estimate that treating male partners will reduce the risk of BV recurrence in females by 40%. This effect size is at the lower end of that reported from consistent condom use and circumcision and is supported by our pilot. Smaller effect sizes are not likely to be of clinical significance or markedly reduce important sequelae.

With a total sample size of 290 heterosexual couples (145 per arm), we will have 80% power to detect a 40% reduction in BV recurrence from 40% in the control group to 24% in the intervention group (2-alpha=5%). Assuming a 12 week loss to follow up of 15% in keeping with our previous trials, 342 couples will be recruited (171 couples per arm) to allow for a well-powered intention to treat (ITT) and per protocol (PP) analysis.

10.2 INTERIM ANALYSIS AND STOPPING PROTOCOL [as published (18)]

An interim analysis will be undertaken when the first 150 randomised couples have reached the 12 week study endpoint and will include the stated primary and secondary measures of interest. This interim analysis will then be reviewed blinded by the independent DSMB (above) who will recommend to the Protocol steering committee that the study should continue unchanged or be amended in light of observed differences between treatment groups (i.e. between intervention and control) or aspects

of study conduct that warrant modification (e.g. poor recruitment, safety concerns, and substantial losses to follow-up).

In agreement with trial chairs, the DSMB may require stopping rules to be implemented that require changes in the conduct of the study should concerns surrounding the safety of patients arise from review of the interim results. A conservative Peto-Prentice stopping rule ($p<0.001$) will be used to judge whether one or both arms should be ceased for inferiority only.

In addition, the DSMB will be provided with information relating to the conduct and management of the study. Unblinded datasets will not be made available for review until final data lock at the conclusion of the study.

10.3 PARTICIPANT CHARACTERISTICS AND BASELINE COMPARISON

PARTICIPANT FLOW

Details of participant flow will be summarised in a CONSORT flow diagram. The final schema will report the number of participants randomised, the number withdrawn or loss to follow up and the number meeting the definition of the primary analysis (modified intention-to-treat (ITT) population) and the per-protocol population.

BASELINE CHARACTERISTICS

The demographic characteristics and sexual risk behaviours of all recruits randomised will form *Table 1* with information reported separately for the two arms. Continuous variables at baseline will be presented using summary statistics (mean or median) and variability (standard deviation, range, or interquartile range). Categorical variables will be presented as frequency counts (n) and as a proportion (%) of participants with available data (N). Baseline variables may include:

- age (years)
- Lifetime sexual partners
- Past BV diagnoses
- Smoking, douching practices
- Contraceptive practices
- Duration of relationship with current regular sexual partner
- Sexual practices with that partner in the last 3 months

There will be no formal comparison (i.e. p-values) comparing randomised groups in terms of baseline characteristics. A baseline characteristics table of those included in the mITT analysis will be provided as supplementary material for comparison.

10.4 STATISTICAL ANALYSIS

All analyses will be undertaken using Stata Version 17 (or higher) and all statistical tests will be interpreted with two-sided $\alpha<0.05$ as statistically significant.

PRIMARY ANALYSIS

Modified-Intention-to-treat

As mentioned above, the Primary Analysis is a mITT analysis. Women will be analysed as randomised and will be eligible to be included in the mITT if they:

- returned for ≥1 clinical visit

- took one or more doses of antimicrobial therapy
- are part of the evaluable population (see definitions above)

Cumulative BV-recurrence rates per 100 person-years (PY) and Poisson 95% confidence intervals will be determined for the whole study population and separately by randomisation-arm within 12 weeks. Time to BV will be summarised by treatment arm using Kaplan-Meier survival curves and treatment arms formally compared using Cox regression. Primary analyses will be simple two-group comparisons of randomised treatment arms, but adjusted comparisons may also be performed if there are imbalances in important baseline covariates.

SECONDARY ANALYSES

Per-protocol-analysis

1. The population of participants who qualify for the mITT population and;
2. They adhered to 10 or more doses of 14 (>70%) dose regimens (oral metronidazole or topical clindamycin cream in males), 5 or more doses of a 7 (>70%) dose regimen (intravaginal clindamycin cream) or all 5 doses of a 5 dose regimen (intravaginal metronidazole gel).

Stratified analyses

Analyses will be performed for the primary outcome comparing the pre-defined strata – i.e. 1) IUD vs no IUD and the primary outcome; and 2) circumcision and no circumcision for the primary outcome. Differences between the intervention groups will be assessed using tests for interactions. If there is consistent evidence of an interaction between the intervention and strata, then an efficacy analysis within each strata will be performed – noting that we will likely be underpowered.

Safety evaluation

Serious adverse events, adverse events leading to cessation of treatment, and all reported clinical adverse events will be summarised. This will include every male who took ≥1 dose of at least one of the investigational drugs.

Factors associated with recurrence

Recurrence rates per 100PY will be calculated for baseline and longitudinal characteristics, with Poisson 95%CIs for primary/secondary outcomes. Cox regression analyses will be performed to calculate Hazard Ratios (HRs) for univariate factors associated with recurrence including demographics, sexual behaviours, contraception use, smoking and other factors previously shown to be associated with BV recurrence.

Multivariable analyses will include covariates associated with recurrence by univariable analysis and adjusted for randomisation-arm.

SENSITIVITY ANALYSIS

We will also investigate treatment efficacy by performing the following sensitivity analyses:

1. In the case of a participant providing no Nugent and/or Amsel data after week 4, and not having reached endpoint at week 4, we will carry week 4 data forward to week 12.
2. In the case of a participant not providing any Nugent score data after day 8, we will carry their day 8 Nugent score forward as their week 4 data, and censor their participation at this time point (i.e. no week 8 or 12 data).

3. Sensitivity analyses will include analyses that take into consideration varying levels of adherence to treatment (for women and/or men).

ASSESSMENT OF NON-ADHERENCE AND ADVERSE EVENTS

As described in the per-protocol analysis, non-adherence is defined as <10 doses (oral metronidazole in females and males or topical clindamycin cream in males), or <5 doses of intravaginal clindamycin cream (females). A table of doses taken by treatment group will be provided.

Descriptive statistics will be used to describe the reporting of adverse effects (presence of: nausea; vomiting; diarrhoea; metallic-taste; headache; and others) using frequency counts and proportions (%) by treatment arm for women, and for all men who took ≥1 dose of treatment. The difference in the proportion reporting adverse events between groups will be calculated using the predictive margins function of logistic regression and robust standard errors will be calculated to account for clustering by recruitment site.

OTHER ANALYSES

Descriptive statistics will be used to describe symptoms, date of last menstrual period, and sexual and contraceptive practices during follow-up.

Continuous variables will be presented using summary statistics (mean or median) and variability (standard deviation, range, or interquartile range). Categorical variables will be presented as frequency counts (n) and as a proportion (%) of participants with available data (N).

11. CONCLUSION

Our pre-specified statistical analysis plan was prepared before completion of data collection and database lock in keeping with best statistical practice. The plan provides a detailed description of the planned analysis and reporting of the trial results, aids transparency, and may assist future study design.

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