Gentamicin for Pharyngeal Gonorrhea – A Demonstration Study

Study Protocol

Version 1.2

January 4, 2018

Executive Summary

The Centers for Disease Control and Prevention has identified antimicrobial-resistant (AMR) *Neisseria gonorrhoeae* (NG) as one of the nation's top three urgent AMR threats. Since the advent of antibiotics in the 1930s, NG has developed resistance to every first-line antibiotic. Parenteral third-generation cephalosporins are now the only class of drug with consistent efficacy against NG. New therapies are urgently needed. Although some novel antimicrobials are under development, reevaluating older drugs is another option for quickly identifying additional treatments for gonorrhea. We propose a demonstration study to test a single dose of gentamicin for the treatment of pharyngeal gonorrhea. We chose to focus on pharyngeal gonorrhea because these infections are common, play an important role in fostering gonococcal resistance, and are harder to eradicate than genital infections. Although gentamicin is 91% efficacious for genital NG, its efficacy at the pharynx may be less since streptomycin, another aminoglycoside previously used to treat gonorrhea, was not effective for pharyngeal NG. It is unknown if streptomycin's poor efficacy is indicative of limitations of aminoglycosides as a class. We plan to enroll 60 men who have sex with men in a demonstration study to be conducted at the Seattle & King County STD Clinic to test the efficacy of 360 mg of gentamicin given intramuscularly for pharyngeal gonorrhea. Secondary objectives include determining the ideal pharmacodynamic criterion (comparing *in vitro* minimal inhibitory concentrations (MIC) of NG to peak gentamicin serum levels), estimating resistance induction among treatment failures, and assessing the tolerability of 360 mg of IM gentamicin.

Objectives

The proposed study aims to evaluate the efficacy of a single intramuscular (IM) dose of gentamicin in the treatment of pharyngeal gonorrhea. Secondary objectives include documenting the efficacy stratified by minimal inhibitory concentration (MIC) compared with the gentamicin peak level in order to estimate a pharmacodynamic criterion. We will also attempt to determine whether gentamicin monotherapy induces antimicrobial resistance among treatment failures. Lastly, we will evaluate the tolerability of 360 mg of IM gentamicin, stratified by subject weight (i.e. weight based dosing). The specific aims are:

1) Determine the proportion of persons whose pharyngeal gonococcal infections are cured with a single dose of 360mg gentamicin intramuscularly alone.
2) Evaluate the *renal safety* and tolerability of 360mg IM of gentamicin.
3) Document mean peak gentamicin levels following 360mg IM of gentamicin stratified by weight.
4) Estimate the best pharmacodynamics criterion (i.e. peak/MIC ratio) for pharyngeal gonorrhea treated with gentamicin using individual and mean peak gentamicin levels and NG isolate MIC.
5) Among treatment failures, conduct exploratory analyses comparing pre- and post-treatment MIC for evidence of induced resistance.

Study Design: Prospective cohort

Study Population & Inclusion Criteria:

Persons diagnosed with pharyngeal gonorrhea who are not yet treated

Exclusion criteria:

Age less than 16 years
Receipt of antibiotics in ≤30 days
Known allergy to any aminoglycoside
History of renal disease (including diagnosis of solitary kidney, chronic renal insufficiency, renal cell carcinoma etc),
Use of concurrent nephrotoxic drugs or muscle relaxants
History of diabetes
History of hearing loss or tinnitus
Concurrent infection with syphilis or chlamydia
Pregnancy and/or nursing
Unable to return for a follow-up visit 4-7 days (+/- 1 day).
Study team's discretion

Intervention: 360mg IM gentamicin x 1

Primary Outcome: Negative gonorrhea culture 4-7 days (+/- 1 day) after treatment

Sample Size: 60 persons

Statistical Analysis Plan:

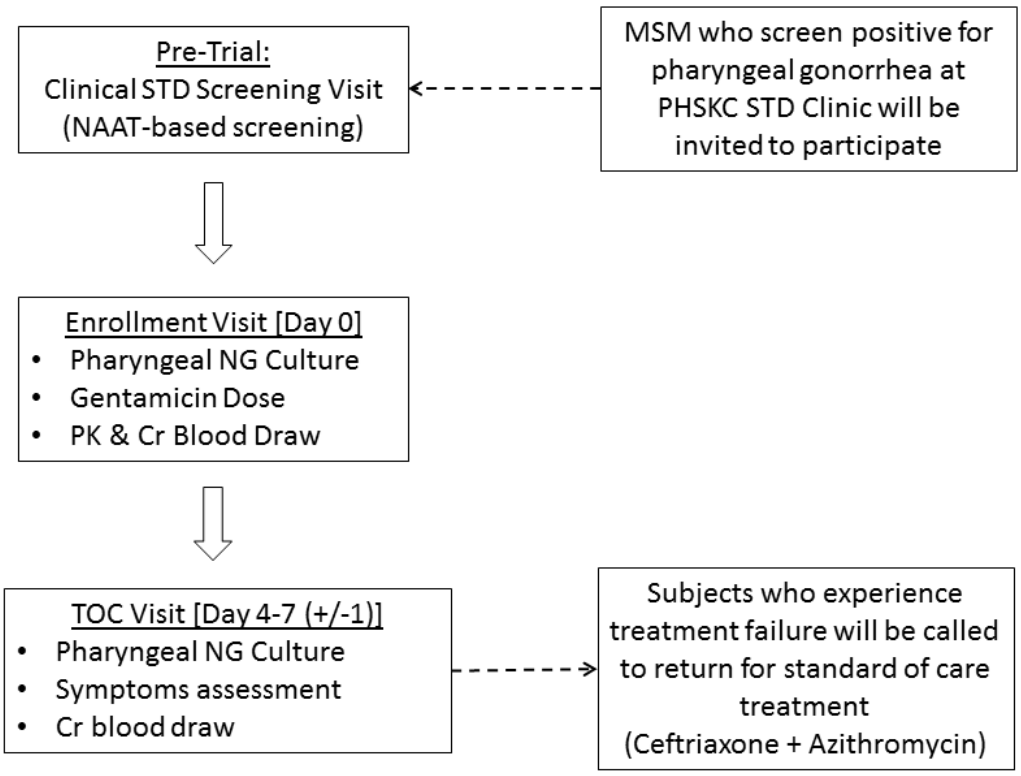
The primary endpoint is the pharyngeal cure rate – that is, the proportion of subjects who return for TOC who clear their infections as evidenced by a negative culture 4-7 (+/-1) days after treatment. Persons who test negative for NG at their enrollment visit (i.e. infection cleared between initial clinical screening test and enrollment) will be excluded from analysis. Treatment failure will be defined as a positive culture 4-7 (+/-1) days after treatment. The cure rate will be calculated as the number of cured subjects divided by the total number of subjects who received treatment and returned for follow-up testing in a modified an intention to treat (mITT) analysis. We will also calculate the 95% CI for this proportion.

We will conduct an interim analysis after we have primary outcome results for 25 subjects. If the cure rate is ≤44% (99% CI 17% – 74%; i.e. 12 persons of 25 persons with a positive culture on treatment date fail treatment), we will stop the study. We will also monitor for renal toxicity at the interim analysis. We plan to obtain pre-treatment and post-treatment Creatinine. Increases >40% will be considered significant. If more than 20% of the study population has a >40% increase in serum Creatinine, we will halt the study. We chose 20% because if 20% of subjects experience renal toxicity it is unlikely to be due to chance (95% CI 7 – 40%).
For our second objective, we will compare the MIC of cures to treatment failures using a stratified approach (Table 2), and we will correlate this data with individual peak gentamicin level in order to estimate the ideal pharmacodynamic (PD) criterion (Obj. 2). For persons who fail treatment we will also compare the pre- and post-treatment genatmicin MIC to estimate whether single dose gentamicin therapy can "induce" resistance (Obj. 3) as an exploratory analysis. We will consider an increase in the MIC by two doubling-dilutions (i.e. from MIC of 2 to 8 μg/mL, or 8 to 32 μg/mL) to represent induced resistance, and we will calculate the

proportion of isolates among those that fail treatment that demonstrate induced resistance following receipt of gentamicin. Lastly, we will report the number and proportion of subjects who report side effects.

We will evaluate subject's tolerability (Obj. 4) using a standardized symptom questionnaire conducted at the TOC visit. The symptoms questioned will include, but not be limited to, rash, tinnitus, decreased hearing, nausea, vomiting, diarrhea, headaches, and decreased appetite. There will also be a section for free response.

Figure 2: Schematic of Clinical Trial



PK: pharmacokinetics, (i.e. gentamicin peak level); Cr: creatinine, marker of kidney function

Responsibilities

Principle Investigator:	Lindley A. Barbee, MD MPH Department of Medicine, Division of Allergy and Infectious Diseases, University of Washington
Study Coordinator:	Angela LeClaire
Study Clinician:	TBD
Medical Monitor:	Ann Collier, MD Professor of Medicine, Division of Allergy and Infectious Diseases University of Washington Director, UW AIDS Clinical Trials Unit Associate Director, Center for AIDS Research University of Washington and Fred Hutch
Study Communication:	Harborview Medical Center – STD clinic 325 9th Ave, Box 359777 Seattle, WA 98104 Phone: (206) 744.2595 Fax: (206) 744.4151 Email: lbarbee@u.washington.edu
Study Site:	Public Health – Seattle & King County STD Clinic 908 Jefferson St, 11th Floor Seattle, WA 98104 Phone: (206) 744.3590

Recruitment and Retention of Subjects

Patients who screen positive for pharyngeal gonorrhea, with or without concomitant GC at other anatomic sites, and have not yet received treatment for this infection or other antibiotics in the past 30 days are eligible for this study. We will not exclude based on gender or HIV status. Recruitment will primarily occur at PHSKC STD Clinic, however, we will also encourage high-diagnosing partner clinics to refer eligible patients as well. We plan to enroll 60 persons with untreated pharyngeal gonorrhea for this study.

Recruitment at PHSKC STD Clinic:

We will employ several strategies to recruit at PHSKC STD Clinic.

1) Patients who screen positive for pharyngeal gonorrhea and are called with their test results to return for treatment will be told on the phone that they may be eligible for a treatment study. (PHSKC STD Clinic providers (ie. ARNP, PA and/or RN) routinely call patients with positive tests results, thus these clinical staff will tell patients about their eligibility for the study, however, PHSKC STD Clinic providers will not conduct formal study eligibility screening nor will they elaborate about study procedures (see script, Appendix A).) If patients indicate interest, the PHSKC provider will make an appointment for the patient with the study coordinator (for more information about the study, eligibility screening and enrollment procedures) and study clinician (for testing and treatment procedures) at their earliest convenience, ideally the next day.
2) Some patient may not receive a phone call with test results prior to coming in for treatment (i.e. they see results electronically on MyChart [patient portal] or they were not informed of the study via a test results phone call, or were referred by an outside clinic to the PHSKC STD Clinic for treatment as their provider does not stock the recommended therapy in their office). Patients who arrive at the PHSKC STD Clinic for treatment of pharyngeal gonorrhea and have not yet been informed about the study will be told by the triage RN or their clinician that they may be eligible and inquire about their interest. If they indicate any interest in the study, they will be referred to the study coordinator and/or study clinician at that time.

Recruitment outside of PHSKC STD Clinic:

We will advertise the Gentamicin Study with high diagnosing providers in the community. Through a large CDC surveillance grant, SURRG, we already have a relationship with many of these providers: Madison Clinic, Dr. Shalit's office, and Capitol Hill Medical Group. We will inform providers in these clinics about the study and provide study info cards (see Appendix B). When they diagnose a patient with untreated pharyngeal gonorrhea, they will inform the patient that they may be eligible for this study, and if the patient is interested, either the provider or patient can call the study coordinator to set up an appointment.

Retention of subjects:

This study consists of two study visits. Once enrolled, subjects need to return 4-7 days (+/- 1day) for a test of cure (TOC) visit. At their enrollment visit, the subject will set up a TOC visit with either the study coordinator or study clinician, hand them an appointment reminder card (see Appendix C). Less than 24 hours prior to the appointment, the study coordinator will call the patient with an appointment reminder call. If the subject does not show up for their appointment, the study coordinator will call them that day to set up another TOC visit appointment.

Study Procedures

Screening & Enrollment

Pre-screening will occur by the PHSKC STD Clinic RN who reviews all patients charts to determine which services and provider(s) are warranted. Patients with untreated pharyngeal gonorrhea will be referred to the study coordinator for screening. Screening will occur in person by the study coordinator, study clinician or PI. We will use a standardized screening eligibility form (see Appendix D) to determine patient's eligibility. Since eligibility hinges on a diagnosis of untreated pharyngeal gonorrhea, for PHSKC STD Clinic patients, we will ask the IRB for a waiver of consent to be able to pre-screen charts to access the subject's medical record to confirm diagnosis prior to approaching the patient. All other eligibility criteria will be based on self-report. Patient will be excluded if they meet any of the following criteria:

- Age less than 16 years
- Receipt of antibiotics in ≤30 days
- Known allergy to any aminoglycoside
- History of renal disease (including diagnosis of solitary kidney, chronic renal insufficiency, renal cell carcinoma etc),
- Use of concurrent nephrotoxic drugs or muscle relaxants
- History of diabetes
- History of hearing loss or tinnitus
- Concurrent infection with syphilis or chlamydia
- Pregnancy and/or nursing (we will screen women not using highly active birth control, i.e. OCPs, IUD or nexplanon, who are more than 28 days from their last menstrual period)
- Unable to return for a follow-up visit 4-7 days (+/- 1 day).
- Study team's discretion

Once it has been determined that the patient is eligible for and interested in the study, the study team member will go through the informed consent with the patient in detail. After the subject has signed the informed consent, formal study procedures will begin.

1. *Intake & Data Entry Form*:
   The study coordinator or clinician will fill out the first section of the data entry form (see Appendix E) which includes subject contact information, demographic information (include sex assigned at birth, current gender identity, sexual orientation, race/ethnicity), HIV status, anatomic sites of infection, height, weight, concurrent medications and medical history.

2. *Diagnostics and Baseline Clinical Data*:

The study clinician will obtain specimens for culture from all anatomic sites that were screen positive for gonorrhea. This will include the pharynx (by inclusion criteria) and possibly the rectum, urethra and endocervix, per routine clinical care. A pharyngeal specimen is taken by gently touching a swab to the posterior aspect of the throat and moving it up and down, and over the tonsils for 5-10 seconds. Rectal swabs involved inserting a small q-tip like swab in the rectum approximately 3 cm and twirling it around approximately five times. To obtain a urethral swabs, clinician use a smaller swab and insert it approximately 0.5 – 1 cm into the urethra and twirl 1-2 times. Endocervical specimen collection requires a pelvic exam with speculum. Once the cervical os is visualized the clinician inserts the swab into the cervical os approximately 1 cm and spins it about 5 times for an adequate sample. Swabs for culture will be plated directly onto Thayer-Martin media and placed in a candle (CO_2) jar. Plates will be transported to the PHSKC lab daily, those that grow *N. gonorrhoeae* will be sent to the Neisseria Reference Laboratory (NRL) for antimicrobial susceptibility testing. The procedures described above are all part of routine clinical care.

The study clinician will also obtain blood to check a baseline creatinine to measure renal toxicity. This blood draw will occur with the PK blood draw 30, 45, or 60 minutes after dosing to avoid obtaining blood from the subject more than once.

3. *Treatment*:
   Once all diagnostic procedures have been completed, the study clinician will administer 360 mg gentamicin IM. The gentamicin injections will be given as 2 injections of 4 mL each, one in each gluteus maximus. The time of injection will be recorded

4. *Pharmacokinetics data:*
   Subjects will be assigned post-injection blood draw at either 30 minutes, 45 minutes or 60 minutes after the gentamicin injection in a sequential fashion, meaning that participant #1 will have a 30 minute draw,#2 a 45 minute and #3 at 60 minute. This one green top tube of blood will be tested for gentamicin concentration as part of Objective 2 and Creatinine.

5. *Counseling*:
   The study clinician will counsel the subject to avoid sexual activity, and most specifically performing oral sex, deep kissing, and oral-anal sex until they return for the TOC visit.

Test of Cure Visit

Subjects will return to the PHSKC STD Clinic to meet with the study clinician 4-7 days (+/- 1 day) after their enrollment visit. The study coordinator will call the subject the day prior to the visit as a reminder. At the TOC visit:

1. *Diagnostics*:
   The study clinician will obtain a pharyngeal culture for *N. gonorrhoeae* and a blood draw for a follow-up creatinine. (Specimen processing per above). If the culture is positive for *N. gonorrhoeae*, the subject will be called to return for standard of care treatment – Ceftriaxone 250mg IM plus Azithromycin 1g orally once.

2. *Behavior and Symptom screen*:
   The study clinician will administer a standardized symptom screening form (see Appendix F) to assess for gentamicin side effects and tolerability. The form will also ask about sexual activity, including kissing, in the time period between treatment and TOC.

Retention Plan

The study coordinator will attempt to schedule the TOC visit at 4 days after the enrollment visit so that if the subject fails to show to that visit, there are still opportunities to reschedule the TOC visit. Additionally, although the subject will be compensated for their time in the study, the will not receive compensation until they return for the TOC visit. This is an incentive for them to keep their follow-up visit.

Data Sources and Data Management

PHSKC STD Clinic Medical Record: We will use gonorrhea test results that occurred prior to enrollment visit from the STD clinic medical record for inclusion criteria. This data will be extracted by chart review during the enrollment visit using subject name and DOB as identifiers. The information will then be entered using a subject ID in the study database. We will also use demographic and sexual behavior data from the medical record.

Neisseria Reference Laboratory: The NRL will provide the antimicrobial susceptibility data for the *N. gonorrhoeae* isolates recovered at both enrollment and TOC visit. The culture specimens are clinical specimens and full antimicrobial susceptibility testing (AST; this includes, beta-lactamase, penicillin, tetracycline, ciprofloxacin, cefixime, ceftriaxone, azithromycin and gentamicin) is done on all isolates. However, AST is not routinely reported to the clinic. Thus, in order to obtain this data, we will submit a list of names and MRN to Dr. Olusegun Soge, the Director of the NRL, to pull the data for us. All personal health information (PHI) will be transferred electronically using secure file transfer technology.

Data Entry Form*:* The study coordinator and study clinician will record key data points on a paper data entry form which will be transcribed into electronic data entry form in RedCap. Data to be collected will come from both the STD Clinic medical record and patient interview. Data points will include: date of visit, gender identity, sex assigned at birth, age, race, height, weight, site of infections pre-enrollment and type of test used to diagnosis; time of administration of gentamicin, time of gentamicin concentration blood draw; results of treatment visit testing (culture results at each site); date of TOC visit, symptoms since treatment; sexual activity since treatment (including kissing, oral-anal, oral-penile, anal-penile, penile-vaginal etc); anatomic sites tested by culture and results; repeat treatment needed etc (see Appendices E and F)

UW Lab Medicine Research Testing Services: RTS will test our blood specimens for pre- and post- treatment Creatinine and gentamicin levels. We will give the specimens to them with study id's and dates on them, and they will provide the data via an excel file with only study ID and results.

Data Management

We will use RedCap to collect and store all study data. Data can be exported from RedCap as a Stata database, which will be used for analysis. Upon export from RedCap, we will remove identifiers except for subject ID and maintain a separate database to link subject identifiers/contact info to subject ID.

Study Confidentiality and Security

We will use study IDs to label data items that only pertain to the study – i.e. data entry form, aliquots for gentamicin level and creatinine. Specimens that are also clinical specimen, will be labeled with the patient's clinic label. Data will be obtained from the medical record and entered into the data entry form which will only be labeled with the Study ID. We will maintain a separate data base in RedCap that links the patient study ID and PHI. RedCap is 21 CFR part 11 compliant. We will destroy the link between the patient's identifying data and study ID after the study has been published or by December 31, 2021, whichever is sooner.

Laboratory Processing

Gentamicin & Creatinine levels:

Draw blood for enrollment day gentamicin level and baseline creatinine at 30, 45 or 60 minutes (timing to be pre-determined by sequential participants; i.e. participant 1 is drawn at 30 minutes, 2 at 45 minutes and 3 at 60 minutes) following administration of gentamicin 360mg IM. Use green top (sodium heparin) tube. Centrifuge and aliquot ~ 2mL into 5ml Sarstedt polypropylene tube. These will be transferred to freezer within 4 hours and frozen at -80 degrees celsius until transferred to UW RTS Lab in monthly batches. UW RTS determines peak Genatmicin levels using Enzyme Immunoassay Technique and Creatinine by Spectrophotometric technique.

N. gonorrhoeae *cultures and Antimicrobial Susceptibility Testing*

All gonorrhea cultures are clinical specimens. The PHSKC STD Clinic's policy is to collect specimens for *N. gonorrhoeae* culture from persons with 1) a NAAT positive test who are not yet treated (i.e. most of our subjects); 2) persons with symptoms of urethritis, or cervicitis; 3) persons who are a contact to gonorrhea, at all exposed anatomic sites; and 4) persons who need a test of cure (TOC). TOC are done for any person with a cefixime, ceftriaxone or azithromycin alert value MIC, or have pharyngeal gonorrhea treated with a non-ceftriaxone containing regimen (i.e. study subjects).

Clinical gonorrhea cultures are first sent to the PHSKC Laboratory for culture and identification, and *N. gonorrhoeae* isolates are then sent to the University of Washington's Neisseria Reference Laboratory (NRL) for antimicrobial susceptibility testing (AST) for surveillance purposes. Once a culture is identified as positive growth for gonorrhea, a report is made into the PHSKC STD Clinic medical record.

The NRL first determines cefixime, ceftriaxone and azithromycin MIC by the etest method initially for prompt clinical action. Then, gonorrhea isolates undergo AST according to the Clinical and Laboratory Standards Institute (CLSI) recommended agar dilution method for further surveillance.[13] AST testing includes: Penicillin (PEN), Tetracycline (TET), Cefixime (CFM), Ceftriaxone (CRO), Ciprofloxacin (CIP), Azithromycin (AZM), and Gentamicin (GEN). We used CLSI[15] breakpoints to interpret MICs for penicillin (≥2.0μg/ml), tetracycline (≥2.0μg/ml), spectinomycin (≥128.0μg/ml), and ciprofloxacin (≥1.0μg/ml),[15] and used GISP alert values for azithromycin (≥2.0μg/ml), cefixime (≥0.25μg/ml), and ceftriaxone (≥0.125μg/ml) as CLSI has not established breakpoints for these antimicrobial agents.[12] Neither CLSI nor CDC have published breakpoints for gentamicin however, other institutes have used the following breakpoints that we will follow in this study: Resistant is ≥32μg/ml; Intermediate susceptible 8-16 μg/ml, and Susceptible ≤4 μg/ml. Full AST results will be reported to the PI.

Data Safety and Monitoring Plan, Resolving & reporting Protocol Deviations, AEs and Unanticipated problems

OVERSIGHT RESPONSIBILITIES

Day-to-day oversight of the trial is provided by the Principal Investigator (PI), Dr. Barbee, along with Dr. Golden (co-I). Dr. Barbee assures that informed consent is obtained prior to performing any research procedures, that all subjects meet eligibility criteria, and that the study is conducted according to the IRB-approved research plan. Drs. Barbee and Golden review all study data and any adverse events (AEs) real-time, and report all SAEs to the Medical Monitor and IRB according to the approved DSMP.

Medical Monitor: Monitoring for the study will be provided by a volunteer Medical Monitor, Dr. Ann Collier, Professor of Medicine in the Division of Allergy and Infectious Diseases, who has over 25 years of experience with clinical trials, but is not directly related to the study team.

MONITORING PROCEDURES

The Medical Monitor, Dr. Collier, will review study conduct including efficacy and renal toxicity and AEs in aggregate on at a single interim analysis after 25 subjects, and at the end of the study. The Medical Monitor will review serious adverse events (SAEs), hearing loss, and serious drug allergies in real-time. Study data are provided to the Medical Monitor prior to the interim and final analysis. Data reports are prepared by the PI, Dr. Barbee.

MONITORING REPORT

The Medical Monitor, Dr. Collier, will provide a written report to the study team with recommendations for study modification, study continuation/discontinuation as relevant. The study team is responsible for forwarding the report to the IRB.

COLLECTION AND REPORTING OF SAEs AND AEs

For this study, the following standard AE definitions are used:

Adverse event: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure.

Serious Adverse Event: Any AE that results in any of the following outcomes:

- Death
- Life-threatening
- Event requiring inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity

AEs are graded according to the following scale:

> **Mild:** An experience that is transient, & requires no special treatment or intervention. The experience does not generally interfere with usual daily activities. This includes transient laboratory test alterations.
>
> **Moderate:** An experience that is alleviated with simple therapeutic treatments. The experience impacts usual daily activities. Includes laboratory test alterations indicating injury, but without long-term risk.
>
> **Severe:** An experience that requires therapeutic intervention. The experience interrupts usual daily activities. If hospitalization (or prolongation of hospitalization) is required for treatment it becomes an SAE.

The study uses the following AE attribution:

> **Not related:** The AE is clearly not related to the study procedures (i.e., another cause of the event is most plausible and/or a clinically plausible temporal sequence is inconsistent with the onset of the event).
>
> **Possibly related:** An event that follows a reasonable temporal sequence from the initiation of study procedures, but that could readily have been produced by a number of other factors.
>
> **Related:** The AE is clearly related to the study procedures.

AEs are identified at the TOC visit using the symptom screen, and with Creatinine monitoring.

SAEs and specific procedure-associated AEs are reported to the Medical Monitor within 24 hours. In addition, all AEs are reported according to the University of Washington AE reporting guidelines.

MANAGEMENT OF RISKS TO SUBJECTS

<u>Expected AEs</u>

Expected AEs associated with gentamicin include:

- Pain at injection site
- Bruising at injection site

<u>AE Management</u>

- Warm compresses as needed
- Tylenol as needed

DATA ANALYSIS PLANS

The Medical Monitor will conduct interim analyses after we have primary outcome results for 10 and 25 subjects. (Enrollment will pause until analyses can be conducted). Stopping rules include the primary end point (i.e. pharyngeal cure rate), and adverse events. If after 10 and 25 subjects, the cure rate is ≤44% (99% CI 17% – 74%; i.e. 11 persons of 25 persons with a positive culture on treatment date fail treatment), we will stop the study. We chose these numbers using the O'Brien-Fleming method of alpha spending for multiple analyses, assuming and alpha of 0.003 for the first analysis and a alpha of 0.049 for the final analysis. Using the 99% CI obtained with 11/25 treatment failures, the upper bound is less than 75%. (Our hypothesis is that gentamicin alone will be 80% effective for pharyngeal gonorrhea, with the goal to use it in combination with a second agent). Alternatively, if at interim analysis more than 20% of subjects (99% CI 20% - 46%) have a greater than 40% increase in their creatinine from baseline, we will halt the study

Data Analysis

The proposed study aims to evaluate the efficacy of a single intramuscular (IM) dose of 360 mg of gentamicin in the treatment of pharyngeal gonorrhea. Secondary objectives include evaluating the renal safety and tolerability of 360mg of gentamicin, *documenting peak serum gentamicin levels by subject weight,* and estimating the ideal pharmacodynamic criterion using a combination of peak gentamicin concentration levels compared to MIC and treatment outcomes. *In exploratory analyses*, we will attempt to determine whether gentamicin-monotherapy induces antimicrobial resistance among treatment failures. The specific aims are:

1) Determine the proportion of persons whose pharyngeal gonococcal infections are cured with a single dose of 360mg gentamicin intramuscularly alone.
2) Evaluate the renal safety and tolerability of 360mg IM of gentamicin.
3) Document mean peak gentamicin levels following 360mg IM of gentamicin stratified by weight.
4) Estimate the best pharmacodynamics criterion (i.e. peak/MIC ratio) for pharyngeal gonorrhea treated with gentamicin *using individual* and mean peak gentamicin levels and NG isolate MIC.
5) Among treatment failures, *conduct exploratory analyses* comparing pre- and post-treatment MIC for evidence of induced resistance.

Analyses
The primary endpoint is the pharyngeal cure rate – that is, the proportion of subjects who return for TOC who clear their infections as evidenced by a negative culture 4-7 (+/-1) days after treatment. The cure rate will be calculated as the number of cured subjects divided by the total number of subjects who received treatment and returned for follow-up testing in a modified an intention to treat (mITT) analysis. We will also calculate the 95% CI for this proportion.

$$\textit{Cure rate} = \frac{\textit{number of subjects with negative pharyngeal culture at TOC}}{\textit{number of subjects with + throat test at enrollment \& received gentamicin}}$$

Persons who test negative for NG at their enrollment visit (i.e. infection cleared between initial clinical screening test and enrollment) will be excluded from analysis. Treatment failure will be defined as a positive culture 4-7 (+/-1) days after treatment.

For our second objective, we will obtain pre- and post-treatment serum creatinine. An elevation of >40% will be considered significant. We will evaluate subject's tolerability (Obj. 2) using a standardized symptom questionnaire (see Appendix F) conducted at the TOC visit. The symptoms questioned will include, but not be limited to, rash, tinnitus, decreased hearing, nausea, vomiting, diarrhea, headaches, and decreased appetite. There will also be a section for free response.

Our third objective is to document the pharmacokinetics of 360mg of IM gentamicin by weight. We will obtain serum gentamicin levels at 30, 45 and 60minutes post dosing based on previous studies that estimated that IM gentamicin peaks between 30-60 minutes post-dose.

We will stratify the results by time post-dose, as well as by subject weight in kilograms (categorized).

In order to estimate the ideal PK/PD criterion, we will compare the MIC of cures to treatment failures using a stratified approach (Table 2), and we will correlate this data with individual peak gentamicin level in order to estimate the ideal pharmacodynamic (PD) criterion (Obj. 4). As these results are ratios and may have a skewed distribution, we will log transform them and calculate a geometric mean. We anticipate that the vast majority of isolates will have a gentamicin MIC ≤8 µg/mL, and that most subjects' peak concentration will be between 16-27 µg/mL giving a ratio of 2-4. Variations in subjects' weight or the gentamicin MICs might alter those predictions. Assuming an 80% cure rate (n=40), and a mean ratio of 3, with a standard deviation of 1, we anticipate a 95% CI of the peak/MIC ratio of 2.7 – 3.3, suggesting that our sample size is adequate to calculate this ratio.

For persons who fail treatment we will also compare the pre- and post-treatment genatmicin MIC to estimate whether single dose gentamicin therapy can "induce" resistance (Obj. 5) in an exploratory analysis. We will consider an increase in the MIC by two doubling-dilutions (i.e. from MIC of 2 to 8 µg/mL, or 8 to 32 µg/mL) to represent induced resistance, and we will calculate the proportion of isolates among those that fail treatment that demonstrate induced resistance following receipt of gentamicin. Should we see a signal for induced resistance, we plan to apply for additional funding to conduct whole genome sequencing to look for genetic determinants of resistance.

Table 2: Proposed Description of Primary Study Findings

GENTAMICIN MIC (µg/mL)	Cure (N=XX)	Treatment Failures (N=XX)	Gentamicin Serum Level (Mean, Range)	Peak/MIC Ratio (Mean, Range)
≤1				
2				
4				
8				
16				
32				
≥64				

As a means to preserve funding and ensure safety, we will conduct interim analyses after we have primary outcome results for 10 and 25 subjects. (Enrollment will pause until analyses can be conducted). Stopping rules include the primary end point (i.e. pharyngeal cure rate), and adverse events. If after 25 subjects, the cure rate is ≤44% (99% CI 17% – 74%; i.e. 11 persons of 25 persons with a positive culture on treatment date fail treatment), we will stop the study. We chose these numbers using the O'Brien-Fleming method of alpha spending for multiple analyses, assuming and alpha of 0.003 for the first analysis and an alpha of 0.049 for the final analysis. Using the 99% CI obtained with 11/25 treatment failures, the upper bound is less than 75%. Alternatively, if at interim analysis 32% or more of subjects (99% CI 9.4% - 63%) have a greater than 40% increase in their creatinine from baseline, we will halt the study.

Appendices

A. Clinician Information and Referral Script

B. Study Info Card

C. Appointment Reminder Card

D. Screening Eligibility Form

E. Enrollment visit data entry form

F. TOC visit data entry form

APPENDIX A

Clinician Informational and Referral Script for The Gent Study

RN or Clinician: You have gonorrhea in your throat. We are conducting a study about a new medication for pharyngeal gonorrhea. Are you interested in learning more?

Patient: No

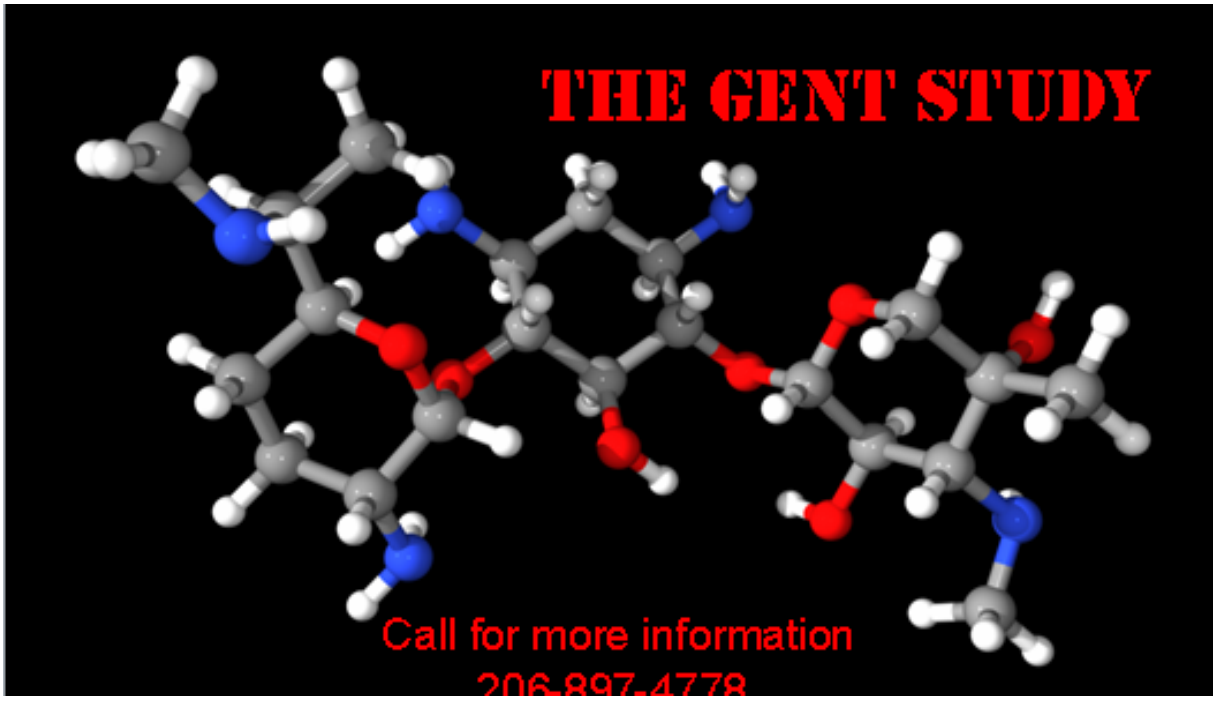
End of discussion

OR

Patient: Yes. What does it entail?

RN or Clinician: Great. I can just give you an overview, but instead of our usual treatment, you will get a different injection called gentamicin. Then you'll have to come back in about a week for a visit to make sure that you cleared the infection. You will earn up to $XXX for participation. If you are still interested, I will call the study coordinator to come get you started.

APPENDIX B



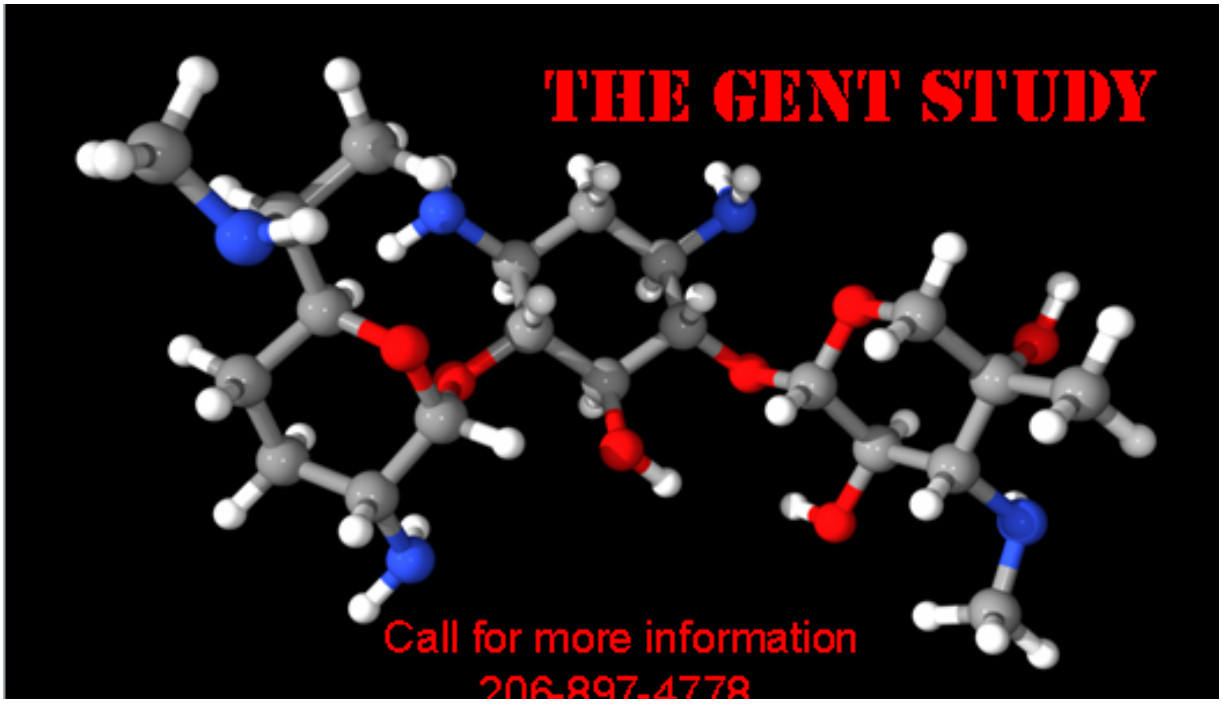
The Gent Study

University of Washington

The Gent Study aims to test an established medication, gentamicin, for the treatment of gonorrhea of the throat. Participants will receive the medicine at the PHSKC STD Clinic and return for a repeat test 4 - 7 days later. Compensation up to $100, depending on completion of study procedures. *To qualify, you must:*

1) Have pharyngeal gonorrhea and not yet been treated
2) Not have diabetes, kidney disease or be on certain medication
3) Have not received antibiotics in the past 30 days.

APPENDIX C



The Gent Study
University of Washington

Appointment Reminder

You have an appointment on:

_____

With _____

At the PHSKC STD Clinic, located on the 11th Floor of the Ninth and Jefferson Building on Harborview's campus.
908 Jefferson St, 11th Floor

Please call ahead if you need to reschedule: 206.897.4778

APPENDIX D

Eligibility Screening Form

From Pre-screening Chart Review:

Pharyngeal Gonorrhea Positive? Y or N
Date of Pharyngeal gonorrhea test: _____
Chlamydial infection? Y or N (*If yes, patient not eligible)*
Syphilis infection? Y or N (*If yes, patient not elgibile)*

Researcher: Is it alright with you if I ask you some questions in order to assess your eligibility for the study? Some of the questions are personal in nature.

☐Yes ☐No

If the potential subject answers yes above, complete the following questions. Please check the boxes at left to indicate the question has been asked. Answer the question to the Right. Eligibility criteria are in italics following the question. You may stop the interview if, based on a single question, they do not meet eligibility criteria

☐ Are you 16 years old or older? *Yes*

☐ Do you speak and read in English? *Yes*

☐Have you taken any antibiotics (e.g. Keflex, bactrim, penicillin etc) in the past 30 days? Y or N *(If yes, they are not eligible)*

☐Are you allergic to any antibiotics? If so, which ones? _____ *(Allergy to gentamicin, spectinomycin, kanamycin, netilmycin, streptomycin, amikacin or tobramycin is an exclusion criteria.)*

☐Do you have kidney disease? Kidney diseases include renal failure (including dialysis); diagnosis of solitary kidney, renal cell carcinoma etc. (*Report of any renal disease is cause for exclusion.)*

☐ Do you have Diabetes? Y or N *(Patients with diabetes are not eligible).*

☐ Do you have a history of hearing loss or tinnitus? Y or N (*Patients with these symptoms are ineligible).*

☐What medications do you take on a regular basis? *(Potentially nephrotoxic drugs such as, Lisinopril, lithium and muscle relaxants, are cause for exclusion. If questions about certain drugs, call study PI.)*

List Here:

☐Are you able to return to the PHSKC STD Clinic in 4-7 days for a TOC visit? (*Yes*)

Study personnel:

☐In your estimation is the potential subject able to comply with the study procedures? *Yes*

Study personnel conducting screening: _____

□□ If female-bodied patient, do urine pregnancy test. Record result here: Positive Negative Ineligible if pregnant.

APPENDIX E

Study ID:____

The Gent Study – Enrollment Data Entry Form

Date: _____ Age: _____ Race/ethnicity: _____

Current Gender: [ ] Male [ ] Female [ ] Trans MTF [ ] Trans FTM [ ] NB/GQ

Sex Assigned at Birth: [ ] Male [ ] Female

Gender of Sex Partners: [ ] Male [ ] Female [ ] Trans MTF [ ] Trans FTM [ ] NB/GQ

HIV Status: [ ] Positive [ ] Negative Number of oral sex partners <2 months: __

Date of last oral sex exposure: _____

Anatomic Sites of Gonorrhea Infection and Date and Test Type:

Pharynx: Test Date: _____ **NAAT** [ ] Yes [ ] No **Culture** [ ] Yes [ ] No

NAAT Type: _____

Rectum: Test Date: _____ **NAAT** [ ] Yes [ ] No **Culture** [ ] Yes [ ] No

NAAT Type: _____

Urethra: Test Date: _____ **NAAT** [ ] Yes [ ] No **Culture** [ ] Yes [ ] No

NAAT Type: _____

Cervix/Vagina: Test Date: _____ **NAAT** [ ] Yes [ ] No **Culture** [ ] Yes [ ] No

NAAT Type: _____

Height: _____ cm Weight: _____ kg

Medical History:

_____

_____

Current Medications (and doses):_____

Date & Time of Administration of Gentamicin: _____

Date & Time of Blood Draw for Gent PK and Cr: _____

Immediate reactions (if any):

APPENDIX F

Study ID: _____

The Gent Study – TOC Visit Data Entry Form

TOC Visit Date: _____ Days since Tx: _____

Symptoms since Tx:

Y or N Headache (if yes, characterize):_____

Y or N Vision Changes (if yes, characterize):_____

Y or N Changes in Hearing (if yes, characterize):_____

Y or N Tinnitus (if yes, characterize):_____

Y or N Urinary Symptoms (if yes, characterize):_____

Y or N Nausea or Vomiting (if yes, characterize):_____

Y or N Diarrhea (if yes, characterize):_____

Y or N Constipation (if yes, characterize):_____

Y or N Rash (if yes, characterize):_____

Y or N Other (if yes, characterize):_____

Sexual Activity Since Treatment:

1) Have you been intimate with any partners since treatment? Y or N
   (Intimacy includes kissing, oral-anal sex, oral-penile sex, anal-penile sex, penile-vaginal sex)

2) Were any of your partners since treatment the same as someone you were intimate with in the 2 months prior to your pharyngeal gonorrhea diagnosis? Y or N

If Yes to #1 above, ask the following questions:

3) How many days did you deep kiss a partner since treatment? _____
4) How many partners did you deep kiss since treatment? _____
5) How many times did you perform oral sex on a penis since treatment? _____

6) How many male-bodied persons did you give oral sex to since treatment? _____
7) How many times did you rim a partner since treatment? _____
8) How many partners did you rim since treatment? _____
9) How many times did you top (i.e. insertive anal intercourse) a partner since treatment? _____
10) How many partners did you top since treatment? _____
11) How many times did you bottom (i.e. have receptive anal intercourse) for a partner since treatment? ____
12) For how many partners did you bottom since treatment? _____
13) How many times did you have vaginal sex with a partner since treatment? _____
14) How many partners did you have vaginal sex with since treatment? _____

TOC Testing Conducted and Results:

Pharynx: NAAT Result [ ] Positive [ ] Negative

Culture Done [ ] Yes [ ] No

Culture Results [ ] Positive [ ] Negative

AST results (in mcg/mL): PCN: ____ TET: ____ CIP: ____

CFX: ____ CTX: ____ AZM: ___ GENT: ____

Rectum: NAAT Result [ ] Positive [ ] Negative

Culture Done [ ] Yes [ ] No

Culture Results [ ] Positive [ ] Negative

AST results (in mcg/mL): PCN: ____ TET: ____ CIP: ____

CFX: ____ CTX: ____ AZM: ___ GENT: ____

Urethra: NAAT Result [ ] Positive [ ] Negative

Culture Done [ ] Yes [ ] No

Culture Results [ ] Positive [ ] Negative

AST results (in mcg/mL): PCN: ____ TET: ____ CIP: ____

CFX: ____ CTX: ____ AZM: ___ GENT: ____

Cervix: NAAT Result [ ] Positive [ ] Negative

Culture Done [ ] Yes [ ] No

Culture Results [ ] Positive [ ] Negative

AST results (in mcg/mL): PCN: ____ TET: ____ CIP: ____

CFX: ____ CTX: ____ AZM: ___ GENT: ____

Repeat Treatment needed? Y or N

Date of Repeat Treatment: _____

Repeat treatment given: [ ] Ceftriaxone 250mg IM + Azithromycin 1g PO

[ ] Ceftriaxone 250mg IM + Doxycycline 100mg PO BID

[ ] Other: _____

APPENDIX G

Identifying Data (to be maintained separately from study data)

Name: _____

Date of birth: _____

PHSKC STD Clinic MRN: _____

Cell Phone Number: _____

Email: _____

Address: _____