Confirmation of pregnancy. A commercial kit to detect hCG levels in urine (KAT Quick HCG, KAT Medical, http://www.katmedical.com; the performance of this kit was validated against another commercial kit, QuickVue, Quidel Corporation, http://www.quidel.com) was used to screen for pregnancy at enrollment and each visit. Women with positive urine pregnancy tests were confirmed by ultrasound.

Other laboratory tests. A vaginal wet mount was examined microscopically at each visit to detect motile trichomonads and candida using standard laboratory methods [10]. We did not test for *N. gonorrhoeae* and *C. trachomatis,* because the prevalence of these organisms has been very low (1%) in recent studies in Malawi [14].

Study Questionnaires and Physical Examination

Demographic, clinical, acceptability, and adherence questionnaires were completed at enrollment and follow-up visits. To monitor adherence, women were asked to return the empty tube and used applicators at the PTE follow-up visits. The amount of gel used in each tube was also assessed.

Adverse Experience Reporting

Trained clinicians and nurses conducted a speculum-aided pelvic examination at each visit to detect mucosal abnormalities. Adverse events (AEs) by severity (grade 1, mild; 2, moderate; 3, severe; 4, life threatening; and 5, death) and relatedness to product use were recorded. Serious AEs were defined based on Code of Federal Regulations ICH Guidelines [15].

Objectives

The primary objective of this study was to determine whether intermittent intravaginal metronidazole gel antibiotic treatment would reduce the frequency of BV among HIV-uninfected and -infected African women. We hypothesized that repeated (intermittent) presumptive treatment with an intravaginal antibiotic regimen would restore normal vaginal flora, enhance BV clearance (conversely, limit persistence), and decrease BV recurrence.

Outcome Measures

The primary endpoint was proportion of women testing positive for BV based on the Nugent score at each visit, both cross-sectionally (between study arms) and longitudinally (within study arms). The secondary outcome measure was the effect of treatment on clearance and recurrence of BV.

Sample Size

In this trial a sample size of 832 HIV-uninfected women and 832 HIV-infected women was assumed adequate based on the longitudinal comparisons to provide a power of 87% or more to detect a reduction of 33% or more in the prevalence of BV from a baseline prevalence of 30% (two treatment arms, type 1 error of $\alpha = 0.05$ and 10% loss during 1 y of follow-up).

Randomization

Block-randomized computer-generated lists were prepared in the US and were stratified by clinic and HIV status. The study product was provided to the site in a sealed envelope (opaque and padded to avoid damage) with no identifiers other than the study and clinic identification numbers. These envelopes were issued from a central pharmacy at the study site after the women had been counseled and screened, and had given their informed consent to enroll. The study pharmacist and coordinator regularly carried out checks on the order of randomization and matched enrollment identification numbers with clinic and HIV status. The packaging and labeling of the study product was performed in the US by an independent team. Investigators, research workers, and participants were masked about the study product. Neither the study pharmacist at the research site in Malawi or study coordinator was aware of the details of the study product. Both treatment and placebo gels had comparable appearance, consistency, and packaging. Participants were randomized at enrollment (baseline) visit (V1.0).

Statistical Methods

Data were checked for completeness and consistency and entered locally in a database. All analyses of the primary outcome were performed separately for HIV-uninfected and -infected women using the intent to treat (rather than as treated) approach. The proportion of events occurring in the two study arms were cross-sectionally compared at each visit. Analyses were also conducted to compare events longitudinally within the same study arms between visits. Chi-square (exact test) and other nonparametric tests were used for these comparisons. Generalized estimating equation log binomial models based on relative risk ratio assessed longitudinal associations of treatment with BV after controlling for other covariates measured at multiple visits to account for repeated visit correlation of these repeated observations. Univariable (unadjusted) relative risks (RRs) and adjusted RRs, and 95% confidence intervals (CIs) are presented. Statistical significance was considered to be two-sided $p \leq 0.05$.

We further analyzed BV clearance and recurrence in the subgroups of women to determine the impact of intravaginal antibiotic treatment among HIV-uninfected and -infected women. We defined BV clearance as a positive BV test at baseline with a negative BV test (first negative test) in a follow-up visit, and BV recurrence as a positive BV test at baseline with a negative BV test (first negative test) in a follow-up visit and becoming BV positive (first positive test) in a subsequent visit. We used Kaplan-Meier survival analyses to estimate median time to clearance and recurrence events and the cumulative probability of these events stratified by study arm among HIV-uninfected and -infected women. Cox proportional hazard models were used to control for various factors and determine the major predictors of these events. Treatment, number of sex partners, frequency of sex, vaginal pH, douching, and *T. vaginalis* were included in these Cox models (all were time-dependent except for treatment). Statistical significance was determined by a p-value ≤ 0.05. All analyses were conducted using SAS (version 8.2; http://www.sas.com).

Study Monitoring

This study was monitored by a five-member Data and Safety Monitoring Board. Study conduct and review of clinical AEs were also monitored by two independent monitors.

Provision of Clinical Care

Routine clinical care for all women (including continuous counseling, provision of condoms, and treatment of STIs) was provided by the project at no cost. HIV-infected women were provided additional support by trained counselors and community educators. Specialized clinical care for HIV-infected women was provided by project clinicians and