A randomized trial of doxycycline chemoprophylaxis for the prevention of sexually transmitted infections in gay, bisexual and other men who have sex with men (gbMSM)

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Confidentiality Statement

This clinical study protocol contains information which is of a confidential, trade-secret or proprietary nature. The protocol is for the use of Dr. Grennan, his study team, and their designated representatives participating in the

investigational trial. It is not to be disclosed to any other person or party without the prior written approval of Dr. Grennan or his delegates.

STATEMENT OF COMPLIANCE

Protocol Title:	A randomized trial of doxycycline chemoprophylaxis for the prevention of sexually transmitted infections in gay, bisexual and other men who have sex with men (gbMSM).
Version No.:	5.0
Date:	05-FEB-2024

This clinical study will be conducted in accordance with the protocol, applicable Health Canada regulations, International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines on current Good Clinical Practice (GCP), Tri-Council Policy Statement Version 2 (TCPS 2) and the Declaration of Helsinki.

I confirm that I have read and understand this protocol and I agree to conduct this clinical study in accordance with the design and specific provisions of the protocol, with the exception of a change intended to eliminate an immediate hazard to participants. Any deviation from the study protocol will be documented in the case report form.

I agree to promptly report to the applicable ethics boards any changes in the research activity and all unanticipated problems involving risks to human participants or others. Additionally, I will not make any changes in the research without prior ethics and sponsor approval, except where necessary to ensure the safety of study participants. Finally, I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator: Printed Name	Signature	Date (dd-mmm-yyyy)

PROTOCOL SYNOPSIS

Full Title	A randomized trial of doxycycline chemoprophylaxis for the prevention of sexually transmitted infections in gay, bisexual and other men who have sex with men (gbMSM)
Short Title	**D**oxycycline **I**ntervention for bacterial **S**TI **C**hemopr**O**phylaxis (DISCO).
Protocol and Version No.	Version 5.0
Sponsor-Investigator	Dr. Troy Grennan
Medical Monitor	To be determined
Number of Centres	9
Study Design	Prospective, multicentre, open label, two-arm, non-inferiority randomized controlled trial
Primary Objective	To establish the non-inferiority of STI PrEP vs. PEP, by examining the efficacy of each intervention in preventing incident bacterial STI cases in gbMSM.
Secondary Objectives	Tolerability/safety endpoints, changes in sexual behaviour, adherence measures, antimicrobial resistance development, superiority of STI PrEP over PEP, as well as some mathematical modeling and economic evaluation work examining the impact of PrEP and PEP on STIs.
Sample Size	N = 560
Study Population / Diagnosis and Main criteria for inclusion	Inclusion criteria 1. Adults, ≥ 18 years of age; 2. Gay, bisexual and other men who have sex with men (both cisgender and transgender) or transgender women 3. Any sexual activity (i.e. oral sex, insertive or receptive anal sex, with or without a condom) with more than one male partner in the preceding 12 months; 4. Intention to remain sexually active with more than one male partner in the next 12 months; 5. At least one prior episode of a previously diagnosed and adequately treated syphilis, gonorrhea or chlamydia infection within 12 months prior to screening; 6. If you are sexually active and capable of becoming pregnant, you will use birth control/contraception during the entire study duration. Exclusion criteria 1. Known allergy to doxycycline or tetracyclines; 2. Existing chronic or intermittent tetracycline or doxycycline use (e.g. for chronic osteomyelitis, acne). 3. Use of medications which could lower doxycycline levels, including barbiturates, phenytoin and carbamazepine. 4. Individuals currently using isotretinoin; 5. Are sexually active, capable of becoming pregnant, and will not use birth control/contraception reliably during the course of the study duration.
Intervention Description	1. STI PrEP arm: doxycycline capsules 100mg daily for 12 months; 2. STI PEP arm: doxycycline 200mg orally once within 24-72 hours following each sexual encounter deemed at risk (i.e. condomless anal or oral sex), to a maximum of six pills (i.e. 600 mg total) per week;

Participant Duration	15 months
Study Duration	48 months (4 years)
Outcome Measures	Primary: a composite endpoint defined as the count of any incident bacterial STIs (syphilis, gonorrhea, and/or chlamydia, including LGV). Secondary: **(1)** Pathogen-specific incident infections: syphilis; gonorrhea; chlamydia; **(2)** Treatment-emergent adverse events and laboratory abnormalities; **(3)** Risk compensation, as defined by increases in condomless sexual acts and number of sexual partners; **(4)** AMR patterns in common commensal organisms in the nasopharynx, as defined by increases in the minimal inhibitory concentrations (MIC) for the organisms in question to tetracyclines; **(5)** Antimicrobial resistance in gonorrhea and syphilis isolates taken from the anus, oral cavity, urethra or vagina; **(6)** adherence to study treatment (i.e. STI PrEP or PEP) by participants.
Statistical Analysis	Intention-to-treat. Primary analysis: Negative binomial regression model to assess the effect of the treatment on the total number of incident STI by month 15. STI PrEP will be declared non-inferior to PEP if the upper bound of the two-tailed 95% confidence interval for the rate ratio comparing PrEP to PEP is no greater than 1.15. Secondary analysis: Descriptive analyses, rates and rate ratios (with 95% CI), as well as longitudinal models for secondary outcomes.

1. BACKGROUND & RATIONALE

1.1 THE BURDEN OF SEXUALLY TRANSMITTED INFECTIONS (STIS) IN CANADA

There is a growing epidemic of the bacterial sexually transmitted infections (STIs) syphilis, chlamydia and gonorrhea worldwide, with the World Health Organization estimating that there are nearly 1 million people infected with a curable STI daily[1]. This has led to a global call to action for the elimination of STIs by 2030[2]. Similarly concerning trends have been noted in Canada, where increases of over 160% have been seen in bacterial STIs over the last decade[3]. Clearly, this has a significant economic and resource impact on our healthcare system, and strains an already-taxed public health system[4]. In Canada, gay, bisexual, and other men who have sex with men (gbMSM) – including those living with HIV – are disproportionately impacted by bacterial STIs[5,6]. Record-high syphilis rates[7–9], are occurring alongside alarming increases in severe syphilis complications[10–12]. While syphilis was at an all-time low in 2000, by 2014 rates in males (mostly gbMSM) had increased 13-fold, and continue on this trajectory, with males now making up 80% of Canada's infectious syphilis cases[3]. In Canada's urban centres, we have shown that two-thirds of syphilis cases are in HIV-positive gbMSM, 20% of syphilis diagnoses are repeat infections[13], and re-infections are most common among HIV-positive gbMSM[3,5,14]. *Neisseria gonorrhoeae*, the causative agent of gonorrhea, is the second most commonly-reported STI in Canada. It disproportionately impacts gbMSM[3], who are a key group for limiting the propagation of antimicrobial resistance (AMR)[15]. Increasing minimum inhibitory concentrations (a measure of AMR to specific antibiotics) make the prospect of untreatable gonococcal infection a real possibility[16]. Meanwhile, the re-emergence of lymphogranuloma venereum (LGV), caused by invasive serovars of *Chlamydia trachomatis*, also disproportionately impact gbMSM[17–19]. Moreover, people living with HIV can be at risk for more serious STI complications. People with syphilis-HIV co-infection are more likely to progress to neurosyphilis[20] even in early-stage syphilis infection[21,22] , and we demonstrated an association between HIV and the development of ocular syphilis[23]. The same is true for LGV, for which serious long-term complications include lymphatic obstruction and fistulae. There is strong evidence that HIV infectiousness is enhanced in syphilis and other STI co-infections, thus promoting HIV transmission[24,25]. These dramatic increases in bacterial STIs, the potential development of serious complications including AMR, and waning effectiveness of the promotion of conventional STI prevention tools (e.g. condoms), signals the need for novel STI prevention strategies and tools to mitigate STI-related complications.

1.2 THE ERA OF PHARMACOTHERAPEUTIC CHEMOPROPHYLAXIS

Momentum is building for the use of doxycycline as a novel STI prevention tool. The magnitude of the bacterial STI epidemic among gbMSM is unprecedented. Decades' worth of prevention efforts such as the promotion of condoms has not worked. It is time for a paradigm-shifting approach. Meanwhile, HIV pre-exposure prophylaxis (PrEP) has revolutionized HIV prevention, making it reasonable to consider STI PrEP. Preliminary data from STI PrEP and post-exposure prophylaxis (PEP) studies[26,27], suggest potential benefit of doxycycline in preventing bacterial STIs. Following the release of those results and in response to community interest[28], online pharmaceutical companies that sell HIV PrEP (primarily in the UK) started making doxycycline available for STI PrEP. This prompted a rapid response from Public Health England and the British Association for Sexual Health and HIV (BASHH), who cautioned that more research needed to be done[28]. Taken together, these developments clearly suggest that off-label, unsupervised use of doxycycline is already occurring in affected communities (a situation anecdotally confirmed by several of our team members), making it all the more urgent that definitive data about efficacy, AMR, and safety be acquired through a well-designed trial.

1.3 CURRENT EVIDENCE FOR STI PEP AND PREP

In the current protocol, doxycycline PEP and PrEP will be used interchangeably with STI PEP and PrEP, respectively. To date, four studies have sought to understand the efficacy of doxycycline in preventing STIs. The first was a pilot RCT of daily doxycycline PrEP for the prevention of syphilis and other bacterial STIs[26]. This study randomized 30 HIV-positive gbMSM to receive the antibiotic doxycycline daily or a monetary incentive to remain STI-free. After 48

weeks, those receiving doxycycline were less likely to be diagnosed with any STI (odds ratio [OR]: 0.27; 95% confidence interval [CI]: 0.09-0.83). In a second open-label RCT that was a substudy of the IPERGAY HIV PrEP study, 232 HIV-negative gbMSM were randomized to doxycycline PEP (200mg of doxycycline within 24-72 hours following condomless sex), or no intervention[27]. The incidence of STIs was halved in those randomized to receive doxycycline (hazard ratio [HR]: 0.53; 95% CI 0.33 – 0.85), with key reductions for syphilis (HR: 0.27; 95% CI 0.07-0.98) and chlamydia (HR: 0.30; 95% CI 0.13-0.70). Additionally, our group recently completed a third open-label RCT in 52 gbMSM concurrently using HIV PrEP who were randomized to receive either immediate daily doxycycline 100mg, or deferred doxycycline beginning 24 weeks later. Chlamydia infection occurred only in the deferred arm during the first 24 weeks (n=10) (rate 0 vs. 81.63/100 PY, p = 0.001), subsequently no other infections occurred in either arm[29]. Moreover, receipt of doxycycline was associated with a reduced likelihood of developing any STI (OR 0.18, 95% CI 0.05 - 0.68) during the first 24 weeks. Finally, the DoxyPEP study, led by Luetkemeyer and colleagues, intended to randomize 780 participants in a 2:1 open-label fashion to doxycycline PEP (DoxyPEP) and standard of care (i.e. no PEP). However, in May 2022, an interim analysis of the 501 enrolled participants resulted in the study's Data Safety and Monitoring Board (DSMB) recommending a stop to enrolment due to significant effectiveness of the DoxyPEP intervention compared to standard of care. Overall, the results demonstrated >60% quarterly reduction in all STIs[30].

Aside from the last study, which is yet-to-be published, none of these trials were adequately powered to fully address the critical questions surrounding doxycycline for STI prevention. A large-scale trial is needed to determine whether these interventions could prevent STIs and their associated complications with minimal harms, as well as determining longer-term acceptability. We will conduct **a rigorous RCT to definitively assess the efficacy, safety, AMR profiles and costs associated with doxycycline-based STI prevention.** A key strength of our design is that we will do a head-to-head comparison of STI PrEP to PEP, the latter of which now has more established data for use in real-world clinical settings. Each of these strategies has potential advantages and disadvantages that must be considered in identifying an optimal STI prevention strategy.

1.4 The potential emergence of antimicrobial resistance (AMR)

Arguably, the greatest potential risk from doxycycline for STI prevention is its unknown potential impact on AMR. Our team has recently completed a systematic review of doxycycline use on AMR[31]. This review yielded 7 articles fulfilling inclusion criteria and most were at moderate/high risk of bias. Various tetracyclines were used for 2-18 weeks, and did demonstrate an increased burden of tetracycline resistance in subgingival, gastrointestinal and upper respiratory normal flora; however, persistent resistance was not seen. Overall, though these limited data suggest an increased burden of AMR, data quality was poor and our team feels this issue signals the need to more comprehensively study the longer-term impact of doxycycline use on AMR. In several doxycycline acne treatment studies, serial monitoring of resistance of various acne-causing organisms did not demonstrate an appreciable increase in resistance over 9-12 months of follow-up[32–34]. Though there have been no confirmed cases of penicillin- or doxycycline-resistant *T. pallidum*[35] (both are curative agents for syphilis), doxycycline does have activity against *N. gonorrhoea* and *C. trachomatis,* which have documented resistance to tetracyclines[36,37] . As a result, there is a profound need to undertake a rigorous assessment of the emergence of resistance in the flora of participants using ongoing therapy with doxycycline.

1.5 The Doxycycline as an Intervention for bacterial STI ChemOprophylaxis (DISCO) study: A potential game-changer

Within the previously-cited clinical indications for doxycycline (e.g. acne treatment, malaria prophylaxis), there is considerable evidence that it is acceptable to patients, primarily owing to its favourable side effect profile[38,39]. Much less is known about the acceptability of doxycycline prophylaxis for STI prevention among those at high STI risk. Although drug tolerability in the above-mentioned studies of doxycycline PEP and PrEP was good, with only 3-7% of participants discontinuing the drug due to adverse events[26,27], conceptual models of acceptability developed in the HIV PrEP literature emphasize that tolerability is only one dimension of acceptability[40]. Other dimensions, including dosing regimen, partner attitudes and community norms (e.g. stigma) may also impact on doxycycline acceptability,

with important potential downstream implications for uptake and adherence. Indeed, despite the high tolerability of doxycycline seen in the pilot PrEP trial, 69% of participants were non-adherent at one or more clinic visit according to measured drug levels.

With the rising rates of STIs among gbMSM populations in Canada, there is an urgent need for novel interventions to prevent significant sequelae (e.g. neurosyphilis) and onward transmission of untreated infections. Despite longstanding public health efforts to encourage conventional strategies of STI prevention, gbMSM continue to bear the burden of bacterial STIs which poses synergistic effects in the transmission of HIV. Our study will add to this field through providing the first methodologically rigorous randomized controlled trial with adequate power to address drug efficacy in the prevention of bacterial STIs. Beyond the determination of efficacy, our trial will provide insight to the unique challenges of medication adherence through assessing the acceptability, tolerability and safety of therapy with doxycycline. This study aims to provide health care providers one additional tool to address the burden of STIs in populations with an increased likelihood of infection.

The DISCO study was originally conceived as a three-arm trial comparing doxycycline PrEP, PEP and standard of care (i.e. no doxycycline). However, following the DSMB's decision to halt the above-mentioned DoxyPEP study due to significant effectiveness of the doxycycline treatment arm, the DISCO study team, steering committee, and the Community Advisory Board (CAB) decided it was no longer appropriate or ethical to include a standard of care arm. Because of this, we have eliminated the standard of care arm so that DISCO is now a two-arm, head-to-head comparison of doxycycline PEP vs. PrEP. To our knowledge, **there is no other clinical trial globally comparing these two interventions directly to each other, such that our trial will make an important contribution to the field regardless of its findings.**

2. OBJECTIVES

2.1 Primary Objective

1. To establish the non-inferiority of STI PrEP vs. PEP, by examining the efficacy of each intervention in preventing incident bacterial STI cases (syphilis, gonorrhea, and/or chlamydia, including LGV)

2.2 Secondary Objectives

2. To describe frequency of syphilis, gonorrhea, and chlamydia incident infections among participants over the study period
3. To determine the tolerability and safety of doxycycline chemoprophylaxis
4. To determine the adherence to study treatment (i.e. STI PrEP or PEP) by participants
5. To evaluate the change in sexual behaviour reported by participants over the study period
6. To evaluate antimicrobial resistance in bacterial STIs and common commensal pathogens over study period
7. To assess for superiority of doxycycline PrEP over PEP (if non-inferiority is shown)

3. METHODS

3.1 STUDY DESIGN

This will be a prospective multicentre, open-label, non-inferiority randomized controlled trial (RCT) of doxycycline-based STI PrEP (daily 100mg doxycycline) versus STI PEP (200mg doxycycline after exposure event) for the prevention of bacterial STIs among gbMSM over 15 months (60 weeks) of follow-up. Of note, participants will receive the intervention for a total of 48 weeks; the week 60 visit is to allow for sufficient follow up time to ascertain for any infections during the window period that may have gone undiagnosed at week 48.

3.1.1 Rationale for study design revision

We are considering doxycycline PEP to be the active control because it is the more established treatment for STI prophylaxis. It has more robust data reported from both above-described trials: the DoxyPEP trial with 501 participants[30], and the IPERGAY substudy with 232 participants that showed it to be superior to the standard of care (no drug)[27]. On the other hand, doxycycline PrEP's performance – though also promising – has only been evaluated in two smaller studies with a total of 82 participants. It is important to note that, despite these results, we still do not know with certainty if one intervention is better than the other from an efficacy standpoint, and we cannot make any assumptions on efficacy or effectiveness. For instance, some may argue that the effectiveness/efficacy of STI PrEP in someone fully adherent must necessarily be as good as the effectiveness/efficacy of PEP if fully adherent. From a pragmatic trial approach, we recognize that daily PrEP adherence may be inconsistent, and it is adherence around the time of sexual exposures that will dictate how likely or unlikely it is that PrEP will be better or worse than PEP. For this reason, we cannot assume effectiveness/efficacy will be the same.

Further, there are also considerations aside from effectiveness/efficacy that must be taken into account, including patient preferences. Within members of our CAB, there was debate and no real consensus about preferences re: PEP vs. PrEP. Whereas some members argued that "less drug is better" and would thus favour PEP, others argued that even with the PEP approach, they would likely already be taking close to the equivalent of daily doxycycline, and would find it easier to just take it daily (e.g. along with their HIV PrEP).

Thus, to summarize, the main reasons for choosing a non-inferiority design with doxycycline PEP as the 'active control' are:

- There is currently more data on doxycycline PEP (n = 733 participants total), and this has been shown to be superior to the 'no doxycycline' arm in the studied groups;
- The data on doxycycline PrEP (n = 82 participants) is promising, but not definitive (e.g. wide confidence intervals around the event rate);
- There has been no head-to-head study of doxycycline PrEP vs. PEP;
- We cannot make any assumptions of benefit of one intervention over the other relating to the following:
    - Efficacy or effectiveness: as discussed above.
    - Antimicrobial resistance (AMR): no good quality data exists in general, and certainly nothing comparing AMR in PrEP vs. PEP.
    - Tolerability or safety: there is ample data showing that doxycycline is safe and well-tolerated, and no appreciable difference in these parameters have been noted between the doxycycline PrEP and PEP studies.
    - Patient preference: there is no option that has been clearly selected as preferred by potential patients, and both approaches have been deemed acceptable in studies.
- Had we selected a superiority trial, and PrEP was not shown to be superior, we would now have strong evidence against its use, thus removing an effective and preferred option for STI prevention in some. With a non-inferiority trial, we have the ability to assess superiority as a secondary objective.
- Finally, the eligibility criteria for DISCO are very similar to the eligibility criteria of the previously-cited DoxyPEP study, which was prematurely halted due to significant effectiveness of the doxycycline PEP arm. This finding clearly bolsters the assumption that doxycycline PEP is better than no treatment in the proposed populations to be studied, which is an important methodological aspect of a non-inferiority study.

3.2 Study Setting and Recruitment

Participants will be recruited from 9 clinical sites with a high proportion of gbMSM clients in Vancouver, Calgary, Toronto, Hamilton, Ottawa, and Montreal. These sites will include: one HIV PrEP and STI clinic (BCCDC STI clinic) in Vancouver, HIV/HIV PrEP clinics in Toronto (St. Michael's Hospital – Positive Care Clinic), two HIV and HIV PrEP clinics in Hamilton (McMaster University), HIV and HIV PrEP clinics in Ottawa (Ottawa Hospital), two HIV and STI clinics in Calgary (Sheldon Chumir Centre and the Southern Alberta Clinic), and two clinic(s) in Montreal (e.g. McGill University Health Centre and CMU du Quartier Latin, Inc.). Other sites may be added in the future. Research coordinators at each site will identify potential participants and screen them for entry into the study. Additionally, recruitment

ads/flyers will also be posted on-site, and online by community organizations (i.e. online recruitment ads/flyers posted their websites and their Facebook and Twitter accounts) and businesses with clientele or servicing gbMSM. The recruitment flyer will also be posted on the study website. We believe all participants can be allocated within two years, given that 3-6 months is typical for both HIV-positive gbMSM receiving standard care and HIV-negative gbMSM undergoing routine STI screening or attending HIV PrEP follow-up visits.

3.3 Eligibility Criteria

3.3.1 Inclusion criteria

The full list of inclusion criteria are as follows:

1. Adults, ≥ 18 years of age;
2. Gay, bisexual and other men who have sex with men (both cis gender and transgender) or transgender women
3. Any sexual activity (i.e. oral sex, insertive or receptive anal sex, with or without a condom) with more than one male partner in the preceding 12 months;
4. Intention to remain sexually active with more than one male partner in the next 12 months;
5. At least one prior episode of a previously diagnosed and adequately treated syphilis, gonorrhea or chlamydia infection within 12 months prior to screening;
6. If you are sexually active and capable of becoming pregnant, you will use birth control/contraception reliably during the entire study duration.

NOTE:

1. Both HIV-positive and HIV-negative participants will be enrolled.

2. Though we are not requiring HIV-negative individuals to be on HIV PrEP to participate in the study, nor are we requiring those living with HIV to be on HIV treatment, we recognize that most participants who would be eligible for this study should most likely be on HIV PrEP, since this is standard-of-care for those at-risk of HIV acquisition. Similarly, HIV treatment is standard-of-care for all individuals living with HIV.

If either of these scenarios arise at baseline or at any point during the study, this information will be captured, which will prompt an immediate alert to the site investigator to have a conversation with the participant about this, provide education as relevant, and refer them to the appropriate provider if required.

3.3.2 Exclusion criteria

The exclusion criteria are as follows:

1. Known allergy to doxycycline or tetracyclines;
2. Existing chronic or intermittent tetracycline or doxycycline use (e.g. for chronic osteomyelitis, acne).
3. Use of medications which could lower doxycycline levels, including barbiturates, phenytoin and carbamazepine.
4. Individuals currently using isotretinoin;
5. Are sexually active, capable of becoming pregnant, and will not use birth control/contraception reliably during the course of the study duration.

3.4 Outcomes

3.4.1 Primary outcome (study objective 1)

Our primary outcome is incident STI cases defined as: a composite endpoint of the count of any incident bacterial STIs (syphilis, gonorrhea, and/or chlamydia, including LGV).

3.4.2 Secondary outcomes (study objective 2-7)

1. Pathogen-specific incident infections: syphilis; gonorrhea; chlamydia
2. Treatment-emergent adverse events and laboratory abnormalities
    a. Proportion of individuals reporting grade 3 or 4 adverse events in each study arm.
    b. Proportion of individuals with adverse event-related study drug discontinuation in each study arm.

3. Adherence ≥ 95% measured via self-report, pill counts and doxycycline drug levels (defined as ≥ 1000 ng/mL) at each study visit.
4. Change in self-reported sexual activity defined as increases in condomless sexual acts and number of sexual partners.
5. Antimicrobial resistance in bacterial STIs and common commensal pathogens over study period
    a. Evidence of tetracycline class resistance in common flora *(Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae*) at 24 and 48 weeks.
    b. Resistance to penicillins, and tetracycline in gonorrhea isolates taken from the anus, oral cavity, urethra or vagina.
    c. Resistance to doxycycline, penicillin and macrolides in syphilis isolated via polymerase chain reaction (PCR) from lesions, anus, oral cavity, or vagina.

3.5 Outcome Assessment and Laboratory Methodology

3.5.1 Outcome assessments

1. Primary Outcome: Each participant will receive laboratory testing for syphilis, gonorrhea and chlamydia at each study visit (and HIV, if HIV-negative). ***Incident syphilis*:** Syphilis diagnosis will be made according to established diagnostic criteria[41]. For those who are syphilis naïve, a new diagnosis will be determined by a combination of reactive serological tests, including screening treponemal enzyme immunoassay (EIA), rapid plasma reagin (RPR) and confirmatory *T. pallidum* particle agglutination (TPPA). In someone who is syphilis-naïve, a reactive EIA and a reactive TPPA are typically sufficient to diagnose a new infection. As these two treponemal tests remain reactive for life, diagnosis of a new syphilis infection in someone who has been previously diagnosed relies primarily on history and the RPR titre[41]. For participants with a history of past syphilis infection, a new infection will be defined as a minimum two-dilution (i.e. 4-fold) increase in RPR (e.g. 1:2 to 1:8), as per standard guidelines. ***Incident gonorrhea and chlamydia**:* New gonorrhea and chlamydia cases will be measured by positive nucleic acid amplification tests (NAAT), which is the standard of care for all sites. Site-directed culture for *N. gonorrhoeae* will only be done in combination with NAAT when participant is symptomatic (the primary purpose of culture is to elucidate AMR and susceptibility) at the study visit. Outcome ascertainment will be exclusively via NAAT. For any equivocal/unclear STI results, a blinded adjudication committee will assist with diagnostic and clinical interpretation.
2. Secondary Outcomes: (1) As above; counts for each separate STI; (2) Adverse events will be captured via self-report and clinical assessment at study visits; they will be graded on a 4-point scale according to the standard scale from the National Institute for Allergy and Infectious Diseases, Division of AIDS[42]; (3) Risk compensation will be assessed via self-completed questionnaire at each study visit, using a brief sexual behaviour questionnaire (e.g. number of sexual partners, number of condomless sexual encounters) that we have successfully used in multiple prior studies [29,43]; (4) Adherence will be assessed using three strategies at each visit: a modified version of the AIDS Clinical Trials Group (ACTG) Adherence Questionnaire[44] and Simplified Medication Adherence Questionnaire(SMAQ)[45] for those participants taking STI PrEP, and a separate questionnaire for those taking STI PEP; pill counts; and, plasma doxycycline levels. For those individuals in the STI PEP arm, a single questionnaire consisting of components from both the modified form of the ACTG and SMAQ questionnaires will be administered, as these questionnaires are meant primarily for those taking daily medication. The doxycycline levels will be performed via mass spectrometry in the laboratory of CoA Cameron, with clinically-relevant 'protective' levels defined as being between 1000-4000ng/mL, based on previous work[26]; (5) Resistance in commensal nasopharyngeal organisms will be measured via serial assessments of minimal inhibitory concentration (MIC; measured in mg/L via disc diffusion or automated susceptibility testing) in cultured nasopharyngeal isolates of commensal organisms *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae* and non-gonococcal *Neisseria* species; (6) Treatment-emergent resistance of *N. gonorrhoeae* will be assessed via culture (oral, anal,

urethral, or vaginal samples); (7) Resistance mutations in *T. pallidum* diagnosed via PCR, and assessed through whole genome sequencing (WGS)

For both the primary outcome and secondary outcome for STI incidence, if participants present to other clinics with new diagnoses, efforts will be made to obtain the relevant documentation through release of clinical information requests.

3.5.2 Laboratory methodology

Evaluation of antimicrobial resistance in common commensal organisms

At baseline, as well as the week 24 and 48 visits, nasopharyngeal swabs will be done in all participants, and these will be cultured for the presence of *Staphylococcus aureus*, *Streptococcus pyogenes,* and *Streptococcus pneumoniae*. Nasal swabs (anterior nares) and throat swabs will be performed using Venturi trans system culture swabs (Copan, Brescia, Italy). Bacterial isolates will be tested for antimicrobial susceptibility using disk diffusion or automated susceptibility testing. Given the relatively high nasopharyngeal colonization rates of commensal organisms in the nose and throat, this represents a reasonable surrogate means by which to monitor antimicrobial resistance[46–50].

Resistance in *Neisseria gonorrhoeae*

This will be assessed according to local laboratory standards/methods, which involve growth of the organism via culture (Note: gonorrhea culture will be done in those participants with symptomatic pharyngitis, urethritis, vaginitis or proctitis), and – from there – the determination of minimal inhibitory concentrations (MIC) via the E-test method of tetracyclines will be made. Tetracycline resistance will be defined by a MIC greater than 1mg/mL, and intermediate resistance as MIC greater than 0.5mg/mL and less than or equal to 1mg/mL[27].

Resistance in Treponema pallidum

Because *T. pallidum* is not generally a culturable pathogen, antibiotic susceptibility testing cannot be performed in the standard way. Rather, an emerging technology for AMR monitoring in syphilis is the identification of resistance mutations using WGS[51]. We do not anticipate a significant number of syphilis infections to emerge within either treatment arms (estimated to be less than 10 infections in the full study population). In those testing positive for syphilis during the study period, when participants return to clinic for treatment, we will perform swabs for *T. pallidum* PCR of lesions in those who are symptomatic, or of mucosal sites (i.e. blind swabs of oral, anal, vaginal) if asymptomatic. These will be sent to the BCCDC Public Health Laboratory for performance of PCR.

Resistance in *Chlamydia trachomatis*

Due to the challenges of culturing chlamydia and the unreliability of molecular techniques for AMR determination, AMR analyses of *Chlamydia trachomatis* will not be performed in the current study. Though this is a limitation, there is ongoing exploratory work from other, ongoing doxycycline PEP studies assessing molecular markers of *C. trachomatis* resistance that will inform the direction of future work in this area.

Characterization of the rectal microbiome

At baseline, 24 and 48 weeks a rectal swab using Copan Floqswabs (Copan Diagnostics, Murietta, CA, USA) will be collected. Swabs will be stored at -80°C or lower for future analyses. The microbiome will be characterized using mass spectrometry and 16S rRNA methodologies as used to assess other microbiome studies[52]. To assess the diversity of the bacterial proteome, relative proteome load of each bacterial genus will be determined in each sample by summing the total number of protein spectral counts assigned to each genus, an approach previously shown to directly correlate with colony-forming units[53]. Bacterial composition will also be evaluated using 16S rRNA.

Plasma doxycycline levels

At each follow up visit starting at week 4, a blood sample for a random doxycycline level will be obtained; however, we will only perform doxycycline levels in a random sample of 20% of the study visits, while ensuring that each participant will be tested at least once, and that all time points (i.e. weeks 4, 12, 24, 36, and 48) will be equally tested. Thus, 20% of visits will be randomly chosen (i.e. one per participant), and this will be stratified by treatment group and time point. This will be determined at the point of randomization (and will be known only to the study team,

and not the participant), and a flag will be placed for that particular study visit where the doxycycline level will be performed. Randomization will occur via an on-line randomization tool developed by the Canadian CIHR HIV Trials Network.

Additionally, we will perform doxycycline levels in samples from individuals testing positive for a bacterial STI at a given study visit. Since all doxycycline level testing will be batched and performed at study end, and STI results will not be immediately available at the study visit, all participants will have a sample drawn for doxycycline levels at each follow-up visit, and these samples will be kept until study end. At study end, the study team will go through all samples, and select the specific samples from the randomized/pre-selected 'doxycycline level' visit, as well as any visits where an STI was diagnosed. These samples will then be sent for analysis, and all other samples will be destroyed.

For the pharmacokinetic sampling of all analytes, 5.0 mL blood samples will be collected to obtain at least 2 mL of plasma in labeled test-tubes (K2-EDTA). The exact times of the most recent study drug dose, as well as the exact time of sampling will be recorded in the case report forms. The blood samples will be mixed and stored in the refrigerator. Blood samples will be centrifuged at about 2500 g (3800 rpm for a normal laboratory centrifuge) for 10 minutes at 4°C. Plasma will be transferred to a labeled polypropylene tube and stored at -80°C or lower within 2 hours after collection. Blood sampling will be done according to the standard operating procedure (SOP) of the Clinical Investigation Unit at the University of Ottawa (Collaborator Dr. B. Cameron). All samples will be labeled with the following information: study ID; patient study number; study day; date and time after dosing. For the purposes of this study, a doxycycline level of ≥1000 ng/mL will be considered therapeutic, and will be our threshold for determining adherence. From previous studies, we know that doxycycline is an antibiotic that is well absorbed orally, with a half-life of 12 hours and a usual steady-state level ranging between 1000-4000 ng/mL based on once-daily 100mg dosing[54]. Doxycycline levels will be performed in participants in both study arms.

4. STUDY PROCEDURES

4.1 INTERVENTIONS

STI PrEP arm: doxycycline capsules 100mg orally daily for 12 months. Though the usual *treatment* dose of doxycycline is 100mg twice daily[36], we chose a single daily *prophylactic* dose based on its known efficacy against other infections (e.g. malaria, leptosopirosis)[55,56], and the use of this dose in the previously-cited pilot study[26] and our DuDHS study. We also believe that once-daily dosing is a reasonable pill burden that will maximize adherence – analogous to the established acceptability of once-daily HIV PrEP[42]. While we are reassured by previous studies (including the leptospirosis work cited above) showing that doxycycline's half-life is long enough to provide protection with as little as weekly dosing, there is currently no evidence on the length of time one needs to continue doxycycline PrEP after an exposure in order to maintain protective levels; thus, we are not placing specific parameters around this as part of the study.

STI PEP arm: doxycycline 200mg orally once within 24-72 hours following each sexual encounter deemed at risk (i.e. condomless anal or oral sex), to a maximum of six pills (i.e. 600 mg total) per week [27].

4.2 RANDOMIZATION

This is a non-inferiority trial that will compare STI PrEP to STI PEP. Participants will be randomized in a 1:1 fashion. Randomization will occur via an on-line randomization tool developed by the Canadian CIHR HIV Trials Network and will be accessed by the study coordinator performing randomization. Subsequent visits will occur 30 days after study drug initiation, 12 weeks following study drug initiation and then on a quarterly basis (weeks 24, 36, 48, and 60).

1. STI PrEP arm: doxycycline capsules 100mg daily for 12 months;

2. STI PEP arm: doxycycline 200mg orally once within 24-72 hours following each sexual encounter deemed at risk (i.e. condomless anal or oral sex), to a maximum of six pills (i.e. 600 mg total) per week.

4.3 STUDY VISITS AND DURATION

Participants will be recruited over two years. Once enrolled, each participant will receive the allocated intervention for 48 weeks and followed for 60 weeks. The week 60 visit is intended to allow for sufficient follow-up time to ascertain for any infections during the window period that may have gone undiagnosed at week 48 (e.g. the incubation period for syphilis can extend to 90 days)[41].

Initially-referred participants will be screened by the site study coordinator using an inclusion/exclusion checklist at a scheduled Screening visit. The Baseline visit can occur on the same day of the Screening visit, if the participant is deemed eligible based on the checklist. All enrollees will then be randomized, and commence a schedule of study visits: baseline, week 4, week 12, week 24, week 36, week 48, and week 60. At baseline, the following will be collected: (1) demographic information (age, sex, ethnicity, etc.); (2) HIV information (*only for participants living with HIV*: year of diagnosis, treatment details, CD4 and viral load data); (3) current medical comorbidities; (4) concomitant medications, including other antimicrobials; and, (5) sexual behaviours over the last 3 months (sex, number and HIV/PrEP status of sexual partners, types of sexual activities [e.g. oral insertive, anal receptive], condom use). At baseline, weeks 24 and 48, rectal swabs for microbiome analysis will be collected and stored for future analysis. All participants will receive a $50 for baseline and final visits, and $20 honorarium for all other visits.

4.3.1 Questionnaires

Data will be collected using standardized questionnaires at baseline, as well as follow-up questionnaires at three-month intervals. The baseline questionnaire will include demographic data, previous use of HIV PrEP, sexual risk behaviour, depression, health status, alcohol and substance use questionnaires. Follow-up questionnaires will include syphilis drug adherence, sexual risk behaviours, depression, health status, and self-reported STIs.

1. Sexual risk behaviour at baseline and subsequent study visits will be assessed via questionnaire focusing on number of partners/type of sex act within last 30 days.
2. Adherence will be assessed at all follow-up visits for the intervention groups using an abbreviated version of the AIDS Clinical Trials Group (ACTG) Adherence Questionnaire[44] and Simplified Medication Adherence Questionnaire (SMAQ)[45]. For those individuals in the STI PEP arm, a single questionnaire consisting of components from both the modified form of the ACTG and SMAQ questionnaires will be administered, as these questionnaires are meant primarily for those taking daily medication.
3. Syndemic assessment. The baseline questionnaire will include the Drug Use Disorders Identification Test (DUDIT) and the Alcohol Use Disorders Identification Test (AUDIT). These instruments have been validated as screening tools for substance dependence and hazardous alcohol use, respectively[57,58]. Depressive symptoms will be assessed at baseline and each follow up visit using the widely utilized nine-item depression component of the Patient Health Questionnaire (PHQ-9). The PHQ-9 has been validated both as a diagnostic tool for depression, as well as a measure of depression severity[59].
4. Health status assessment. Health-related quality of life (HRQoL) information will be collected at baseline and all follow-up visits by using a validated tool, EQ-5D-5L[60] (with five domains) and summarized into a single score (range 0-1), which represents health utility. Health utility can be used to calculated quality-adjusted life years, a single measure capturing length and quality of life. Additionally, data will be collected at each follow up visit on clinic, emergency room visits, and hospital admissions between study visits (including frequency of visits and the reasons for those visits).

Scores on the PHQ-9, DUDIT, and AUDIT questionnaires will be calculated at the time of the visit, and any referrals will be made at this time as well. Individuals with scores on the PHQ-9 in keeping with depression will be offered referral to a trained mental health counsellor or to a general practitioner for further counselling and/or assessment for anti-depressant therapy. Individuals with scores on DUDIT and/or AUDIT reflecting problematic drug or alcohol

dependence or those interested in assistance with drug or alcohol use will be offered a referral to a trained provider for further assessment, and/or counselling/treatment.

Individuals who test positive at screening for syphilis or any other STI including HIV will be referred immediately to a treating physician, and public health authorities will be notified as per legal requirements in the relevant province.

4.3.2 Screening and Baseline visits

All individuals who are approached for potential participation will be recorded in the Study Screening and Enrolment Log. This will form the denominator for those who are eligible and consent to participate. If all eligibility criteria have been met, the following baseline investigations and procedures will be performed: Randomization will occur via an on-line randomization tool developed by the Canadian CIHR HIV Trials Network and will be accessed by the study coordinator performing randomization. The following data will be collected at the screening/baseline visit:

- Informed consent process (At Screening Visit)
- Inclusion/Exclusion checklist (At Screening Visit)
- Randomization to study arm and release of study medication.
- Randomization to study visit where doxycycline level will be performed.
- Demographics (date of birth, ethnicity, self-identified gender)
- Medical history (including concurrent medical diagnoses and, for participants living with HIV, year of HIV diagnosis, CD4 count, viral load)
- Focused physical exam
- Concurrent medication review
- Alcohol, smoking, and drug use history
- Routine bloodwork
    - Complete blood count with differential
    - Creatinine
    - ALT
- HIV serology (If HIV-negative)
- Screen for STIs
    - Syphilis screen (i.e. EIA ± RPR, TPPA)
    - Chlamydia and gonorrhea nucleic acid amplification testing (NAAT) at each of the following sites: rectal and throat swabs, and urine (and vagina, if relevant).
        - If found symptomatic for gonorrhea at the study visit, culture swab will be performed for gonorrhea resistance
        - If test result is positive for gonorrhea (post-visit), participant will receive treatment and culture swab will be performed (standard of care)
- Nasopharyngeal swabs for *S. aureus, S. pyogenes, S. pneumoniae*
- Rectal swab for rectal microbiome characterization
- Questionnaires:
    - Sexual risk behavior questionnaire
    - Depression: PHQ-9
    - Alcohol use questionnaire: AUDIT-C Questionnaire
    - Drug use questionnaire: DUDIT Questionnaire
    - Health status questionnaire: EQ-5D-5L

4.3.3 Weeks 4 and 12 visits (+/- 7 days)

These visits will be to review adherence and adverse events. During these visits, the following assessments will be performed:

- Adverse event assessment
- Concurrent medication review

- Focused physical exam
- Routine bloodwork
    - Complete blood count with differential
    - Creatinine
    - ALT
- HIV serology (If HIV-negative)
- Screen for STIs
    - Syphilis screen (i.e. EIA ± RPR, TPPA)
    - Chlamydia and gonorrhea nucleic acid amplification testing (NAAT) at each of the following sites: rectal and throat swabs, and urine (and vagina, if relevant).
        - If found symptomatic for gonorrhea at the study visit, culture swab will be performed for gonorrhea resistance
        - If test result is positive for gonorrhea (post-visit), participant will receive treatment and culture swab will be performed (standard of care)

- Plasma doxycycline level blood draw; doxycycline level to be performed in random, 20% sample and in those testing positive for STI.
- Adherence assessment
    - Pill count
    - Acceptability questionnaire (both STI PrEP and STI PEP participants)
    - Simplified medication adherence questionnaire (for those on STI PrEP)
    - Adherence questionnaire (for those on STI PEP)

- Questionnaires:
    - Sexual risk behavior questionnaire (Week 12 only)
    - Alcohol use questionnaire: AUDIT-C Questionnaire (Week 12 only)
    - Drug use questionnaire: DUDIT Questionnaire (Week 12 only)
    - Depression: PHQ-9
    - Health Status: EQ-5D-5L

4.3.4 Week 24 visit (+/- 14 days)

At this follow-up visit, the following assessments will take place:

- Adverse event review profile
- Concurrent medication review
- Focused physical exam
- Routine bloodwork
    - Complete blood count with differential
    - Creatinine
    - ALT
- HIV serology (If HIV-negative)
- Screen for STIs
    - Syphilis screen (i.e. EIA ± RPR, TPPA)
    - Chlamydia and gonorrhea nucleic acid amplification testing (NAAT) at each of the following sites: rectal and throat swabs, and urine (and vagina, if relevant).
        - If found symptomatic for gonorrhea at the study visit, culture swab will be performed for gonorrhea resistance
        - If test result is positive for gonorrhea (post-visit), participant will receive treatment and culture swab will be performed (standard of care)

- Nasopharyngeal swabs for *S. aureus, S. pyogenes, S. pneumoniae*
- Rectal swab for rectal microbiome characterization

- Plasma doxycycline level blood draw; doxycycline level to be performed in random, 20% sample and in those testing positive for STI.
- Adherence assessment
  - Pill count
  - Acceptability questionnaire (both STI PrEP and STI PEP participants)
  - Simplified medication adherence questionnaire (for those on STI PrEP)
  - Adherence questionnaire (for those on STI PEP)

- Questionnaires:
  - Sexual risk behavior questionnaire
  - Alcohol use questionnaire: AUDIT-C Questionnaire
  - Drug use questionnaire: DUDIT Questionnaire
  - Depression: PHQ-9
  - Health Status: EQ-5D-5L

4.3.5 Week 36 visit (+/- 14 days)

This visit will be to review adherence and adverse events. During this visit, the following assessments will be performed:

- Adverse event assessment
- Concurrent medication review
- Focused physical exam
- Routine bloodwork
  - Complete blood count with differential
  - Creatinine
  - ALT
- HIV serology (If HIV-negative)
- Screen for STIs
  - Syphilis screen (i.e. EIA ± RPR, TPPA)
  - Chlamydia and gonorrhea nucleic acid amplification testing (NAAT) at each of the following sites: rectal and throat swabs, and urine (and vagina, if relevant).
    - If found symptomatic for gonorrhea at the study visit, culture swab will be performed for gonorrhea resistance
    - If test result is positive for gonorrhea (post-visit), participant will receive treatment and culture swab will be performed (standard of care)

- Plasma doxycycline level blood draw; doxycycline level to be performed in random, 20% sample and in those testing positive for STI.
- Adherence assessment
  - Pill count
  - Acceptability questionnaire (both STI PrEP and STI PEP participants)
  - Simplified medication adherence questionnaire (for those on STI PrEP)
  - Adherence questionnaire (for those on STI PEP)

- Questionnaires:
  - Sexual risk behavior questionnaire
  - Alcohol use questionnaire: AUDIT-C Questionnaire
  - Drug use questionnaire: DUDIT Questionnaire
  - Depression: PHQ-9
  - Health Status: EQ-5D-5L

4.3.6 Week 48 visit (+/- 14 days)

This visit will be to review adherence and adverse events. During this visit, the following assessments will be performed:

- Adverse event review profile
- Concurrent medication review
- Focused physical exam
- Routine bloodwork
    - Complete blood count with differential
    - Creatinine
    - ALT
- HIV serology (If HIV-negative)
- Screen for STIs
    - Syphilis screen (i.e. EIA ± RPR, TPPA)
    - Chlamydia and gonorrhea nucleic acid amplification testing (NAAT) at each of the following sites: rectal and throat swabs, and urine (and vagina, if relevant).
        - If found symptomatic for gonorrhea at the study visit, culture swab will be performed for gonorrhea resistance
        - If test result is positive for gonorrhea (post-visit), participant will receive treatment and culture swab will be performed (standard of care)

- Nasopharyngeal swabs for *S. aureus, S. pyogenes, S. pneumoniae*
- Rectal swab for rectal microbiome characterization
- Plasma doxycycline level blood draw; doxycycline level to be performed in random, 20% sample and in those testing positive for STI.
- Adherence assessment
    - Pill count
    - Acceptability questionnaire (both STI PrEP and STI PEP participants)
    - Simplified medication adherence questionnaire (for those on STI PrEP)
    - Adherence questionnaire (for those on STI PEP)

- Questionnaires:
    - Sexual risk behavior questionnaire
    - Alcohol use questionnaire: AUDIT-C Questionnaire
    - Drug use questionnaire: DUDIT Questionnaire
    - Depression: PHQ-9
    - Health Status: EQ-5D-5L

4.3.7 Week 60 visit (+/- 14 days)

This visit is intended to capture any possible new syphilis infection occurring within the window period (as described above). At this visit, the following will be performed:

- Adverse event assessment (for late emerging AEs)
- Concurrent medication review
- Medical history
- Focused physical exam
- Routine bloodwork
    - Complete blood count with differential
    - Creatinine
    - ALT
- HIV serology (If HIV-negative)
- Screen for STIs
    - Syphilis screen (i.e. EIA ± RPR, TPPA)

- Chlamydia and gonorrhea nucleic acid amplification testing (NAAT) at each of the following sites: rectal and throat swabs, and urine (and vagina, if relevant).
  - If found symptomatic for gonorrhea at the study visit, culture swab will be performed for gonorrhea resistance
  - If test result is positive for gonorrhea (post-visit), participant will receive treatment and culture swab will be performed (standard of care)

- Questionnaires:
  - Sexual risk behavior questionnaire
  - Depression: PHQ-9
  - Health Status: EQ-5D-5L

4.4 Schedule of Events Table

Study Weeks		**W0**	**W4**	**W12**	**W24**	**W36**	**W48**	**W60**
Study Visits	**Screening**	**Baseline (+/- 3 d)**	**V3 (+/-7d)**	**V4 (+/- 7d)**	**V5 (+/- 14d)**	**V6 (+/- 14d)**	**V7 (+/- 14d)**	**V8 (+/- 14d)**
Visit Duration (hours)	0.5	1	1	1	1	1	1	1
Eligibility Criteria	X[a]							
Informed Consent Form	X							
Randomization to intervention		X						
Medical History		X						X
Alcohol, Drug, & Smoking History		X						
Concurrent Medication Review		X	X	X	X	X	X	X
Adverse Event Review			X	X	X	X	X	X
Focused Physical Exam		X	X	X	X	X	X	
Routine bloodwork (CBCD, creatinine, ALT)		X	X	X	X	X	X	X
HIV serology (if HIV-negative)		X	X	X	X	X	X	X
STI screening: syphilis serology, GC/CT NAAT (urine, pharynx, rectum) and/or GC culture (urethra, pharynx, rectum, vagina)[b]; if resistance present, swab anogenital lesions for CT/GC NAAT		X	X	X	X	X	X	X
Plasma doxycycline level			X	X	X	X	X	
Rectal swab for microbiome[d]		X			X		X	
Nasopharyngeal swabs for *S. aureus, S. pneumoniae, S. pyogenes, Neisseria spp.*		X			X		X	
Adherence pill count[c]			X	X	X	X	X	
Adherence questionnaires: • For STI PrEP participants :Acceptability Questionnaire[c] & SMAQ • For STI PEP participants: Acceptability Questionnaire[c] & STI PEP adherence questionnaire			X	X	X	X	X	
Sexual History Questionnaire		X		X	X	X	X	X
AUDIT & DUDIT Questionnaires		X		X	X	X	X	
PHQ-9 Questionnaire		X	X	X	X	X	X	X
Health Status Questionnaire		X	X	X	X	X	X	X

Abbreviations: ALT, alanine aminotransferase; CBCD, complete blood count with differential; CT, Chlamydia trachomatis; GC, Neisseria gonorrhoeae; NAAT, nucleic acid amplification test; STI, sexually transmitted infection.
[a] If a participant does not meet eligibility criteria, they will not be asked to provide remaining information for the baseline visit and will not be randomized.
[b] STI screening may also be performed at times outside of study visits (e.g. if participant is symptomatic, or was named as a contact to a diagnosed case); for asymptomatic individuals, only NAAT for chlamydia and gonorrhea will be performed; for symptomatic individuals (e.g. urethritis, proctitis, pharyngitis, vaginitis), gonorrhea culture will also be performed.
[c] For intervention arms.
[d] Though there are no budgetary funds being allocated to microbiome analyses, these samples will be stored for future analyses.

4.5 Alternate Methods - Due to the COVID-19 Pandemic

Researchers have an ongoing ethical obligation to bring to participants' attention any changes to the clinical trial that may affect them. Thus, the Study Coordinator shall disclose changes to the risks or potential benefits of the research. This includes the alternate methods used for the completion of study visit assessments to minimize risk and exposure to COVID-19 aligned with physical distancing measures.

To minimize the risk and maximize the safety of study participants during the COVID-19 pandemic, the study procedures will be conducted in compliance with the guidance notes/memos issued by the institutional, ethical, and health regulatory bodies on clinical research operations and clinical trial management. Certain study visit procedures will be completed remotely with a virtual visit (either by phone call or videoconferencing via "Zoom for Healthcare" or similar secure platform), if possible, and upon the permission received from the participant and the collection of their personal email address and/or phone number. The participant will be well-informed of the risks associated with attending an in-person visit as well as the completion of study visit procedures via a virtual visit. Virtual visits will be conducted when necessary and feasible, and sufficient to assure the safety of trial participants. Procedures and assessments that can be completed remotely or during a virtual visit include obtaining fully informed consent, completing clinic questionnaires (online), data collection of demographic information, health history, adverse event review, concomitant medication review, return and review of completed vaccine diaries and honorarium payment via bank e-transfer. Other procedures such as physical exam and biospecimen collection will require an in-person visit. Laboratory evaluations (i.e. routine bloodwork) can be completed with lab requisitions provided for testing at central laboratories.

Documentation of the nature of alternate approaches to obtain consent, complete assessments (missed or completed) or visits (e.g. missed study visits or study discontinuations due to COVID-19) will be noted. This will include details on the specific limitation imposed by COVID-19 leading to the inability to perform the protocol-specific assessment.

4.6 Early Termination or unscheduled Visit

4.6.1 *Participation in the study will be terminated early if:*

i. Voluntary withdrawal from the study protocol. The participant refuses further treatment and/or follow-up evaluations. Withdrawal will not prejudice future therapy by the study physicians.
ii. Adverse event related. The study staff or participant's medical provider determines that further participation in the study would be detrimental to the participant's health or well-being due to potential adverse event related to study medications (See Section below for definitions).
iii. The participant is non-compliant with the study requirements in a manner that is either detrimental to their health or interferes with the validity of the study results.

4.6.2 Criteria for discontinuing allocated intervention

The interventions should be completed at their prescribed schedule. However, any of the following would be reasonable grounds for which to discontinue the allocated intervention:

- Suspected hypersensitivity to study drug;
- Other serious or life-threatening adverse event, as per clinician opinion;
- Participant desire to opt out of trial;
- Clinician opinion that intervention caused other significant harm or cessation of trial involvement is in the best interest of the participant.

Of note, all serious adverse events will be reported to the principal investigator, as well as to the necessary regulatory authorities.

4.6.3 Early Termination Visit due to Voluntary withdrawal/Adverse Event

If a participant withdraws early from the study, documentation for the reason of early termination is required. The participant should be instructed to discontinue all study treatments. If possible, perform the assessments listed below.

- Medication review
- Study medication return
- Physical exam
- Hematological, and biochemical lab work (CBC with differential, ALT, creatinine)
- STI screens.
- Repeat nasal and throat swabs for *S. aureus*, *S. pyogenes* and *S. pneumoniae* in those receiving doxycycline.
- Repeat rectal swab for characterization of the rectal microbiome.

4.7 UNSCHEDULED VISIT

In the event that a study participant requires a study visit outside the planned study visits (and if outside the study window period) outlined in the protocol but otherwise required for the study, the following study assessments will occur as per the investigator's discretion and approval:

- Adverse event assessment
- Medication review
- Physical exam
- Hematological, and biochemical lab work (CBC with differential, ALT, creatinine)
- HIV serology ***(if HIV-negative)***
- STI screens.
- Plasma doxycycline levels
- Adherence assessment
  - Pill count

- Depression: PHQ-9
- Health Status: EQ-5D-5L

5. STATISTICAL CONSIDERATIONS

5.1 STATISTICAL ANALYSIS

Descriptive statistics (primarily frequency tabulations) will be used to describe the study population at baseline in terms of socio-demographic makeup, clinical characteristics, prior STI diagnoses, DUDIT, AUDIT, PHQ-9, EQ5D-5L, and sexual risk behaviour.

All analyses will be performed using SAS software (SAS 9.4, Cary, NC).

All analyses will be performed using the intention-to-treat principle. Subgroup analyses will be performed according to HIV status, recreational drug use history, and HIV PrEP use. The rationale for these subgroup analyses is primarily to test for effect modification (i.e. would a benefit from the interventions differ according to these variables).

Objective 1: Establish the non-inferiority of STI PrEP vs. PEP, by examining efficacy of both interventions in preventing incident bacterial STI cases (syphilis, gonorrhea, and/or chlamydia, including LGV)

We will fit a negative binomial regression model to assess the effect of the treatment on the annual rate of incident STI by month 15. PrEP will be declared non-inferior to PEP if the upper bound of the two-tailed 95% confidence interval for the rate ratio comparing PrEP to PEP is no greater than 1.15. To account for recurrent events (i.e. re-infection) and differential periods of time-at-risk during the follow-up due to re-infection (i.e. a participant will not be at risk of re-infection while he is being treated for an infection), an offset equal to the natural log of person-years will be also included in the model for proper calculation of rates and rate ratios and 95% confidence intervals. For participants who are lost-to-follow-up, they will also be included in the analysis up to the time of the loss. As an alternative approach, we will consider analyzing recurrent events using the Andersen-Gill intensity model, which is a generalization of the Cox proportional hazards model, to estimate the treatment effect as the hazard ratio and 95% confidence interval.

Objective 2-7: Evaluate STI incidence, tolerability /safety endpoints, changes in sexual behaviour, antimicrobial resistance development, STI PEP and PrEP acceptability over the study period, and superiority of STI PrEP over PEP.

The following will be presented using descriptive analyses, rates and rate ratios (with 95% CI), as well as longitudinal models (mixed models or generalized estimating equations):

1. incident syphilis, gonorrhea and chlamydia diagnoses
2. drug-related adverse events;
3. changes in the number of sexual partners and condomless sexual encounters;
4. counts of documented AMR occurring for the assessed commensal organisms and the STI pathogens;
5. acceptability of STI PrEP and PEP.

Adjustment for multiple comparisons will be considered, where we will apply a conservative adjustment to the p-value. We will censor participants lost-to-follow-up at their last visit. We will address missing data via multiple imputation. In order to mitigate the misclassification of events during the 12-15 months period (i.e. the possibility that STI diagnosed during the ascertainment period following the end of interventions did not occur prior to month 12), sensitivity analyses will be performed.

Additional Analysis: To examine the economic impact of PrEP and PEP on STIs

A full economic evaluation will be conducted for each doxycycline strategy (as STI PrEP; as STI PEP) using data from the trial. We will use two methods performed under best practice guidelines[61-65]: (1) net-benefit regression; and (2) Markov-based microsimulation modeling. From the health system perspective, we will estimate an incremental cost-utility ratio (i.e., incremental cost per additional quality-adjusted life year) and an incremental cost-effectiveness ratio (i.e., an incremental costs per bacterial STI (syphilis, or gonorrhea or chlamydia, including LGV) prevented at 15 months) for each doxycycline strategy compared to standard of care. We will also complete budget impact analyses. Costs will include capital cost (overhead, training), and the per-visit clinical costs of clinical visits, laboratory tests, and drug (doxycycline). Cost data will be collected within the trial by combining the clinical case report forms with provincial billing codes for physicians, and time-stamps for health-care visits. See Appendix 4 for a full description of these planned analyses.

5.2 Sample Size

The necessary revisions to our study involved transitioning it from a three-arm study, to a two-arm non-inferiority trial of STI PrEP vs. STI PEP. This necessitated a change to the sample size. Based on data from the existing studies on STI PEP, the STI incidence (for all STIs) in individuals on STI PEP is assumed to be 45 per 100person years (95% CI: 37.4-54.9)[27]. The STI incidence (for all STIs) in individuals on STI PrEP from our DuDHS study was 31 per 100 person-years (95% CI: 8.4-80.3)[66]. However, given the wide confidence interval around our DuDHS PrEP event rate, we aimed to be more conservative with this, in order to ensure a more appropriate sample size. We therefore decided that a PrEP rate 20% lower than the PEP rate (i.e. PrEP rate of 36 per 100 person-years) was a reasonable compromise. Thus, assuming participants are followed for 15 months (1.25 years), a PEP event rate of 45 per 100 person-years, and a PrEP rate of 36 per 100 person-years, and a non-inferiority margin of 15%, the sample size required to establish non-inferiority with 80% power is 476 participants (238 per arm), based on a two-tailed 95% confidence interval to judge non-inferiority. This calculation assumes the counts are Poisson distributed. We have proposed a negative binomial regression for the primary analysis as a safeguard against overdispersion; however, our analyses from the DuDHS study indicated an ignorable level of overdispersion. Conservatively accounting for an attrition rate of 15% (based on our DuDHS study, where there were <10% dropouts), the revised sample size is 560 **participants (280 per arm)**.

Aside from feasibility, the reason our non-inferiority margin was set at 15% was based on agreement by our study Steering Committee, as well as our study CAB that a 20% difference in STI incidence between arms was at the upper limit of what did not represent a significant difference. Accordingly, we then opted for a slightly lower margin of 15%. For our revised sample size, we translated this percentage into an absolute difference in numbers of infections between arms (which, for an event rate of 45 per 100 person-years, would equate to ~7 infections) for our CAB, and based on the data presented their feedback to us was that this was an allowable difference to still be considered non-inferior from their perspective. In order to 'test out' whether our revised sample size calculation was reasonable, different sample size requirements were calculated based on different parameters. The approach taken was based on testing various STI event rates for STI PEP (based on the published confidence intervals), and varying the PrEP STI event rates (i.e. X% lower, the same, X% higher). See **Table** below. For each scenario, a sample size is calculated for each of the non-inferiority margins of 10%, 15%, and 20%. These calculations provided reassurance to our team that our revised sample size calculation of 560 participants is appropriate. Examining the sample size calculations for the various 'PrEP is better than PEP' scenarios using a 15% non-inferiority margin, all values fell within reasonably close proximity of our calculated N of 238 per arm.

Scenario	Non-inferiority margin for PrEP vs. PEP	PEP STI event rate (per 100py)	PrEP STI event rate (per 100py)	Abs diff (PrEP-PEP)	N (80% power)
Revised DISCO calculation	1.1	45.0	36.0	-9	310
	1.15	**45.0**	**36.0**	**-9**	**238**
	1.2	45.0	36.0	-9	191
DISCO sample size based on literature	1.1	37.0	31.0	-6	503
	1.15	37.0	31.0	-6	372
	1.2	37.0	31.0	-6	**289**
Low rate, PrEP 20% less	1.1	25.0	20.0	-5	557
	1.15	25.0	20.0	-5	429
	1.2	25.0	20.0	-5	344
Low rate, PrEP the same	1.1	25.0	25.0	0	5530
	1.15	25.0	25.0	0	2572
	1.2	25.0	25.0	0	1511
Low-rate, PrEP 10% more	1.1	25.0	27.5	2.5	NA
	1.15	25.0	27.5	2.5	24266

	1.2	25.0	27.5	2.5	6333
Mid-low rate, PrEP 20% less	1.1	37.0	29.6	-7.4	377
	1.15	37.0	29.6	-7.4	290
	1.2	37.0	29.6	-7.4	232
Mid-low rate, PrEP the same	1.1	37.0	37.0	0	3736
	1.15	37.0	37.0	0	1738
	1.2	37.0	37.0	0	1021
Mid-low rate, PrEP 10% more	1.1	37.0	40.7	3.7	NA
	1.15	37.0	40.7	3.7	16396
	1.2	37.0	40.7	3.7	4279
Mid-high rate, PrEP the same	1.1	45.0	45.0	0	3072
	1.15	45.0	45.0	0	1429
	1.2	45.0	45.0	0	840
Mid-high rate, PrEP 10% more	1.1	45.0	49.5	4.5	NA
	1.15	45.0	49.5	4.5	13481
	1.2	45.0	49.5	4.5	3519
High rate, PrEP 20% less	1.1	54.0	43.2	-10.8	258
	1.15	54.0	43.2	-10.8	199
	1.2	54.0	43.2	-10.8	159
High rate, PrEP the same	1.1	54.0	54.0	0	2560
	1.15	54.0	54.0	0	1191
	1.2	54.0	54.0	0	700
High rate, PrEP 10% more	1.1	54.0	59.4	5.4	NA
	1.15	54.0	59.4	5.4	11234
	1.2	54.0	59.4	5.4	2932

5.3 Blinding and Bias

This is an open-label trial. It would be impractical to keep both study arms fully blinded, as this would require every participant to take multiple pills (both a daily pill and two post-exposure pills). Thus, participants and site trial investigators will be unblinded to treatment allocation. All other study personnel (i.e. laboratory technicians, data managers/data entry staff, statisticians) will be blinded, and a blinded adjudication committee will be convened for equivocal or difficult to interpret STI cases. To assess for selection bias, persons who decline enrolment will have their age, ethnicity, and STI history noted in a refusal log, to compare participants to non-participants.

6. Safety Assessments

6.1 Harms

There are several potential concerns surrounding the use of a daily or intermittent antimicrobial for STI prevention, even if only as a time-limited intervention targeted to those at significant STI risk. These relate primarily to (1) the potential development of AMR, which we will systematically assess in common bacterial flora and diagnosed STI pathogens; (2) the potential side effects of doxycycline when used for STI prevention; and (3) sexual risk compensation. The use of any antibiotic is potentially compromised by the development of resistance in targeted organisms to this compound[67]. Though it is reassuring that the previously-cited data observed a lack of tetracycline-driven resistance development, and that our recently-completed systematic review[31] did not demonstrate

persistence of resistance, we are committed to monitoring for any signal indicating potential AMR development, including resistance of STI organisms and commensal organisms, which would represent a significant public health concern. Doxycycline is generally a safe drug, though some adverse effects can occur, including gastrointestinal (GI) effects (e.g. abdominal discomfort, gastroesophageal reflux, esophagitis, nausea, vomiting, diarrhea) and dermatologic manifestations (e.g. photosensitivity). Diarrhea occurs in <5% of users, with the other GI effects occurring in <1-2%[68]. Most side effects tend to subside once the drug is discontinued. In the above-cited syphilis PrEP study[26], one participant (of 15 receiving doxycycline) developed gastroesophageal reflux; no other adverse events were reported. In the above-cited STI PEP study, there were no differences in serious adverse events between the 'PEP' and 'No PEP' groups (4% vs. 9%, respectively; p = 0.18), though the PEP group did have higher rates of drug-related GI adverse events (25% vs 14%, p = 0.03), with 8/116 (7%) discontinuations in the PEP group. Another concern is 'risk compensation': that individuals taking doxycycline may perceive their STI risk level to be lower, and engage in behaviours placing them at higher risk of STI acquisition. Risk compensation has not been seen in any of the HIV PrEP clinical trials[69,70] , nor was it seen in the syphilis PrEP pilot study described[26]. However, in the PREPARATORY-5 study, Canada's first HIV PrEP demonstration project led by our team, we found modest evidence of risk compensation; with an increase in bacterial STIs among participants (48% experienced at least one bacterial STI)[43]. Given these conflicting findings, our team will assess sexual behaviours at each visit.

6.2 Adverse Events

Stable chronic conditions which are present prior to clinical study entry and do not worsen are not considered adverse events and will be accounted for in the participant's medical history.

Any AE and SAE that occurs between baseline and the time the participant departs the study at the end of the final follow-up visit (or at the time of early discontinuation of the participant from the study for any reason) will be captured and recorded in the source documentation. At each contact with the participant, the investigator (or designate) must seek information on adverse events by specific questioning and, as appropriate, by examination.

Adverse events that had previously been reported by the study participant will also be reassessed for duration, intensity and possible reoccurrence. Assessment of safety will include clinical observation and monitoring of routine hematology and biochemistry.

Adverse events will be defined using standard definitions as per Division 5 of the Food and Drug Regulations as follows:

Adverse event	means any adverse occurrence in the health of a clinical trial participant who is administered a drug that may or may not be caused by the administration of the drug, and includes an adverse drug reaction
Adverse drug reaction (ADR)	means any noxious and unintended response to a drug that is caused by the administration of any dose of the drug.
Serious ADR	means an adverse drug reaction that requires in-patient hospitalization or prolongation of existing hospitalization, that causes congenital malformation, that results in persistent or significant disability or incapacity, that is life threatening or that results in death
Serious unexpected ADR	means a serious adverse drug reaction that is not identified in nature, severity or frequency in the risk information set out in the investigator's brochure or on the label of the drug

Toxicities will be graded on a 4-point scale according to standard guidelines outlined in the "Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events" (https://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables)[42].

Any AE that occurs between baseline and the last study visit at Week 60 (or at the time of early discontinuation of the participant from the study for any reason) will be recorded in the source documentation. If an AE is ongoing at

the time a participant discontinues/completes the trial the AE will be followed until the Investigator agrees that the event is satisfactorily resolved, becomes chronic, or that no further follow-up is required.

Participants who have an adverse event should be evaluated, followed and treated by a clinician until the abnormal parameter or symptom has resolved or stabilized. It is up to the Investigator to determine that the AE is either resolved or that is has reached a stable state, after which no further follow-up is necessary. There should also be source documentation to support this determination.

For all collected AEs (including SAEs), the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and their clinical judgment. The degree of certainty about causality will be graded using the categories below:

Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.
Probably Related: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time sequence to administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition.
Possibly Related: There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an adverse drug event may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
Not related: The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

6.3 Safety Monitoring Plan and Reporting Procedures

6.3.1 Safety Monitoring: The Study will undergo periodic review (on a quarterly basis) by an External Study Monitor (separate to the Study Team). The External Study Monitor will consist of an impartial, independent clinician and/or the Data Safety Board of the Canadian HIV Trials Network. The External Monitor will evaluate rates of AEs between study arms and will receive reports on all SAEs.

6.3.2 SAE Reporting: The Investigator or designee must report any SAE occurring in a patient receiving study drug to the Principal Investigator within 24 hours, even if the SAE is not drug-related. This should be done by telephone and by sending a faxed copy of the Serious Adverse Event Form plus other supporting documentation, as required.

All additional follow-up evaluations must also be reported as soon as possible in the same manner. All SAEs will be followed until the Investigator agrees that the event is satisfactorily resolved, becomes chronic, or that no further follow-up is required.

The Sponsor or designee will be responsible for notifying the relevant authorities of any reportable SAE as outlined in the Canadian Food and Drug Regulations and ICH Guidelines. Health Canada (Fax 613 941 2121) will be notified for serious unexpected adverse events within seven days if the event was fatal or deemed to be life-threatening, and within 15 days if neither fatal nor life-threatening.

The Investigator is responsible for ensuring that their Research Ethics Board (REB) is notified of any SAEs, as well as the External Study Monitor.

7. DATA MANAGEMENT AND CONFIDENTIALITY

7.1 STUDY MANAGEMENT

Site investigators will supervise site personnel, obtain Research Ethics Board approval, and supervise study activities. The research coordinator will identify participants, obtain informed consent, randomize participants via the online system, ensure participants have regular follow-up, encourage adherence, and collect data into case report forms (CRFs). A study operations manual will outline all relevant trial methods. The CTN will support all sites and assist with the day-to-day trial management, communication strategies and also assign a national study coordinator and national data manager to the project. A computerized study database will be created by an appointed Data Manager and managed at the data management center of the CIHR Canadian HIV Trials Network. Electronic CRFs will be used. A study personnel log will be created with designated roles and activities for all study personnel.

7.2 PARTICIPANT IDENTIFICATION

Participant Identification (ID) numbers will be assigned sequentially to each participant who is eligible to participate in the study. Participants who are not eligible for the study will not be assigned a Participant ID Number. A refusal log will be kept to keep track of those approached and refusing participation. The Participant ID Number will consist of a three digit centre number and a three digit number according to chronological order of entry at each centre. Details for assigning Participant ID Numbers will be provided in the Operations Manual. Log(s) should be completed by each site to capture all participants who have consented and who have been assigned a participant identification number. If a patient discontinues from the study the Participant ID Number will not be reused.

The Participant ID Numbers will be used for identification of participants in the source documentation, CRFs, and laboratory samples. This will ensure that participant data and laboratory samples leaving the study sites will be identified and tracked.

7.3 DATA COLLECTION

Site research coordinators will capture all data onto CTN-produced electronic case report forms (CRFs) and participants will be examined by a physician or nurse at each study visit.

Any data recorded on a Case Report Form will be considered source data. Please see **section 4.4 Schedule of Events Table** for a timeline of key milestones and deliverables. Participants initially referred to the study will be screened by the site study coordinator using a screening checklist. All enrolled participants will then be randomized, and commence a schedule of study visits at the following times: baseline, weeks 4, 12, 24, 36, 48, and 60.

To help ensure optimal adherence and monitoring of subjects during the trial, we will deploy the WelTel patient engagement and virtual care service (See www.weltelhealth.com) that is evidence-based to improve antiretroviral medication adherence[71,72] and retention in care for HIV PrEP[73,74]. The service can also be used as a way for participants to report any issues they have during the trial real-time via their mobile phones or complete surveys.

7.4 Data Storage

All participant-related information including Case Report Forms including laboratory specimen reports will be kept strictly confidential. All paper records will be kept in a secure, locked location at the BCCDC, and only accessible to research staff. The Investigator will maintain adequate and accurate source documents upon which CRFs for each participant are based. They are to be separate and distinct from CRFs. These records should include detailed notes on:

- Oral and written communication with participant regarding the study
- Participation in study and signed and dated informed consent forms
- Inclusion and exclusion criteria details
- Visit dates
- Results of relevant examinations
- Laboratory printouts
- Concomitant medications (if applicable)
- Reason for premature discontinuation (if applicable)
- Enrollment number
- Compliance/noncompliance protocol deviation information

A master list will be kept with name and identification number, and this will be stored securely at each respective study site. The storage cabinet will be in a locked room, and the entire facility is identity-card entry only. The computerized database will identify participants by numeric codes only, and will be password protected, behind a firewall and accessible only to designated analysts at the CTN Data Centre. The CTN Data Centre is housed at St Paul's Hospital in a locked wing, with identity-card entry only.

Upon request, and in the presence of the investigator or their representative, participant records will be made available to the study sponsor, monitoring groups, representative of the study sponsor, representatives of funding groups, and applicable regulatory agencies for the purpose of verification of clinical study procedures and/or data, as is permissible by local regulations. The Investigator will maintain all study records according to local institutional requirements. If the Sponsor-Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. No destruction of medical records should occur without the written approval of the Sponsor-Investigator. Records will be kept for a minimum of 15 years, in accordance with Health Canada requirements for a clinical trial.

7.5 Data monitoring

The study will be monitored by the Data Safety Monitoring Committee (DSMC) of the CTN. This standing committee, established in 1990, is composed of clinicians, methodologists and a representative of the affected community, meeting every six months and on an *ad hoc* basis, if required. It has an existing charter and monitors safety, recruitment, protocol adherence, and ongoing ethical issues. The DSMC will also conduct safety analyses on serious adverse events every 6 months.

8. Ethical Considerations

8.1 Research Ethics Board

The Research Ethics Board (REB) will review all appropriate study documentation to safeguard the rights, safety, and well-being of the participants. The study will be conducted only at sites where ethics approval has been obtained. A copy of the protocol (including protocol amendments), all versions of informed consent forms, other information to be completed by participants such as survey instruments or questionnaires, and any proposed advertising/

recruitment materials must be reviewed and approved by the REB prior to implementation of the study. The investigator will be responsible for obtaining REB approval of the annual Continuing Review throughout the duration of the study.

8.2 Protocol amendments and Deviations

8.2.1 Protocol Amendments: All protocol amendments will be reviewed and approved and if applicable submitted to the applicable regulatory agencies for prior approval or notification. The Investigator must sign and date the amendment prior to implementation. All protocol amendments must also be submitted to the local Research and Ethics Board.

8.2.2 Protocol Deviations: No deviations from this protocol will be permitted without the prior written approval of the sponsor, except when the modification is needed to eliminate an immediate hazard or hazards to participants. Any deviations that may affect a participant's informed consent, especially those increasing potential risks, must receive prior approval from the REB unless performed to remove an immediate safety risk to the participants. In this case, it will be reported to the REB and the Sponsor immediately thereafter. Any departures from the protocol must be documented.

8.3 Informed Consent

All participants will be given detailed oral and written information about the study. Consent forms describing in detail the study procedures and risks will be given to each participant and written documentation of informed consent is required prior to starting study procedures. Participants must sign an informed consent document that has been approved by a participating centre's REB/IRB prior to any procedures being done specifically for the study. Each participant should have sufficient opportunity to discuss the study, have all of their questions addressed and consider the information in the consent process prior to agreeing to participate. Participants may withdraw consent at any time during the course of the study without prejudice. The informed consent form will be signed and dated by the participant and the investigator. The original signed informed consent form will be retained in the participant's study files and a copy will be provided to the participant.

The informed consent process must be conducted and form signed before the participant undergoes any screening procedures that are performed solely for the purpose of determining eligibility for the study.

8.4 Confidentiality

All participant-related information including Case Report Forms, laboratory specimens, evaluation forms, reports, etc. will be kept strictly confidential. All records will be kept in a secure, locked location and only accessible to research staff. Participants will be identified only by means of a coded number specific to each participant. All computerized databases will identify participants by numeric codes only, and will be password protected.

Upon request, and in the presence of the investigator or their representative, participant records will be made available to the study sponsor, monitoring groups representative of the study sponsor, representatives of funding groups, and applicable regulatory agencies for the purpose of verification of clinical study procedures and/or data, as is permissible by local regulations.

9.0 Dissemination

Our results will be targeted to four main groups of stakeholders: clinicians, public health, researchers, and community. Results have the potential to revolutionize STI prevention in a population of individuals who are particularly vulnerable to STIs and their associated complications. Our comprehensive analyses of possible adverse outcomes can inform their eventual rollout. To facilitate knowledge translation and exchange (KTE) for this trial, we

will assemble a community advisory board (CAB). The CAB will be made up primarily of 'patient-partners', and will provide useful interpretation of adverse event data, acceptability data, and specific concerns held by participants. This will enable us to better frame these issues when disseminating to broader community audiences. At trial-end, a lay summary report will be produced to promote community awareness. The Community-Based Research Centre for Gay Men's Health, the Canadian AIDS Treatment Information Exchange (CATIE), and the AIDS Committee of Toronto will help facilitate KTE locally and nationally. We will disseminate our findings to a large, international audience of clinicians and public-health professionals by publishing in high-impact peer-reviewed journals.

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