STATISTICAL ANALYSIS PLAN

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Amendments and Dates

CRF Date:

STUDY TITLE:

PrEP and dPEP: Doxycycline post-exposure prophylaxis for prevention of sexually transmitted infections among Kenyan women using HIV pre-exposure prophylaxis

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PROTOCOL SUMMARY

PREP AND DPEP: DOXYCYCLINE POST-EXPOSURE PROPHYLAXIS FOR PREVENTION OF SEXUALLY TRANSMITTED INFECTIONS AMONG KENYAN WOMEN USING HIV PRE-EXPOSURE PROPHYLAXIS

Design: Open-label 1:1 randomized clinical trial of doxycycline PEP (dPEP) to reduce bacterial STIs – *Neisseria gonorrhoeae, Chlamydia trachomatis, and Treponema pallidum* (syphilis) – among Kenyan women taking PrEP. We will also use quantitative questionnaires, focus group discussions, SMS, and in-depth interviews to study acceptability, adherence, and changes in sexual behavior due to dPEP.

Study Population: 446 Kenyan women aged ≥18 and ≤30 years old taking PrEP randomized to dPEP and standard of care vs. standard of care alone

Study **Sites:** KEMRI RCTP – Lumumba clinic

Primary Study Objectives:

1. Evaluate the effectiveness of doxycycline post-exposure prophylaxis (dPEP) to reduce STI infections in HIV-uninfected Kenyan women taking HIV PrEP
2. Assess the safety, tolerability, and acceptability of dPEP
3. Assess adherence to dPEP
4. Investigate the impact of dPEP on tetracycline resistance in *N. gonorrhoeae* and *C. trachomatis*
5. Measure the cost of dPEP and estimate the cost per case averted, budget impact, and affordability.

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LIST OF ABBREVIATIONS

Table 1: List of Abbreviations

AE	Adverse event
AMR	antimicrobial resistance
ART	Antiretroviral therapy
CDC	Centers for Disease Control
CT	*Chlamydia trachomatis*
DAIDS	Division of AIDS
DALY	Disability-adjusted life year
dPEP	Doxycycline post-exposure prophylaxis
DSMB	Data safety and monitoring board
FGD	Focus group discussion
GEE	Generalized estimating equations
HAL	Hair Analytical Laboratory
HIV	Human Immunodeficiency virus
HSV	Herpes simplex virus
IATA	International Air Transport Association
ICER	Incremental cost effectiveness ratio
IDI	In-depth interviews
IH	Intracranial hypertension
IRB	Institutional Review Board
IPV	Intimate partner violence
KEMRI	Kenya Medical Research Institute
LARC	Long-acting reversible contraceptives
LMP	Last menstrual period
MOH	Ministry of Health
MSM	Men who have sex with men
NAAT	Nucleic acid amplification test
NASCOP	National AIDS & STI Control Programme
GC	*Neisseria gonorrhoeae*
PCR	Polymerase chain reaction
PEP	Post-exposure prophylaxis
PID	Pelvic inflammatory disease
PLWH	People living with HIV
RPR	Rapid Plasma Reagin
SAE	Serious Adverse Event
SMS	Short message service

STIs	Sexually transmitted infections
TDF/FTC	Tenofovir/Emtricitabine
*tet*R	Tetracycline resistant
UCSF	University of California San Francisco
UW	University of Washington
VCT	Voluntary counseling and testing

1. INTRODUCTION

The purpose of this document is to provide details on study populations and on how the variables will be derived, how missing data will be handled as well as details on statistical methods to be used to analyze the safety and efficacy data. This SAP only details the analysis of primary and key secondary objectives.

The document may evolve over time, for example, to reflect the requirements of protocol amendments or regulatory requests. However, the final SAP will be finalized, approved by the Protocol Chairs, and placed on file before database is locked.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Study Objectives

2.1.1. Primary Efficacy and Safety Objectives

1. Evaluate the effectiveness of doxycycline post-exposure prophylaxis (PEP) to reduce STI infections in HIV-uninfected Kenyan women taking HIV PrEP
2. Assess the safety, tolerability, and acceptability of dPEP

2.1.2. Secondary objectives

1. Assess adherence to dPEP
2. Investigate the impact of dPEP on tetracycline resistance in *N. gonorrhoeae* and *C. trachomatis*
3. Measure the cost of dPEP and estimate the cost per case averted, budget impact, and affordability

2.2. Study Endpoints

2.2.1. Primary Efficacy Endpoints

Effectiveness of doxycycline

The primary endpoint is assessed using the following STI endpoints at month 3,6, 9 and 12 visits:

- Documented positive vaginal/cervical GC
- Documented positive vaginal/cervical CT
- Early syphilis infection based on four-fold increase in non-treponemal titers.

The primary trial endpoint is any newly diagnosed STI, defined as any one of the above after the enrollment visit, with the exception of re-diagnosis of the same infection at a test of cure visit within 28 days of first treatment of a baseline or incident infections (which is considered a non-cured infection and re-treated). Any infection newly diagnosed at an interim visit will be counted as a primary endpoint for the subsequent scheduled visit i.e., if a participant is diagnosed with new GC infection at an interim visit 40 days after their 6 month visit, this will be analysed as a primary 9 month endpoint. Endpoints are reviewed by committee, blinded to study arm, and primary endpoints designated in that review.

2.2.2. Secondary Efficacy Endpoints

Efficacy of doxycycline for individual STIs

Effectiveness of doxycycline will also be assessed for CT and GC separately, using an incident STI definition that parallels the above for the specific infection.

2.2.3. Safety, tolerability and acceptability of dPEP

- Safety:
  - serious adverse events (SAEs),
  - all related AEs (assessed in the dPEP arm only)
- Tolerability:
  - dPEP discontinuation (assessed in the dPEP arm only)
  - Candida yeast infection treatment (documented as fluconazole on STI treatment CRF)
  - Doxycycline-specific side effects (assessed via standardized symptom assessment: includes reports of nausea, pill esophagitis, photosensitivity)
- Acceptability:
  - Acceptability and Optimism questionnaire (FDAO) (13 question on a 5-point Likert scale)

2.2.4. Adherence

Adherence is only assessed in the dPEP arm

- Weekly SMS questions

  Weekly questions collected in participants who opted in to SMS.
  - Number of days in the last 7 days when dPEP was taken
  - Number of days in the last 7 days when participants had sex or “danced”).
- Hair samples

  Collected quarterly
  - Quantitative amount of doxycycline in 1cm segments from cut end to tip which corresponds with a 4-week period of dPEP use (starting 1 week ago).
- Self-report of coverage of sexual contacts in the prior 14 days to 3 days in the dPEP arm:

  Quarterly (timeline follow-back) adherence questionnaire.

2.2.5. Tetracycline resistance

- Molecular markers of resistance for CT after enrollment (positive CT swabs collected at Enrollment, Month 3, 6, 9 and 12, as well as any test of cure visits)
- Molecular markers of resistance for GC after enrollment (positive GC swabs collected at Enrollment, Month 3, 6, 9 and 12, as well as any test of cure visits)

3. STUDY DESIGN

3.1. Summary of Study Design

Open label 1:1 randomized trial of dPEP versus standard of care. Participants assigned to dPEP will be instructed to take doxycycline 200 mg (two 100mg capsules) orally within 24 hours and up to 72 hours after each condomless sex act as frequently as daily if indicated but not more than once daily.

At Months 0/3/6/9, women randomized to dPEP will receive doxycycline, sufficient for nearly daily use for 3 months (i.e., 100 capsules). Unused capsules will be counted at each follow-up visit (Months 3/6/9/12) and additional doxycycline will be given to total up to 180 capsules if needed. The medication, doxycycline hyclate, will be purchased from a quality-controlled supplier and with consultation by the Kenya National AIDS and STI Control Programme. Participants will also be offered single- or multi-dose pill carriers for ease of dosing dPEP following exposures. All participants will receive quarterly visits for standard of care prevention services and collection of clinical and behavioral data, for a total of 12 months of follow-up.

Study visits: Every 3 months, study visits will include STI screening, behavioral questionnaires, hair sample collection, and swab collection for archived samples. Women may return to the study clinic for interim visits for any reason and will undergo STI testing and treatment if STI symptoms are present. Participants with a new STI diagnosis will return for prompt treatment, provided on-site. Laboratory testing will be conducted by staff blinded to randomization assignment, and STIs will be reviewed by an Endpoint Adjudication Committee blinded to treatment arm. All participants diagnosed with an STI will either receive same day treatment or return to the research clinic for treatment, using WHO/Kenya standard therapies, avoiding doxycycline options, and a second visit two weeks following completion of STI treatment will be done for test of cure; this mitigates potential risk to participants and the community if resistance is selected.

Study population: This study will enroll approximately 446 Kenyan women aged 18 and 30 years old taking PrEP. PrEP use is an eligibility criterion for enrollment because we hypothesize that women engaging in PrEP are a priority population for prevention of curable STIs, given high incidence, interest in longitudinal preventative services, and willingness to take pills for prevention. Aligning with Kenya's national PrEP services, we propose an evaluation of the benefits, risks, and costs of dPEP among women, in the first trial of this intervention for this population. However, participants may opt to stop PrEP use at any time during the study without affecting their involvement in the study. Any HIV-uninfected participants who subsequently seroconvert will be managed clinically by the study site according to local practice (appropriate counseling, clinical evaluation and immediate linkage to clinical and psychosocial services). These participants will also be retained in the study unless they choose to discontinue study participation

3.2. Definition of Study Drugs

The study drug is open-label doxycycline 200 mg to be taken ideally within 24 hours but no later than 72 hours after condomless sexual contact (oral or anal). 200 mg of doxycycline will be taken at most once per 24 hour period regardless of the number of sexual acts occurring during this time period

3.3. Sample Size Considerations

3.3.1. Sample Size Justifications

The study is a 1:1 randomized superiority design, comparing dPEP to standard of care. The primary trial outcome will be the combined incidence of incident *C. trachomatis, N. gonorrhoeae*, and syphilis infections, compared between the randomization arms, to mirror the IPERGAY and Doxy PEP approach; analyses will also test CT and NG alone. Sample size was determined using an event driven approach (N = 66). Assuming 10% loss-to-follow-up, incidence of 22% for any STI in the SOC arm and a two-sided alpha of 0.05, a sample size of n=446 (n=223 per arm) has 80% power to detect a 50% reduction in time to first STI, i.e. ~11% in the dPEP arm (n=44 vs. 22 cases).

The trial intends to have sufficient power to detect 50% effectiveness in CT alone, and thus not stop before incident diagnosis of CT has occurred in 66 women (resulting in more than the minimum number of composite events for the endpoint of any STI).

Sample sizes were computed using nQuery for time to first STI within one year of follow-up

Table 2 Power Table for N = 446; 1:1 randomization dPEP: SOC

Control arm incidence	Power to detect 50% reduction (Number of infections for 80% power = 66)		Power to detect 65% reduction (Number of infections for 80% power = 52)	
	Intervention arm incidence	Power	Intervention arm incidence	Power
50%	25.0%	99%	17.5%	99%
33%	15.5%	93%	11.6%	99%
22%	11.0%	80%	7.7%	96%
18%	9.0%	71%	6.3%	92%
15%	7.5%	63%	5.2%	87%

3.3.2. Sample Size Re-estimation

At the point when enrollment is near completion, the study team will evaluate the expected power of the trial, based on the observed rate of infections. If the rate of the primary endpoint is substantially lower than anticipated, sample size re-estimation would be used to restore power to the extent possible, through additional enrollments, increase in person-time, or modification of risk criteria.

3.4. Randomization

Participants will be randomized 1:1 to dPEP versus standard of care; randomization will be done in variable- sized blocks and using a computer based randomization system, Randomize.net, following consent and eligibility confirmation.

3.5. Clinical Assessments

Safety assessments are limited to the following:

In dPEP arm only:

- All grade 2 and higher AEs that are judged related to dPEP, in the opinion of the site clinician, that result in an interruption of study drug
- All grade 3 and 4 adverse events judged to be related to doxycycline

In both arms:

- All AEs meeting SAE definition regardless of relationship with dPEP

In this open label trial, for those randomized to receive doxycycline PEP, all AE's and SAE's have attribution recorded as doxycycline-related or not doxycycline-related, in the judgment of the site investigator.

4. PLANNED ANALYSES

4.1. Interim Analyses

The trial will be monitored by an independent data monitoring committee approximately every 6 months. The trial plans to continue until we have established definitive efficacy results, whether effective or ineffective, for both CT alone and for any STI. Two formal interim monitoring times are planned, when 1/3 and 2/3 of the follow-up visits have been completed, with stopping rules based on consideration of both the primary outcome and the CT outcome alone. Because the outcomes are highly correlated, no adjustment for multiple comparisons is planned. O'Brien-Fleming boundaries will be used, with stopping based on the alpha spending corresponding to observed p-values in the efficacy analyses. Stopping for both efficacy and lack of efficacy will be considered; however, a decision to stop for efficacy will need to balance the need for evidence assessing the potential harm of acquired tetracycline resistance.

4.2. Final Analyses

The final analysis will be conducted after database lock of all final (12 month) study visits.

5. GENERAL CONSIDERATIONS FOR DATA ANALYSES AND HANDLING

5.1. Data Presentation Conventions

Continuous variables (e.g. age) are summarized using descriptive statistics (the number of subjects with available data, the mean, standard deviation (SD), median and minimum and maximum). Categorical variables (e.g. preferred language) are summarized using counts and percentages. Percentages are calculated using the total subjects per treatment group.

5.2. Analysis Populations

5.2.1. Screened population

All subjects who give informed written consent for screening but are not enrolled will be characterized based on reason for screen failures (e.g., lost-to-follow-up, did not meet entry criteria, administrative) in summary tables. These subjects will neither contribute to other data presentations nor participate in formal analyses.

5.2.2. ITT Population

The intent to treat (ITT) population comprises all randomized participants.

5.2.3. dPEP Safety Population for dPEP

The 'Safety Population' is defined as all subjects who were dispensed at least one dose of study medication. This population is expected to be identical to participants randomized to dPEP for this study.

5.2.4. GC positive Population

Post-baseline GC positive participants assessed for TCN resistance. All participants with positive GC will be assessed for molecular resistance

5.2.5. CT positive Population

Post-baseline CT positive participants assessed for TCN resistance. All participants with positive CT will be assessed for molecular resistance

5.2.6. Hair population

Participants in the dPEP arm who have hair assessed for quantitative PK assays.

5.2.7. Asymptomatic STI population

Participants who have tested positive for an STI (CT, NG, and/or TP) and "Per national guidelines, do abnormal findings require immediate STI treatment?" is answered "no"

5.2.8. SMS responder population

Participants who have responded to at least 4 SMS surveys

5.2.9. Subgroups

The following subgroups will be defined by baseline characteristics

- Age: ≤ vs > 24 years old at enrollment
- Nulliparous: No previous live births
- Using contraceptives: IUD, implants, DMPA, oral contraceptives
- Any STI at baseline: Any laboratory confirmed diagnosis of GC, CT or incident syphilis during enrollment visit(s).
- Transactional sex: exchanged sex for goods, services, or money in the last 3 months

5.3. Handling of Missing Data

5.3.1. Missing Efficacy Endpoints

Complete case analysis will be used for the primary efficacy analysis

6. STUDY POPULATION

6.1. Participant Disposition

A consort diagram will describe the number screened, randomized, retained by visit, and contributing to the primary analysis endpoints by arm. Reason for screen-out and non-inclusion in the primary outcome will be summarized.

6.2. Screen Failures

Reason for screen failures will be summarized by arm

6.3. Protocol Deviations

A listing or summary of major protocol deviations will be provided

6.4. Study Termination Status

Reason for early study termination or study withdrawal will be enumerated and detailed. Study disposition at the time of a report will list those remaining in study follow-up, completed study follow-up, early withdrawal (including deaths).

6.5. Demographic and Baseline Characteristics

6.5.1. Demographics and risk characteristics

The following baseline demographics and risk characteristics will be presented separately by arm for the ITT cohort.

- Age in years
- Education
- Sexual risk (i.e. number of partners, transactional sex, condom use with last sex)
- Living situation
- Preferred language
- Marital status
- Number of live births
- Earns her own income (Y/N)

6.5.2. Baseline Laboratory Data

Baseline bacterial STI diagnoses (GC/CT, Syphilis) detected by lab-based study testing will be reported for each cohort separately, by arm and by study site.

7. EFFICACY

7.1. General Considerations

Tabulations will be by study arm, for each quarter of study follow-up (or at visits where evaluated). These will be reported in only the closed report during the study.

Inferential statistical tests will be two-sided and will be performed at alpha levels of 0.05 and 0.10 to declare the significance of main effects and interaction effects, respectively.

7.2. Statement of the Null and Alternate Hypotheses

For enrolled women:

- H_o: Use of Doxycycline PEP does not have a differential effect on incidence of GC, CT and syphilis infections compared to the standard of care

$$RR_{\text{any STI}} \text{ (Doxy Arm/SOC Arm)} = 1$$

- H_A: Use of Doxycycline PEP decreases the incidence of GC, CT and syphilis infections compared to the standard of care.

$$RR_{\text{any STI}} \text{ (Doxy Arm/SOC Arm)} \neq 1$$

7.3. Subgroup Analyses

Subgroup analysis of the efficacy of the intervention will be conducted for

- Age subgroups (≤/> 24)
- Nulliparous (N/Y)
- Using contraceptives (N/Y)
- Any STI at baseline (N/Y)
- Transactional sex (N/Y)

7.4. Multiple Comparisons and Multiplicity

No adjustments are planned for multiple comparisons

7.5. Analysis of the Primary Efficacy Endpoint

7.5.1. Primary Efficacy Analysis

The primary analysis of efficacy will be conducted in the ITT cohort

Endpoint definitions:

Primary endpoint: **Any incident STI at Month 3,6 9 or 12**. Repeat positive tests for the same STI up to 28 days after treatment of an incident infection (the window for the test of care visit) will not be counted as incident infections. Incident infections detected at interim visits more than

28 days after treatment will be treated in the analysis as if they would have been detected at a scheduled visit (months 3, 6, 9 and 12). Therefore, an STI diagnosed at an interim visit outside the treatment window is carried forward to the next scheduled visit. If a participant has more than one positive STI (different bacterial infections) at a scheduled visit it will be counted as one incident "any STI" event for that visit interval. If scheduled visits are missed, infections will be assigned to the next scheduled visit (i.e., the first missed scheduled visit).

Secondary endpoints: **Any GC, Any CT at Month 3, 6, 9 and 12**. The same approach as defined for the primary endpoint will be used, restricted to each specific bacterial infection.

The criteria for an incident syphilis case will include a four-fold increase in non-treponemal titers (e.g., the RPR) and/or a consistent clinical presentation.

Descriptive analysis

- The number of participants with any bacterial STI, and with each type of STI, will be summarized at each scheduled visit by arm
- The number of STIs reported by each participant will be summarized at each scheduled visit by arm. Detail of testing and results at interim visits will also be summarized.
- Occurrence and results of test of care visits will be summarized.

Statistical analysis

Comparison of STI incidence by arm will be conducted by estimating the relative risks of any STI over the 3, 6, 9 and 12 month visits using a modified Poisson model fitted using GEE methods to account for repeated observations from each participant, assuming an independent covariance structure, with study arm as the only covariate[1]. The test for significance will be a two-sided alpha = 0.05. 95% confidence intervals will be computed using robust standard errors.

The same analysis will be repeated for the individual STIs.

7.5.2. Sensitivity Analyses of the Primary Efficacy Results

Several sensitivity analyses are planned:

1. Analysis restricted to time eligible for dPEP (i.e., no active pregnancy). Time in both arms will be censored at the study visit when a participant was determined to be not pregnant (from visit with first positive pregnancy test until exit without re-entry into risk set)
2. Time to first STI (from randomization) using Kaplan Meier and Cox PH regression. All women will be treated as at risk for all STIs at randomization. It is acknowledged that women diagnosed with an STI at a visit are treated until cure, and thus are not immediately at risk. However, women re-enter the risk set when cured, thus are at risk prior to the next quarterly visit.
3. Defining an STI as incident, in the case where the same STI was previously present, only if a negative test for the same pathogen is recorded before the positive result. The

primary outcome will be modified to exclude any repeat STI without a preceding negative result, and the statistical analysis for the primary analysis repeated.

4. Analysis counting all individual STIs diagnosed in a quarterly interval A Poisson model fitted using GEE methods to account for multiple events from each participant will be used to estimate the change in rate of infections between arms. If necessary to achieve a better more fit, a zero-inflated Poisson model may be used.

7.5.3. Analyses of the Primary Efficacy Results while on study drug (Per protocol)

For the per-protocol analysis, for each cohort the dPEP arm will be restricted to study time prior to the first discontinuation (> 2 weeks) of study drug documented on the study dPEP CRF. The evaluation of the impact of doxycycline on Any STI will use the same model as specified for the primary analysis, but also potentially adjusted for age, baseline STI, baseline and follow-up sexual risk (number of partners, nulliparous, contraceptive use, transactional sex).

7.6. Study drug use and time on study drug in the dPEP arm

- Study drug dispensation (number of participants and number of pills) will be summarized for each study visit over time by cohort.
- Longitudinal exposure to doxycycline will be assessed using hair collection, with a semi-quantitative evaluation in 10% the control arm (doxycycline present vs. absent) and quantitative evaluation in the 100% of dPEP arm (doxycycline concentration value).
- Proportion of time on study drug in the dPEP arm, based on discontinuations documented on study CRFs will be tabulated for each quarter and overall by study arm within each cohort.
- Concomitant use of antibiotic use as documented in the antibiotic use log, will be summarized for the control and dPEP arms

7.7. Analysis of Tetracycline resistance

7.7.1. Analysis of TCN resistance in GC cases

TCN resistance will be assessed in GC cases only. TCN resistant GC will be defined by the detection of plasmid-medicated *tet*M gene conferring high-level tetracycline resistance.

Descriptive statistics: Tabulations of resistance (No GC, GC: TNC resistance testing = missing, not resistant, resistant) will be reported at baseline, 3, 6, 9 and 12 month visits in GC cases in each arm.

Statistical analysis: Assessment of odds of resistance by arm will be assessed by estimating the odds of GC infection with resistance among cases detected after enrollment using logistic regression, adjusted for visit.

Sensitivity analysis: molecular chromosomal mutations may lead to expression of tetracycline resistance. Tabulations of number of expressed chromosomally-mediated tetracycline resistance determinants (mtrR, rpsJ, porB, and pilQ) will be reported at baseline, 3, 6, 9, and 12-

month visits in GC cases, among those with or without plasmid-mediated tetracycline resistance (i.e., detection of *tet*M), in each arm.

7.7.2. Analysis of TCN resistance in CT cases

The analysis of TCN resistance in CT cases will include all positive CT cases after baseline. TCN resistant CT will be defined by the detection of *tet*C gene cassette.

Descriptive statistics: Tabulations of TCN resistance in CT cases (TNC resistance testing = missing, not resistant, resistant) will be reported at baseline, 3, 6, 9 and 12 month visits in each arm.

Statistical analysis: Assessment of difference in odds of TCN resistance by arm across all post baseline visits with infection will be assessed by estimating the odds of TCN resistance using logistic regression, with repeated measures methods (GEE) if repeat infections occur.

8. SAFETY AND TOLERABILITY

Statistical tests are not planned for adverse event comparisons between arms. Safety and tolerability will be assessed in the ITT cohort, given the open label nature of the cohorts.

8.1. Adverse Events and Deaths

8.1.1. AE Definitions

The following AEs are recorded on eCRFs:

- All SAEs
- All AEs attributed to doxycycline in the opinion of the site investigator

8.1.2. Adverse Event Summary Tables

The number of SAEs will be tabulated by grade and arm. For the dPEP arm, related AEs will be tabulated by grade (see below of definition of relatedness).

8.1.3. Listings of Serious Adverse Events (SAE), Adverse Event Dropouts, and Death

SAE data will be reported by related and not related.

Participant data listing for SAEs will be by grade and arm, sorted by reported date within participant. Unique information included in the listing contains SAE onset and resolution dates and times; verbatim description of SAE; relationship to study medication, action taken, and outcome.

Participant data listing for related AEs will be by grade, sorted by reported date within participant. Unique information included in the listing contains AE onset and resolution dates and times; verbatim description of AE; action taken, and outcome.

A listing will present participants who discontinue study drug in the dPEP arm due to an adverse event, and the reasons for discontinuations:

- Requirement for prohibited concomitant medications or other contraindication to doxycycline
- Pregnancy
- Occurrence of an adverse event requiring discontinuation of doxycycline
- Request by participant to terminate study treatment
- Clinical reasons believed life threatening by the physician, even if not addressed in the toxicity section of the protocol
- Requirement for chronic tetracycline use ($\geq$14 days)

8.2. PrEP use

Tabulation of time on PrEP

Tabulations of PrEP will use the following assessments:

- Residual hair samples of participants will be tested for quantitative tenofovir drug levels to measure monthly use of tenofovir disoproxil fumarate/lamivudine PrEP.
- Reported time on PrEP prior to enrollment
- Tabulation of PrEP use at each visits
  - Self-report of PrEP adherence (How well have you taken PrEP in the past month? How often do you take PrEP?)
  - PrEP dispensing (PrEP discontinued prior to visit or on PrEP prior to the visit. Has PrEP/PrEP dispensed)

8.3. Tolerability

Tolerability is assessed for participants on the dPEP arm through

- a self-reported response to whether a participant did not take dPEP because of side-effects.

Descriptive: The Yes/No responses of dPEP side effects will be tabulated for each study visit among dPEP participants

- Symptoms from the side-effect questionnaire

Descriptive: Symptoms, collected at each visit, will be tabulated for each arm

8.4. Acceptability

Acceptability is assessed in the dPEP arm only via a 7 question acceptability questionnaire using a 5 point Likert scale.

Descriptive: The scales will be converted to an acceptability score, the dPEP Acceptability and Optimism CRF, (using reverse coding where appropriate), and summarized by visit. Individual questions will also be reported in detail. This will be supplemented with qualitative interviews for a mixed methods analysis.

8.5. Adherence

Adherence is assessed as coverage of sex acts for this event-driven dosing and may have impact on effectiveness of medication.

8.5.1. Coverage of sex acts

Assessed in the dPEP arm only:

Measures

- Last 14 days (up to 3 days prior to visit) calendar reporting of days taking dPEP and days with sex.
- SMS: Self-reported coverage of last week: last 7 days # days with “dance” (SMS term used to mean sex), and # days taking dPEP.

Definition: Acceptable coverage for a participant is defined as on 80% of days with sex, the participant self-reports use of dPEP within 72 hours.

Descriptive:

EchoMobile SMS data: Past-week use of dPEP by reported sex acts among dPEP study arm participants in a table over all follow-up time, excluding periods where participant is on a documented study drug hold.

Timeline follow-back calendar: Past 2-week (day -14 to -3) participant-reported dPEP coverage among those with reported sex, by scheduled visit and over all follow-up time, excluding visits where participant is on a documented study drug hold.

8.5.2. Longitudinal adherence-effectiveness analysis:

Hair samples collected at quarterly follow-up study visits for all treatment group participants will be analyzed for quantitative hair drug levels. Based on HIV PrEP studies, we assume that dPEP will either be used regularly or not at all and that underdosing will be a rare occurrence. Because quantifying doxycycline drug hair levels is not yet standardized, we will not describe true event-based adherence but rather use vs. non-use.

1. Doxycycline use (defined as detectable doxycycline in a hair sample – binary variable) will be analyzed using GEE to measure effect of dPEP use compared to nonuse in preventing STI cases (quarterly measure of any incident STI – binary variable).

Secondary analyses

2. Describe the relationship between quantitative tertials of doxycycline hair drug levels (low, medium, and high) and the number of dPEP doses reported in SMS in the 4-week time period of hair sample (5 weeks prior to study visit until 1 week prior to study visit) by generating a scatterplot and reporting the mean number of doses reported per quantitative category.

3. The mean hair drug concentration among hair samples collected at study visits when an incident STI (CT, NG, or TP, combined and per infection) was detected will be described and compared to study visits when no incident STI was detected among treatment group.

9. ADDITIONAL SECONDARY ENDPOINT ANALYSIS

9.1. Changes in sexual frequency

SMS surveys collected weekly ask all participants how many days of the last week they had sexual intercourse with responses ranging from 0-7. We will compare frequency of sexual activity over time to investigate whether they differ by arm using a GEE with Poisson distribution.

9.1.1 Changes in sexual risk behavior

Survey data collected quarterly, asking: “Was a condom used at last sex (N/Y)?” We will compare no sex/used condom at last sex over time to investigate whether they differ by arm using GEE with Poisson distribution.

9.2. Contamination and non-use:

Hair samples were collected at all study visits. A random 10% subset of all participants at enrollment visit and 10% subset of samples from follow up study visits from the standard of care group will be analyzed for doxycycline drug concentrations to summarize instances of doxycycline detection at study enrollment (prior to dPEP initiation) or in the standard of care group. All hair samples collected in follow up study visits in the treatment group will be analyzed for instances of doxycycline non-detection, i.e., non-use (excluding participants with no sexual exposure reported in the last month). The percentage of participants with doxycycline detected in standard of care group will be compared with percentage of participants with doxycycline detected in dPEP arm over time, quarterly.

9.3. STI Incidence

Describe overall incidence rate of any STI (CT, NG, and TP) and individual STI by study arm.

9.4. Prevalence and incidence of asymptomatic STIs

The standard of care in Kenya is syndromic management of STIs. To better understand the role of asymptomatic STIs in this trial, symptom questions and physical exam findings will be used to define symptomatic vs. asymptomatic STIs (defined in 5.2.7), and the proportion of STIs that are detected at the time of symptoms and asymptomatic will be reported at baseline and at each quarter over time by study arm.

10. REVISIONS TO THE SAP

Version	Changes	Date
1.0		16 March 2021
2.0	Correction of interim monitoring plan to 2 interim reviews at 1/3 and 2/3 of follow-up visits.	10 April 2021
3.0	Addition of sensitivity analysis plans following 2/3 interim analysis DSMB	June 25
4.0	Clarification of SAP as end of data collection approaches	Sept 12

11. APPENDIX

11.1. Table of Contents for Data Reports

This section will be completed in the next SAP version.

12. REFERENCES

1. Yelland LN, Salter AB, Ryan P. Performance of the modified Poisson regression approach for estimating relative risks from clustered prospective data. Am J Epidemiol. 2011;174(8):984-92. Epub 2011/08/16. doi: 10.1093/aje/kwr183. PubMed PMID: 21841157.

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Final Audit Report 2022-11-05

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