STATISTICAL ANALYSIS PLAN

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STUDY TITLE:

Weekly doxycycline DOT for STI prevention among cisgender women taking HIV PrEP in Kisumu, Kenya

SIGNATURE PAGE

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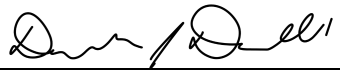
Deborah Donnell		25 Feb 2026
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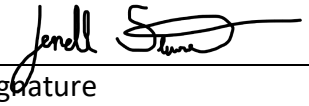
Jenell Stewart		02/24/2026
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PROTOCOL SUMMARY

WEEKLY DOXYCYCLINE DIRECTLY OBSERVED THERAPY FOR PREVENTION OF SEXUALLY TRANSMITTED INFECTIONS AMONG CISGENDER WOMEN USING HIV PRE-EXPOSURE PROPHYLAXIS IN KISUMU, KENYA

Design: Open-label one arm trial of 200 mg doxycycline PEP (dPEP) given orally, once weekly at the study clinic or within the community with a trained study staff as DOT to reduce bacterial STIs – *Neisseria gonorrhoeae, Chlamydia trachomatis, and Treponema pallidum* (syphilis) – among Kenyan women taking PrEP. We will also use quantitative questionnaires to study acceptability, adherence, and changes in sexual behavior due to weekly doxycycline prophylaxis.

Study Population: 60 Kenyan women aged ≥18 and ≤30 years who are sexually active and using PrEP for HIV prevention.

Study Site: KEMRI RCTP – Lumumba clinic

Primary Study Objectives:

1. Assess the acceptability of once weekly doxycycline prophylaxis DOT
2. Assess persistence in once weekly doxycycline DOT

Secondary Study Objectives:

1. Evaluate incidence rate of STIs in women given once-weekly doxycycline DOT
2. Assess the safety and tolerability of once weekly doxycycline prophylaxis

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LIST OF ABBREVIATIONS

Table 1: List of Abbreviations

AE	Adverse event
AMR	antimicrobial resistance
ART	Antiretroviral therapy
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
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 Principal Investigators, and placed on file before database is locked.

2. STUDY OBJECTIVES

2.1. Study Objectives

2.1.1. Primary Objectives

1. Assess the acceptability of once weekly doxycycline prophylaxis
2. Assess persistence in once weekly doxycycline DOT

2.1.2. Secondary Objectives

1. Evaluate incidence rate of STIs in women given once-weekly doxycycline DOT
2. Assess the safety and tolerability of once weekly doxycycline prophylaxis

3. STUDY DESIGN

3.1. Summary of Study Design

1.1. Study Evaluations

An overview of study specimen and data collection is provided in Table 2. Participants will also have weekly visits as described below.

Table 2. Schedule of study procedures

Study Visit	S	E	3	6
Surveys				
Demographic information	X	X		
Acceptability		X	X	X
Sexual behavior		X	X	X
Clinical care				
Medication review	X	X	X	X
General symptom assessment		X	X	X
WHO pregnancy checklist	X		X	X
STI symptom assessment	X	X	X	X
Clinical Testing				
HIV testing (rapid test)	X		X	X
Urine pregnancy testing	(X)	X	(X)	(X)
CT/NG testing Xpert (endocervical swab)		X	X	X
Syphilis testing (serum RPR)		X	X	X
Archived Sample Collection				
Hair collection for drug level testing (stored)			X	X
Aptima CT/NG endocervical swab (stored)		X	X	X
Endocervical swabs for resistance (stored)		X		X
Rectal swabs for resistance (stored)		X		X
Vaginal swabs for microbiome (stored)		X		X

3.2. Definition of Study Drugs

The study drug is open-label doxycycline 200 mg to be taken DOT weekly.

3.3. Clinical Assessments

Safety assessments are limited to the following:

- All grade 2 and higher AEs that are judged related to doxycycline, 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
- All AEs meeting SAE definition regardless of relationship with doxycycline

In this open label trial, 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. Statistical Analyses

The final analysis will be conducted after database lock of all final (6 month) study visits.

4.1.1. Primary Endpoints

Acceptability and persistence of once-weekly doxycycline prophylaxis

4.1.2. Secondary Endpoints

Evaluate the incidence rate of STIs in women given once-weekly doxycycline DOT

4.1.3. Safety, tolerability and acceptability of once weekly doxycycline prophylaxis

- Safety:
  - serious adverse events (SAEs),
  - all related AEs (assessed in the doxyDOT group only)
- Tolerability:
  - doxycycline discontinuation (assessed in the doxyDOT group only)
  - Candida yeast infection treatment (documented as fluconazole on STI treatment CRF)
  - Doxycycline-specific side effects (assessed via standardized symptom assessment: includes nausea, pill esophagitis, photosensitivity)

4.2. Demographic and Baseline Characteristics

4.2.1. Demographics and risk characteristics

The following baseline demographics and risk characteristics will be presented separately by study group (doxyDOT vs dPEP Kenya SOC group) for the included cohort of each.

- 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
- Contraceptive use (e.g., OCP, IUD, depo IM, implant, vaginal ring)

- Earns her own income (Y/N)

4.2.2. Baseline Laboratory Data

Baseline bacterial STI diagnoses (GC/CT, Syphilis) detected by lab-based study testing will be reported for each cohort separately, by study (doxyDOT vs dPEP SOC).

4.3. Analysis Primary Endpoint

4.3.1. Persistence endpoint

Objective: *Assess the persistence of directly observed once weekly doxycycline prophylaxis*	
Estimand: Proportion of women who receive 80% of weekly DOT doses for 6 months	
Treatment: Directly observed once weekly doxycycline for the prevention of STIs	
ESTIMAND	ANALYSIS
Target population *Kenyan women aged ≥18 and ≤30 years who are sexually active and using PrEP for HIV prevention*	Analysis set *All enrolled women*
Variable *Receipt of study drug DOT*	Outcome measure *Receiving 80% of all expected weekly DOT doses defined as 7 days after prior dose within +/- 1 day.*
Handling of intercurrent events *Early drop out; clinical discontinuation of dPEP.*	Handling of missing data *Any week with no DOT within a day of expected, missed visit or early drop out will be treated as not receiving a DOT dose. Women who have any dose held for clinical reason (e.g. pregnant) will be assessed up to the time of first hold* *Visits that are more than a day late/early based on 7 days from prior dose will be considered as a missing dose* *Sensitivity analysis* .: Women who terminate early from the study for reasons unrelated to the DOT procedures will be censored at the time of drop out. A women's compliance will be assessed only over the period when they are active on study.

Population-level summary measure	Analysis approach
Persistence: Proportion receiving at least 80% of DOT doses for 6 months	The proportion of women who receive at least 80% of all expected doses on time in the course of their participation*. Each women will be assessed separately for the proportion of doses received (p_i = number doses received on time/total number of doses expected). The proportion of women meeting or exceeding 80% and the 95% confidence limit of will be reported. Confidence limits wil be based on a binomial distribution.* *A graphical display will show the proportion of women receiving the dose on time by week through 6 months. Proportions will be assessed across all women for each expected dose.* <u>*Sensitivity analyses*</u>: *The proportion of women who received 80% of the expected DOT doses while remaining in study followup*

4.3.2. Acceptability endpoint

Objective: *Assess the acceptability of directly observed once weekly doxycycline prophylaxis*	
Estimand *Proportion of the enrolled cohort who self report once weekly doxycycline to be acceptable*	
Treatment: Directly observed once weekly doxycycline for the prevention of STIs	
ESTIMAND	ANALYSIS
Target population *Kenyan women aged <u>≥</u>18 and <u><</u>30 years who are sexually active and using PrEP for HIV prevention and on dPEP*	Analysis set *Enrolled women who are on doxycycline for at least one follow-up visit (3 or 6 m) with an acceptability questionnaire* *[Is participant currently on a dPEPhold/discontinuation? Questions only asked if participant on dPEP]*

Variable	Outcome measure
Primary acceptability question: (assessed at 3 and 6 mo) *I do not like taking dPEP* *Secondary acceptability questions: (assessed at Enrollment, 3 and 6 mo)* *I am worried about how people will react if they see me taking dPEP* *Taking dPEP is too demanding* *I worry that dPEP will make me sick.*	Agree/Somehwat agree./Neither agree nor diagree/Somewhat disagree/Dsagree. *Answers == Disagree and Somewhat disagree will be considered acceptable.* *Acceptibility will be assessed for each woman at each visit (month 3 and 6 for primary, and Enrollmetn, 3 and 6 mo for secondary).*
Handling of intercurrent events Missing visits or missing acceptability data at a visit	Handling of missing data *Missing acceptibility: Women will be omitted from visit acceptibility assessment if visit is missed for any reason or if the data on acceptibility is missing.* <u>*Sensitivity analysis*</u>: Missing visits are coded as not acceptable and corresponding summaries produced. Visits missing data because the participant is on study drug hold will continue to be omitted
Population-level summary measure *Proportion of women self- reporting DOT dosing as acceptable*	Analysis approach The proportion of women reporting the intervention is acceptable at Month 3 and Month 6, and a 95% CI based on the normal approx to the binomial. <u>*Sensitivity analyses*</u>: *A graphical display will show the proportion reporting in each level of response for each acceptibility question and an overall mean score for each question, for each visit separately*

4.4. Secondary Objectives

4.4.1. STI endpoint

Descriptive analysis

- The number of participants with any bacterial STI, and with each type of STI, will be summarized by group (DoxyDOT and dPEP Kenya SOC), including person years accumulated and incidence rates, with 95% confidence limits based on a Poisson distribution.

Statistical analysis

Objective: C*omparing STI incidence for women on weekly doxycycline DOT with being assigned to standard of care in recently completed dPEP Kenya Study*	
Estimand: Relative incidence rates between the two studies, based on a 'doubly robust' method	
Treatment: Weekly DOT doxycycline prophylaxis	
ESTIMAND	ANALYSIS
Target population *Kenyan women aged ≥18 and ≤30 years who are sexually active and using PrEP for HIV prevention*	Analysis set *Weekly doxycycline DOT study: All participants with at least one STI assessment at Month 3 or Month 6* *dPEP Kenya study: women assigned to the standard of care arm with at least one STI assessment at Month 3 or Month 6*
Variable *STI infection* *CT infection*	Outcome measure *Incident C. trachomatis, N. gonorrhoeae, or syphilis infection. Visits with multiple STIs will be treated as a single event of incident STI. Infections present at baseline are omitted and must have documented completion of treatment for subsequent infections to be defined as incident.* *Incident C. trachomatis infection. CT Infections present at baseline are omitted and must have documented completion of treatment for subsequent infections to be defined as incident.*
Handling of intercurrent events	Handling of missing data

Intercurrent Event: 1. *Clinical hold* 2. *Missing DOT dPEP dose* 3. *Drop out or missed visit* 4. *Late doses*	 *Visits occurring while on a clinical hold in doxyDOT are omitted* *Visits occuring while pregnant in dPEPKenya are omitted* *All visits are included irrespective of missed doses (this does not include clinical hold)* *No imputation for missed visits* *Late doses are not treated differently than on-time doses.*
Population-level summary measure *Incidence rate ratio for doxyDOT vs SOC*	Analysis approach *The analysis data will combine the first 6 months of follow-up of dPEP Kenya (through the Month 6 study visit) with all follow-up in doxyDOT.* 1. *Unadjusted analysis* *IRR is estimated using repeated-measure Poisson analysis of STI incidence with study membership as the only predictor, offset of (log) study time (in years) between visits.* 2. *'Doubly robust' analysis* *Relative risks are estimated using a weighted, repeated measures modified Poisson regression for the outcome using generalized estimating equations (GEE) to account for recurrent STIs within individual participants. The model includes dPEP Kenya/doxyDOT study membership with offset of (log) study time (in years) between visits, and is adjusted for baseline covariates: age (18-24 vs > 24), transactional sex, multiple partners, any contraceptive use, and STI diagnosis. The weights are inverse propensity scores (accounting for differences in study cohort characteristics). The propensity score model is a logistic regression for the outcome study membership using the same set of baseline*

	characteristics. Propensity score weights are assigned as 1 for women in doxyDOT and 1/p_i for women in dPEP Kenya.

4.4.2. Analyses of STI while on study drug (Per protocol)

For the per-protocol analysis, the doxyDOT group will be restricted to study time prior to the first missed dose (defined by >8 days without a dose of doxycycline from the prior dose) of study drug documented on the study dPEP CRF. The dPEP Kenya dataset will remain the same (i.e. excluding time while pregnant). The evaluation of the impact of doxycycline on Any STI/Any CT will use the same model as specified for the primary analysis.

5. 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.

5.1. Adverse Events and Deaths

5.1.1. AE Definitions

The following AEs are recorded on eCRFs:

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

5.1.2. Adverse Event Summary Tables

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

5.1.3. 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)

5.2. PrEP use

Tabulation of time on PrEP

Tabulations of PrEP will use the following assessments:

- 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?)

5.3. Tolerability

Tolerability is assessed for participants on the doxyDOT arm through

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

Descriptive: The Yes/No responses to possible doxycycline side effects will be tabulated for each study visit among DoxyDOT participants and SOC group among dPEP participants

- Symptoms from the symptom/side-effect questionnaire (includes nausea, vomiting, pill esophagitis, photosensitivity, empiric treatment with clotrimoxazole or fluconazole for yeast infection, empiric treatment with metronidazole for vaginitis symptoms)

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

5.4. Adherence

Adherence is assessed as completion of weekly dose in this analysis. Additionally, we will describe coverage of sex acts for comparison with on-demand dosing strategy which may have impact on effectiveness of medication.

5.4.1. Coverage of sex acts

Assessed in the doxyDOT cohort only:

Measures

- Last 14 days (up to 3 days prior to visit) calendar reporting dates with sex and days with condomless sex.
- Weekly DOT CRFs reporting date of doxycycline doses

Definition: Coverage for a participant is defined as though doxycycline dosing per on-demand dosing schedule, or percentage of days with sexual activity followed by a weekly DOT within 72 hours of each sexual exposure in 14 day period.

Descriptive:

Timeline follow-back calendar: Past 2-week (day -14 to -3) 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.

6. ADDITIONAL SECONDARY ENDPOINT ANALYSIS

6.1. Changes in sexual frequency

Quarterly CRFs collected ask all participants how many times of the last 3 months they had vaginal sexual intercourse. We will compare frequency of sexual activity over time to investigate whether they differ by study cohort 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 study cohort using GEE with Poisson distribution.

6.2. STI Incidence

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

6.3. 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 protocol section 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 cohort.

7. 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.