A Phase 4 Comparative Trial of Benzathine Penicillin G 2.4 Million Units Administered as a Single Dose versus Three Successive Weekly Doses for Treatment of Early Syphilis in Subjects with or without HIV Infection

DMID Protocol Number: 17-0101

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DMID Scientific Lead: Carolyn Deal, PhD

Version Number: 4.0

22 June 2020

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- International Conference on Harmonisation: Good Clinical Practice (ICH) E6; 62 Federal Register 25691 (1997); and future revisions
- National Institutes of Health (NIH) Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
BPG	Benzathine Penicillin G
CFR	Code of Federal Regulations
CMS	Clinical Material Services
CoC	Certificate of Confidentiality
CROMS	Clinical Research Operations and Management Support
CSR	Clinical Study Report
DFA-TP	Direct Fluorescence Antibody-*Treponema pallidum*
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EMR	Electronic Medical Record
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
IATA	International Air Transport Association
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICON	ICON Clinical Research, Inc.
IDES	Internet Data Entry System
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intent-to-Treat
IV	Intravenous
MedDRA®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSM	Men who have Sex with Men
MU	Million Units
N	Number (typically refers to subjects)
NAT	Nucleic Acid Test
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OER	Office of Extramural Research, NIH, DHHS
OHRP	Office for Human Research Protections
P&S	Primary and Secondary
PASS	Power Analysis and Sample Size Software
PCR	Polymerase Chain Reaction

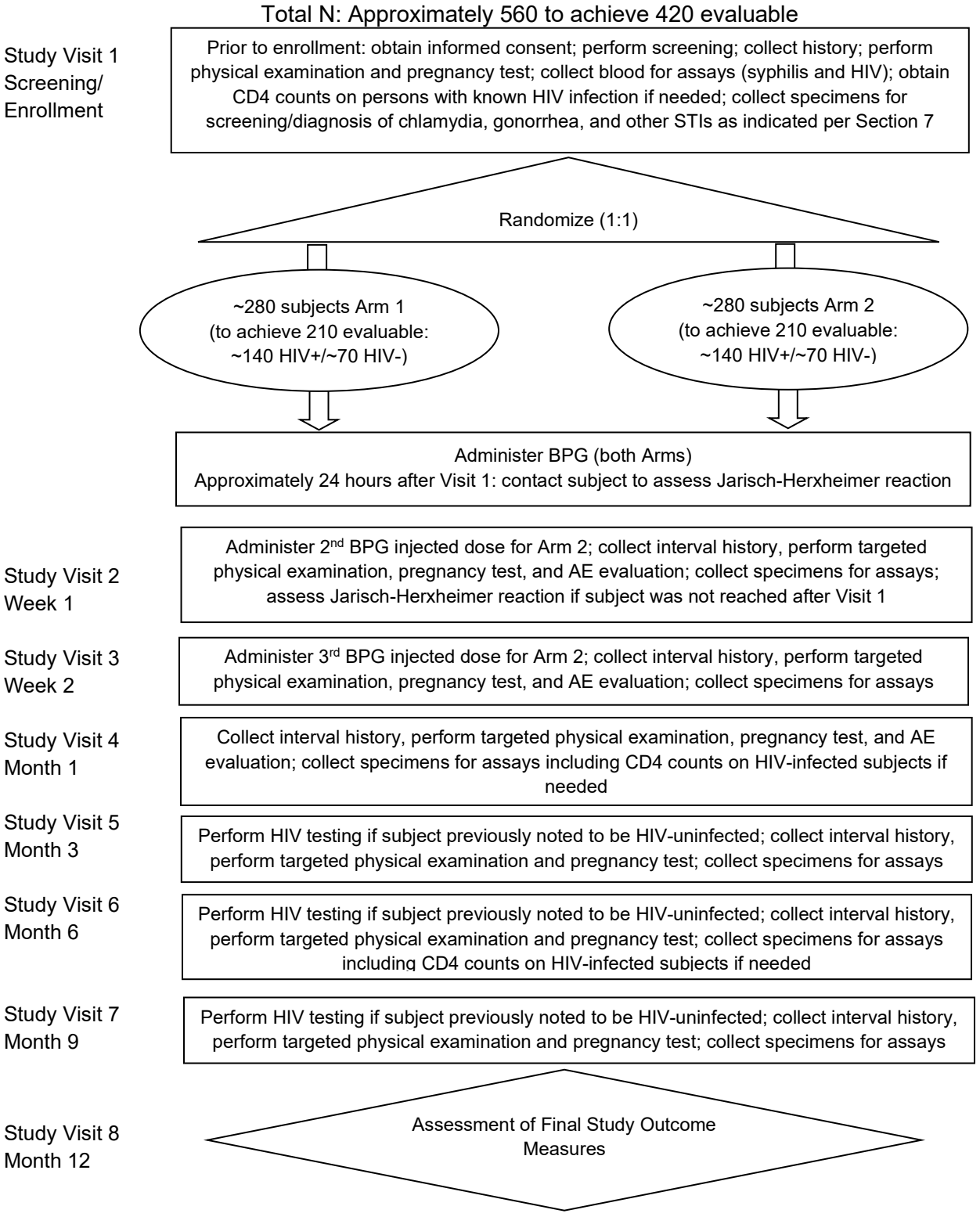
PI	Principal Investigator
RCT	Randomized Controlled Trial
RPR	Rapid Plasma Reagin
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SAS	Statistical Analysis System
SDCC	Statistical and Data Coordinating Center
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infection
STI CTG	Sexually Transmitted Infections Clinical Trials Group
STS	Serological Test for Syphilis
TRI	Technical Resources International, Inc.
US	United States

PROTOCOL SUMMARY

Title:	A Phase 4 Comparative Trial of Benzathine Penicillin G 2.4 Million Units Administered as a Single Dose versus Three Successive Weekly Doses for Treatment of Early Syphilis in Subjects with or without HIV Infection
Phase:	4
Population:	Approximately 560 adults aged 18 years or older with untreated early (primary, secondary, or early latent) syphilis (to achieve 420 evaluable subjects)
Number of Sites:	Ten
Study Duration:	48 months
Subject Participation Duration:	12 months
Description of Agent or Intervention:	Benzathine penicillin G (BPG) 2.4 million units (MU) injected once (Arm 1) or weekly for three successive weeks (Arm 2)
Objectives:	Primary: • To compare the serological response to therapy in subjects with early (primary, secondary, or early latent) syphilis treated with BPG 2.4 MU once or weekly for three successive weeks Secondary: • To determine if the difference in response to therapy between treatment arms by Month 6 differs among subjects with or without HIV infection • To determine the impact of multiple BPG injected doses on subject compliance with study product and adherence to the corresponding scheduled visits • To determine the incidence and manifestations of the Jarisch-Herxheimer reaction among subjects treated for early syphilis with BPG • To collect prospective data up to Month 12 on the serological response to therapy in subjects treated for early syphilis with either BPG regimen • To compare epidemiological characteristics of early syphilis among subjects with or without HIV infection

Description of Study Design:	BPG 2.4 MU injected either once (Arm 1) or weekly for three successive weeks (Arm 2) as determined by randomization, with follow-up visits at Months 1, 3, 6, 9, and 12 for safety, clinical, and laboratory assessments
Estimated Time to Complete Enrollment:	36 months

Schematic of Study Design



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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Despite syphilis being a major public health problem for well over a century, and its treatment recommendations being unchanged for nearly 50 years, questions about syphilis and its management are amongst the most common questions to arise regarding sexually transmitted infections (STIs). The human immunodeficiency virus (HIV) epidemic has compounded these problems, as syphilis is now appreciated as both a risk marker and risk factor for HIV acquisition, and case reports and uncontrolled case series have led to recurring, unresolved questions regarding the optimal management of syphilis with respect to treatment regimens, serological follow-up, and risks for treatment failure [1]. Syphilis in the US is resurging, with rates of primary and secondary (P&S) syphilis increasing by 74% from 2012 to 2016 and disproportionately among HIV-infected persons [2]. Further rising P&S rates in women led to a 28% increase in congenital syphilis cases in the US between 2015 and 2016 [3]. No large prospective studies in the US have provided data on outcomes of recommended single-dose penicillin therapy versus widely-used but unstudied multiple-dose therapy for persons with early syphilis and HIV co-infection for over 30 years. In addition, there are no contemporary data describing serological responses to syphilis therapy at >6 months following therapy, raising questions as to how to best follow the ~20% of "serofast" patients who fail to serologically respond at >6 months following therapy. These questions represent a pressing public health need that is best addressed through a multicenter, randomized clinical trial (RCT).

Infections due to *Treponema pallidum* are the third most commonly reported infection in the US and are a global public health priority. Currently, P&S syphilis rates in the US are increasing faster than for any other reportable STI, and over half of P&S syphilis cases in men who have sex with men (MSM), the group that accounts for most new cases, are occurring among persons with HIV co-infection [3]. Untreated syphilis is a significant cause of morbidity in children born to mothers with untreated infection, may cause serious neurological or cardiovascular disease, and is both a proven risk factor and risk marker for HIV acquisition. Syphilis also remains a glaring example of STI health disparities, with P&S syphilis rates among African Americans in the US being more than six times those of non-Hispanic whites [4].

Penicillin is currently the only recommended treatment for syphilis (alternative therapies may be used in persons with penicillin allergy [5]). However, recent studies have shown that ~20% of persons treated for early syphilis with recommended, single-dose BPG 2.4 MU are "serofast" (do not show the desired 4-fold decline in serological test for syphilis titers 6 months following therapy) [5, 6], and concerns have been raised as to whether this regimen adequately treats early syphilis in HIV-infected persons. A well-conducted, multicenter RCT in the 1990s showed that higher doses of penicillin given in combination with the recommended BPG regimen did not

significantly change the serological response to therapy at 3, 6, or 12 months [7], but there are no RCTs evaluating whether a longer duration of BPG therapy improves the serological response to therapy among persons with or without HIV infection. While this earlier study addressed questions regarding daily penicillin doses for early syphilis treatment, the question of duration remains unresolved. As a result, there is substantial variation in the treatment of early syphilis, with many providers using multiple injected doses of BPG or treating serofast persons who have no evidence of active infection.

2.2 Rationale

Epidemiologically, syphilis infections tend to shift from one risk group to another. Past syphilis epidemics in the US have occurred in heterosexual men and women, or MSM. More recently, syphilis rates in the US have more than doubled over the past decade and now occur disproportionately among HIV-infected persons [4].

While penicillin is generally regarded as highly effective for syphilis treatment, whether or not currently recommended therapy is equally effective for persons with or without HIV infection remains unclear. In addition, recent appreciation of the fact that ~20% of persons treated for early syphilis are serofast has raised concerns regarding the significance of this observation [6]. Some clinicians believe that the serofast state signifies treatment failure, while others believe it represents failure to serologically respond to therapy in successfully treated patients. Each of these concerns may lead to inadequate therapy or over treatment in persons with early syphilis, as well as much confusion among clinicians about appropriate therapy.

The primary aims of this study are to determine whether subjects with or without HIV infection benefit from a longer duration of therapy (BPG 2.4 MU weekly for three successive weeks instead of as a single dose), and whether subjects with or without HIV infection differ in their response to BPG therapy for early syphilis.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The risks associated with participation in the study are small. Penicillin is widely used to treat syphilis and other infections and is relatively well tolerated. All subjects in the trial will receive at least the currently recommended therapy. The most common adverse events (AEs) caused by penicillin include rash and hypersensitivity reactions. Therapeutic outcome is not a major concern, as all subjects will receive at least the Centers for Disease Control and Prevention-recommended dose of BPG for early syphilis. Subjects randomized to receive two additional injected doses of BPG will be at risk for additional discomfort and bruising at injection sites. Subjects may feel temporary pain or discomfort during blood draws, and there is a small risk of

infection at the site of the blood draw.

Information concerning subjects' sexual history is necessarily detailed given the study's objectives and may provoke some minor psychological or emotional stress when requested; subjects who receive a new diagnosis of an STI during the study may also experience minor psychological or emotional stress. Female subjects will be cautioned about the potential hazard of becoming pregnant and advised to use adequate birth control methods for the entire duration of their participation in the trial.

2.3.2 Known Potential Benefits

Subjects may benefit from this study by finding out more about the specific cause of symptoms they may be experiencing. Subjects may benefit from pre- and post-test counseling, treatment, and referrals, as necessary. It has previously been observed that subjects are empowered by knowing, for example, more about what syphilis is and how it can be prevented.

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary Objective

- To compare the serological response to therapy in subjects with early (primary, secondary, or early latent) syphilis treated with BPG 2.4 MU once or weekly for three successive weeks

3.1.2 Secondary Objectives

- To determine if the difference in response to therapy between treatment arms by Month 6 differs among subjects with or without HIV infection
- To determine the impact of multiple BPG injected doses on subject compliance with study product and adherence to the corresponding scheduled visits
- To determine the incidence and manifestations of the Jarisch-Herxheimer reaction among subjects treated for early syphilis with BPG
- To collect prospective data up to Month 12 on the serological response to therapy in subjects treated for early syphilis with either BPG regimen
- To compare epidemiological characteristics of early syphilis among subjects with or without HIV infection

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measure

- The proportion of subjects in each treatment group with a 4-fold decline in rapid plasma reagin (RPR) titers or seroreversion, simultaneously measured in sera collected throughout study participation at a single reference laboratory, by Month 6

3.2.2 Secondary Outcome Measures

- The proportion of subjects in each treatment group with a 4-fold decline in RPR titers or seroreversion by Month 6 among subjects with or without HIV infection
- The proportion of subjects in each treatment group with a 4-fold decline in RPR titers or seroreversion by Month 12
- The proportion of subjects in each treatment group with a 4-fold decline in RPR titers or seroreversion by Month 12 among subjects with or without HIV infection
- The proportion of subjects (overall and in each treatment group) who receive all assigned doses within the assigned visit windows
- The proportion of subjects in each treatment group who report Jarisch-Herxheimer reaction manifestations (fever, intensification of rash, myalgia, and other systemic symptoms) occurring within approximately 24 hours of initial BPG administration
- Demographics, sexual history, and socio-epidemiologic characteristics at baseline and sexual history through Months 6 and 12 among subjects with or without HIV infection and overall

4 STUDY DESIGN

This is a Phase 4, open-label, multicenter trial to evaluate the efficacy of a single injected dose of BPG 2.4 MU (Arm 1) compared to three successive weekly injected doses of BPG 2.4 MU (Arm 2) for treatment of early syphilis in HIV-infected and HIV-uninfected subjects. Subjects will be aged 18 years or older with untreated early syphilis (primary, secondary, or early latent as defined in prior syphilis treatment trials of the Sexually Transmitted Infections Clinical Trials Group (STI CTG)). The study will be conducted at 10 sites in the US and will enroll approximately 560 subjects to achieve 420 evaluable.

The study will involve a screening/enrollment visit and seven scheduled follow-up visits (Weeks 1 and 2, and Months 1, 3, 6, 9, and 12) over a 12-month period. At the enrollment visit, after providing informed consent, all subjects will undergo a brief sexual and medical history and a directed physical examination; all women of childbearing potential will have a urine or serum pregnancy test performed as part of qualification for study participation; all subjects will undergo phlebotomy for serological testing for syphilis and HIV according to the study and clinic protocols. All subjects who have not been tested for chlamydia and gonorrhea since last sexual activity and all subjects who have been sexually active in the past 14 days will have specimens collected for chlamydia and gonorrhea testing. Subjects will have specimens collected for other STI testing as indicated by local standard of care and subject history. All eligible subjects will be randomized to Arm 1 or Arm 2 and will receive an injected dose of BPG 2.4 MU.

Study personnel will attempt to contact subjects approximately 24 hours after Visit 1 to assess for symptoms of a Jarisch-Herxheimer reaction as described in Section 8.1. At the second and third visits (Weeks 1 and 2 of follow-up), subjects randomized to Arm 2 will receive injected doses of BPG 2.4 MU.

At all follow-up visits, subjects will have an interval history obtained, undergo a directed physical examination, and have repeat specimens collected for STI testing (based on the subject history and clinic protocols). At all follow-up visits, subjects will undergo phlebotomy for serological testing for syphilis and serum storage. At the Month 3, Month 6, Month 9, and Month 12 follow-up visits, consenting HIV-uninfected subjects will be tested for HIV infection using a 4th generation serological test for HIV. At Visit 1, study staff will swab consenting subjects' oral cavities and primary or secondary lesions (if lesions are present per Section 5.1); see Section 8.2.2.

Safety will be measured by subject report and physical examination (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations). All AEs (including solicited reactogenicity AEs and other unsolicited AEs) will be recorded through Month 1. Safety oversight will be provided by a

Data and Safety Monitoring Board (DSMB) and site Independent Safety Monitors (ISMs) as described in Section 9.6.

While sites will perform their own serological testing as needed for subject management, serological testing to determine study outcomes and serum banking will be performed at the Central Laboratory (University of Alabama at Birmingham).

The duration of the study for each subject will be 12 months. Enrollment is expected to be completed in 36 months. For additional details on study procedures/evaluations and study schedule by study visits, see Sections 7 and 8 and Appendix A.

5 STUDY ENROLLMENT AND WITHDRAWAL

To achieve 420 evaluable subjects, approximately 560 adults aged 18 years or older who meet all inclusion criteria and no exclusion criteria will be enrolled. No exemptions are granted on inclusion/exclusion criteria in trials sponsored by the Division of Microbiology and Infectious Diseases (DMID). Subjects may be recruited from STI clinics, HIV clinics, student health centers, and the public via advertising (e.g., flyers, radio, newspaper, social media). To enhance recruitment and retention, sites may seek institutional review board (IRB) permission to contact subjects by phone, text messaging, and/or email as appropriate. Any communication with subjects must be made using devices, methods, and services allowed by the local IRB and must be documented appropriately. As noted in Section 7.1, each subject may indicate his/her preferred method of contact to the study staff.

5.1 Subject Inclusion Criteria

Subjects eligible to enroll in this study must meet all inclusion criteria:

1. Subject is aged 18 years or older.
2. Subject has provided informed consent.
3. Subject has untreated primary*, secondary**, or early latent*** syphilis.
   *Primary syphilis is characterized by the presence of an ulcerative lesion at a potential site of inoculation (while classically solitary, shallow, painless and with an indurated, clean base, primary lesions may be multiple, may vary considerably in appearance, and/or may not be painless) or by darkfield, acceptable polymerase chain reaction (PCR), or direct fluorescence antibody-*T. pallidum* (DFA-TP) positive ulcers.
   **Secondary syphilis is characterized by classical palmar/plantar rash, condylomata lata, mucous patches, etc. or by darkfield, acceptable PCR, or DFA-TP positive lesions.
   ***Early latent syphilis is characterized by current reactive serologic tests for syphilis (STS) and a documented non-reactive STS, or documented sexual exposure to an individual known to have primary, secondary, or early latent syphilis diagnosed within the last 12 months.
4. Subject either has a newly reactive non-treponemal test (such as an RPR test) or a history of syphilis and a current increase in RPR titer of two or more dilutions (i.e., four-fold).
5. If subject is of childbearing potential, subject has a negative urine or serum pregnancy test.
6. Subject is willing to have an HIV test, participate in HIV counseling, and return to clinic for follow-up.
7. In the opinion of the investigator, subject is able and willing to comply with study procedures, including receipt of three BPG injected doses if randomized to Arm 2.

8. If female, subject must be of non-childbearing potential* or must be using an acceptable method of birth control** to avoid becoming pregnant.

   *Non-childbearing potential is defined as being post-menopausal for at least 1 year, status after bilateral tubal ligation, or status after bilateral oophorectomy, or status after hysterectomy.

   **Subject must agree to avoid becoming pregnant by using one of the following acceptable methods of birth control for the entire duration of participation in the trial:

   - Intrauterine contraceptive device; OR
   - Oral contraceptives; OR
   - Hormonal injections; OR
   - Hormonal implants; OR
   - Contraceptive patches; OR
   - Monogamous relationship with vasectomized partner; OR
   - Exclusively same-sex relationships; OR
   - Use of condoms by the male partner; OR
   - Abstinence

5.2 Subject Exclusion Criteria

Subjects eligible to enroll in this study must not meet any exclusion criteria:

1. Subject previously enrolled in this trial.
2. Subject has latent syphilis of unknown duration, late latent syphilis, or evidence of neurosyphilis, including ocular syphilis.*

   *e.g., eye pain/redness, recent ocular change, and/or changes in visual acuity
3. Subject has a known or suspected allergy or hypersensitivity to penicillin or other beta-lactam antibiotics.
4. Subject has a known or suspected STI other than syphilis requiring treatment with a drug active against *T. pallidum*.
5. Subject has used antibiotics* active against *T. pallidum* in the preceding 30 days.

   *Note: the use of antimicrobials known to NOT be effective against *T. pallidum* (e.g., quinolones, sulfonamides, trimethoprim, metronidazole, spectinomycin) will be allowed.
6. Subject has suspected or known ongoing drug use that might interfere with study participation and follow-up treatment.
7. Subject is breastfeeding.
8. Subject has used an investigational drug in the past 30 days that might interfere with safety or efficacy assessment.*

   *If the subject has used any investigational drugs in the past 30 days, contact the Principal Investigator, DMID Clinical Project Manager, DMID Medical Officer, and FHI 360 to confirm eligibility.
9. Subject has any other condition that, in the opinion of the investigator, would interfere with participation in the study.

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

Enrollment of subjects will be done online using the enrollment module of Emmes Advantage eClinical®. Subjects will be randomized at a 1:1 ratio to receive one of the two BPG treatments after informed consent is obtained and their eligibility is confirmed.

The study will use a stratified, permuted block-randomization scheme. Permuted block randomization is used to avoid the potential for serious imbalance in the number of subjects assigned to each group, an imbalance that can occur in the simple randomization procedures. Stratification will be by study site. While HIV status may not be known at the time of randomization, subjects will be classified as HIV-infected or HIV-uninfected to assess the secondary HIV status subgroup objective, and randomization will be limited as follows:

- If 280 subjects who were HIV-infected at baseline are confirmed evaluable, additional subjects who are known to be HIV-infected will not be allowed to enroll thereafter, while HIV-uninfected subjects and subjects of unknown HIV status may continue to enroll.

- If 140 subjects who were HIV-uninfected at baseline are confirmed evaluable, only subjects who are known to be HIV-infected will be allowed to enroll thereafter.

The list of randomized treatment assignments will be prepared by statisticians at Emmes and included in the enrollment module of its Internet Data Entry System (IDES). IDES will assign each subject a treatment code from the list after demographic and eligibility data have been entered.

Instructions for use of the enrollment module are included in the IDES User's Guide. Manual back-up randomization procedures are provided in the Manual of Procedures (MOP) for use in case a site temporarily loses access to the Internet or the online enrollment system is unavailable.

5.3.2 Masking Procedures

Neither subjects nor providers will be masked in this study. The outcome (serological response to therapy) is objective—furthermore, laboratory staff will be blinded to subject ID and treatment assignment—and it would not be ethical to give subjects placebo injections.

5.3.3 Reasons for Withdrawal from Study Participation/Discontinuation of Study Treatment

Subjects may voluntarily withdraw their consent for further study participation or may request to discontinue study treatment at any time and for any reason without penalty or prejudice to future

medical care.

In addition to voluntarily requesting to discontinue study treatment as noted above, subjects randomized to Arm 2 may also be discontinued from additional treatment after discussion with the investigator for the following reasons:

- AE(s) judged to be Grade 3 and related to study drug
- SAE(s) judged to be related to study drug
- Investigator's discretion
- Violation of eligibility criteria
- Significant deviation from the treatment plan specified in the protocol (e.g., incorrect administration of BPG, failure to attend study visits)
- Receipt of antibiotic therapy active against *T. pallidum* for treatment of syphilis infection

Subjects in either arm who require off-study retreatment for syphilis infection will be withdrawn from the study after completing an Early Termination Visit.

5.3.4 Handling of Withdrawals from Study Participation/Discontinuations of Study Treatment

Subjects who withdraw from study participation, or are excluded from any efficacy endpoint analysis, or are lost to follow-up after signing the informed consent form (ICF), randomization, and receipt of the first dose of BPG will not be replaced. Subjects who withdraw consent after signing the ICF and randomization but before receipt of the first dose of BPG may be replaced.

If the subject requests to discontinue study treatment at any time after the first dose of BPG, the subject will be asked to continue scheduled study procedures including safety and efficacy evaluations, if possible, and be given appropriate care under medical supervision if symptoms of any AE related to participation in the study are continuing. The subject will be followed until the AE is resolved or until the subject's condition becomes stable.

Subjects who request to discontinue study treatment at any time after the first dose of BPG will be reminded of the importance of continuing in the study for safety evaluations. Subjects will be encouraged to complete the Early Termination Visit if they choose not to complete the remaining study visits (i.e., if they withdraw from study participation). The Early Termination Visit procedures are listed in Section 7.3. Subjects who choose to withdraw from study participation will no longer be contacted for follow-up.

In the case of subjects who fail to appear for a follow-up safety assessment, extensive effort (e.g., three documented contact attempts via phone calls, e-mails, etc., made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. Subjects who cannot be located after extensive effort will no longer be contacted for follow-up. These efforts will be documented in the subject's records.

If a subject is withdrawn from the study due to an AE that prohibits continued participation in the study, s/he will be given appropriate care and treatment under medical supervision until the condition has resolved or becomes stable.

Safety and efficacy data will be collected on any subject who is excluded from any efficacy endpoint analysis. Refer to Section 11 for details on how subjects who are withdrawn will be handled during analysis.

5.3.5 Termination of Study

Although the study sponsor has every intention of completing the study, the sponsor reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to DSMB review and recommendation or at the discretion of DMID.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

Penicillin G benzathine injectable suspension (BPG) is prepared by the reaction of dibenzylethylene diamine with two molecules of penicillin G. It occurs as a white crystalline powder and is very slightly soluble in water and sparingly soluble in alcohol. BPG (Bicillin® L-A) is available as a suspension for deep intramuscular (IM) injection.

6.1.1 Acquisition

BPG (Bicillin® L-A) is manufactured by Pfizer Laboratories and will be purchased from the study budget. Upon request by DMID, BPG will be transferred to:

DMID Clinical Materials Services (CMS)
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-[redacted]
Fax: 240-[redacted]

BPG will be shipped from the DMID Clinical Materials Services (CMS) to the investigational sites upon request and approval by DMID.

6.1.2 Formulation, Packaging, and Labeling

BPG contains penicillin G benzathine in aqueous suspension with sodium citrate buffer and, as weight/volume, approximately 0.5% lecithin, 0.6% carboxymethylcellulose, 0.6% povidone, 0.1% methylparaben, and 0.01% propylparaben. Bicillin® L-A suspension in the disposable-syringe formulation is viscous and opaque, and is available in a 2-mL size containing 1.2 MU BPG per syringe. It is labeled for deep **IM injection only**, not for intravenous (IV) use.

6.1.3 Product Storage and Stability

Refer to the protocol-specific MOP for guidance on BPG storage.

6.2 Dosage, Preparation, and Administration of Study Intervention/Investigational Product

At each site, a Research Pharmacist or clinician may be delegated the responsibility of BPG dispensation. The Research Pharmacist must be a licensed, registered pharmacist and is the

preferred healthcare practitioner to be delegated to perform this activity. If a Research Pharmacist is not available, a physician, nurse practitioner, physician assistant, registered nurse, or other authorized healthcare practitioner who is a member of the clinical study staff may be delegated to dispense BPG. These personnel must be licensed, trained, and qualified to prepare an investigational study product and must be authorized to dispense it under state and local rules and regulations.

BPG will be administered as a deep IM injection in the upper, outer quadrant of the buttock. Subjects will be randomized to one of two treatment assignments:

- Arm 1: BPG 2.4 MU injected once as two 2-mL injections (one in each buttock)
- Arm 2: BPG 2.4 MU injected weekly as two 2-mL injections (one in each buttock) for three successive weeks

6.3 Accountability Procedures for the Study Intervention/Investigational Product(s)

After receiving the BPG, the site principal investigator (PI) is responsible for its distribution and disposition and has ultimate responsibility for its accountability. The site PI may delegate to a site Research Pharmacist or an appropriately qualified staff member the responsibility for BPG accountability. The designee will be responsible for maintaining complete records and documentation of BPG receipt, accountability, dispensation, temperature and storage conditions, and final disposition. All BPG, whether administered or not, must be documented on the appropriate study drug accountability record or dispensing log. The sponsor's monitoring staff will verify the participating clinical sites' study drug accountability records and dispensing logs per the study monitoring plan.

Upon completion or termination of the study and after the final monitoring visit, final disposition of the unused BPG will be determined by DMID and communicated to the sites by the DMID Clinical Project Manager.

6.4 Assessment of Subject Compliance with Study Intervention/Investigational Product

All BPG injections will be directly observed by the administering clinician. Compliance with study product for all subjects will be defined as whether subjects receive all assigned doses within the assigned visit windows.

6.5 Concomitant Medications/Treatments

Administration of any medications, therapies, or vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all medications taken 30 days before initiating study treatment through Month 12 or early termination, whichever occurs first. Prescription and over-the-counter drugs will be included, as well as herbs, vitamins, and supplements. Previously recorded medications will be updated as appropriate.

Subjects who have received BPG and are subsequently diagnosed with a concomitant infection that requires systemic antibiotics will receive treatment according to the local clinic's standard protocols. Likewise, subjects who have received BPG through the study and subsequently require retreatment for syphilis infection will receive treatment according to the local clinic's standard protocols and will be withdrawn from the study per protocol Section 5.3.3.

At the discretion of the site PI, use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition.

Medications that might interfere with the evaluation of BPG should not be used unless absolutely necessary.

Refer to Section 5 (Subject Inclusion and Exclusion Criteria) for medications that are prohibited for study eligibility and throughout study participation.

7 STUDY SCHEDULE

Study visit information is listed in this section, Section 8, and the Schedule of Events (Appendix A). Further instructions are described in the protocol-specific MOP.

7.1 Visit 1 – Screening and Enrollment/Baseline Visit (Day 1)

- The potential subject will be provided with a description of the study (purpose and study procedures) and asked to read the ICF or have it read to him/her. The ICF must be signed before any screening or study procedures are performed.
- Demographic information will be collected from the subject.
- Eligibility criteria will be reviewed with the subject.
    - Complete medical history will be obtained by interviewing subjects (and reviewing medical records for the past 14 days, if available) to assure eligibility. If available in the medical record, the most recent viral load result from the past six months prior to enrollment should be recorded.
    - Sexual history for the past 60 days will be collected.
    - A targeted physical examination will be performed by a qualified study clinician (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations).
    - Study staff will swab consenting subjects' oral cavities and primary or secondary lesions (if lesions are present per Section 5.1); see Section 8.2.2.
    - A urine or serum pregnancy test will be performed on all subjects of childbearing potential and must be negative prior to randomization.
    - All concomitant medications taken in the last 30 days prior to initiating BPG treatment will be recorded on the appropriate data collection form.
    - Blood will be collected for RPR test, storage, and HIV assays (including a CD4 count for persons with known HIV infection whose medical records do not include a CD4 count in the past 30 days, and an HIV test for persons who do not have a previously documented positive HIV test result). Specimens will be collected at sites of exposure for screening/diagnosis of gonorrhea and chlamydia for all subjects who have not been tested since last sexual activity and for all subjects who have been sexually active in the past 14 days and for other STIs as indicated by local standard of care and subject history. Clinicians will provide pre- and post-test counseling, treatment, and referrals per local standard of care.

At this juncture, if a potential subject is not eligible, the reason for screening failure will be recorded and the subject will not be enrolled.

- Subject will be enrolled in Advantage eClinical® and randomly assigned to Arm 1 or Arm 2.

- BPG will be administered, and the subject will be observed per clinic standard operating procedure (SOP).

- Study personnel will discuss with subjects and assess and record all AEs/SAEs (including solicited reactogenicity AEs and other unsolicited AEs).

- Protocol requirements will be reviewed with the subject.

- Contact information will be collected, the subject's preferred method of contact will be noted, and the Week 1 visit will be scheduled.

- The Jarisch-Herxheimer checklist will be distributed and the instructions reviewed with the subject. The subject will be asked to have the completed checklist available for reference during the non-visit contact approximately 24-48 hours after Visit 1 and to bring it to Visit 2. Study personnel will instruct the subject to use a thermometer to take temperature reading(s) at home, if possible, and note the highest temperature if s/he feels feverish any time before being contacted to assess for symptoms of a Jarisch-Herxheimer reaction as described in Section 8.1. Study personnel will attempt to contact subjects by phone call, text message, or email approximately 24 hours after Visit 1 to make this assessment. Note that data will be recorded on the appropriate electronic case report forms (eCRFs) based on subject interview; the Jarisch-Herxheimer checklist completed by the subject will not be retained.

7.2 Follow-up

7.2.1 Visit 2 – Week 1 (Day 7 to 13)

- Subject will be evaluated for resolution of any clinical signs of infection and will be asked specific questions on medication tolerance. If the subject was not contacted following Visit 1, s/he will be evaluated for symptoms of a Jarisch-Herxheimer reaction at Visit 2 as described in Section 8.1.

- Medical history will be reviewed (including review of interval medical records and local laboratory results, if available) and updated as appropriate. If available in the medical record, the most recent viral load result since enrollment should be recorded. If the local laboratory results indicate a need for retreatment for syphilis infection, the subject will receive treatment according to the local clinic's standard protocols and will be withdrawn from the study per protocol Section 5.3.3.

- Interim sexual history since the last study visit will be collected. Specimens will be collected at sites of exposure for STI screening/diagnosis as indicated by local standard of care and subject history.

- A urine pregnancy test will be performed on all subjects of childbearing potential.
- All concomitant medications taken since the last study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- A targeted physical examination will be performed by a qualified study clinician (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations).
- Blood will be collected for follow-up RPR tests and storage.
- Arm 2 only: second dose of BPG will be administered, and the subject will be observed per clinic SOP.
- Study personnel will discuss with subjects and assess and record all AEs/SAEs (including solicited reactogenicity AEs and other unsolicited AEs).
- Contact information will be reviewed, and the Week 2 visit will be scheduled.

7.2.2 Visit 3 – Week 2 (6 to 12 days after Visit 2)

- Subject will be evaluated for resolution of any clinical signs of infection.
- Medical history will be reviewed (including review of interval medical records and local laboratory results, if available) and updated as appropriate. If available in the medical record, the most recent viral load result since enrollment should be recorded. If the local laboratory results indicate a need for retreatment for syphilis infection, the subject will receive treatment according to the local clinic's standard protocols and will be withdrawn from the study per protocol Section 5.3.3.
- Interim sexual history since the last study visit will be collected. Specimens will be collected at sites of exposure for STI screening/diagnosis as indicated by local standard of care and subject history.
- A urine pregnancy test will be performed on all subjects of childbearing potential.
- All concomitant medications taken since the last study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- A targeted physical examination will be performed by a qualified study clinician (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations).
- Blood will be collected for follow-up RPR tests and storage.

- Arm 2 only: third dose of BPG will be administered, and the subject will be observed per clinic SOP.

- Study personnel will discuss with subjects and assess and record all AEs/SAEs (including solicited reactogenicity AEs and other unsolicited AEs).

- Contact information will be reviewed, and the Month 1 visit will be scheduled.

7.2.3 Visit 4 – Month 1 (Day 30 ± 7 days)

- Medical history will be reviewed (including review of interval medical records and local laboratory results, if available) and updated as appropriate. If available in the medical record, the most recent viral load result since enrollment should be recorded. If the local laboratory results indicate a need for retreatment for syphilis infection, the subject will receive treatment according to the local clinic's standard protocols and will be withdrawn from the study per protocol Section 5.3.3.

- Interim sexual history since the last study visit will be collected. Specimens will be collected at sites of exposure for STI screening/diagnosis as indicated by local standard of care and subject history.

- A urine pregnancy test will be performed on all subjects of childbearing potential.

- All concomitant medications taken since the last study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.

- Study personnel will discuss with subjects and assess and record all AEs/SAEs (including solicited reactogenicity AEs and other unsolicited AEs).

- A targeted physical examination will be performed by a qualified study clinician (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations).

- Blood will be collected for follow-up RPR tests, storage, and CD4 counts on persons newly found to have HIV infection from Visit 1 to Visit 4 (inclusive) whose medical records do not include a CD4 count in the past 30 days. Clinicians will provide pre- and post-test counseling, treatment, and referrals per local standard of care.

- Contact information will be reviewed, and the Month 3 visit will be scheduled.

7.2.4 Visit 5 – Month 3 (Day 90 ± 21 days)

- Medical history will be reviewed (including review of interval medical records and local laboratory results, if available) and updated as appropriate. If the local laboratory results indicate a need for retreatment for syphilis infection, the subject will receive treatment

according to the local clinic's standard protocols and will be withdrawn from the study per protocol Section 5.3.3.

- Interim sexual history since the last study visit will be collected. Specimens will be collected at sites of exposure for STI screening/diagnosis as indicated by local standard of care and subject history.
- A urine pregnancy test will be performed on all subjects of childbearing potential.
- All concomitant medications taken since the last study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- A targeted physical examination will be performed by a qualified study clinician (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations).
- HIV testing will be performed if the subject was previously noted to be HIV-uninfected. Clinicians will provide pre- and post-test counseling, treatment, and referrals per local standard of care.
- Blood will be collected for follow-up RPR tests and storage.
- Contact information will be reviewed, and the Month 6 visit will be scheduled.

7.2.5 Visit 6 – Month 6 (Day 180 ± 21 days)

- Medical history will be reviewed (including review of interval medical records and local laboratory results, if available) and updated as appropriate. If the local laboratory results indicate a need for retreatment for syphilis infection, the subject will receive treatment according to the local clinic's standard protocols and will be withdrawn from the study per protocol Section 5.3.3.
- Interim sexual history since the last study visit will be collected. Specimens will be collected at sites of exposure for STI screening/diagnosis as indicated by local standard of care and subject history.
- A urine pregnancy test will be performed on all subjects of childbearing potential.
- All concomitant medications taken since the last study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- A targeted physical examination will be performed by a qualified study clinician (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations).
- HIV testing will be performed if the subject was previously noted to be HIV-uninfected.

Clinicians will provide pre- and post-test counseling, treatment, and referrals per local standard of care.

- Blood will be collected for follow-up RPR tests, storage, and CD4 counts on persons found to have HIV infection whose medical records do not include a CD4 count in the past 30 days.

- Contact information will be reviewed, and the Month 9 visit will be scheduled.

7.2.6 Visit 7 – Month 9 (Day 270 ± 28 days)

- Medical history will be reviewed (including review of interval medical records and local laboratory results, if available) and updated as appropriate. If the local laboratory results indicate a need for retreatment for syphilis infection, the subject will receive treatment according to the local clinic's standard protocols and will be withdrawn from the study per protocol Section 5.3.3.

- Interim sexual history since the last study visit will be collected. Specimens will be collected at sites of exposure for STI screening/diagnosis as indicated by local standard of care and subject history.

- A urine pregnancy test will be performed on all subjects of childbearing potential.

- All concomitant medications taken since the last study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.

- A targeted physical examination will be performed by a qualified study clinician (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations).

- HIV testing will be performed if the subject was previously noted to be HIV-uninfected. Clinicians will provide pre- and post-test counseling, treatment, and referrals per local standard of care.

- Blood will be collected for follow-up RPR tests and storage.

- Contact information will be reviewed, and the Month 12 visit will be scheduled.

7.2.7 Visit 8 – Month 12 (Day 360 ± 28 days) (Final Study Visit)

- Medical history will be reviewed (including review of interval medical records and local laboratory results, if available) and updated as appropriate. If the local laboratory results indicate a need for retreatment for syphilis infection, the subject will receive treatment according to the local clinic's standard protocols.

- Interim sexual history since the last study visit will be collected. Specimens will be

collected at sites of exposure for STI screening/diagnosis as indicated by local standard of care and subject history.

- A urine pregnancy test will be performed on all subjects of childbearing potential.
- All concomitant medications taken since the last study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- A targeted physical examination will be performed by a qualified study clinician (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations).
- HIV testing will be performed if the subject was previously noted to be HIV-uninfected. Clinicians will provide pre- and post-test counseling, treatment, and referrals per local standard of care.
- Blood will be collected for follow-up RPR tests, storage, and CD4 counts on persons found to have HIV infection whose medical records do not include a CD4 count in the past 30 days.
- Contact information will be reviewed.

7.3 Early Termination Visit

The Visit 8 assessments and eCRFs (see Section 7.2.7: Visit 8 – Month 12 (Final Study Visit)) must be completed at the end of each subject's participation in the study if discontinued prior to Visit 8. If a subject withdraws between scheduled visits, the subject must come to the clinic to perform the Visit 8 assessments.

7.4 Unscheduled Visit

Subjects will be permitted to return for unscheduled visits between scheduled visits as needed to address issues related to AEs, continuing or new symptoms, or other problems as they arise. Any of the following activities may be performed at the discretion of the site PI (note that some items are only relevant to a subset of subjects):

- Medical history will be reviewed (including review of interval medical records and local laboratory results, if available) and updated as appropriate. If the local laboratory results indicate a need for retreatment for syphilis infection, the subject will receive treatment according to the local clinic's standard protocols and will be withdrawn from the study per protocol Section 5.3.3.
- Interim sexual history since the last study visit will be collected. Specimens will be collected at sites of exposure for STI screening/diagnosis as indicated by local standard of care and subject history.

- A urine pregnancy test will be performed on all subjects of childbearing potential.
- All concomitant medications taken since the last study visit will be recorded on the appropriate data collection form. Previously recorded medications will be updated as appropriate.
- Study personnel will discuss with subjects and assess and record all AEs/SAEs. Previously recorded AEs/SAEs will be updated as appropriate.
- A targeted physical examination will be performed by a qualified study clinician (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations).
- Contact information will be reviewed.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Complete medical history will be obtained by interviewing the subjects at Visit 1 (and reviewing medical records for the past 14 days, if available) and will be updated at each clinic visit. At follow-up visits, an interim medical history will be obtained by interviewing the subjects (and reviewing interval medical records, if available), noting any changes since the previous visit.

Sexual history for the past 60 days will be obtained by interviewing the subjects at Visit 1. At follow-up visits, an interim sexual history will be obtained by interviewing the subjects, noting any changes since the previous visit.

Medications history (concomitant medications) will include a review of all current medications and medications taken 30 days before initiating study treatment. Prescription and over-the-counter drugs will be included as well as vitamins, herbs, and supplements. Assessment of eligibility will also include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Section 5).

A targeted physical examination (vital signs (temperature, heart rate, respiration rate, and blood pressure); genital, rectal, oral, skin, and lymph node examinations) will be performed at each visit. All physical examinations will be performed by a qualified study clinician.

Evaluation for symptoms of a Jarisch-Herxheimer reaction

Approximately 24 hours after Visit 1, the subjects will be contacted as noted in Section 7.1 and evaluated for symptoms of a Jarisch-Herxheimer reaction. During this assessment, subjects should refer to the completed Jarisch-Herxheimer checklist distributed at Visit 1. Subjects will be asked about fever (confirmed by thermometer at home close to the time of fever onset, if possible), chills, myalgia, weakness, flushing, worsening of skin rash, tachycardia (i.e., fast heartbeat), heart palpitations, arthralgia, nausea, headache, and dizziness, including time of onset and time of resolution of each symptom reported. If subjects cannot be reached after Visit 1, the evaluation for symptoms of a Jarisch-Herxheimer reaction will be conducted at Visit 2 (per Section 7.1, all subjects will be asked to bring the completed checklist with them to Visit 2). Data will be recorded on the appropriate eCRF(s) based on subject interview during the non-visit contact or at Visit 2 if the contact is not successful; the Jarisch-Herxheimer checklist completed by the subject will not be retained.

Evaluation for resolution of clinical signs of syphilis infection

At Visit 2 and Visit 3, subjects will be evaluated for resolution of signs of syphilis documented at Visit 1, as noted in Section 7.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

Diagnostic laboratory tests for HIV and other STIs as indicated will be performed locally according to local protocols. Phlebotomy for serological testing and serum storage to determine study outcomes will be performed locally with testing carried out at the Central Laboratory. Serological testing may also be conducted locally according to local protocols.

A pregnancy test will be performed at all visits on all subjects of childbearing potential; a urine or serum pregnancy test is permitted at Visit 1, while a urine pregnancy test is to be performed at all other visits. Results must be negative and known prior to randomization at Visit 1.

8.2.2 Special Assays or Procedures

At Visit 1, study staff will swab consenting subjects' oral cavities and primary or secondary lesions (if lesions are present per Section 5.1). Swabs will be stored in study-specific medium and shipped to the Giacani laboratory at the University of Washington for whole-genome sequencing of *T. pallidum* isolates. Collecting oral swabs and swabs of lesions (if present) is optional, and additional details can be found in the protocol-specific swab guidance manual.

8.2.3 Specimen Preparation, Handling, and Shipping

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Specimen preparation, handling, and storage will be done according to local clinic SOPs. Additional details can be found in the protocol-specific MOP.

8.2.3.2 Specimen Shipment

Specimen shipment to the Central Laboratory will occur according to all applicable International Air Transport Association (IATA) requirements. Additional details can be found in the protocol-specific MOP.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety will be monitored throughout the study by physical examination (including vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations) and subject reporting. Safety will be assessed by the frequency and severity of AEs/SAEs (including solicited reactogenicity AEs and other unsolicited AEs) occurring from the time of study product administration through the Month 1 visit.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

Adverse Event:

ICH E6 GCP defines an AE as any untoward medical occurrence in a clinical research subject administered a study drug regardless of its causal relationship to the study drug. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study drug. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study subject presenting for medical care or upon review by a study monitor. Since the safety profile of BPG (both 1 and 3 doses) is well established, and this study is not powered to detect new, unknown safety signals, non-serious AEs will not be reported to the DSMB.

All AEs not meeting the criteria for SAEs should be captured on the appropriate eCRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study drug (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the subject is screened should be considered as a baseline finding and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to the study drug.

The US Food and Drug Administration (FDA) defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Severity of Event: All AEs will be assessed by the clinician using a protocol-defined grading system (see Appendix B).

Changes in the severity of an AE should be documented to allow an assessment of its duration at each level of intensity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Drug: The clinician's assessment of an AE's relationship to the study drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, it should be reported. All AEs must have their relationship to the study drug assessed using the terms "related" or "not related," as defined below. In a clinical trial, the study drug must always be suspect.

- Related – There is a reasonable possibility that the study drug caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study drug and the AE.

- Not Related – There is not a reasonable possibility that administration of the study drug caused the AE.

9.2.2 Reactogenicity

Reactogenicity events are AEs that are common and known to occur for the study drug being studied. For this protocol, the following reactogenicity events are expected to occur among some subjects:

- Systemic reactions: Jarisch-Herxheimer reaction (as described in protocol Section 8.1)

- Local injection site reactions: pain or tenderness, erythema or redness, induration or swelling

Reactogenicity events (as solicited AEs), along with unsolicited AEs, will be assessed at the visits specified in protocol Section 7 and will be graded using a protocol-defined grading system (see Appendix B).

9.2.3 Serious Adverse Events

Serious Adverse Event (SAE):

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death,

- a life-threatening AE*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

* An AE is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

All SAEs will be:

- Assessed for severity and relationship to the study drug and alternate etiology (if not related to the study drug) by a licensed study physician.
- Recorded on the appropriate SAE form and eCRF.
- Followed through resolution by a licensed study physician.
- Reviewed and evaluated by the DSMB (periodic review unless related), DMID, and the IRB.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

SAEs will be followed until resolution even if this extends beyond the study reporting period. Resolution of an SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. [redacted]
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-[redacted] (US) or 1-301[redacted] (outside US)
SAE FAX Phone Number: 1-800-[redacted] (US) or 1-301[redacted] (outside US)
SAE Email Address:[redacted]

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The DMID Medical Monitor and Clinical Protocol Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to the study drug, the investigator will report the event to the DMID Pharmacovigilance Group.

9.3.2 Regulatory Reporting for Studies Not Conducted Under DMID-Sponsored IND

As this study will not be conducted under an Investigational New Drug application (IND), MedWatch will be used only to report SAEs that are both unexpected and deemed related to the study drug. DMID should be copied simultaneously when an alternate method of reporting is utilized.

9.3.3 Reporting of Pregnancy

Subjects of child-bearing potential will be counseled to continue using acceptable forms of birth control for the entire duration of their participation in the trial. If a subject becomes pregnant during the study, dosing will be discontinued immediately, and early termination assessments will be performed. A pregnancy reporting form will be completed for any study subject who becomes pregnant during study participation. The site will maintain contact with pregnant study subjects to obtain pregnancy outcome information. The pregnant subject will be followed until delivery or until the end of pregnancy (in the case of miscarriage or pregnancy termination). Infants born to these study subjects will also be monitored for SAEs (congenital anomalies or other birth defects) and other complications for up to 2 months after birth. Pregnancy reporting forms will be limited to collecting data on the following information:

- prior maternal history including congenital abnormalities or pregnancy complications;

- estimated date of conception;
- estimated and actual date of delivery or end of pregnancy;
- pregnancy outcome (live birth, stillbirth, miscarriage, or elective termination);
- mode of delivery;
- maternal complications; and
- neonatal complications (i.e., lethal or nonlethal congenital abnormality).

Pregnancies occurring in subjects will be reported via Advantage eClinical® on the Pregnancy Report form.

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

All AEs and SAEs will be followed from the time of study treatment through resolution even if this extends beyond the study reporting period (i.e., the Month 1 visit). Resolution of an AE/SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5 Halting Rules

Further enrollment will be halted for DSMB review if any of the following are reported:

- If one or more subjects experience an SAE judged by an investigator to be related to the study drug
- An overall pattern of clinical events that the DMID Medical Monitor or DSMB consider associated with the study drug and that may appear minor in terms of individual events, but that may collectively represent a serious potential concern for safety

If any of the halting rules are met, the study will not continue with the remaining enrollments or study treatments without a review by and recommendation from the DSMB to proceed.

DMID retains the authority to suspend additional enrollment and administration of the study drug during the entire study, as applicable.

9.6 Safety Oversight (ISM plus DSMB)

An ISM at each clinical research site will oversee the safety of research subjects at that site and will provide independent written evaluation of SAEs and related Grade 3 AEs to the PI, DMID Clinical Project Manager, and DMID Medical Monitor. The ISM will serve as an independent consultant for the site PI on subject-related issues. The ISM will communicate with the site PI and study PIs to resolve any issues.

Safety oversight will be conducted by a DSMB that is an independent group of experts that monitors subject safety and advises DMID. The DSMB members will be separate and independent of study personnel participating in this trial and should not have scientific, financial, or other conflict of interest related to the study. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial.

The DSMB will review safety and enrollment data when half of the study subjects have been enrolled and observed for SAEs through the Month 1 Visit.

The DSMB will review study progress and subject clinical and safety data at the following time points:

- At least annually after the first subject is enrolled.
- Ad hoc when a halting rule is met, for immediate concerns regarding observations during the study, or as needed.
- During a final closeout meeting, held at the end of the study after the database is locked to review cumulative study data prior to finalizing the Clinical Study Report (CSR).

The DSMB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study administrations (as applicable), and to continue, modify, or terminate the study.

DMID or the DSMB chair may convene the DSMB on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the study. The DMID Medical Monitor is empowered to stop enrollment and study treatment if SAEs that meet the halting criteria are reported. The DMID Medical Monitor will be responsible for reviewing SAEs in real time. The DSMB will review SAEs on a regular basis and *ad hoc* during the study.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

DMID, the sponsoring agency, or its designee will conduct site monitoring visits as detailed in the clinical monitoring plan and in accordance with DMID policies. Site monitoring is conducted to ensure that the human subject protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor and ICH/GCP guidelines and applicable regulations and that the study is conducted in accordance with the protocol, protocol-specific manual of procedures, and applicable sponsor SOPs. Monitoring visits will include, but are not limited to, inspection of study facilities; review of regulatory files, accountability records, eCRFs, ICFs, printouts of medical and laboratory reports from the electronic medical records (EMR) system; and review of protocol and GCP compliance. Site monitors will have access to the study site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with PIs to discuss any problems and actions to be taken and document visit findings and discussions.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

The primary objective of the study is to compare the serological response to therapy in subjects with early (primary, secondary, or early latent) syphilis treated with BPG 2.4 MU once or weekly for three successive weeks. In particular, the objective is to demonstrate noninferiority of the one-dose regimen compared to the three-dose regimen and to also assess noninferiority within the HIV status subgroups (HIV-infected and HIV-uninfected subjects). See Section 11.4.4 for the definition of serological response.

The null hypothesis for the primary objective is that the difference in serological response rate between the three-dose and one-dose groups is at least 10%, and the alternative hypothesis is that the difference in response rates is less than 10%. The null and alternative hypotheses for the secondary HIV status subgroup objective are defined similarly.

Note that tests will be performed and p-values will be reported for secondary analyses for informational purposes and not to make definitive claims. The study is intended and designed to formally test the primary hypothesis only, and so no corrections for multiplicity are planned.

11.2 Sample Size Considerations

Sample size calculations were performed using PASS 2008 and SAS 9.4. The following parameters were used for determining the required sample size for the primary objective:

- 1:1 allocation ratio between one-dose and three-dose arms
- Test the difference in response rate between the one-dose and three-dose arms (Farrington-Manning test, unadjusted for HIV status)
- Null hypothesis response rate in one-dose arm: 0.688
- Null hypothesis response rate in three-dose arm: 0.788
- Alternative hypothesis response rate in both arms: 0.788
- One-sided 0.05 alpha level

To achieve 80% power for the primary noninferiority comparison, 420 evaluable subjects (210 in each treatment arm) are needed. It is assumed that 25% of subjects will be ineligible for the primary analysis, and so 560 enrolled subjects are needed to reach the target of 420 evaluable subjects.

The study is not powered to assess the secondary noninferiority comparisons in the HIV status subgroups. As an illustration, power calculations were performed to assess the power available for these subgroup analyses using a sample size of 420. The following parameters were used for the calculations:

- Test the difference in response rate between the one-dose and three-dose arms within each HIV status stratum.
- Null hypothesis response rate in one-dose arm: 0.738 (HIV-infected); 0.638 (HIV-uninfected)
- Null hypothesis response rate in three-dose arm: 0.838 (HIV-infected); 0.738 (HIV-uninfected)
- Alternative hypothesis response rate in both arms: 0.838 (HIV-infected); 0.738 (HIV-uninfected)
- One-sided 0.05 alpha level
- 280 evaluable subjects in the HIV-infected stratum (140 in one-dose arm, 140 in three-dose arm).
- 140 evaluable subjects in the HIV-uninfected stratum (70 in one-dose arm, 70 in three-dose arm).

The HIV-infected noninferiority comparison has 72% power, and the HIV-uninfected noninferiority comparison has 38% power.

11.3 Planned Interim Analyses

11.3.1 Safety Review

The study will be monitored to determine if any of the safety halting rules described in Section 9.5 are met. The DSMB will also review study progress and subject safety data at specified times during the study and hold a study closeout meeting, as defined in the DSMB Charter.

11.3.2 Immunogenicity or Efficacy Review

There are no planned interim analyses of efficacy data.

11.4 Final Analysis Plan

A separate statistical analysis plan document will be generated that will contain the details of the analyses. This section outlines the major components of the analyses.

11.4.1 Analysis Populations

Intent-to-Treat (ITT) Population: This analysis population includes all randomized subjects.

Modified Intent-to-Treat (mITT) Population: This analysis population includes all randomized subjects who are eligible at the baseline visit and whose TPPA result is positive at the baseline visit and/or at Visit 5 (Month 3), if repeat testing was performed.

Safety Population: This analysis population includes all randomized subjects who received at least one dose of study treatment.

Evaluable Population: This analysis population includes all randomized subjects who are eligible at the baseline visit, have a known HIV status determined at or before the baseline visit, have positive TPPA results at the baseline visit and/or Visit 5 (Month 3) (if repeat testing was performed), received all assigned doses of BPG, have adequate RPR titer data available at baseline and applicable follow-up visit(s), and (for HIV-uninfected subjects only) HIV-uninfected status persists through applicable follow-up visit(s).

If a subject receives antibiotic(s) active against *T. pallidum* for reasons other than syphilis between enrollment and the applicable follow-up visit(s), the subject will be included in ITT analyses but excluded from Evaluable analyses. If a subject receives antibiotic(s) active against *T. pallidum* between enrollment and the applicable follow-up visit(s) due to local RPR testing results, the subject will be included in all analyses.

A blinded case review committee will review subjects' data to determine if treatment with drug(s) known to be active against *T. pallidum* for reasons other than syphilis infection excludes the subject from the Evaluable analysis population.

11.4.2 Baseline Characteristics

Baseline and demographic characteristics will be summarized overall and by formulation. For both continuous and categorical variables, appropriate summary statistics will be applied. For continuous variables, descriptive statistics will include the number of non-missing values, mean, standard deviation, median, minimum, and maximum. For categorical variables, descriptive statistics will include counts and percentages per category.

11.4.3 Safety Analysis Plan

Safety evaluations will be based on the incidence, severity, and type of AEs and SAEs, as detailed in Section 9. In addition, the incidence of systemic reactions and local injection site reactions will be evaluated. Safety variables will be tabulated and presented for all subjects in the safety population, grouped by treatment.

Reactogenicity events will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) and using exact confidence intervals to summarize the reactogenicity rates. Tabular and graphical summaries of events will be presented for each solicited event, by type (local/systemic), severity (none, mild, moderate, severe), and time point post-dose. The proportion of subjects who experience a Jarisch-Herxheimer reaction will be summarized overall and by treatment. Logistic regression analyses may be used to determine the association of treatment with Jarisch-Herxheimer reaction after adjusting for HIV co-infection status and time point.

Unsolicited AEs and SAEs will be coded by MedDRA® for preferred term and system organ class. The rate and exact 95% confidence intervals of AEs and SAEs in aggregate, as well as by MedDRA® categories, will be computed. The number of SAEs will be reported by a detailed listing showing the type, MedDRA® coding, relevant dates (treatment dosing date(s) and SAE onset and resolution dates), severity, relatedness, and outcome for each event.

11.4.4 Efficacy Analysis Plan

11.4.4.1 Primary Efficacy Analysis

For the primary analysis, serological response to therapy by Month 6 will be defined as follows:

- 4-fold or greater decline in RPR titer by Month 6 compared to baseline, OR
- RPR-negative by Month 6 (i.e., seroreversion).

For the primary analysis, subjects who do not meet either of the above criteria by Month 6 will be classified as not experiencing serological response. If a subject has an observed serological response prior to meeting one of the Evaluable analysis population exclusion criteria, the subject will be included in the analyses as a responder. For subjects who have received BPG through the study and subsequently require retreatment for syphilis infection (i.e., per the local clinic's standard protocols), data collected following retreatment will not be used for analyses. Exploratory analyses of the primary outcome will consider alternative classifications of subjects (see Section 11.4.4.2).

For the comparison of efficacy of one dose versus three doses of BPG 2.4 MU, the number and proportion of subjects with serological response will be summarized overall and by treatment.

The point estimates for the treatment-specific proportions and difference in proportions as well as corresponding 95% confidence intervals will be reported. A hypothesis test will be conducted using the Farrington-Manning test at the 5% one-sided level of significance to formally compare the treatment arms. The primary analysis will be performed in the ITT analysis population and repeated as a secondary analysis in the mITT and the Evaluable analysis populations.

As the ITT analyses will include subjects who may not have adequate serological data to determine their response status for any reason, the response will need to be imputed. Multiple methods for imputation will be explored (e.g., Last Observation Carried Forward); details will be provided in the Statistical Analysis Plan.

As a secondary corroborative analysis, the Cochran-Mantel-Haenszel Chi-square test will be used to compare the two treatment arms with respect to response rate across HIV status subgroups (HIV-infected, HIV-uninfected). The Breslow-Day test for the homogeneity of odds ratios will be used to determine if the difference between the two treatment groups with respect to response rates varies by HIV status.

11.4.4.2 Secondary Efficacy Analysis

The secondary efficacy analyses will be performed in the ITT and Evaluable analysis populations. For subjects who have received BPG through the study and subsequently require retreatment for syphilis infection (i.e., per the local clinic's standard protocols), data collected following retreatment will not be used for analyses.

For the comparison of efficacy of one dose versus three doses of BPG 2.4 MU by Month 6 in each of the HIV status subgroups, the number and proportion of subjects with serological response will be summarized overall and by treatment within each HIV status subgroup. The point estimates for the treatment-specific proportions and difference in proportions as well as corresponding 95% confidence intervals will be reported. For each HIV status subgroup, a hypothesis test will be conducted using the Farrington-Manning test at the 5% one-sided level of significance to formally compare the treatment arms.

The analyses comparing one dose versus three doses by Month 12, overall and within HIV status subgroups, will follow similarly to the above. In addition, geometric mean titers and fold declines from baseline will be summarized by time-point for each treatment group, overall and within HIV status subgroups.

For the comparison of compliance between the one dose and three dose groups, the number and proportion of subjects who receive all assigned doses within the assigned visit windows will be summarized overall and by treatment. The point estimates for the treatment-specific proportions and difference in proportions as well as corresponding 95% confidence intervals will be reported. A hypothesis test will be conducted using a Chi-square test at the 5% two-sided level of significance to formally compare the treatment arms.

Demographic, sexual history, and other characteristics of subjects will be presented overall and within HIV status subgroups at baseline and throughout follow-up. HIV status subgroups will be compared with respect to epidemiological characteristics using the Pearson Chi-square test for categorical measures and the t-test for independent samples or Wilcoxon rank sum test for continuous measures.

11.4.4.3 Exploratory Efficacy Analysis

Any exploratory or sensitivity analyses to be performed to support any of the primary or secondary objectives will be specified in the Statistical Analysis Plan. As an example, the following alternative serological response classifications will be explored:

- Response: 4-fold decline in RPR titer compared to baseline, or RPR-negative (i.e., seroreversion).
- Non-Response: Less than 4-fold decline in RPR titer compared to baseline, and RPR-positive
- Failure: 4-fold or greater increase in RPR titer without a clear history of re-exposure

A blinded case review committee will review subjects' data to determine cases of re-exposure. If a subject has an observed serological response prior to meeting one of the Evaluable analysis population exclusion criteria, the subject will be included in the analyses as a responder. For subjects who have received BPG through the study and subsequently require retreatment for syphilis infection (i.e., per the local clinic's standard protocols), data collected following retreatment will not be used for analyses.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. Each participating site will permit authorized representatives of DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical study records for the purposes of quality assurance reviews, audits, monitoring, and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept in the pharmacies, laboratories, and medico-technical departments involved in this clinical trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of this trial.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the participating site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site PI will provide direct access to all trial-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site PI will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The Statistical and Data Coordinating Center (SDCC) will implement QC procedures beginning with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The site PI will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The site PI's institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

14.2 Institutional Review Board

Prior to enrollment of subjects into this trial, the approved protocol and ICF will be reviewed and approved by the appropriate IRB listed on its FWA.

The responsible official for the IRB will sign the IRB letter of approval of the protocol before the start of this trial, and provide a copy to DMID. The IRB FWA number will be provided to DMID.

Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the site PI for submission to the IRB.

14.3 Informed Consent Process

The site PI, or designated study staff, will choose subjects in accordance with the eligibility criteria detailed in Section 5. Before any study procedures are performed, subjects must sign an ICF that complies with the requirements of 21 CFR Part 50, 45 CFR Part 46, and the local IRB.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, subjects will receive a comprehensive explanation of the proposed study procedures and study drug, including the nature and risks of the trial, alternate therapies, any known AEs, the investigational status of the components, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their specimens. Subjects will be allowed sufficient time to consider participation in the trial, after having the nature and risks of the trial explained to them, and will have the opportunity to discuss the trial with their family, friends, or legally authorized representative or think about it prior to agreeing to participate.

ICFs describing in detail the study drug, study procedures, risks, and possible benefits are given to subjects. The ICF must not include any exculpatory statements. ICFs will be IRB-approved, and subjects will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the site PI (or designee) will explain the research study to subjects and answer any questions that may arise. Subjects must sign the ICF, and written documentation of the informed consent process is required prior to starting any study procedures/interventions being done specifically for the trial, including administering BPG.

DMID will provide the site PI, in writing, any new information that significantly affects the subjects' risk of receiving BPG. This new information will be communicated by the site PI to subjects who consent to participate in the trial in accordance with IRB requirements. The ICF will be updated, and subjects will be re-consented per IRB requirements, if necessary.

Study personnel may employ IRB-approved recruitment efforts before obtaining the subject’s consent; however, before any study procedures are performed to determine protocol eligibility, an ICF must be signed. Subjects will be given a copy of all ICFs that they sign.

By signing the ICF, subjects agree to complete all evaluations required by the trial, unless the subject withdraws voluntarily or is terminated from the trial for any reason.

The rights and welfare of subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from this trial.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of female and male adults who meet the subject inclusion/exclusion criteria, regardless of religion or ethnic background. Should the outcome of this trial be deemed acceptable, additional trials may be initiated in other populations.

14.5 Subject Confidentiality

Subjects will have code numbers and will not be identified by name. Subject confidentiality is strictly held in trust by the participating site PIs, their study personnel, the sponsor(s), and their agents. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the trial or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All information provided by the sponsor and all data and information generated by the participating site as part of the trial (other than a subject's medical records) will be kept confidential by the site PI and other study personnel to the extent permitted by law. This information and data will not be used by the site PI or other study personnel for any purpose other than conducting the trial. These restrictions do not apply to: (1) information that becomes publicly available through no fault of the site PI or other study personnel; (2) information that is necessary to disclose in confidence to an IRB solely for the evaluation of the trial; (3) information that is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results that may be published as described in Section 16. If a written contract for the conduct of the trial that includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

The study monitor, applicable regulatory authorities, such as the FDA, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the site PI. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this trial. The participating sites will permit access to such records.

To protect privacy, a Certificate of Confidentiality (CoC) has been obtained. With this CoC, the researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the CoC to resist any demands for information that would identify the subject, except as explained below.

The CoC cannot be used to resist a demand for information from personnel of the US Government that is used for auditing or evaluation of federally funded projects, like this trial, or for information that must be released in order to meet the requirements of the FDA.

A CoC does not prevent subjects from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the CoC to withhold that information.

The CoC does not prevent the researchers from reporting, without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported, including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

14.6 Study Discontinuation

If the trial is discontinued, subjects who sign the ICF and are randomized and treated will continue to be followed for safety assessments. No further study drug will be administered

14.7 Future Use of Stored Specimens

Subjects will be consented to have leftover serum stored for future testing. Storage of samples for future testing is optional and is not a requirement for participation in the study. If the subject consents to storage and future use of specimens, specimens will be kept at the Central Laboratory (University of Alabama at Birmingham). Subjects will not be contacted with the results of these future research studies. Future testing on specimens will only occur to the extent authorized in each study site's ICF or as otherwise authorized under applicable law and after review and approval by DMID and the IRB of the researcher requesting the specimens.

Archived specimens will be identified only by the subject number and visit number, which will allow linkage of the specimens to study data but not to any personal identifiers.

There will be no direct benefit to subjects from allowing specimens to be stored and used for future purposes. However, the results may provide information that will help in the diagnosis or treatment of future patients.

Subjects' specimens will be kept until they are used up or destroyed. They may be used to develop new tests or products. In some instances, these may have potential commercial value. If a subject decides at any time that s/he does not want specimens stored for future research, s/he must contact the study staff who will then notify the laboratory/specimen archive staff, who will mark the specimens by adding a "destroy" label. Labeled specimens will be destroyed at the end of this study or will be removed from storage and destroyed as soon as possible.

15 DATA HANDLING AND RECORD KEEPING

The site PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRFs and provided by the SDCC to record and maintain data for each subject enrolled in the study. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRFs derived from the data collection forms should be consistent with the data collection forms, or the discrepancies should be explained.

The sponsor and/or its designee will provide guidance to site PIs and other study personnel on making corrections to the data collection forms and eCRFs.

15.1 Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. AEs must be recorded on the appropriate data collection form, assessed for severity and relationship, and reviewed by the site PI or appropriate sub-investigator.

Data collection is the responsibility of the study personnel at the participating sites under the supervision of the respective site PI. During the study, the site PI must maintain complete and accurate documentation for the study.

The SDCC for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical (including, but not limited to, AEs/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values), and immunogenicity data will be entered into a 21 CFR 11-compliant Internet Data Entry System, Advantage eClinical®, provided by the SDCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms completed by the study personnel.

15.3 Types of Data

Data for this study will include clinical, safety, and outcome measures (e.g., clinical laboratory values and immunogenicity data).

15.4 Timing/Reports

A final report will be prepared following the availability of all the safety and laboratory data. Interim statistical reports may be generated as deemed necessary and appropriate by DMID. Safety and laboratory data summary reports may be generated for the DSMB.

After full analysis and final CSR reporting is complete, and upon request and DMID approval, the SDCC will provide the participating sites with a summary of results by treatment group. The participating sites requesting such information to share with study subjects must do so in compliance with their respective IRB guidelines.

15.5 Study Records Retention

Records and documents pertaining to the conduct of this study, including data collection forms, source documents, ICFs, laboratory test results, and medication inventory records, must be retained by the investigator for at least 2 years following the completion of this study. No records may be destroyed without written permission from the sponsor.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site PI, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, section 5.1.1

5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site PI to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via the SDCC's Advantage eClinical®.

Note: Those sites participating in trials with a designated ‘central unit’ will follow the reporting requirements specified in their protocols and MOPs. The ‘central unit’ will be responsible for submission of the protocol deviation information to TRI-ICON/DMID-CROMS.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the regulatory file, as well as in the subject’s study chart. Protocol deviations must be sent to the local IRB per their guidelines. The site PI and other study personnel are responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH-funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH generally need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting, a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial, the responsible party is DMID, which will register the trial and post results.

The responsible party does not plan to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

17 LITERATURE REFERENCES

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2. *CDC Sexually transmitted disease surveillance 2016*. 2017, U.S. Department of Health and Human Services: Atlanta.
3. *CDC Sexually transmitted disease surveillance 2015*. 2016, U.S. Department of Health and Human Services: Atlanta.
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5. Workowski, K.A. and G.A. Bolan, *Sexually transmitted diseases treatment guidelines, 2015.* MMWR Recomm Rep, 2015. **64**(Rr-03): p. 1-137.
6. Sena, A.C., et al., *Predictors of serological cure and serofast state after treatment in HIV-negative persons with early syphilis.* Clin Infect Dis, 2011. **53**(11): p. 1092-9.
7. Rolfs, R.T., et al., *A randomized trial of enhanced therapy for early syphilis in patients with and without human immunodeficiency virus infection. The Syphilis and HIV Study Group.* N Engl J Med, 1997. **337**(5): p. 307-14.

APPENDIX A: SCHEDULE OF EVENTS

Procedures		**Visit 1 – Screening/ Enrollment** (Day 1)	**Contact** (approx. 24 hours after Visit 1)	**Visit 2** (Week 1 – Day 7 to 13)	**Visit 3** (Week 2 – 6 to 12 days after Visit 2)	**Visit 4** (Month 1 – Day 30 ± 7 days)	**Visit 5** (Month 3 – Day 90 ± 21 days)	**Visit 6** (Month 6 – Day 180 ± 21 days)	**Visit 7** (Month 9 – Day 270 ± 28 days)	**Visit 8** (Month 12 – Day 360 ± 28 days)	**Early Termination**	**Unscheduled Visit**[15]
Informed consent		X										
Demographics		X										
Eligibility criteria		X										
Medical history[1]		X		X	X	X	X	X	X	X	X	X
Sexual history[2]		X		X	X	X	X	X	X	X	X	X
Targeted physical exam[3]		X		X	X	X	X	X	X	X	X	X
Pregnancy test[4]		(X)		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Concomitant medications[5]		X		X	X	X	X	X	X	X	X	X
Specimen collection	RPR titer	X		X	X	X	X	X	X	X	X	
	Storage	X		X	X	X	X	X	X	X	X	
	CD4 count[6]	(X)				(X)		(X)		(X)	(X)	
	HIV testing[7]	(X)					(X)	(X)	(X)	(X)	(X)	
	Other STI screening/ diagnosis[8]	X		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
	Oral cavity and lesions (if present) swabbed[9]	(X)		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Randomization		X										
BPG administered[10]		X		(X)	(X)							
Contact information collected/reviewed[11]		X		X	X	X	X	X	X	X	X	X
Jarisch-Herxheimer checklist[12]		X	X	(X)								
Jarisch-Herxheimer reaction assessed[13]			X	(X)								
Resolution of syphilis signs assessed				X	X							
AEs/SAEs assessed[14]		X		X	X	X						X

(X) – As indicated/appropriate. Refer to footnotes below and protocol Section 7.

[1] At Visit 1, collect complete medical history (including review of medical records for the past 14 days, if available). At subsequent visits, review medical history (including interval medical records, if available) and update as appropriate.
[2] At Visit 1, collect sexual history for the past 60 days. At subsequent visits, collect interim sexual history since last visit.
[3] Targeted physical exam includes vital signs: temperature, heart rate, respiration rate, and blood pressure; and genital, rectal, oral, skin, and lymph node examinations.
[4] Perform on subjects of childbearing potential. At Visit 1, urine or serum pregnancy test is permitted. At subsequent visits, perform urine pregnancy test.
[5] At Visit 1, record concomitant medications taken in the last 30 days before initiating BPG. At subsequent visits, record all concomitant medications taken since the last visit and update previously recorded medications as appropriate.
[6] Collect for subjects with known HIV infection whose medical records do not include a CD4 count in the past 30 days; at Visit 4, this is only applicable to subjects newly found to have HIV infection from Visit 1 to Visit 4 (inclusive).
[7] Perform for subjects who do not have a previously documented positive HIV test result using locally available tests (e.g., nucleic acid tests (NATs), antibody/antigen tests).
[8] At Visit 1, collect specimens at sites of exposure for screening/diagnosis of chlamydia and gonorrhea for all subjects who have not been tested since last sexual activity and for all subjects who have been sexually active in the past 14 days and for other STIs as indicated by local standard of care and subject history. At subsequent visits, collect specimens at sites of

exposure for STI testing per local standard of care and subject history.
[9]It is optional to swab subject's oral cavity and lesions, if lesions are present. See Section 8.2.2.
[10] At Visit 1, administer BPG to all subjects. At Visits 2 and 3, administer BPG to subjects in Arm 2 only.
[11]At Visit 1, collect contact information. At subsequent visits, review contact information and update if needed.
[12] Distribute checklist at Visit 1. Subject refers to completed checklist during Jarisch-Herxheimer assessment; see Sections 7 and 8.1.
[13]Assess per protocol Section 8.1. If subject is not reached, assess at Visit 2.
[14] Assess and record all AEs/SAEs (including solicited reactogenicity AEs and other unsolicited AEs).
[15] At Unscheduled Visits, any of the specified evaluations that are relevant to the subject may be performed at the discretion of the site PI.

APPENDIX B: TABLE FOR GRADING THE SEVERITY OF ADVERSE EVENTS

PARAMETER	GRADE 1 - MILD	GRADE 2 - MODERATE	GRADE 3 - SEVERE
Clinical adverse event **NOT** identified below	Mild symptoms causing no or minimal interference with usual social and functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social and functional activities with intervention indicated	Severe symptoms causing inability to perform usual social and functional activities with intervention or hospitalization indicated
Vital signs			
Temperature	38.0 to <38.6°C	38.6 to <39.3°C	≥39.3°C
Blood pressure	140 – 159 mmHg systolic OR 90 – 99 mmHg diastolic	160 – 179 mmHg systolic OR 100 – 109 mmHg diastolic	≥180 mmHg systolic OR ≥110 mmHg diastolic OR hospitalization indicated
Pulse	101-115 or 50-54 or 45-50 if baseline <60bpm	116-130 or 45-49 or 40-44 if baseline <60bpm	>130 or ventricular dysrhythmias or <45 or <40 if baseline <60bpm
Respiration	23-25	26-30	≥30
Local injection site			
Pain or tenderness (report only one)	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities
Erythema or redness (report only one)	2.5 to <5 cm in greatest diameter AND Symptoms causing no or minimal interference with usual social and functional activities	≥5 to <10 cm in greatest diameter OR Symptoms causing greater than minimal interference with usual social and functional activities	≥10 cm in greatest diameter OR Ulceration OR Secondary infection OR Sterile abscess OR Symptoms causing inability to perform usual social and functional activities
Induration or swelling (report only one)	2.5 to <5 cm in greatest diameter AND Symptoms causing no or minimal interference with usual social and functional activities	≥5 to <10 cm in greatest diameter OR Symptoms causing greater than minimal interference with usual social and functional activities	≥10 cm in greatest diameter OR Ulceration OR Secondary infection OR Sterile abscess OR Symptoms causing inability to perform usual social and functional activities

Systemic			
Anaphylaxis **	--	--	Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema or angioedema; hypotension
***Definition*: A disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis, and loss of consciousness, and may lead to death.**			
Acute allergic reaction	Localized urticaria with no intervention indicated	Localized urticaria with intervention indicated OR Mild angioedema with no intervention indicated	Generalized urticaria OR Angioedema with intervention indicated OR Symptoms of mild bronchospasm
Hypersensitivity	Transient flushing or rash	Rash; flushing; urticaria; dyspnea	Symptomatic bronchospasm with or without urticaria; parenteral medication indicated; allergy-related edema or angioedema; hypotension
Fever	38.0 to <38.6°C or 100.4 to <101.5°F	≥38.6 to <39.3°C or ≥101.5 to <102.7°F	≥39.3°C or ≥102.7°F
Chills	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities
Headache	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities
Myalgias (generalized)	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities
Fatigue or Malaise (report only one)	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities

Decreased appetite	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss
Dermatological			
Skin rash	Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site
Gastrointestinal			
Nausea	Transient (< 24 hours) or intermittent AND No or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24-48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Rehydration indicated (e.g., IV fluids)
Vomiting	Transient (< 24 hours) or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or minimal dehydration	Persistent vomiting resulting in orthostatic hypotension OR Rehydration indicated (e.g., IV fluids)
Diarrhea	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4-6 stools over baseline per 24-hour period	Increase of ≥ 7 stools over baseline per 24-hour period OR IV fluid replacement indicated
Abdominal discomfort	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities