STATISTICAL ANALYSIS PLAN

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BASED ON:

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Amendments and Dates

CRF Date:

STUDY TITLE:

DoxyPEP: Prospective, randomized, open-label, clinical trial to evaluate the impact of doxycycline post-exposure prophylaxis on the occurrence of bacterial STIs among men who have sex with men (MSM) and transgender women (TGW) PrEP users and people living with HIV

SIGNATURE PAGE

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PROTOCOL SUMMARY

Design:

Open label randomized clinical trial of doxycyline PEP to reduce bacterial STIs (*N. gonorrhea* [GC]*, C. trachomatis* [CT]*,* and *T. pallidum* [syphilis]) among men who have sex with men (MSM) and transgender women (TGW) living with HIV (PLWH) or on HIV PrEP.

Study Population:

390 PLWH participants and 390 participants on HIV pre-exposure prophylaxis (PrEP), for a total sample size of 780

Study Sites:

1. HIV/AIDS Clinic ("Ward 86"), Zuckerberg San Francisco General Hospital, UCSF, San Francisco
2. San Francisco City Clinic, San Francisco Department of Public Health, San Francisco (municipal STD clinic)
3. Madison HIV/AIDS Clinic, Harborview Medical Center University of Washington, Seattle
4. Public Health-Seattle & King County STD Clinic at Harborview Medical Center, Seattle

Primary Study Objectives:

1. Evaluate the effectiveness of doxycycline post-exposure prophylaxis (PEP) to reduce STI infections in two populations: a) MSM/TGW living with HIV and b) MSM/TGW taking HIV PrEP.
2. Investigate the impact of doxycycline PEP on development of culture-based tetracycline (TCN) resistance in *N. gonorrhea* (GC) and among nasopharyngeal carriers of *S. aureus.*

Secondary Study Objectives:

1. Assess the safety, tolerability, and acceptability of doxycycline PEP.
2. Investigate the impact of doxycycline PEP on development of molecular markers of tetracycline (TCN) resistance in *C. trachomatis* (CT), and *T. pallidum* (syphilis).
3. Evaluate the development of phenotypic tetracycline resistance among nasopharyngeal carriers of commensal *Neisseria* species.
4. Measure the proportion of sex acts which are covered by doxycycline PEP.
5. Assess the effect of doxycycline PEP on the gut resistome through measuring the abundance of tetracycline resistance genes in a subset of 50 MSM/TGW living with HIV and 50 MSM/TGW on PrEP assigned to receive doxycycline PEP as well as rectal swabs from all participants, stored for future evaluation.

Exploratory objectives:

1. Evaluate the diversity of the gut microbiome and quantity and breadth of drug resistance genes in participants on doxycycline PEP and not on PEP.
2. Assess the association between meningococcal vaccination and incident GC infection.
3. Evaluate the incidence of nongonococcal, non-chlamydial urethritis and the proportion of urethritis in which *Mycoplasma genitalium* (MG) is detected.
4. Assess the rate of detection of *Mycoplasma genitalium* (MG) in urine in each arm and the presence of tetracycline and other antimicrobial resistance in MG in each arm.
5. Evaluate the incidence of lymphogranuloma venereum (LGV) in those diagnosed with rectal chlamydia in each arm.
6. Evaluate whether doxycycline PEP is associated with PrEP persistence among persons on PrEP and HIV virologic suppression among persons on ART by comparison of arms and within the doxycycline PEP arm by adherence to doxycycline PEP.

Approach:

Eligible participants randomized to receive PEP will receive open-label doxycycline 200 mg to be taken ideally within 24 hours but no later than 72 hours after each condomless sexual contact (receptive anal, insertive anal, vaginal, or oral, or use of shared sex toys). Participants are instructed to take no more than 200 mg in each 24-hour period. Sexual activity and doxycycline PEP usage will be recorded using a smartphone application and assessment at study visits. At enrollment and quarterly for 12 months of follow-up, GC and CT testing will be conducted on samples from the oropharynx, rectum and urine and a blood specimen for syphilis serology will be collected. Resistance testing will be conducted on specimens that test positive for GC using phenotypic methods, and molecular techniques for CT and syphilis (for syphilis, *T. pallidum* DNA will attempt to be obtained from mucosal or lesional swabs). Nares and pharyngeal swabs will be obtained to evaluate tetracycline resistance among nasopharyngeal carriers of *S. aureus* and commensal *Neisseria* species (oropharyngeal swab only). Stool samples from 50 MSM/TGW living with HIV and 50 on PrEP in the doxycycline PEP arm at three time points will undergo metagenomic sequencing for tetracycline resistance genes. Rectal swabs from all participants who consent will be archived for future evaluation of the enteric microbiome and resistome, as well as for presence of MG.

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LIST OF ABBREVIATIONS

Table 1: List of Abbreviations

Abbreviation	Term
AE	Adverse Event
ART	Antiretroviral therapy
CASI	Computer Assisted Self-Interview
CRF	Case Report Form
CT	Chlamydia trachomatis
GC	Neisseria gonorrhea
GCP	Good Clinical Practices
ITT	Intent-to-Treat Population
NAAT	Nucleic acid amplification test
PEP	Post-exposure prophylaxis
PrEP	Pre-exposure prophylaxis
PP	Per-Protocol Population
s.d.	Standard Deviation
SAE	Serious Adverse Event
TCN	Tetracycline

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.

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 the database is locked.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Study Objectives

2.1.1. Primary Objective

1. Evaluate the effectiveness of doxycycline post-exposure prophylaxis (PEP) to reduce STI infections in two populations: a) MSM/TGW living with HIV and b) MSM/TGW taking HIV PrEP.
2. Investigate the impact of doxycycline PEP on development of culture-based tetracycline (TCN) resistance in *N. gonorrheae* (GC) and among nasopharyngeal carriers of *S. aureus.*

2.1.2. Secondary Objective

1. Assess the safety, tolerability, and acceptability of doxycycline PEP
2. Investigate the impact of doxycycline PEP on development molecular markers of tetracycline (TCN) resistance C. trachomatis (CT), and T. pallidum (syphilis).
3. Evaluate the development of phenotypic tetracycline resistance among nasopharyngeal carriers of commensal Neisseria species.
4. Measure the proportion of sex acts which are covered by doxycycline PEP
5. Assess the effect of doxycycline PEP on the gut resistome through measuring the abundance of tetracycline resistance genes in a subset of 50 MSM living with HIV and 50 MSM/TGW on PrEP assigned to receive doxycycline PEP as well as rectal swabs from all participants, stored for future evaluation.

2.2. Study Endpoints

2.2.1. Primary Endpoints

2.2.1.1. Effectiveness of doxycycline

The primary endpoint is any of the following STI endpoints post-enrollment:

- Documented positive GC by NAAT
- Documented positive CT by NAAT
- Early syphilis infection based on four-fold increase in non-treponemal titers or positive darkfield on exudate from a lesion.

STIs are assessed at month 3,6, 9 and 12 visits in all participants, in addition to testing at interim visits, if indicated (e.g. symptomatic). A primary endpoint event is defined as occurrence of any one of the above STIs diagnosed within each quarter, with the exception that repeat diagnosis of the same STI within 28 days of a quarterly visit is assumed to be the same infection (i.e. not an incident infection). Any infection diagnosed at an interim visit (outside the 28 days) will be counted as a primary endpoint for that quarter i.e., if a participant is diagnosed with GC

at 40 days after their 6 month visit, this will be analysed as a primary 9 month endpoint. All endpoints will be reviewed by the endpoint adjudication committee, blinded to arm assignment, for final determination, using most current CDC STI guidance.

2.2.1.2. Impact of doxycycline on TCN resistance

1. Resistance of GC to TCN detected in positive GC cultures. Those with positive GC NAAT results will have a swab obtained from the involved anatomic site and other sites of sexual contact in past 90 days (to include throat, rectum, urine) for GC culture-based phenotypic testing. Swabs for GC culture will be collected at time of suspected GC or in patients recalled after positive GC NAAT result reported.
2. Tetracycline resistance detected in *S. aureus* culture-positive nasopharyngeal swabs, collected at baseline, six, and 12 months

2.2.2. Secondary Endpoints

2.2.2.1. Efficacy of doxycycline for individual STIs

Effectiveness of doxycycline will also be assessed for each of the STIs separately, for both cohort combined, using endpoints as defined above.

2.2.2.2. Safety, tolerability and acceptability of doxycycline PEP

- Safety: All grade 2 and higher hematologic and hepatic laboratory abnormalities, all grade 3 and 4 adverse events judged to be associated with doxycycline (possibly, probably, or definitely related), and all SAEs.
- Tolerability: in person quarterly assessments of discontinuations of doxycycline, discontinuation of PEP, symptom assessment, and self-reported side effects questionnaire at 6 and 12 months by CASI (computer assisted self-interview),

  In person assessment by research staff at scheduled visits to include:

  - All permanent doxycycline discontinuation and reasons for discontinuation;
  - For all temporary doxycycline interruptions ≥7 days, reason for interruption; and
  - Side effects:
    - presence/absence of the following symptoms and if present, whether attributed to doxycycline (if taking) by a) participant and b) investigator: rash, sunburn, headache, change in vision, pain with swallowing, diarrhea, nausea, vomiting, abdominal pain, or other.

  CASI questions related to tolerability

- At least once in the past 3 months, I did not take doxycycline within 3 days after sex because I wanted to avoid side effects. (Reported using 5-point Likert scale)
- Were there any other reasons that you did not take doxycycline within 3 days after sex? Yes/No (Y/N) and open-ended response if yes is selected.
- In the past 3 months, do you feel that taking doxycycline affected your sexual experience? (Reported using 3-point scale: negative, neutral, positive effect).

- Acceptability: self-reported acceptability questionnaire by CASI for all doxycycline PEP recipients administered at month 6 and 12.

  Answer to the following questions, reported using a 5-point Likert scale, unless otherwise indicated

  - Doxycycline is easy to take.
  - I'm worried that taking doxycycline will cause antibiotic drug resistance. (reverse coded)
  - I'm worried that doxycycline will hurt my body. (reverse coded)
  - Taking doxycycline PEP will reduce my condom use. (reverse coded)
  - Doxycycline has other good effects for me.
  - Taking doxycycline PEP has helped me take my PrEP or ART more regularly.
  - A sexual partner has expressed negative feelings about me taking doxycycline. (reverse coded)
  - A sexual partner has expressed positive feelings about me taking doxycycline
  - Taking doxycycline only when needed after sex and not every day is... (5-point scale from very unacceptable to very acceptable)
  - In my community, taking doxycycline to prevent STDs is considered... (5-point scale from very unacceptable to very acceptable)
  - I would continue to take doxycycline to reduce sexually transmitted infections if available to me at no cost after the study.
  - I would recommend doxycycline PEP to a friend. (Y/N)

2.2.2.3. Sex acts covered by doxycycline PEP

Sex acts covered by doxycycline will be recorded by quarterly CASI assessment and medication reconciliation as well as optional usage of an ongoing smartphone app (Blackbook).

- CASI: Coverage of sexual contacts and accompanying doxycycline use assessed at month 6 and 12 in the doxycycline PEP arm assessed by the following questions:

  - In the past 3 months, I missed taking doxycycline within 3 days after sex at least once. Y/N
  - In the past 3 months, when you have had anal or vaginal/frontal without using a condom the whole time, how often did you take doxycycline PEP within 72 hours after sex? 5-point scale

- In the past 3 months, when you have given or received oral sex, how often did you take doxycycline PEP within 72 hours after oral sex? 5-point scale
- -- Of the TIMES you had anal sex or vaginal/frontal sex without a condom, how many TIMES did you take doxycycline PEP within 72 hours after sex? Numeric value
- The last time you had anal or vaginal/frontal sex without using a condom the entire time, did you take doxycycline? Y/N
- Of these times you had oral sex, how many TIMES did you take doxycycline PEP within 72hrs? Numeric value
- The last time you had oral sex did you take doxycycline. Y/N
- How many times did you take doxycycline PEP more than 72 hours after anal or vaginal sex?
- How many times did you take doxycycline PEP more than 72 hours after oral sex?

- Quarterly review of doxycycline dispensing and remaining tablets:
  - Number of doxycycline pills returned at each quarterly visit.
  - Bottles of doxycycline dispensed at each visit.
- Smartphone app: Coverage of sex acts by doxy will also be assessed by use of app, which will prompt participants to record all sexual activity (oral, anal, vaginal), use of condoms, and whether doxycycline was taken after qualifying sexual contact (proportion of sex acts covered).

2.2.2.4. Tetracycline resistance

- Molecular markers of resistance for CT
- Molecular markers of resistance to syphilis
- Phenotypic resistance in nasopharyngeal swabs culture positive for Neisseria
- Abundance of TCN resistant genes in rectal swabs from MSM/TGW receiving doxycycline PEP (based on total Tet C and Tet M genes detected, normalized for the quantity of bacterial present, not isolated from specific flora)

3. STUDY DESIGN

3.1. Summary of Study Design

Open label randomized clinical trial of doxycyline PEP to reduce bacterial STIs (*N. gonorrheae* [GC]*, C. trachomatis* [CT]*,* and *T. pallidum* [syphilis]) among MSM/TGW) living with HIV (PLWH) or on HIV PrEP. The trial is powered to separately assess impact for MSM/TGW living with HIV and MSM/TGW on PrEP.

Eligible participants randomized 2:1 to doxycycline PEP will receive open-label doxycycline 200 mg to be taken ideally within 24 hours but no later than 72 hours after condomless sexual contact ((receptive anal, insertive anal, vaginal, or oral, or use of shared sex toys).). 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. Sexual activity will be recorded for both arms of the study (doxycycline PEP and control condition) by the participant using a smartphone application that will be adapted for study use; this will enable comparable assessment of risk in the two arms. PEP pill-taking will also be measured by the app to enable assessment of coverage of sex acts by PEP. Sexual activity and adherence will also be assessed in person at quarterly visits.

STI testing will be conducted quarterly from three anatomic sites (pharyngeal, rectal, and urine samples for GC and CT NAAT testing) and blood obtained for syphilis testing, following CDC guidelines for serologic diagnosis of syphilis.

Participants with a positive STI test will return for STI treatment and for swabs of the affected site for resistance testing; culture- and molecular-based for GC and molecular methods for GC, CT and syphilis. Those with signs and symptoms suggestive of syphilis infection and those with a reactive syphilis serologic test that indicates a new syphilis infection will have swabs of any current active lesion (if present) as well as mucosal swabs from the oropharynx to obtain DNA for molecular testing.

To assess tetracycline resistance in a common, sometimes pathogenic bacteria, swabs from the anterior nares and oropharynx will be obtained at baseline, 6 and 12 months to identify *S. aureus* carriers in whom tetracycline resistance will be evaluated. To assess tetracycline resistance that could be transferred to oropharyngeal GC from commensal *Neisseria* species, oropharyngeal swabs will be obtained at baseline and 12 months to evaluate tetracycline resistance in a carrier.

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. Participants will be instructed to take 200 mg of doxycycline 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 sample size of 390 MSM/TGW in each cohort (MSM/TGW living with HIV and MSM/TGW on PrEP) has 80% power with 0.05 two-sided Type 1 error, for the endpoint of any lab-detected incident early syphilis, GC and/or CT infection, based on quarterly assessments for each person, 10% annual loss to follow-up and an intra-class correlation of 0.2. With these assumptions, a cohort of 390 of MSM/TGW living with HIV and 390 of MSM/TGW on PrEP (130 in the control and 260 in the intervention for each cohort) will provide 80% power to detect a decrease in quarterly STI prevalence from 10% to 5%, which corresponds to an annual reduction in combined STI incidence from 34% to 19%, assuming effective treatment.

We expect in the control arm approximately 5% of participants to have GC, 5% CT and 2% early syphilis at each quarterly visit; some will be diagnosed with more than one infection. We anticipate effectiveness of doxycycline PEP to be 35% for GC, 65% for CT and 65% for syphilis.

Sample sizes were computed using nQuery for repeated measures for two proportions, assuming four visits for each participant, and ratio estimators for the specified reduction. Approximately 10% annual loss to follow-up is assumed, because of partial information expected at some visits, this is approximated with sample size of 370.

Table 2 Power Table for N = 390; 2:1 randomization doxycycline PEP: SOC

Control arm quarterly prevalence	Intervention arm quarterly prevalence	Power to detect 50% reduction		Power to detect 65% reduction	
		ICC = 0.2	ICC = 0.1	ICC = 0.2	ICC = 0.1
10%	5%	80%	87%	96%	99%
7.5%	3.75%	68%	76%	90%	95%
5%	2.5%	51%	59%	77%	85%
3%	1.5%	35%	41%	57%	65%
2%	1%	26%	30%	43%	50%

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, estimating intra-class correlation and retention to date in the trial. 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

The randomization is 2:1 for assignment to doxycycline PEP versus standard of care (no doxycycline PEP). Randomization is stratified by four sites, two primarily enrolling HIV-infected, and two HIV-uninfected participants, with block sizes selected with size randomly selected from between 15 to 30.

3.5. Clinical Assessments

Safety assessments are limited to the following:

- All grade 2 and higher hematologic and hepatic laboratory abnormalities that are attributed to doxycycline in the opinion of the site investigator
- All AEs meeting SAE definition
- All grade 3 and 4 adverse events judged to be associated with doxycycline (possibly, probably or definitely related)

In this open label trial, for those randomized to receive doxycycline PEP, all AEs and SAEs 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. A single formal interim monitoring time is planned, when approximately half of the planned total enrollment have completed the 6-month visit, with stopping rules based on the achieved accrual of primary outcomes using the O'Brien-Fleming boundaries. Each cohort will be monitored separately. 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 doxycycline-associated side effects.

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, median and minimum and maximum). Categorical variables (e.g., race) are summarized using counts and percentages. Percentages are calculated using the total subjects with data per treatment group.

5.2. Analysis Populations

5.2.1. ITT population

The intent-to-treat (ITT) population comprises all randomized participants. There are two ITT populations: HIV-uninfected MSM and TGW on PrEP; HIV-infected MSM and TGW.

5.2.2. Safety population

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

5.2.3. Blackbook responders

The subset of the ITT population that participates (i.e. contributes data) in the Blackbook app.

5.2.4. GC positive population

Post-baseline GC positive participants assessed for TCN resistance. Participants with positive GC will be assessed for culture based TCN resistance and for molecular resistance.

5.2.5. CT positive population

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

5.2.6. Syphilis positive population

Post-baseline Syphilis positive participants assessed for TCN resistance. Participants with incident syphilis will be assessed for molecular-based TCN resistance.

5.2.7. Nasal or oropharyngeal S. aureus carrier Population

Participants with nasopharyngeal cultures with *S. Aureus* growth assessed for TCN resistance.

5.2.8. Neisseria positive population

Participants with nasopharyngeal *Neisseria* growth assessed for oropharyngeal TCN resistance.

5.2.9. Hair population

Participants who have hair assessed for presence of doxycycline.

5.2.10. Gut resistome Population

50 HIV positive MSM and 50 HIV negative MSM/TGW assigned to the doxycycline PEP arm assessed for tetracycline resistance using rectal swabs.

5.2.11. Subgroups

The following are subgroups of interest:

- MSM only
- Age (< 30 vs. > 30)
- City (San Francisco/Seattle)
- Use of HIV PrEP (Event driven/Daily)

5.3. Handling of Missing Data

5.3.1. Missing Efficacy Endpoints

Complete case analysis will be used for the primary efficacy analysis, unless substantial imbalance (> 8%) in missing primary endpoint by arm occurs. Appropriate missing data methods (e.g. imputation) or causal estimation of direct effects will be conducted to assess the potential influence of differential missingness by arm.

6. STUDY POPULATION

6.1. Participant Disposition

A consort diagram will describe for each cohort separately 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 and by site.

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, and early withdrawal (including deaths).

6.5. Demographic and Baseline Characteristics

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

- Age in years
- Race/ethnicity
- Education
- Gender identity
- Sexual preference
- Sexual risk
  - Number and HIV serostatus of partners
  - Frequency of oropharyngeal, penile, and rectal exposure with and without condoms
  - STI(s) in year before enrollment
- alcohol use
- meth use
- IDU
- recreational drug use
- Living situation
- Health insurance
- Currently taking PrEP (Daily vs. event driven)/ART

6.5.1. 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. Infections will be tabulated by sites(s) of infections

7. EFFICACY

7.1. General Considerations

Tabulations will be by study arm within each cohort, for each quarter of study follow-up (or at visits where evaluated). These will be reported in only the closed report during the study. Parallel tabulations will be given by study site only if substantive differences are observed between sites (which are not expected).

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.

Analyses will be conducted separately for HIV-infected and HIV-uninfected cohorts for the primary endpoint, and for the combined cohort. All secondary endpoints will be conducted in the combined cohort.

7.2. Statement of the Null and Alternate Hypotheses

For both HIV-uninfected MSM/TGW on PrEP and HIV-infected MSM/TGW separately:

- 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 does have differential effect 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 (</> 30).
- Report of STI diagnosis in prior year.
- Baseline viral suppression (< 50 copies) for HIV-infected men.
- On PrEP at baseline (Daily/Event driven/No) for HIV-uninfected men.

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 STI** in the first, second, third or fourth quarter of follow-up, detected in post-baseline testing up to and including STI testing at months 3,6,9 and 12. Any STI diagnosed within 28 days of the same STI present at a quarterly visit will not be defined as an incident infection (suggesting incomplete treatment or persistent NAAT positivity without active infection, rather than re-infection). Incident infections detected and treated at interim visits after 28 days will be defined as incident infections for the current quarter. To avoid potential assessment bias, these will be handled 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 40 days after the month 6 visit will be carried forward as a primary event at the next scheduled visit (month 9). If a participant has more than one positive STI (either of the same or different bacterial infections at a scheduled visit it will be counted as one primary event for that quarter. If scheduled visits are missed, infections will be assigned to the next scheduled visit.

All endpoints will be reviewed by the endpoint adjudication committee, blinded to doxycycline PEP assignment, for final determination, using most current CDC STI guidelines.

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 (e.g., characteristic chancre in primary syphilis which would be confirmed by a darkfield or reactive RPR without the requirement for a four-fold increase in titer).

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.
- For CT and GC, the anatomical site of infections will be detailed.

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 site and study arm as the only covariates[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, potentially also by anatomical site.

7.5.2. Sensitivity Analyses of the Primary Efficacy Results

Several sensitivity analyses are planned:

1. Time to first STI using Kaplan-Meier and Cox PH regression.

2. Including all STIs diagnosed, irrespective of timing. The primary outcome will be modified to include any STI re-diagnosed within 28 days of the scheduled quarterly visits, and the statistical analysis for the primary analysis repeated.
3. Analysis counting each separate STI diagnosis as an event. 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 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 doxycycline PEP arm will be restricted to study time prior to the first permanent discontinuation of study drug by self-report or documented on the study CRFs.

First discontinuation is defined as first of

- Product discontinuation
- Missed quarterly visit

Descriptive: Occurrence and reasons for first discontinuation will be tabulated (number of participants).

Analysis: The evaluation of the impact of non-disrupted use of doxycycline on any STI will use the same model as specified for the primary analysis, but also potentially adjusted for age, race/ethnicity, baseline STI, and baseline and follow-up sexual risk (number of partners, unprotected anal sex, employment). For the HIV negative cohort, PrEP at baseline; for the HIV positive cohort, viral suppression at baseline will also be considered.

7.5.3.1. Study drug use and time on study drug in doxycycline PEP arm

The following descriptive tabulations will be completed for each measure:

- Study drug dispensation (number of participants and number of pills) will be summarized for each study visit over time by cohort.
- Proportion of sex acts and condomless sex acts where doxycycline was taken, based on self-report will be tabulated for each quarter and overall within each cohort. Evaluated for both the mobile app (Blackbook cohort only) and CRF calendar data.
- Semiquantitative assessment (high/low) of concentrations in hair will be tabulated for each quarter and overall within each cohort (Hair cohort only).
- Concomitant use of doxycycline, as documented in the antibiotic use log, will be summarized for the control and doxycycline arms

Descriptive comparisons are planned to evaluate the consistency of measures across different measurement modalities

- Mobile app, hair, and CASI data will be compared using variables defined to approximate the same time period.

7.5.3.2. Assessment of doxycycline in hair

Longitudinal exposure to doxycycline assessed using hair collection.

- A semiquantitative evaluation in the control arm (doxycycline present vs. absent).
- A semiquantitative assessment in the PEP arm (doxycycline concentration high vs. low vs. none).

Descriptive: The proportion of samples with doxycycline present, and the concentration levels will be tabulated for each quarter and overall by study arm within each cohort.

7.5.4. Secondary analysis of efficacy for each site of infection

Secondary endpoints:

- Any GC.
- Any CT.
- Any new early syphilis diagnosis.
- Site of GC infection (pharyngeal GC compared to urine or rectal GC).
- Site of CT infection (pharyngeal CT compared to urine or rectal CT).

Analysis: For secondary endpoints, the same approach as defined for the primary endpoint will be used, restricted to the specific bacterial infections or infection site.

7.5.5. Analysis of Tetracycline resistance

7.5.5.1. Primary analysis of TCN resistance in GC cases

The primary analysis of TCN resistance in GC will be conducted in the GC positive.

Descriptive statistics: Tabulations of resistance amongst cases (GC: TCN resistance testing missing, not resistant, resistant) will be reported at baseline, month 3, 6, 9, and 12 (and potentially interim visits) in each arm, for any infection site, and broken out by infection site. Additional descriptive information may also be included (i.e. change in MIC in GC cultures amongst those with repeat infections)

Statistical analysis: Assessment of odds of resistance amongst GC cases with resistance results will be assessed by arm using logistic regression with arm as exposure, adjusted for city. To account for repeated infections, repeated measures methods (GEE) will be used. Analysis comparing arms also potentially adjusted for age, race/ethnicity, baseline STI, baseline and follow-up sexual risk (number of partners, unprotected anal sex, employment). For the HIV-negative cohort, PrEP at baseline; for the HIV-positive cohort, viral suppression at baseline will also be considered.

7.5.5.2. Analysis of TCN resistance in CT cases

The analysis of TCN resistance in CT cases will be conducted in all persons with a positive CT diagnosis after baseline.

Outcome: Molecular TCN resistance will be defined as presence of mutations associated with TCN resistance in the CARD database.

Descriptive statistics: Tabulations of TCN resistance in CT cases (TCN 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 prevalence of TCN resistance by arm across all post-baseline visits combined will be assessed by estimating the relative risk of TCN resistance using logistic regression. The model will assess the relative odds of resistance by arm, adjusted for study site.

7.5.5.3. Analysis of TCN resistance in Syphilis cases

The analysis of TCN resistance in Syphilis cases will be conducted in all persons with an incident syphilis cases after baseline.

Outcome: Molecular TCN resistance will be defined as presence of mutations associated with TCN resistance in the CARD database.

Descriptive statistics: Tabulations of TCN resistance in Syphilis cases (TCN 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 prevalence of TCN resistance by arm across all post-baseline visits combined will be assessed by estimating the relative risk of TCN resistance using modified Poisson regression. The model will assess the relative risk of resistance by arm, adjusted for study site.

7.5.5.4. Analysis of TCN resistance in *S. aureus* carriers

The primary analysis of TCN resistance will be conducted in *S. aureus* cases detected at Baseline, 6- and 12-month visits.

Outcome: TCN resistance will be a dichotomous outcome defined as sensitive or resistance, based on e-Test cut-offs.

Descriptive statistics: Tabulations of TCN resistance in *S. aureus* cases (TCN resistance testing missing, not resistant, resistant) will be reported at baseline, 6- and 12-month visits in each arm. (site of *S. aureus*, if found, will not be known).

Statistical analysis: Assessment of difference in prevalence of TCN resistance by arm at each of 6 and 12 months will be assessed by estimating the relative risk of TCN resistance using modified Poisson regression. The model will assess the relative risk of resistance by arm, adjusted for study site at each time point. If an effect is found, assessment of a trend in time will be assessed by fitting a linear trend over study time. If prevalence of resistance is < 5%, analysis will pool across visits.

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, or the doxycycline PEP arm only, given the open label nature of the cohorts.

8.1. Adverse Events and Deaths

8.1.1. AE Definitions

Only the following AEs are recorded on eCRFs:

- All grade 2 and higher hematologic and hepatic laboratory abnormalities.
- All SAEs.
- In those randomized to doxycycline PEP arm, all grade 3 and 4 AEs with attribution to doxycycline in the opinion of the site investigator.

8.1.2. Adverse Event Summary Tables

The number of Grade 2 and higher hematologic and hepatic AEs data will be tabulated by grade and arm. Subjects will be counted only once for each hematologic and hepatic report. For the doxycycline PEP arm, relatedness will be tabulated (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 as related and not related. The two categories will be defined as follows:

- Related combines the options definitely, probably, and possibly
- Not related is not related.

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

A tabulation will present participants who discontinue study drug prematurely for the doxycycline PEP arm due to an adverse event, and the reasons for discontinuations:

- Requirement for prohibited concomitant medications or other contraindication to doxycycline.
- 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 (>14 days).

8.2. Laboratory Data

Laboratory data collected in this study consist of hematologic and liver function tests at months 3 and 9 in those on doxycycline PEP arm:

- Complete blood count (CBC) (white count, hemoglobin, platelets).
- Liver function testing (AST, ALT, total bilirubin and alkaline phosphatase)

The presentation of laboratory results focuses on the change from baseline to 3 and 9 months. All participants in the doxycycline PEP arm who have a baseline and at least one follow-up laboratory assessment, unless permanently discontinued doxycycline PEP, will be included in the presentation of the laboratory data. For each laboratory test, descriptive statistics will summarize the results at baseline, 3-, and 9-month assessment, and the change from baseline to 3- and 9-months assessments. The summary statistics are N, mean, s.d., median, and range. The within-group p-value from the paired t-test on the mean change from baseline and the 95% confidence interval limits for the mean change from baseline are also included with the change descriptive statistics. Figures will also be used to display change from baseline to 3 and 9 months.

The quantitative laboratory data for participants who have a baseline and follow-up laboratory assessment may be presented as scattergrams plotting the change from baseline as a function of the baseline value.

This analysis will omit values occurring after a participant has permanently discontinued study drug.

8.3. Concomitant and Other Medications

8.3.1.1. Tabulation of time on PrEP and ART

Tabulations of PrEP and ART will use the following assessments:

- Adherence to HIV medications as reflected in viral suppression among participants living with HIV, assessed by chart review of HIV RNA levels conducted as standard clinical care and reported on CRFs.

8.4. Tolerability

Tolerability is assessed through:

- Self-reported response to whether a participant did not take doxycycline because of side effects.
- Report of early termination due to side effects of study drug.

Descriptive: The Likert scale responses will be tabulated for each study visit for each cohort.

8.5. Acceptability

Acceptability is assessed in the doxycycline PEP arm only via a seven-question acceptability questionnaire using a 5-point Likert scale.

Descriptive: The scales will be converted to an acceptability score—using reverse coding where appropriate—and summarized by cohort, site, and visit. Individual questions will also be reported in detail.

9. ADDITIONAL SECONDARY ENDPOINT ANALYSIS

9.1. Coverage of sex acts

Coverage of sexual contacts and accompanying doxycycline use is assessed by CASI at 6 and 12 months, and also assessed in the mobile app.

These self-report endpoints reflect coverage:

1) In the past 3 months, I missed taking doxycycline within 3 days after sex at least once. Y/N
2) In the past 3 months, when you have had anal or vaginal/frontal without using a condom the whole time, how often did you take doxycycline PEP within 72 hours after sex? 5-pointscale
3) In the past 3 months, when you have given or received oral sex, how often did you take doxycycline PEP within 72 hours after oral sex? 5-point scale
4) The last time you had anal or vaginal/frontal sex without using a condom the entire time, did you take doxycycline? Y/N
5) The last time you had oral sex did you take doxycycline. Y/N

Descriptive: Each endpoint will be tabulated by visit for the doxycycline PEP arm. Five-point scales will be dichotomized by combining “always/most of the time” vs. categories that are less often.

Blackbook: App-acquired data will be used to compute the proportion of sex acts covered by doxycycline PEP for each participant for all periods when the app was used, and the participant remained on study drug.

Descriptive:

1) Participant use of the app will be summarized: number/proportion using the app, number of weeks in use, compared to number of weeks of doxycycline PEP, proportion of time described by App use; also summarized by study quarter.
2) Proportion of sex acts covered by doxycycline PEP for all person-time collected in each quarter will be summarized.

9.2. Gut resistome sub-study

Analysis of the gut resistome will be described in a separate statistical analysis plan.

10. REVISIONS TO THE SAP

Not Applicable

11. APPENDIX

11.1. Table of Contents for Data Reports

Appendix to be completed

Title		Population	Comments
Study Population Section			
		ITT	Unique
		ITT	Unique
		ITT	Unique
		ITT	Repeat 9.3.1
Efficacy Section			
Figures			
		ITT	Unique
		ITT	Unique
Tables			
		ITT	Unique
		ITT	Unique
		ITT	Repeat 12.1.2.1
		ITT	Unique
		ITT	Unique
		ITT	Unique
Safety Section			
		Safety	Unique
		Safety	Unique
		Safety	Unique
		Safety	Unique
		Safety	Unique

Listings

Title		Population	Comment
	Subject Enrollment Information	Enrolled	Unique
	Subject Disposition	Safety	Unique
	Subjects who did not Satisfy Inclusion/Exclusion Criteria (if applicable)	Screening Failure	Unique
	Protocol Deviations	Safety	Unique
	Listing of Relationship of Serious Adverse Event Body Systems, Group Terms, and Verbatim Text	Safety	Unique

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.